INFLUENCES OF FRAGMENTATION ON FLUVIAL-SPECIALIST BLACK BASS SPECIES

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Abstract:

North America's fluvial fish fauna are becoming increasingly imperiled, primarily by habitat degradation, non-native species invasions, and fragmentation. The present study was conducted to understand how these conservation threats have affected native, fluvial-specialist black bass (genus *Micropterus*) species that support popular sport fisheries and that can be used as umbrella species for the conservation of other aquatic organisms. Species distribution models illustrated that Shoal Bass (*M. cataractae*) were potentially distributed across up to 84% of the available stream length in their native basin, but that fragmentation by dams and large impoundments, as well as a potential asymmetric relationship with non-native congeners, has contributed to range loss. A range-wide genetic survey demonstrated that although the Shoal Bass has been described as potamodromous, appreciable population structure exists. Five distinct genetic clusters were recovered at the uppermost hierarchical level, each generally corresponding to natural isolating mechanisms (e.g., the Fall Line). Some substructure was detected within these clusters, which was likely related to recent fragmentation (i.e., impoundments) and variable recruitment. Finer-scale case studies of the conservation-genetic influences of impoundments and non-native congener fisheries revealed that impoundments generate propagule pressure that encourages invasion and introgression of non-native alleles into native black bass populations inhabiting upstream tributaries, but whether impoundments serve as barriers to gene flow for native populations was somewhat unclear. Quantifying local-scale population dynamics of Shoal Bass inhabiting three isolated tributaries of the upper Chattahoochee River basin revealed that these populations grew slower, lived longer, and experienced lower annual mortality than other studied populations, which may be adaptations to variable recruitment or lower over-winter survival of age-0 fish. The Big Creek population appears at risk of extirpation because of its isolated nature, low numbers of adults, and greater variation in recruitment. Overall, results provide novel insights into the factors influencing range loss, a framework for management units to conserve existing genetic diversity, a characterization of non-native invasion and hybridization in impoundment tributaries, and quantified population dynamics of several isolated Shoal Bass populations inhabiting the northern extent of the species' range.

TABLE OF CONTENTS

CHAPTER I

POTENTIAL AND RESTRICTED GEOGRAPHIC DISTRIBUTION ESTIMATES: EXAMINING FACTORS ASSOCIATED WITH RANGE LOSS OF A FLUVIAL-SPECIALIST FISH SPECIES

Abstract

Fluvial fishes face increased imperilment from a number of anthropogenic activities, including land-use and water-use activities, introduction of non-native species, and fragmentation of fluvial habitats. Species distribution models (SDMs) can help disentangle numerous biologically-relevant factors to discern those contributing to range declines. The Shoal Bass (*Micropterus cataractae*) is a fluvial-specialist species suffering from continual range loss; however, the anthropogenic factors contributing most to range loss are unknown. We estimated this species' potential distribution with a presence-background modeling approach using program Maxent and produced a series of current restricted distributions based on a variety of fragmentation stressors (e.g., dams, impoundments), non-native species, and land-use activities thought to be influencing Shoal Bass range loss. The potential model estimated environmental suitability for Shoal Bass throughout much of their native basin. Restricted-range models suggested that fragmentation and non-native congeners have contributed most to range declines. Our results also revealed that as fragmentation intensity increased, the apparent relationship between Shoal Bass and non-native congener species changed from coexistence to asymmetry in favor of non-native congeners. Land cover appeared to contribute less to range loss, but was most influential to Shoal Bass probability of suitability at segment-specific stream buffers rather than at a watershed scale. Results are relevant to Shoal Bass conservation by directing actions toward those stressors most influencing Shoal Bass probability of suitability. For example, response curves and distribution estimates can be used as biological criteria for barrier removal prioritization. Similar distribution modeling efforts may prove useful for prioritizing conservation efforts for other imperiled fluvial fishes throughout the globe.

Introduction

Freshwater fishes are a diverse group with an increasingly jeopardized future, particularly for fluvial species that require free-flowing fluvial habitats to persist. Of approximately 700 freshwater and diadromous fishes in North America, 39% are considered imperiled (Jelks *et al.*, 2008) and that rate is expected to steadily increase (Ricciardi & Rasmussen, 1999; Burkhead, 2012). The imperilment of native freshwater fishes is particularly acute in areas of high endemism and biodiversity, like the southeastern U.S. (Warren Jr. & Burr, 1994). Although a myriad of factors are involved in the widespread imperilment of native fluvial fishes, there are several major contributors: anthropogenic land use, flow regime alterations, introductions of non-native species, and fragmentation of populations (Allendorf & Lundquist, 2003; Nilsson, 2005; Jelks *et al.*, 2008).

Anthropogenic activities within the riverine landscape have cascading effects on aquatic ecosystems and fluvial fishes (Fausch *et al.*, 2002). Agricultural and urban land-use practices lead to destabilization of stream banks, warmer water temperatures, and increases in channelization, sediment loads, and pollution (Allan, 2004). Water withdrawals to support these land-use practices alter natural flow regimes that regulate ecological processes in streams (Poff *et al.*, 2007). Additionally, construction of dams and impoundments alter stream habitats and flow regimes. Upstream of dams, stream habitats are converted into impoundments that further facilitate water removal and alter stream fish communities (Taylor *et al.*, 2001; Guenther & Spacie, 2006). Alterations downstream of dams include changes in the natural flow regime (Poff *et al.*, 2007), increases in bank erosion and bed scour (Kondolf, 1997), and changes in the thermal regime (Olden & Naiman, 2010). Native fluvial fishes that are sensitive to these alterations often experience local extirpations or extinction (Allan, 2004; Nilsson, 2005; Poff & Zimmerman, 2010).

In altered stream ecosystems, non-native and invasive species have increased opportunity to expand their range and negatively affect native fluvial fishes. Non-native fishes often use altered stream habitats and impoundments as invasion vectors within river systems (Bunn & Arthington, 2002; Johnson *et al.*, 2008). As non-native fishes invade, they threaten native fluvial fishes through predation, competition, hybridization, disease transmission, and habitat modification (Gozlan *et al.*, 2010). Habitat alteration and non-native invasions result in homogenized stream fish communities, as native fluvial fishes are lost and replaced by a few, but relatively dominant, non-native tolerant species (Rahel, 2002; Freeman & Marcinek, 2006).

Fluvial fish populations are fragmented by the severing or interruption of the longitudinal, upstream-downstream connectivity within stream networks, increasing their vulnerability to local extinctions. Causes of fragmentation vary with location and species, but can include physical barriers like road crossings (Warren Jr. & Pardew, 1998), stream desiccation (Perkin & Gido, 2011), dams (Porto *et al.*, 1999; Nilsson, 2005), and less obvious non-physical barriers like impounded waters, thermal gradients, and pollution (Pringle, 1997; Noatch & Suski, 2012). Fragmented populations are more susceptible to anthropogenic disturbance, demographic stochasticity (Wilcox & Murphy, 1985), inbreeding depression (Frankham, 1995), and interactions with non-native species (Sakai *et al.*, 2001). The effects of fragmentation in river systems can be characterized at multiple spatial scales and in both upstream and downstream directions (Cooper *et al.*, 2016). Although effects of fragmentation vary across study areas and species, fragmentation is regularly identified as a driver of imperilment of fluvial fish faunas worldwide (Nilsson, 2005).

Fluvial black bass species (*Micropterus* spp.) are facing threats emblematic of fluvial fishes worldwide (Birdsong *et al.*, 2010; Tringali *et al.*, 2015). The Shoal Bass (*M. cataractae*) is one of these species, considered vulnerable to extinction because of habitat loss and introductions of non-native species (Jelks *et al.*, 2008). Shoal Bass are endemic fluvial specialists of the Apalachicola-Chattahoochee-Flint (ACF) Basin of Georgia, Florida, and Alabama (Williams & Burgess, 1999) that are typically encountered in shoal habitats, which are areas of increased flow velocity with exposed bedrock and boulder substrates (Taylor & Peterson, 2014). Adults often display migratory behavior during the spawning season (Sammons, 2015), and dense spawning aggregations appear to form at specific complexes of shoal habitat (Sammons, 2015; Taylor & Peterson, 2015), suggesting that shoal habitat connectivity is important for long-term population persistence. The Shoal Bass occupies mainstem rivers and larger tributaries of the ACF Basin, however its exact distribution remains unknown because the species was cryptic among sympatric congeners until the late 1960s, not formally described until 1999, and inhabits areas that are inherently difficult to sample (Williams & Burgess, 1999; Taylor & Peterson, 2014). At present, the species is thought to be in continual decline within its native range (Williams & Burgess, 1999; Taylor & Peterson, 2014). Recent studies have documented functional extirpations of Shoal Bass populations in four tributary streams to the Chattahoochee River (Wacoochee, Halawakee, Osanippa, and Little Uchee creeks; (Stormer & Maceina, 2008) and in an 80-km reach of the Chattahoochee River below Lake Lanier, Georgia (Long & Martin, 2008). Other declines and range losses have likely gone undocumented, as population assessment data are lacking (Taylor & Peterson, 2014).

Researchers have suggested that anthropogenic land-use and water-use activities, non-native species invasions, and fragmentation are the main factors contributing to the imperilment of Shoal Bass. Habitat alteration from land-use activities and extensive damming of the ACF Basin has been thought to contribute most to range loss (Williams & Burgess, 1999; Taylor & Peterson, 2014). Where non-native congener species such as Alabama Bass (*M. henshalli*), Spotted Bass (*M. punctulatus*), and Smallmouth Bass (*M. dolomieu*) have been introduced, the long-term persistence and genetic integrity of Shoal

Bass populations are threatened by introgressive hybridization (Alvarez *et al.*, 2015; Dakin *et al.*, 2015; Sammons & Early, 2015) and, possibly, interspecific competition (Sammons, 2012). Because Shoal Bass exhibit movements of up to 200 km in free-flowing habitats during the spawning season (Sammons, 2015), fragmentation may further jeopardize long-term viability of isolated populations. Despite the continual decline of Shoal Bass, no range-wide efforts have been made to quantify the factors contributing most to range losses and how they relate to species presence.

Species distribution models (SDMs) provide a promising approach towards disentangling the most influential factors contributing to range losses of fluvial fishes like Shoal Bass. SDMs produce a quantitative prediction of species distribution using biologically-relevant variables that can represent natural landscape features, anthropogenic activities, non-native species distributions, fragmentation measures, and climate change forecasts (Guisan & Thuiller, 2005; Elith *et al.*, 2011). Thoughtfully-constructed SDMs can identify factors most influential to a species' distribution from a list of potential factors by quantifying the relative contribution of predictor variables and their respective relationships with species suitability (Rodríguez *et al.*, 2007; Elith *et al.*, 2011). Furthermore, modeling results can provide baseline information necessary for prioritizing management and conservation efforts, as well as fostering predictive conservation efforts rather than typical reactive efforts (Worthington *et al.*, 2014).

To determine what factors have contributed to declines of Shoal Bass within fluvial habitats of their native range, we employed a presence-background species distribution modeling approach. We built several SDMs to estimate two distinct distributions: the potential distribution based solely on natural abiotic factors and the current restricted distribution based on abiotic, biotic, and movement factors of both natural and anthropogenic origins. By incorporating a number of biologically-relevant predictor variables, we explored how fragmentation, non-native species interactions, and land-use activities have contributed to potential range loss of Shoal Bass.

Materials and methods

Methods used to complete our SDMs included defining the study area, obtaining and preparing presence data and predictor variables, and data analyses. A workflow diagram of the methods used to complete this research is detailed in Fig. 1.

I. Study area

Defining the study extent to areas accessible to the species and time period(s) of interest is fundamental to proper model construction (Barve *et al.*, 2011). We restricted our study extent to the native range of the Shoal Bass within the ACF Basin (Fig. 2a). The ACF Basin provides an interesting contrast in fragmentation between its two major river systems. The Flint River flows unimpeded for over 320 km in the upstream portion before reaching the first of only two mainstem dams, and then flows to its confluence with the Chattahoochee River where it is impounded by Lake Seminole. The Chattahoochee River, on the other hand, is riddled with 15 mainstem dams along its entire 702-km length. The study area included only third-order and larger stream segments (modified Strahler classification; McKay *et al.*, 2012). We did not include off-channel lentic habitats (e.g., ponds), as Shoal Bass are not known to inhabit such areas (Williams and Burgess 1999).

II. Data collection and preparation

Presence records – We compiled a comprehensive dataset of presence records from several agencies and online databases. We obtained museum and field collection records for Shoal Bass from the Georgia Museum of Natural History, Auburn Museum of Natural History, Florida Museum of Natural History, Georgia Department of Natural Resources (GADNR) Fisheries Management Section, and GADNR Nongame Conservation Section. We also downloaded presence records for Shoal Bass from several online databases: the Multistate Aquatic Resources Information System (http://www.marisdata.org), Biodiversity Information Serving our Nation (http://bison.usgs.ornl.gov), and the Global Biodiversity Information Facility (http://www.gbif.org). Finally, we included several personal field collection records and two verifiable records in Sweetwater Creek, Georgia from detailed online angler reports (http://www.forum.gon.com).

After obtaining this comprehensive presence dataset, we performed several quality assurance measures to create our final presence dataset for Shoal Bass. First, we identified and removed duplicate records as well as any records that represented introductions outside the ACF Basin. Duplicate records were considered those that shared a common sampling date and locality. We georeferenced 51 Shoal Bass records that were lacking coordinates using the web-based GEOLocate client (Rios & Bart, 2010) and the estimated spatial uncertainty of these georeferenced locations averaged 1.5 km (SD 1.5 km). Records with georeferenced uncertainty estimates > 8.6 km, or featuring exceedingly vague locality descriptions (e.g., “Flint River, Georgia”), were not used. We removed 13 records of Shoal Bass in impoundments because our goal was to include presence locations indicative of fluvial areas in which Shoal Bass may persist. Shoal Bass do not persist in impoundments (Williams & Burgess, 1999; Sammons & Early, 2015), and they quickly vacate impounded waters following translocation (Ingram *et al.*, 2013; Taylor & Peterson, 2015). We also did not include seven Shoal Bass records from first- and second- order streams because they were outside the study area. Afterwards, we plotted all remaining records in ArcMap 10.2 (ESRI, 2015) to visually inspect location of presence records. Following these quality assurance measures, 355 unique Shoal Bass presence records from 1952-2013 were retained. Where multiple presence records occurred within an individual stream segment, the stream segment was considered "present" in the modeling dataset one time, so that each unique stream segment was represented in the models without duplication.

Modeling environments – To estimate Shoal Bass distributions, we built several species distribution model environments that incorporated different combinations of predictor variables and presence records. First, the potential distribution model approximated the abiotically-suitable area for Shoal Bass given unlimited dispersal abilities and the absence of any negative biotic interactions (*see* Soberón & Nakamura, 2009). The potential distribution was estimated in one modeling environment that contained natural abiotic landscape and riverscape factors (Brewer *et al.*, 2007). Because the Shoal Bass was not formally described until 1999 (Williams & Burgess, 1999), presence records were sparse for Shoal Bass until recent decades, necessitating the use of all 355 records from 1952-2013 to estimate the potential distribution of the species (138 unique stream segments represented as present). This approach assumes that the current range of the species is restricted within its former range and the species' niche has not shifted appreciably during the timespan records. The potential distribution conceptually aligns with the fundamental niche of Hutchinson (1957), as estimated using scenopoetic variables (Grinnell, 1917). Because we used presence records that were influenced by anthropogenic alterations and interactions with non-native congeners, the fundamental niche produced may be an incomplete or under-representative estimate (*sensu* Peterson *et al.*, 2011, pg. 139).

Second, the restricted distribution models approximated the current (2000-2013) occupied distribution area for Shoal Bass by including abiotic, biotic, and movement-related factors (Soberón & Nakamura, 2009). For these models, we used presence records from 2000-2013 (202 unique records representing 78 unique stream segments as present) that approximate the current restricted distribution of the species following documented (Long & Martin, 2008; Stormer & Maceina, 2008) and suspected range losses. We created three modeling environments that included a mix of natural abiotic landscape and riverscape factors, streamflow, land cover, fragmentation, and non-native congener suitability. Each of the three modeling environments differed in the intensity of fragmentation: 1) no fragmentation, 2) fragmentation by dams only, and 3) fragmentation by dams and large impoundments. These restricted distributions approximate the realized niche of Hutchinson (1957) using both scenopoetic (Grinnell, 1917) and bionomic (Elton, 1927) variables.

Modeling base layer – To create a modeling base layer, we modified the NHDPlusV2 flowline layer that features a unique common identifier number (COMID) for identification of individual stream segments (McKay *et al.*, 2012). To allow for comparison of results among models featuring differing levels of fragmentation in their modeling environment, we modified the NHDPlusV2 stream segments and COMID system so that stream segment lengths and their common identifier numbers were consistent across all SDMs. First, we created a dendritic network of stream segments in ESRI Arcmap using NHDPlusV2 attributes to retain segments ≥ 3rd order, exclude disjunct tidal channels, and remove side channels and bifurcations that would inhibit connectivity analyses. Within this dendritic network, some stream segments were split by locations of both dams and large impoundments (the highest fragmentation intensity incorporated). We then created a second common identifier ("COMID2") attribute for the modified dendritic network, which we used to label individual segments split by fragmentation features. The final COMID2 layer had 6,135 stream segments totaling 8,005 km.

Predictor variables – We included only biologically-relevant predictor variables in our SDMs (Elith & Leathwick, 2009), and all variables were obtained or derived from publicly available datasets (Table 1). Predictor variables encompassed five broad categories: landscape and riverscape context, streamflow, land cover, fragmentation, and non-native species.

i. Landscape and riverscape – Coarse-scale factors influencing fish presence include natural abiotic landscape and riverscape characteristics (Hynes, 1975). Total drainage area (km^2), maximum elevation (m), slope (km/km), and mean annual precipitation (mm) were obtained from the National Hydrology Dataset Plus version 2 (NHDPlusV2) for individual stream segments (McKay *et al.*, 2012). We used CONUS-Soil polygon datasets (Miller & White, 1998) to characterize mean depth to bedrock (cm), mean permeability of topsoil layer (cm/hr), and mean percentage of rock volume in the top 250 cm of soil for each stream segment. Geology was characterized by the predominant type within each HUC-12 watershed (*sensu* Wuellner *et al.*, 2013; Stewart *et al.*, 2016) using U.S. Geological Survey state geological maps (Dicken *et al.*, 2007) reclassified into five categories: sand/alluvium, clay/mud, sandstone/shale, limestone/dolomite, and crystalline rock (igneous and mafic; *sensu* Olivero & Anderson, 2008). We also included a categorical variable accounting for the presence of natural lentic waterbodies (swamps and marshes within the NHDPlusV2 waterbody dataset) that intersected stream segments, as Shoal Bass do not persist in lentic habitats (Williams & Burgess, 1999).

ii. Streamflow – We used mean annual discharge (m^3/s) and mean annual flow velocity (m/s) to characterize streamflow conditions. Values for both variables were calculated using the Extended Unit Runoff Method (EROM) and reported by stream segment in the NHDPlusV2 dataset. We used reference gage regressions of both values to represent "natural" historic conditions (1952-2000), whereas we used gage-adjusted values to estimate "current" flow conditions (2000-2013) (McKay *et al.*, 2012; Worthington *et al.*, 2014).

iii. Land cover – We used Landsat satellite based land cover from the 2001 National Land Cover Database (NLCD; Homer *et al.*, 2007) to derive several predictor variables. We reclassified land cover types into five classes: urban/suburban, agriculture, forested/herbaceous, barren, and water/wetlands. Because land use affects species presence at multiple spatial scales (Allan, 2004), we calculated the proportion of urban/suburban, agriculture, and forested/herbaceous cover within two different spatial scales: HUC-12 watersheds and segment-specific 300-m stream buffers. Although state laws in our study area typically enforce minimum riparian buffer widths between 7 and 15 m, stream buffer widths of up to 50 m are needed to ameliorate nutrient from agricultural practices entering streams (Osborne & Kovacic, 1993), and stream buffers of up to 300 m may be necessary to maintain natural microclimates near streams (Brosofske *et al.*, 1997); overall, suggesting a 300-m buffer width could provide a relevant characterization of land cover. We considered presence records from 2000-2013 to be contemporaneous with land cover obtained circa 2001, as reclassified land cover in the ACF Basin changed < 3% between the NLCD 2001 and 2011 datasets (Homer *et al.*, 2007, 2015).

iv. Fragmentation – To better elucidate how fragmentation relates to changes in Shoal Bass probability of suitability, we incorporated different fragmentation intensities into our restricted SDMs based on different assumptions of what constitutes a barrier. The three fragmentation intensities included no fragmentation, fragmentation by dam locations only, and fragmentation by dam locations and large impoundments. To calculate predictor variables relevant to each level of fragmentation intensity, we first created a comprehensive layer of dams from the National Inventory of Dams (NID; http://nid.usace.army.mil/), a dataset of small reservoirs in the ACF Basin (Ignatius & Stallins, 2011), and local knowledge of two of us (ATT and JML). We used aerial imagery in Google Earth (https://www.google.com/earth/) to verify dam locations and remove off-stream dams. As dam construction dates ranged from 1830 to 1996 in our dataset, we considered any fragmentation effects to be contemporaneous and relevant to presence data from 2000-2013. Additionally, we retained two dams on the Chattahoochee River (Eagle and Phenix Dam and City Mills Dam) that were removed in

2012-13 because presence data near those locations were recorded prior to dam removal. To account for the potential effects of large impoundments as barriers, we included eight mainstem impoundments with surface areas > 5.5 km^2. The Barrier Analysis Tool (BAT; Martin & Apse, 2011) was used to build functional stream networks between barriers (Hoenke *et al.*, 2014). We calculated the following predictor variables to characterize the potential effects at each fragmentation intensity: completion year of downstream dam, free-flowing fragment length (km), maximum accessible total drainage area (km^2), number of tributary junctions within each fragment, and presence of large impoundments (> 5.5 km^2).

v. Non-native species – Presence of non-native congeners (Alabama Bass and Spotted Bass) is thought to negatively affect Shoal Bass presence. We queried the same online databases as we did for Shoal Bass records to obtain Spotted Bass and Alabama Bass presence records. Both species have been introduced into the ACF Basin and have subsequently expanded their ranges; however, the two species are difficult to distinguish, some specimens appear intermediate (Baker *et al.*, 2008), and biologists have customarily referred to both species as "spotted bass". Because of these difficulties, we combined records for both species into one "non-native congener" presence dataset for use in our SDMs. To clean these records, we retained only georeferenced records and removed any records > 100 m away from base layer flowlines. Available presence data for non-native congeners from 2000-2013 did not appear to adequately depict their invaded range within individual stream segments; thus, we constructed SDMs to predict the probability of suitability for these species in all stream segments. SDMs were constructed using the same variables, modeling environments, and methods as used to build the current restricted Shoal Bass SDMs. Three different predictions of non-native congener probability of suitability were created and used as predictor variables for Shoal Bass, one within each fragmentation intensity (no fragmentation, fragmentation by dam locations only, and fragmentation by dam locations plus large impoundments). This approach represents asymmetric competition between Shoal Bass and non-native congeners, wherein Shoal Bass are assumed to have no effect on non-native congener distribution, but non-natives are assumed to be superior competitors and may influence Shoal Bass distribution (*sensu* Leathwick & Austin, 2001). Results of the three non-native congener models are reported in Appendix I.

Input data – We related presence records and predictor variables to stream segments in ESRI ArcMap and exported attribute tables containing predictor variables into a Microsoft Access database that related predictor variable values to each stream segment via the unique COMID and "COMID2" identifiers. We queried this database to generate samples-with-data (SWD) input files for the modeling algorithm (Maxent, see below).

III. Data analyses

We employed a presence-background approach to species distribution modeling with Maxent version 3.3.3k (Phillips *et al.*, 2006), because the presence data used in this study were not collected in a systematic manner that would allow for inference about detectability of Shoal Bass (Pearce & Boyce, 2006). Maxent is a machine-learning tool that seeks to minimize the relative entropy between predictor variable values associated with known presence locations and values associated with a random background sample

(Elith *et al.*, 2011). The Maxent algorithm minimizes relative entropy by applying a number of pre-defined transformations to the predictor variables (Phillips *et al.*, 2006; Elith *et al.*, 2011). In a comprehensive comparison of predictive performance among 16 distribution modeling methods, Maxent was consistently one of the best performing methods (Elith *et al.*, 2006).

Several considerations were made during model construction to ensure model performance. To account for issues arising from multicollinearity amongst continuous predictor variables, we selected a suite of variables for each model that had Pearson's product-moment correlation coefficients of $|r| \leq 0.7$ (Dormann *et al.*, 2013). To avoid model over-fitting, we removed any predictor variable contributing < 2.0 % gain in model fit to a preliminary model run for each distribution model. Predictive errors resulting from geographic sampling biases are presumed to be negligible in our models because multiple presence records situated within one stream segment are represented as one stream segment denoted "present" in our model, instead of multiple (*see* Boria *et al.*, 2014). Unless otherwise specified, we used the default settings in Maxent, which have been tuned and validated to perform well on diverse datasets (Phillips & Dudík, 2008). Several predictor variables had missing data (denoted -9999), so we enabled the option in Maxent to use samples with some missing data. We employed a 10-fold cross validation, which uses held-out presences to estimate errors around model predictive performance and fitted functions (Elith *et al.*, 2011). We also used the jackknife option to assess variable importance and chose to create response curves for predictor variables.

We followed existing conventions in interpreting model results. Maxent's logistic output provided estimates of the probability of suitability for Shoal Bass based on similarity of environmental conditions (i.e., predictor variable values) with known presence locations (Elith *et al.*, 2011). To assess relative importance of predictor variables, we used the percent contribution of each variable to the gain in model fit of a given distribution model. Possible collinearity of predictor variables can confound the interpretation of ‘marginal’ response curves created in Maxent, whereas ‘single variable’ response curves are created with all other variables excluded (Phillips, 2005). As several predictor variable combinations had high correlation ($0.50<|r|<0.70$), we used ‘single variable’ response curves to infer relationships between predictor variables and Shoal Bass probability of suitability.

To assess model predictive performance, we used the threshold-independent receiver operating characteristic (ROC) area under the curve (AUC) and threshold-dependent omission rates of testing data within the 10-fold cross validation. ROC AUC values of 0.5 indicate model prediction no better than random chance, whereas values of 1.0 indicate perfect prediction (Fielding & Bell, 1997). However, AUC may not appropriately measure changes in the discriminatory power of models (Jiménez-Valverde, 2014). Binary (i.e., suitable/unsuitable) distributions are beneficial because they provide a measure of discrimination through the calculation of omission rates (Jiménez-Valverde, 2014). We applied two thresholds to convert continuous Maxent logistic output into a binary, suitable/unsuitable format: one at 0% omission of training location data and another at 10% omission of training data. The 0% omission threshold (“minimum training presence” in Maxent) is reasonable because we carefully vetted presence data within the narrow distribution of Shoal Bass (Pearson *et al.*, 2007; Anderson & Gonzalez Jr., 2011). The 10% omission threshold provides a more conservative estimate of environmental suitability by retaining the top 90% of training presence locations and excluding some potentially erroneous locations (Anderson & Gonzalez Jr., 2011). Mean omission rates of testing data at each threshold were calculated from the 10-fold cross validation replicates, providing a threshold-dependent measure of model predictive accuracy.

We performed an outside validation by comparing results of our SDMs to an independent dataset of directed Shoal Bass surveys in 17 tributaries of the middle Chattahoochee River basin (Katechis, 2015). These surveys targeted streams in which Shoal Bass were thought to occur and were repeated over two summers (2013-14), wherein Shoal Bass were absent (or not detected) at 8 sites and present at 9 sites (Katechis, 2015). We compared the mean (weighted by stream segment length) and standard error (SE) of probability of suitability estimated by each distribution model for sites considered absent (or not detected) to those considered present.

To visualize estimated distributions, we created distribution maps in ESRI ArcMap that combined both binary, suitable/unsuitable omission thresholds. To compare estimated range loss, we calculated differences in stream segment length (km) predicted present by each distribution model.

Results

The potential species distribution model included six predictor variables (Table 1, Fig. 2b), and was influenced mostly by natural mean annual flow velocity (30.6% model contribution), natural mean annual discharge (29.4%), mean percentage of rock volume in soil (19.1%), and predominate geology type in HUC-12 watershed (13.0%; Table 1). At the 0% omission threshold, much of the ACF Basin was predicted suitable (6,533 km or 84.5% of total available segment length, Table 2), whereas the 10% omission threshold depicted suitability only in mainstem rivers and lower portions of select tributary streams (Fig. 3a; 2,415 km or 31.2%, Table 2). The potential model had good predictive performance, with a mean (SD) ROC AUC of 0.87 (0.05) among cross-validation replicates. Cross-validation also suggested strong discriminatory power, with mean (SD) omission rates of the testing data at 0% omission and 10% omission thresholds of 0.02 (0.03) and 0.15 (0.12), respectively (Table 2).

The three restricted SDMs included 7-8 predictor variables (Fig. 2b, c, d). The restricted distribution model for Shoal Bass with no fragmentation was influenced mostly by probability of suitability of non-native congeners (33.6% model contribution), current mean annual discharge (27.8%), mean percentage of rock volume in soil (16.3%), and predominate geology type in HUC-12 watershed (12.2%; Table 1). With no riverscape fragmentation, Shoal Bass suitability was related in a positive linear fashion with non-native congener suitability (Fig. 4). Proportion of forested/herbaceous cover in the 300-m buffer was the only land cover variable that influenced the models (3.4% contribution), and this variable had a positive linear relationship with Shoal Bass probability of suitability (Fig. 5). The estimated distribution was more conservative than the potential distribution model, with fewer tributary streams predicted suitable and much of the lower Chattahoochee River drainage predicted unsuitable (Fig. 3b). At the 0% omission threshold, 5,574 km were predicted suitable (72.1% of total available segment length), whereas only 1,526 km (19.7%) were predicted suitable at the 10% omission threshold

(Table 2). This model had improved predictive performance, with a mean (SD) ROC AUC of 0.92 (0.06) among cross-validation replicates. Cross-validation also suggested improved discriminatory power over the potential model, with mean (SD) omission rates of the cross-validation testing data at 0% and 10% omission thresholds at 0.01 (0.04) and 0.15 (0.15), respectively (Table 2).

The restricted distribution model for Shoal Bass with fragmentation by dams was influenced mostly by current mean annual discharge (31.2% model contribution), mean percentage of rock volume in soil (18.5%), probability of suitability of non-native congeners (15.5%), and predominate geology type in HUC-12 watershed (12.3%; Table 1). Response curves suggested that Shoal Bass suitability increased with non-native congener suitability at low probabilities of suitability for both species; however, as non-native congener probability of suitability increased beyond 0.70, Shoal Bass probability of suitability began to plateau (Fig. 4). The fragmentation metric of free-flowing fragment length contributed 8.2% and its response curve revealed that fragment lengths less than approximately 100 km were not suitable for Shoal Bass (Fig. 6). Proportion of forested/herbaceous cover in the 300-m buffer contributed 7.3% and had the same positive linear relationship with Shoal Bass probability of suitability as seen in the model with no fragmentation (Fig. 5). Estimated distributions illustrated an increasingly restricted Shoal Bass distribution with further range loss in tributary streams, particularly in several of the larger tributaries of the Flint River (Fig. 3c). At the 0% omission threshold, 4,184 km were predicted suitable (54.1% of total available segment length), whereas only 1,474 km (19.1%) were predicted suitable at the 10% omission threshold (Table 2). Predictive and discriminatory abilities were similar to the restricted model with no fragmentation, as mean (SD) ROC AUC was 0.92 (0.05) among cross-validation replicates, and mean (SD) omission rates of the cross-validation testing data at 0% and 10% omission thresholds were 0.03 (0.08) and 0.19 (0.18), respectively (Table 2).

The restricted distribution model for Shoal Bass with fragmentation by dams and impoundments was influenced mostly by current mean annual discharge (28.1% model contribution), probability of suitability of non-native congeners (18.2%), mean percentage of rock volume in soil (14.1%), and predominate geology type in HUC-12 watershed (12.7%; Table 1). Response curves relating Shoal Bass suitability to non-native congener suitability suggested that Shoal Bass probability of suitability is negatively affected when probability of suitability of non-native congeners is approximately ≥ 0.75 (Fig. 4). Free-flowing fragment length contributed 10.5% and the response curve again suggested a minimum threshold of approximately 100 km for Shoal Bass suitability and fragment lengths > 600 km had a positive relationship with Shoal Bass suitability (Fig. 6). Presence/absence of large impoundments contributed 7.3%, with impounded segments having a mean probability of suitability of approximately 0.05, compared to 0.50 in unimpounded segments. Proportion of forested/herbaceous cover in the 300-m buffer contributed 4.6% and had a positive linear relationship Shoal Bass probability of suitability (Fig. 5). Mapped predictions illustrated the most extensive range loss, with further reductions in range in impounded mainstem rivers and in tributaries to large impoundments (Fig. 3d). At the 0% omission threshold, 4,146 km were predicted suitable (53.6% of total available segment length), whereas only 1,272 km (16.4%) were predicted suitable at the 10% omission threshold (Table 2). Predictive and discriminatory abilities were similar to other restricted SDMs, as mean (SD) ROC AUC was 0.94 (0.04) among cross-validation replicates, and mean (SD) omission rates of the cross-validation testing data at 0% and 10% omission thresholds were 0.01 (0.04) and 0.16 (0.17), respectively (Table 2).

Outside validation of species distribution modeling results with Katechis (2015) surveys indicated that mean probability of suitability was higher at sites where Shoal Bass were present compared to sites where Shoal Bass were absent or not detected for all SDMs (Fig. 7). Mean probability of suitability was consistently 0.12-0.15 higher at sites where Shoal Bass were considered present by Katechis (2015), and SE of means ranged from 0.03-0.06.

Discussion

Mean annual discharge was one of the two highest-contributing variables in all SDMs considered, and although the “natural” and “current” datasets used herein did not represent a change substantial enough to affect Shoal Bass probability of suitability, future alteration could greatly affect population size and stability. Migratory and potamodromous fishes like Shoal Bass are especially vulnerable to declines in streamflow (Beatty *et al.*, 2014; Sammons, 2015). Concerns over streamflow allocations and stream drying are particularly acute given the potential effects of increasing anthropogenic water consumption demands and global climate change projections of longer and more severe droughts in some regions (Knapp *et al.*, 2008; Lynch *et al.*, 2016). Within the native range of the Shoal Bass, for example, growing water demands of Atlanta, GA – one of the top ten most-populated, and second fastest-growing, metropolitan areas in the U.S. from 2000 to 2010 (Mackun & Wilson, 2011) – have resulted in an ongoing "tri-state water war" concerning water allocation (Stephenson, 2000; Ruhl, 2005). Balancing anthropogenic water demands with ecological needs of stream ecosystems will be a key challenge for conservation of fluvial fishes (Poff *et al.*, 1997; Baron *et al.*, 2002; Richter *et al.*, 2003).

Coarse-scale geology and soil characteristics also contributed highly to model predictions. In our models of a shoal-habitat specialist (Wheeler & Allen, 2003; Stormer & Maceina, 2009; Sammons & Early, 2015), mean percent rock volume in the soil and predominate geology type provided adequate coarse-scale surrogates for shoal habitat. However, some shoal habitats have been altered at finer-scale resolutions than these variables represent, like the destruction of shoals in lower portions of the ACF Basin for navigational purposes (Williams & Burgess, 1999). Because in-stream habitat alteration and loss is one of the leading contributors to fluvial fish imperilment (Allan & Flecker, 1993; Ricciardi & Rasmussen, 1999), incorporating finer-scale habitat and substrate data (*e.g.,* from side-scan sonar; Kaeser & Litts, 2010) into SDMs could improve model results as well as provide additional information for management.

The relationship between Shoal Bass and non-native congener probabilities of suitability changed as fragmentation intensified, suggesting an asymmetric interspecific relationship that favored non-native congeners at the highest fragmentation intensity. The introduction of non-native black bass species has often been implicated in the loss of native forms (Barwick *et al.*, 2006; Stormer & Maceina, 2008). Although non-native species are considered a major threat to fluvial fish conservation in general (Allan & Flecker, 1993; Gozlan *et al.*, 2010), habitat alteration is often identified as an underlying driver of the competitive replacement of native fluvial fishes with non-native species (Bunn & Arthington, 2002; Johnson *et al.*, 2008). The potential for competitive exclusion by non-natives is particularly acute in our example, as the large impoundments that fragment fluvial-specialist Shoal Bass also function as 'stepping stone' vectors that allow non-native congeners to invade nearby streams (Johnson *et al.*, 2008). As such, efforts to curb introductions and subsequent invasions of non-native fishes may provide greater conservation benefits to native fluvial fishes if paired with efforts to maintain intact habitats and restore altered habitats.

Although the invasion of non-native congeners appears to have played an important role in restricting the range of the Shoal Bass, there are several difficulties preventing a better understanding of interspecies relationships. Being able to properly identify cryptic, non-native species and understand particular biological and ecological differences among them is a recurring challenge in conserving native fluvial fishes (Cook *et al.*, 1992; Moyer *et al.*, 2005; Haynes *et al.*, 2012). In such scenarios, genetic identification surveys can provide range data for cryptic non-natives, as well as the ability to quantify hybridization rates with natives (*see, for examples* Moyer *et al.*, 2005; Alvarez *et al.*, 2015). Furthermore, finer-scale studies with more directed and intensive sampling efforts, like two-species occupancy modeling (Wagner *et al.*, 2013), could help account for potential differences in detection among species and better separate the potential effects of non-native species from influences of habitat fragmentation and alteration.

Habitat loss and subdivision through dam construction and impounding free-flowing water are independent, yet synergistic in their effects in restricting fluvial fishes

(Hoagstrom, 2015). Longer free-flowing fragments generally support larger fluvial fish populations, larger effective population sizes (i.e., greater allelic diversity), longer population persistence, and more diverse fluvial fish communities (Hilderbrand & Kershner, 2000; Perkin *et al.*, 2015). Although the biological mechanisms behind the benefits of longer fragments are often unknown (Hoagstrom, 2015), we hypothesize that longer fragments may be imperative for multiple life stages of habitat specialists, like Shoal Bass, because these areas confer access to critical habitats that are unevenly distributed within river systems. Distribution estimates and response curves generated by SDMs can help prioritize barrier removal.

The effects of fragmentation inferred from our SDMs have population and population-genetic level implications. Our results suggest that Shoal Bass populations could have potentially been interconnected throughout much of the ACF Basin, but suitable areas now appear confined to smaller, disjunct areas within their native range. In a metapopulation context, fragmentation severs connectivity among subpopulations and local extinctions are more likely to occur (Jager *et al.*, 2001). Fluvial fishes typically experience reduced gene flow and lower genetic diversity when fragmented by dams (Neraas & Spruell, 2001; Laroche & Durand, 2004) and impounded waters (Fluker *et al.*, 2014). Quantifying demographic and genetic connectivity (*see* Lowe & Allendorf, 2010) among Shoal Bass populations is warranted to better understand the extent that fragmentation affects their gene flow and long-term viability.

By incorporating predictor variables characterizing land cover at multiple spatial scales, we were able to discern which land cover types contributed most to segment-scale suitability and at what spatial scale land cover was most influential. The proportion of forested/herbaceous cover in the 300-m stream buffer was the only land cover variable to contribute ≥ 2.0% to the restricted SDMs for Shoal Bass, and the response curve suggested that forested buffers confer increases in suitability. Forested buffer zones minimize sediment runoff, create stream shading that regulates temperature, and contribute large woody debris into streams (Osborne & Kovacic, 1993). Local-scale buffers are also more feasible to implement than catchment-wide management and can provide significant benefits (Saunders *et al.*, 2002; Allan, 2004). Regardless of spatial scale, land cover variables contributed relatively little to model predictive ability compared to other factors. However, inherent difficulties with relating land cover variables to in-stream biotic changes (*see* Allan, 2004) may have underestimated the importance of land cover-related effects at both catchment and buffer scales in our study.

Although estimated distributions have some degree of omission and commission error, model validation can provide context for the applicability of model results to management and conservation. Decisions made during model construction likely influence the degree of omission and commission in model results (Barry & Elith, 2006). In our modeling exercise, excluding 13 presence records (4% of total records) from impoundments may have led to underestimation of suitability in impoundments, increasing the potential of omission error despite a preponderance of evidence that Shoal Bass do not persist in or naturally inhabit impoundments (Williams & Burgess, 1999; Ingram *et al.*, 2013; Sammons & Early, 2015; Taylor & Peterson, 2015). Similarly, we did not include first- and second- order streams in our models, but seven presence records (2% of total records) associated with these areas suggest that our distribution estimates may be somewhat conservative. Omission and commission error could also have been introduced if the two subsets of presence records used to build the potential (1952-2013 records) and restricted (2000-2013) distributions did not accurately represent the desired distributions. Despite these possible sources of model error, suitability estimates aligned well with targeted sampling used for outside validation (Katechis, 2015), suggesting real-world applicability for management and conservation. To better characterize extant ranges, elucidate causes of prediction errors, and refine model construction, systematic range-wide surveys are indispensable. In particular, intensive surveys that incorporate standardized efforts to account for imperfect detection, like occupancy modeling, could improve understanding of factors driving both species presence and absence (MacKenzie, 2006; *see* Albanese *et al.,* 2013).

Imperilment rates of fluvial fishes across the globe are high and only expected to increase in the coming decades (Ricciardi & Rasmussen, 1999; Dudgeon *et al.*, 2006). Unfortunately, a lack of understanding of biological requirements and responses to disturbances has hindered conservation of black basses and many other fluvial fishes in the U.S. (Jelks *et al.*, 2008; Birdsong *et al.*, 2010), as well as freshwater biota across the world (Abell, 2002; Dudgeon *et al.*, 2006). Species distribution modeling approaches, like the one employed herein (*see, for other examples* Albanese *et al.*, 2013; Breece *et al.*, 2013; Liang *et al.*, 2013; Worthington *et al.*, 2014), can help disentangle the factors driving range loss of fluvial fishes and deliver imperative insights into their biology and conservation needs.

References

Abell R (2002) Conservation biology for the biodiversity crisis: a freshwater follow-up. *Conservation Biology*, **16**, 1435–1437.

Albanese B, Litts T, Camp M, Weiler DA (2013) Using occupancy and species distribution models to assess the conservation status and habitat use of the Goldline Darter (*Percina aurolineata*) in Georgia, USA. *Ecology of Freshwater Fish*, **23**, 347–359.

Allan JD (2004) Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 257–284.

Allan JD, Flecker AS (1993) Biodiversity conservation in running waters. *BioScience*, **43**, 32–43.

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, **17**, 24–30.

Alvarez AC, Peterson DL, Taylor AT, Tringali MD, Barthel BL (2015) Distribution and amount of hybridization between Shoal Bass and the invasive Spotted Bass in the lower Flint River, Georgia. In: *Black Bass Diversity: Multidisciplinary Science for Conservation* (eds Tringali MD, Long JM, Birdsong TW, Allen MS), pp. 503–521. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Anderson RP, Gonzalez Jr. I (2011) Species-specific tuning increases robustness to sampling bias in models of species distributions: An implementation with Maxent. *Ecological Modelling*, **222**, 2796–2811.

Baker WH, Johnston CE, Folkerts GW (2008) The Alabama Bass, *Micropterus henshalli* (Teleostei: Centrarchidae), from the Mobile River Basin. *Zootaxa*, **1861**, 57–67.

Baron JS, Poff NL, Angermeier PL et al. (2002) Meeting ecological and societal needs for freshwater. *Ecological Applications*, **12**, 1247–1260.

Barry S, Elith J (2006) Error and uncertainty in habitat models. *Journal of Applied Ecology*, **43**, 413–423.

Barve N, Barve V, Jiménez-Valverde A et al. (2011) The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling*, **222**, 1810–1819.

Barwick HD, Oswald KJ, Quattro JM, Barwick RD (2006) Redeye Bass (*Micropterus coosae*) and Alabama Spotted Bass (*M. punctulatus henshalli*) hybridization in Keowee Reservoir. *Southeastern Naturalist*, **5**, 661–668.

Beatty SJ, Morgan DL, Lymbery AJ (2014) Implications of climate change for potamodromous fishes. *Global Change Biology*, **20**, 1794–1807.

Birdsong T, Krause D, Leitner J, Long JM, Robinson S, Sammons S (2010) *A Business Plan for the Conservation of Native Black Bass Species in the Southeastern U.S.* National Fish and Wildlife Foundation, Washington, D.C.

Boria RA, Olson LE, Goodman SM, Anderson RP (2014) Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. *Ecological Modelling*, **275**, 73–77.

Breece MW, Oliver MJ, Cimino MA, Fox DA (2013) Shifting distributions of adult Atlantic Sturgeon amidst post-industrialization and future impacts in the Delaware River: a maximum entropy approach. *PLOS ONE*, **8**, e81321.

Brewer SK, Rabeni CF, Sowa SP, Annis G (2007) Natural landscape and stream segment attributes influencing the distribution and relative abundance of riverine Smallmouth Bass in Missouri. *North American Journal of Fisheries Management*, **27**, 326–341.

Brosofske KD, Chen J, Naiman RJ, Franklin JF (1997) Harvesting effects on microclimatic gradients from small streams to uplands in western Washington. *Ecological Applications*, **7**, 1188–1200.

Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management*, **30**, 492–507.

Burkhead NM (2012) Extinction rates in North American freshwater fishes, 1900–2010. *BioScience*, **62**, 798–808.

Cook JA, Bestgen KR, Propst DL, Yates TL (1992) Allozymic divergence and systematics of the Rio Grande Silvery Minnow, *Hybognathus amarus* (Teleostei: Cyprinidae). *Copeia*, **1992**, 36–44.

Cooper AR, Infante DM, Wehrly KE, Wang L, Brenden TO (2016) Identifying indicators and quantifying large-scale effects of dams on fishes. *Ecological Indicators*, **61**, 646–657.

Dakin EE, Porter BA, Freeman BJ, Long JM (2015) Hybridization threatens Shoal Bass populations in the upper Chattahoochee River Basin. In: *Black Bass Diversity:*

Multidisciplinary Science for Conservation (eds Tringali MD, Long JM, Birdsong TW, Allen MS), pp. 491–501. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Dicken CL, Nicholson SW, Horton JD, Foose MP, Mueller JAL (2007) Preliminary integrated geologic map databases for the United States.

Dormann CF, Elith J, Bacher S et al. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 27–46.

Dudgeon D, Arthington AH, Gessner MO et al. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**, 163–182.

Elith J, Leathwick JR (2009) Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 677–697.

Elith J, Graham CH, Anderson RP et al. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ (2011) A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, **17**, 43–57.

Elton CS (1927) *Animal Ecology*. Sidgwick and Jackson, London.

ESRI (2015) *ArcGIS Desktop: Realease 10.2.* Environmnetal Systems Research Institute, Redlands, CA.

Fausch KD, Torgersen CE, Baxter CV, Li HW (2002) Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience*, **52**, 483–498.

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, **24**, 38–49.

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.

Frankham R (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305–327.

Freeman MC, Marcinek PA (2006) Fish assemblage responses to water withdrawals and water supply reservoirs in Piedmont streams. *Environmental Management*, **38**, 435–450.

Gozlan RE, Andreou D, Asaeda T et al. (2010) Pan-continental invasion of *Pseudorasbora parva*: towards a better understanding of freshwater fish invasions. *Fish and Fisheries*, **11**, 315–340.

Grinnell J (1917) The niche-relationships of the California Thrasher. *The Auk*, **34**, 427–433.

Guenther CB, Spacie A (2006) Changes in fish assemblage structure upstream of impoundments within the Upper Wabash River Basin, Indiana. *Transactions of the American Fisheries Society*, **135**, 570–583.

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993–1009.

Haynes GD, Gongora J, Gilligan DM, Grewe P, Moran C, Nicholas FW (2012) Cryptic hybridization and introgression between invasive Cyprinid species *Cyprinus carpio* and *Carassius auratus* in Australia: implications for invasive species management. *Animal Conservation*, **15**, 83–94.

Hilderbrand RH, Kershner JL (2000) Conserving inland Cutthroat Trout in small streams: how much stream is enough? *North American Journal of Fisheries Management*, **20**, 513–520.

Hoagstrom CW (2015) Habitat loss and subdivision are additive mechanisms of fish extinction in fragmented rivers. *Global Change Biology*, **21**, 4–5.

Hoenke KM, Kumar M, Batt L (2014) A GIS based approach for prioritizing dams for potential removal. *Ecological Engineering*, **64**, 27–36.

Homer C, Dewitz J, Fry J et al. (2007) Completion of the 2001 National Land Cover Database for the conterminous United States. *Photogrammetric Engineering and Remote Sensing*, **73**, 337.

Homer CG, Dewitz JA, Yang L et al. (2015) Completion of the 2011 National Land Cover Database for the conterminous United States - representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*, **81**, 345–354.

Hutchinson GE (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

Hynes HBN (1975) Edgardo Baldi memorial lecture. The stream and its valley. In: *Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie*, pp. 1–15.

Ignatius A, Stallins JA (2011) Assessing spatial hydrological data integration to characterize geographic trends in small reservoirs in the Apalachicola-Chattahoochee-Flint River Basin. *Southeastern Geographer*, **51**, 371–393.

Ingram TR, Tannehill JE, Young SP (2013) Post-release survival and behavior of adult Shoal Bass in the Flint River, Georgia. *North American Journal of Fisheries Management*, **33**, 717–722.

Jager HI, Chandler JA, Lepla KB, Winkle WV (2001) A theoretical study of river fragmentation by dams and its effects on White Sturgeon populations. *Environmental Biology of Fishes*, **60**, 347–361.

Jelks HL, Walsh SJ, Burkhead NM et al. (2008) Conservation status of imperiled North American freshwater and diadromous fishes. *Fisheries*, **33**, 372–407.

Jiménez-Valverde A (2014) Threshold-dependence as a desirable attribute for discrimination assessment: implications for the evaluation of species distribution models. *Biodiversity and Conservation*, **23**, 369–385.

Johnson PT, Olden JD, Vander Zanden MJ (2008) Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment*, **6**, 357–363.

Kaeser AJ, Litts TL (2010) A novel technique for mapping habitat in navigable streams using low-cost side scan sonar. *Fisheries*, **35**, 163–174.

Katechis CT (2015) *Black bass habitat use and availability at multiple scales in middle Chattahoochee River tributaries*. MS Thesis, Auburn University, Auburn, AL, 86 pp.

Knapp AK, Beier C, Briske DD et al. (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**, 811–821.

Kondolf GM (1997) Hungry water: effects of dams and gravel mining on river channels. *Environmental Management*, **21**, 533–551.

Laroche J, Durand JD (2004) Genetic structure of fragmented populations of a threatened endemic percid of the Rhone river: *Zingel asper*. *Heredity*, **92**, 329–334.

Leathwick JR, Austin MP (2001) Competitive interactions between tree species in New Zealand’s old-growth indigenous forests. *Ecology*, **82**, 2560–2573.

Liang L, Fei S, Ripy JB, Blandford BL, Grossardt T (2013) Stream habitat modelling for conserving a threatened headwater fish in the upper Cumberland River, Kentucky. *River Research and Applications*, **29**, 1207–1214.

Long JM, Martin C (2008) The evolution of fisheries management of the upper Chattahoochee River in response to changing water management. In: *Balancing Fisheries Management and Water Uses for Impounded River Systems.* (eds Allen MS, Sammons SM, Maceina MJ), pp. 21–36. American Fisheries Society, Symposium 62, Bethesda, Maryland.

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology*, **19**, 3038–3051.

Lynch AJ, Myers BJE, Chu C et al. (2016) Climate change effects on North American inland fish populations and assemblages. *Fisheries*, **41**, 346–361.

MacKenzie DI (2006) *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence*. Academic Press, 344 pp.

Mackun P, Wilson S (2011) *2010 Census Briefs. Population Distribution and Change: 2000 to 2010*. U.S. Census Bureau.

Martin EH, Apse CD (2011) *Northeast Aquatic Connectivity: An Assessment of Dams on Northeastern Rivers*. The Nature Conservancy, Eastern Freshwater Program.

McKay L, Bondelid T, Dewald T, Johnston J, Moore R, Rea A (2012) NHDPlus Version 2: User Guide. Available at: http://www.horizon-systems.com/nhdplus/NHDPlusV2_documentation.php (accessed 29 February 2016).

Miller DA, White RA (1998) A conterminous United States multilayer soil characteristics dataset for regional climate and hydrology modeling. *Earth Interactions*, **2**, 1–26.

Moyer GR, Osborne M, Turner TF (2005) Genetic and ecological dynamics of species replacement in an arid-land river system. *Molecular Ecology*, **14**, 1263–1273.

Neraas LP, Spruell P (2001) Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. *Molecular Ecology*, **10**, 1153–1164.

Nilsson C (2005) Fragmentation and flow regulation of the world’s large river systems. *Science*, **308**, 405–408.

Noatch MR, Suski CD (2012) Non-physical barriers to deter fish movements. *Environmental Reviews*, **20**, 71–82.

Olden JD, Naiman RJ (2010) Incorporating thermal regimes into environmental flows assessments: modifying dam operations to restore freshwater ecosystem integrity. *Freshwater Biology*, **55**, 86–107.

Olivero AP, Anderson MG (2008) *Northeast Aquatic Habitat Classification System*. The Nature Conservancy, Eastern Regional Office, Boston, Massachusetts.

Osborne LL, Kovacic DA (1993) Riparian vegetated buffer strips in water-quality restoration and stream management. *Freshwater Biology*, **29**, 243–258.

Pearce JL, Boyce MS (2006) Modelling distribution and abundance with presence-only data. *Journal of Applied Ecology*, **43**, 405–412.

Pearson RG, Raxworthy CJ, Nakamura M, Townsend Peterson A (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.

Perkin JS, Gido KB (2011) Stream fragmentation thresholds for a reproductive guild of Great Plains fishes. *Fisheries*, **36**, 371–383.

Perkin JS, Gido KB, Cooper AR, Turner TF, Osborne MJ, Johnson ER, Mayes KB (2015) Fragmentation and dewatering transform Great Plains stream fish communities. *Ecological Monographs*, **85**, 73–92.

Peterson AT, Soberón J, Pearson RG, Anderson RP, Martínez-Meyer E, Nakamura M, Araújo MB (2011) *Ecological Niches and Geographic Distributions*. Princeton University Press, 330 pp.

Phillips S (2005) *A Brief Tutorial On Maxent*. AT&T Research, Florham Park, New Jersey.

Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161–175.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

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.

Poff NL, Allan JD, Bain MB et al. (1997) The natural flow regime. *BioScience*, **47**, 769–784.

Poff NL, Olden JD, Merritt DM, Pepin DM (2007) Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Sciences*, **104**, 5732–5737.

Porto LM, McLaughlin RL, Noakes DLG (1999) Low-head barrier dams restrict the movements of fishes in two Lake Ontario streams. *North American Journal of Fisheries Management*, **19**, 1028–1036.

Pringle CM (1997) Exploring how disturbance is transmitted upstream: going against the flow. *Journal of the North American Benthological Society*, **16**, 425–438.

Rahel FJ (2002) Homogenization of freshwater faunas. *Annual Review of Ecology and Systematics*, **33**, 291–315.

Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. *Conservation Biology*, **13**, 1220–1222.

Richter BD, Mathews R, Harrison DL, Wigington R (2003) Ecologically sustainable water management: managing river flows for ecological integrity. *Ecological Applications*, **13**, 206–224.

Rios NE, Bart HL (2010) *GEOLocate*. Tulane University Museum of Natural History, Belle Chasse, Louisiana.

Rodríguez JP, Brotons L, Bustamante J, Seoane J (2007) The application of predictive modelling of species distribution to biodiversity conservation. *Diversity and Distributions*, **13**, 243–251.

Ruhl JB (2005) Water wars, eastern style: divvying up the Apalachicola-Chattahoochee-Flint River Basin. *Journal of Contemporary Water Research & Education*, **131**, 47–54.

Sakai AK, Allendorf FW, Holt JS et al. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Sammons SM (2012) Diets of juvenile and sub-adult size classes of three *Micropterus* spp. in the Flint River, Georgia: potential for trophic competition. *Southeastern Naturalist*, **11**, 387–404.

Sammons SM (2015) First evidence of potadromy and partial migration in black basses: Shoal Bass *Micropterus cataractae* (Actinopterygii, Centrarchidae) in the Upper Flint River, USA. *Hydrobiologia*, **751**, 135–146.

Sammons SM, Early LA (2015) Movement and habitat use of Shoal Bass in a regulated portion of the Chattahoochee River, Alabama-Georgia, USA. In: *Black Bass*

Diversity: Multidisciplinary Science for Conservation (eds Tringali MD, Long JM, Birdsong TW, Allen MS), pp. 249–261. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Saunders DL, Meeuwig JJ, Vincent ACJ (2002) Freshwater protected areas: strategies for conservation. *Conservation Biology*, **16**, 30–41.

Soberón J, Nakamura M (2009) Niches and distributional areas: concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences*, **106**, 19644–19650.

Stephenson DS (2000) The tri-state compact: falling waters and fading opportunities. *Journal of Land Use & Environmental Law*, **16**, 83–109.

Stewart DR, Walters AW, Rahel FJ (2016) Landscape-scale determinants of native and non-native Great Plains fish distributions. *Diversity and Distributions*, **22**, 225–238.

Stormer DG, Maceina MJ (2008) Relative abundance, distribution, and population metrics of Shoal Bass in Alabama. *Journal of Freshwater Ecology*, **23**, 651–661.

Stormer DG, Maceina MJ (2009) Habitat use, home range, and movement of Shoal Bass in Alabama. *North American Journal of Fisheries Management*, **29**, 604–613.

Taylor AT, Peterson DL (2014) Shoal bass life history and threats: a synthesis of current knowledge of a *Micropterus* species. *Reviews in Fish Biology and Fisheries*, **24**, 159–167.

Taylor AT, Peterson DL (2015) Movement, homing, and fates of fluvial-specialist Shoal Bass following translocation into an impoundment. *Southeastern Naturalist*, **14**, 425–437.

Taylor CA, Knouft JH, Hiland TM (2001) Consequences of stream impoundment on fish communities in a small North American drainage. *Regulated Rivers: Research & Management*, **17**, 687–698.

Tringali MD, Long JM, Birdsong TW, Allen MS (eds.) (2015) *Black Bass Diversity: Multidisciplinary Science for Conservation.* American Fisheries Society, Symposium 82, Bethesda, Maryland.

Wagner T, Deweber JT, Detar J, Sweka JA (2013) Landscape-scale evaluation of asymmetric interactions between Brown Trout and Brook Trout using two-species occupancy models. *Transactions of the American Fisheries Society*, **142**, 353–361.

Warren Jr. ML, Burr BM (1994) Status of freshwater fishes of the United States: overview of an imperiled fauna. *Fisheries*, **19**, 6–18.

Warren Jr. ML, Pardew MG (1998) Road crossings as barriers to small-stream fish movement. *Transactions of the American Fisheries Society*, **127**, 637–644.

Wheeler AP, Allen MS (2003) Habitat and diet partitioning between Shoal Bass and Largemouth Bass in the Chipola River, Florida. *Transactions of the American Fisheries Society*, **132**, 438–449.

Wilcox BA, Murphy DD (1985) Conservation strategy: the effects of fragmentation on extinction. *The American Naturalist*, **125**, 879–887.

Williams JD, Burgess GH (1999) *A new species of bass, Micropterus cataractae (Teleostei: Centrarchidae), from the Apalachicola River basin in Alabama, Florida, and Georgia.* University of Florida.

Worthington TA, Brewer SK, Grabowski TB, Mueller J (2014) Backcasting the decline of a vulnerable Great Plains reproductive ecotype: identifying threats and conservation priorities. *Global Change Biology*, **20**, 89–102.

Wuellner MR, Bramblett RG, Guy CS, Zale AV, Roberts DR, Johnson J (2013) Reach and catchment-scale characteristics are relatively uninfluential in explaining the occurrence of stream fish species. *Journal of Fish Biology*, **82**, 1497–1513.

Table 1. Summary of predictor variables used to generate Shoal Bass (*Micropterus cataractae*) species distribution models (SDMs), including the mean, standard deviation, and percent contribution of variables to each SDM environment. Key: “c” – variable removed because Pearson correlation coefficient |r| ≥ 0.70 with another variable; “x” – variable removed because it contributed < 2.0% to a preliminary model run; * - variable with incomplete data coverage. Continued on next page.

			SDM environment				
Predictor variables (units)	**mean**	**SD**	**Pote-ntial**	**Restr. no frag**	**Restr. dams only**	**Restr. dams and impnds.**	**data source**
Natural Abiotic Variables							
Total drainage area (km^2)	2598.86	7914.51	c	c	c	C	NHDplusV2; Attributes TotDASqKm
Maximum elevation (m)	132.42	94.60	5.4	x	x	X	NHDplusV2; ElevSlope MaxElevSmo
Slope (km/km)*	0.00	0.01	x	4.2	4.9	4.4	NHDplusV2; ElevSlope SLOPE
Mean annual precipitation (mm)*	1342.88	102.99	x	x	x	X	NHDplusV2; EROM PPT0001
Mean depth to bedrock (cm)	131.34	42.71	2.4	x	x	X	Derived from CONUS-Soil polygons
Mean permeability of topsoil (cm/hr)	12.76	7.70	x	x	x	X	Derived from CONUS-Soil polygons
Mean percentage of rock volume in soil	31.09	12.01	19.1	16.3	18.5	14.1	Derived from CONUS-Soil polygons
Predominant geology of HUC-12 watershed (5 classes)	N/A	N/A	13.0	12.2	12.3	12.7	Derived from reclassified USGS Geologic map
Natural lentic waterbodies (0/1)	N/A	N/A	x	x	x	X	Derived from NHDplusV2 Waterbody polygons
Streamflow Variables							
"Natural" mean annual discharge (m^3/s)	29.98	81.15	29.4				NHDplusV2; EROM Q0001C
"Natural" mean annual flow velocity (m/s)	0.35	0.12	30.6				NHDplusV2; EROM V0001C
"Current" mean annual discharge (m^3/s)	31.55	92.03		27.8	31.2	28.1	NHDplusV2; EROM Q0001E
"Current" mean annual flow velocity (m/s)	0.35	0.12		2.5	2	x	NHDplusV2; EROM V0001E

Table 1. Continued from previous page.

Land Cover Variables (proportions)							
Agricultural cover in HUC-12 watershed	0.18	0.14		x	x	x	Derived from reclassified NLCD 2001
Urban/suburban cover in HUC-12 watershed	0.08	0.12		c	c	c	Derived from reclassified NLCD 2001
Forested/herbaceous cover in HUC-12 watershed	0.62	0.19		x	x	x	Derived from reclassified NLCD 2001
Agricultural cover in 300m segment buffers	0.07	0.10		x	x	x	Derived from reclassified NLCD 2001
Urban/suburban cover in 300m segment buffers	0.04	0.10		x	x	x	Derived from reclassified NLCD 2001
Forested/herbaceous cover in 300m segment buffers	0.46	0.26		3.4	7.3	4.6	Derived from reclassified NLCD 2001
Fragmentation Variables							
Completion date of downstream dam (year)*	1946.25	22.86			x	x	Derived from functional network analyses
Dams Only							
Free-flowing fragment length (km)	701.22	540.95			8.2		Derived from functional network analyses
Maximum accessible total drainage area (km^2)	14164.60	14023.93			x		Derived from functional network analyses
Number of tributary junctions within fragment	143.30	125.26			c		Derived from functional network analyses
Dams and Impoundments							
Free-flowing fragment length (km)	379.55	437.57				10.5	Derived from functional network analyses
Maximum accessible drainage area (km^2)	9814.07	13207.89				x	Derived from functional network analyses
Number of tributary junctions within fragment	72.78	83.02				c	Derived from functional network analyses
Large impoundment waterbodies (0/1)	N/A	N/A				7.3	Derived from NHDplusV2 Waterbody polygons
Non-native Species Variables							
Non-native congener probability of suitability 1*	0.15	0.19		33.6			Maxent results within given SDM environment
Non-native congener probability of suitability 2*	0.15	0.18			15.5		Maxent results within given SDM environment
Non-native congener probability of suitability 3*	0.14	0.18				18.2	Maxent results within given SDM environment
Total Number of Variables in Each SDM			**6**	**7**	**8**	**8**	

Table 2. Predictive performance, accuracy measures, and summary data for species distribution models (SDMs). Test sample omission rates calculated by 10-fold cross validation (CV), and outside validation with 17 directed tributary surveys (Katechis 2015). The receiver operating characteristic area under the curve (ROC AUC) is a threshold-independent metric of model predictive performance.

	SDM environment			
Model prediction and performance data	**Potential**	**Restricted: no frag**	**Restricted: dams only**	**Restricted: dams and impoundments**
Mean (SD) of ROC AUC	0.87 (0.05)	0.92 (0.06)	0.92 (0.05)	0.94 (0.04)
10-fold Cross Validation (CV) settings				
CV training samples mean count	124.20	70.20	70.20	70.20
CV test samples mean count	13.80	7.80	7.80	7.70
0% Omission of Training Samples Threshold				
CV test sample omission rate mean (SD)	0.02 (0.03)	0.01 (0.04)	0.03 (0.08)	0.01 (0.04)
Logistic threshold mean (SD)	0.02 (0.01)	0.01 (0.00)	0.03 (0.01)	0.02 (0.01)
Length of segments (km) predicted present	6533.39	5574.49	4184.02	4145.82
% of total segment length present in base layer	84.46	72.07	54.09	53.60
10% Omission of Training Samples Threshold				
CV test sample omission rate mean (SD)	0.15 (0.12)	0.15 (0.15)	0.19 (0.18)	0.16 (0.17)
Logistic threshold mean (SD)	0.21 (0.02)	0.15 (0.02)	0.17 (0.01)	0.19 (0.02)
Length of segments (km) predicted present	2414.58	1526.43	1474.32	1271.88
% of total segment length present in base layer	31.21	19.73	19.06	16.44

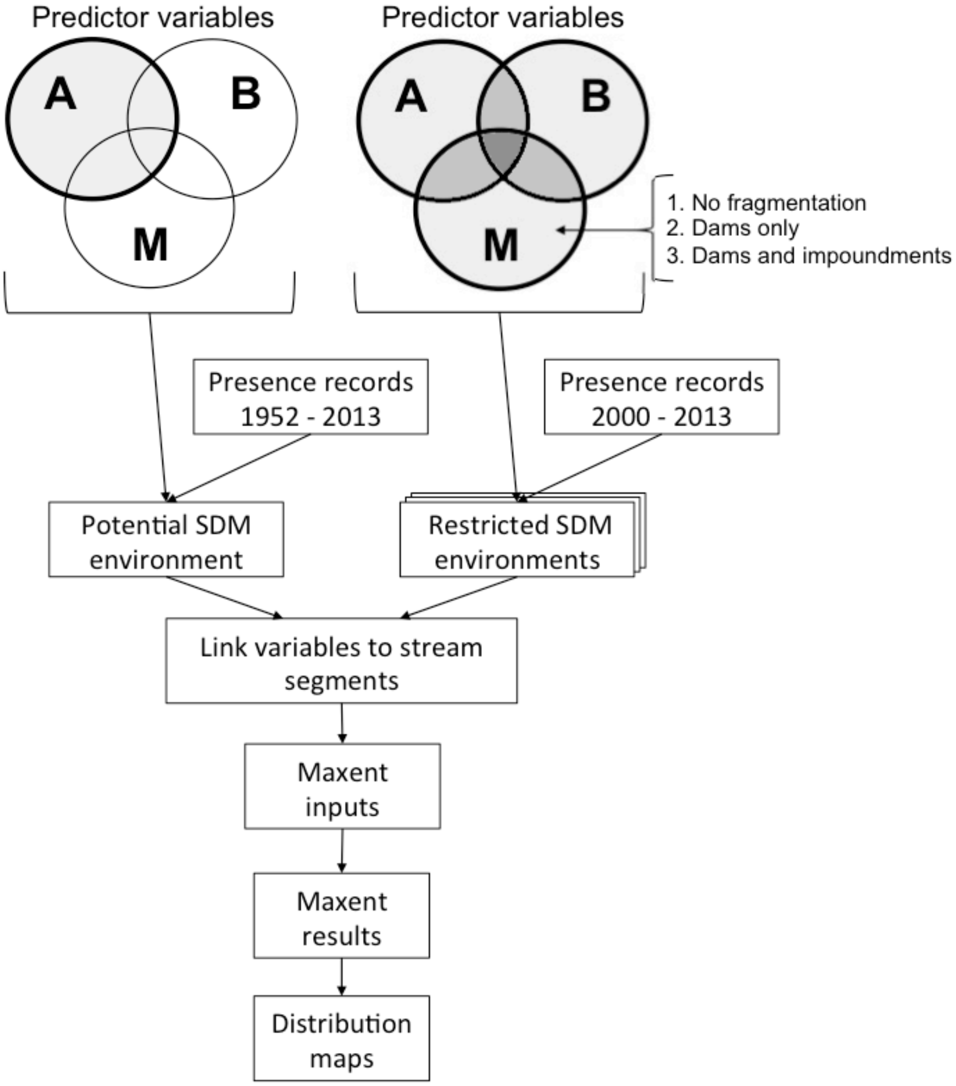

Figure 1. Workflow of species distribution model (SDM) construction. The Venn diagrams illustrate the Abiotic (A), Biotic (B), and Movement (M) elements that influence species distributions (Soberón & Nakamura, 2009), wherein shaded circles illustrate elements that were characterized by predictor variables for a given SDM environment. Three restricted SDM environments were created, one for each fragmentation intensity used to characterize the Movement element. Presence records and predictor variables of each SDM environment were related to individual stream segments to create input files for Maxent. Maxent results were then used to create distribution maps for each SDM environment.

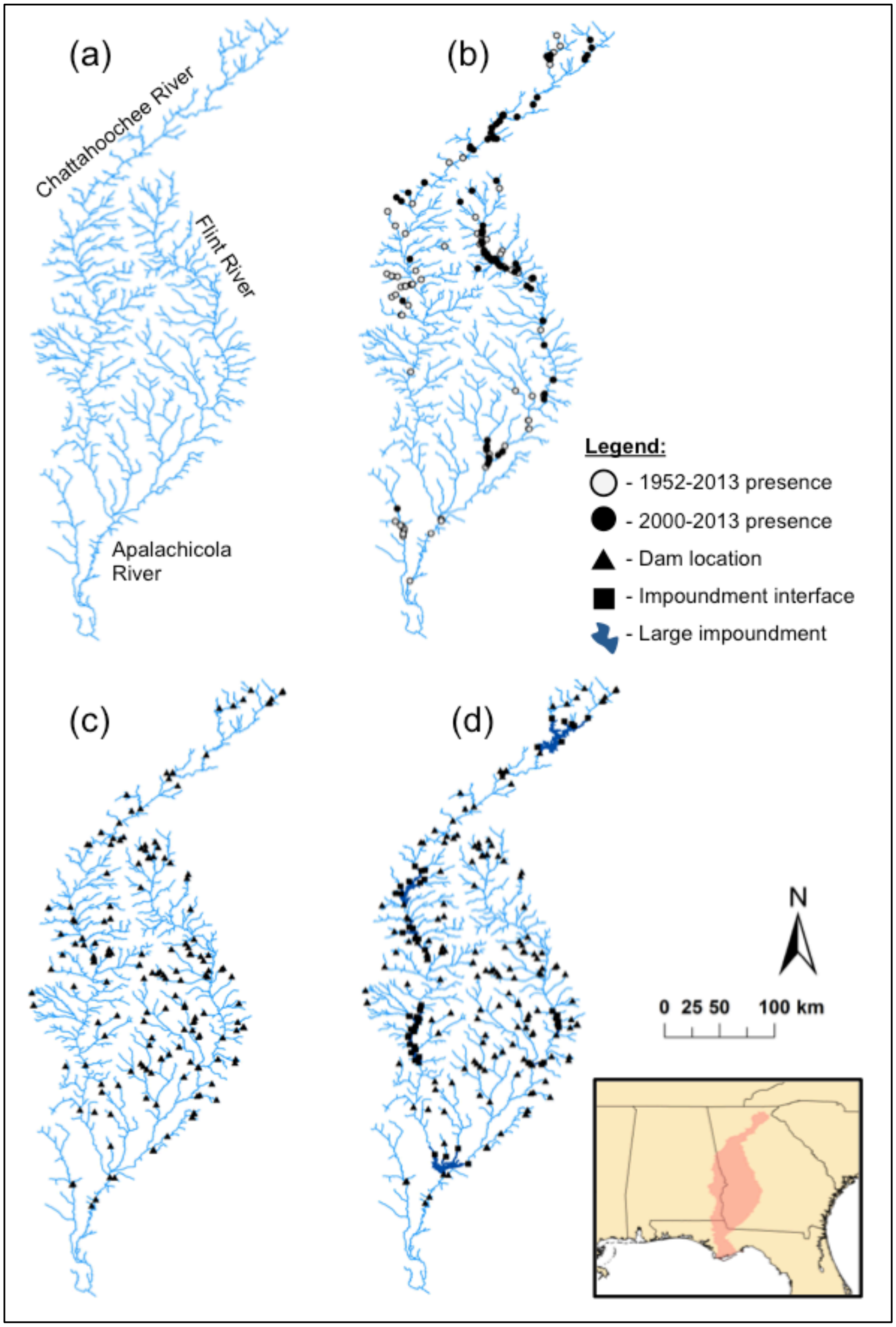

Figure 2. Maps depicting the Apalachicola-Chattahoochee-Flint Basin model base layer (a); 355 unique Shoal Bass (*Micropterus cataractae*) presence locations used to build species distribution models (SDMs; b); dam locations used to build the restricted SDM fragmented by dams only (c); dam locations and large impoundments used to build the restricted SDM with fragmentation by dams and large impoundments (d).

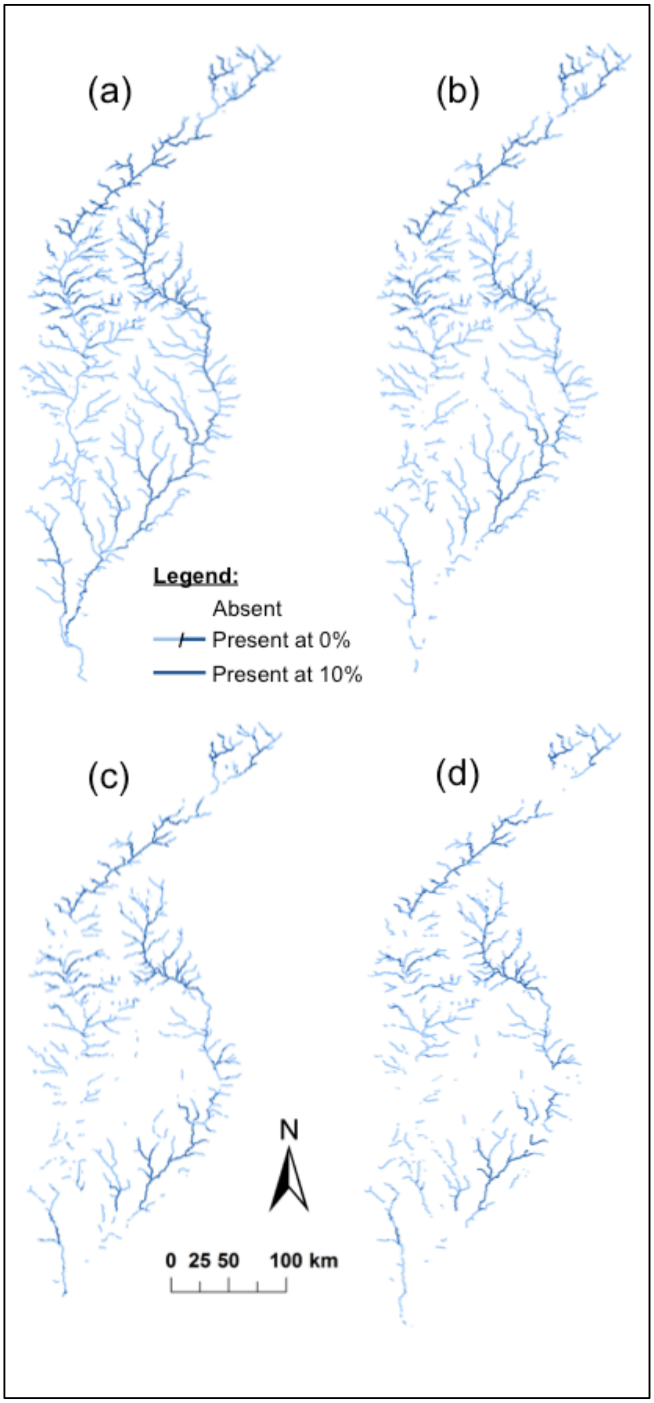

Figure 3. Estimated distributions of Shoal Bass (*Micropterus cataractae*) from species distribution models (SDMs) estimating the potential distribution (a), current restricted distribution with no fragmentation (b), restricted distribution with fragmentation by dams only (c), and restricted distribution with fragmentation by dams and large impoundments (d). Predicted Maxent probability of suitability values were reclassified to binary, suitable/unsuitable predictions using thresholds of 0% omission and 10% omission of the training data.

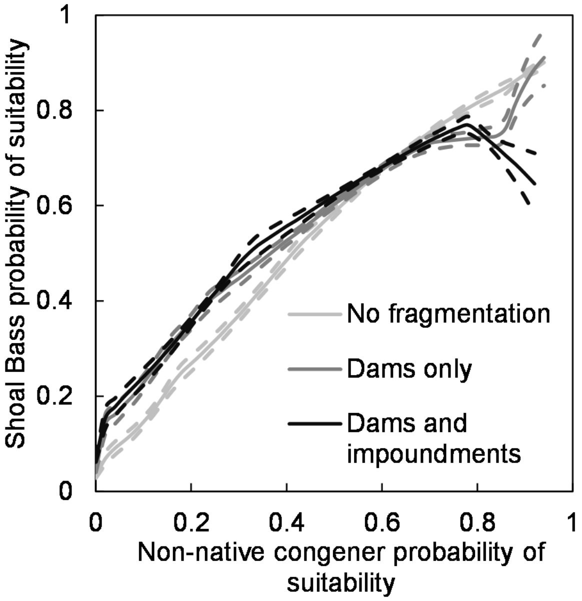

Figure 4. Response curves from three restricted species distribution models (SDMs) relating Shoal Bass (*Micropterus cataractae*) probability of suitability to probability of suitability of two non-native congener species, Spotted Bass (*M. punctulatus*) and Alabama Bass (*M. henshalli*). As fragmentation intensity increased within the restricted SDMs, the apparent relationship between Shoal Bass and the non-native congeners changed. Solid lines represent the average model response curve across the cross-validation SDM replicates ± 1 SD (dashed lines).

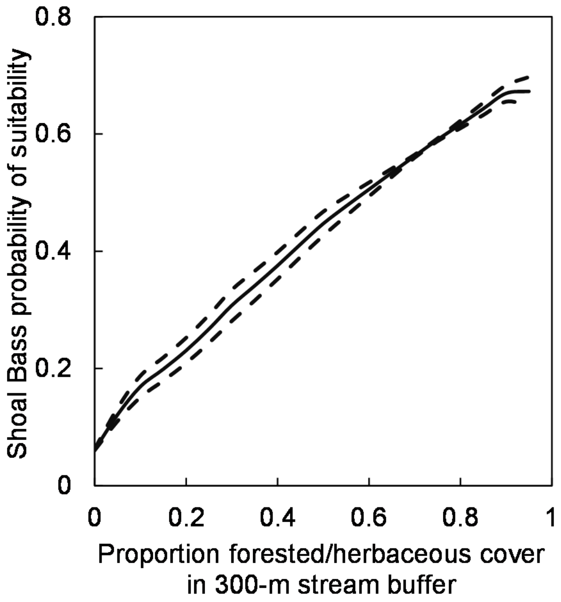

Figure 5. Response curve relating Shoal Bass (*Micropterus cataractae*) probability of suitability to the proportion of forested/herbaceous cover in segment-specific 300 m stream buffers. Similar response curves were produced in each of three restricted species distribution models (SDMs) that contained different fragmentation intensities (not shown). The solid line represents the average model response curve across the cross-validation SDM replicates ± 1 SD (dashed lines).

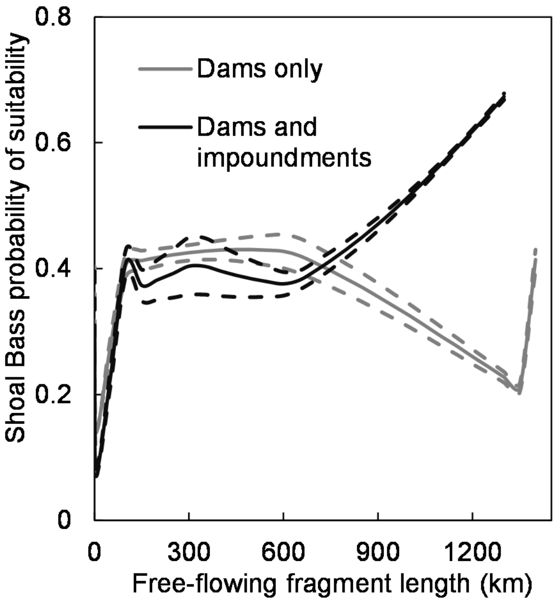

Figure 6. Response curves relating Shoal Bass (*Micropterus cataractae*) probability of suitability to free-flowing fragment length (km) of functional networks between fragmentation features. As fragmentation intensified in the restricted species distribution models (SDMs), probability of suitability of Shoal Bass increased in longer free-flowing fragment lengths. Solid lines represent the average model response curve across the cross-validation SDM replicates ± 1 SD (dashed lines).

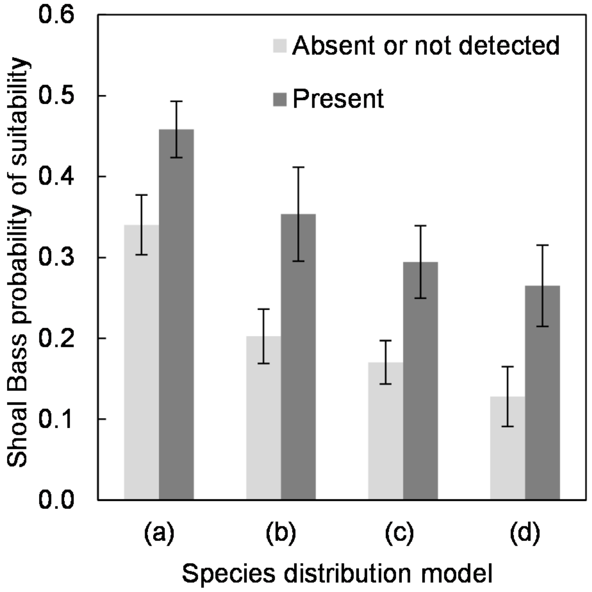

Figure 7. Outside validation of species distribution model estimates of probability of suitability for Shoal Bass (*Micropterus cataractae*) compared to directed tributary surveys by Katechis (2015), wherein 8 sites were considered absent (or not detected) and 9 sites were considered present. Mean and standard error (SE) of probability of suitability were compared among sites considered absent (or not detected) to sites considered present for each model: (a) potential distribution, (b) restricted distribution with no fragmentation, (c) restricted distribution with fragmentation by dams only, and (d) restricted distribution with fragmentation by dams and large impoundments.

Appendix I. Non-native congener (Spotted Bass *Micropterus punctulatus* and Alabama Bass *M. henshalli*) species distribution model results.

Maxent models estimating non-native congener probability of suitability had good predictive performance, with overall ROC AUC averages between 0.86 and 0.87 for all three models (SD 0.03 – 0.04) and similar predicted ranges among all three fragmentation intensities (Fig. Aa, b, c, d). Percent contribution of predictor variables to the non-native congener SDMs was similar among the four highest-contributing variables regardless of fragmentation intensity (Table A): "current" mean annual discharge contributed 21.9-26.5%, proportion urban/suburban land cover in 300 m buffer contributed 17.5-18.6%, mean depth to bedrock contributed 9.2-11.3%, and mean permeability of topsoil contributed 8.2-9.0%. Interestingly, non-native congener probability of suitability had a curvilinear response to proportion of urban/suburban land cover in the 300 m stream buffer that indicated a positive relationship until the proportion exceeded approximately 0.50, wherein the relationship became negative.

Table A. Percent contribution of predictor variables to each species distribution model (SDM). * – indicates that variable values differed among SDM environments.

	% Contribution by SDM environment		
Predictor variable (units)	**Restricted: no frag**	**Restricted: dams only**	**Restricted: dams and impoundments**
"Current" mean annual discharge (m^3/s)	26.5	24.7	21.9
Proportion urban/suburban cover in 300m buffer	18.6	18.2	17.5
Mean depth to bedrock (cm)	11.3	10.6	9.2
Mean permeability of topsoil (cm/hr)	9.0	8.3	8.2
"Current" mean annual flow velocity (m/s)	6.9	6.5	6.4
Maximum elevation (m)	6.3	5.6	3.8
Mean annual precipitation (mm)	5.0	4.6	4.0
Natural lentic waterbodies (0/1)	4.6	4.4	4.0
Proportion agricultural cover in 300m buffer	3.3	3.2	2.8
Proportion agricultural cover in HUC-12 watershed	3.2	2.7	2.9
Proportion forested/herbaceous cover in 300m buffer	3.0	3.2	3.0
Predominate geology of HUC-12 watershed	2.3	2.6	2.4
Free-flowing fragment length (km)*		3.5	5.8
Maximum accessible total drainage area (km^2)*		1.9	8.1
Total number of variables:	**12**	**14**	**14**

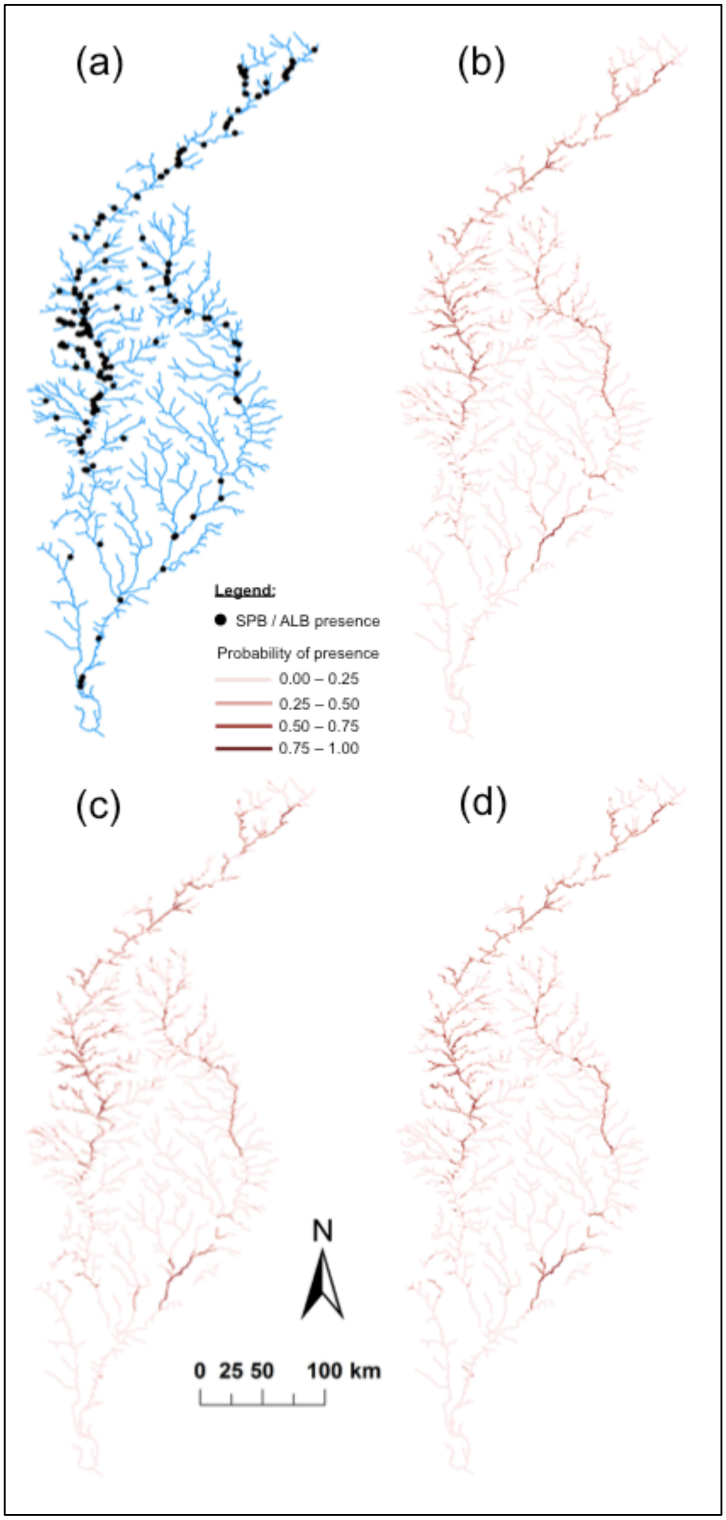

Figure A. Presence locations of Spotted Bass (*Micropterus punctulatus*; "SPB") and Alabama Bass (*M. henshalli*; "ALB"; a) used in species distribution models (SDMs), and estimations of their current distribution with no fragmentation (b), with fragmentation by dams only (c), and with fragmentation by dams and large impoundments (d).

CHAPTER II

POPULATION STRUCTURE OF THE SHOAL BASS: INFORMING RANGE-WIDE MANAGEMENT AND CONSERVATION OF A FLUVIAL SPORTFISH

Abstract

The Shoal Bass *Micropterus cataractae* is a fluvial-specialist sportfish considered vulnerable to extinction because of continued habitat alteration and loss within its native range. The species has been described as potamodromous in free-flowing stream reaches, but differences in movement patterns and life-history characteristics have been documented across the species' range. Because of continued population declines, restorative stocking efforts are becoming an increasingly relevant management strategy; however, whether population-genetic structure exists within the species is currently unknown. Leveraging a range-wide collaborative effort to collect and genotype samples with 16 microsatellite loci, our objective was to characterize hierarchical population structure and genetic differentiation of Shoal Bass, including an examination of structuring mechanisms such as relatedness and inbreeding levels. Our results revealed appreciable population structure, with five distinct Shoal Bass populations identifiable at the uppermost hierarchical level. Additional substructure was recovered within several of these populations, wherein differences in relatedness and inbreeding levels contributed to structure and appeared related to spatial isolation and temporal variation in recruitment strength. An analysis of molecular variance revealed that 4% of the variation in our dataset was accounted for among larger river drainages, whereas 87% was within sample sites. Results provide a population-genetic framework that can inform future management and stocking activities so that genetic diversity within- and among-populations is conserved and overall adaptability of the species is maintained.

Introduction

The Shoal Bass *Micropterus cataractae* is a fluvial-specialist black bass species endemic to the Apalachicola-Chattahoochee-Flint (ACF) Basin of the southeastern US (Williams and Burgess 1999; Sammons et al. 2015). In contrast to congener species like Largemouth Bass *M. salmoides* and Spotted Bass *M. punctulatus*, Shoal Bass are rarely encountered in lentic habitats and do not support impoundment fisheries (Williams and Burgess 1999; Sammons et al. 2015). Shoal Bass occur in larger rivers and streams, and are typically encountered near shoal habitats – areas characterized by high flow velocities, bedrock outcrops, and boulder substrates (Williams and Burgess 1999; Johnston and Kennon 2007; Taylor and Peterson 2014). Populations inhabiting these fluvial habitats support popular sport fisheries that have gained national attention among angling groups (Taylor and Peterson 2014). However, Shoal Bass have been eliminated from many formerly-suitable areas, spurring the American Fisheries Society Endangered Species Committee to list the Shoal Bass as "vulnerable" to extinction (Jelks et al. 2008).

A growing interest in Shoal Bass has spurred research and management during the past decade (Birdsong et al. 2010; Tringali et al. 2015b), yet the long-term conservation of the species appears uncertain (Taylor and Peterson 2014).

Throughout their historic native range in the ACF Basin, Shoal Bass populations are presumed to have suffered dramatic losses (Williams and Burgess 1999). Recent documented examples include the virtual elimination of Shoal Bass from a 58-km reach of the upper Chattahoochee River after completion of Buford Dam in 1956 (Long and Martin 2008) and the functional elimination of populations in several western tributaries to the middle Chattahoochee River in Alabama (Halawakee, Osanippa, and Wacoochee creeks; Stormer and Maceina 2008; Sammons and Maceina 2009). Habitat alteration, particularly from the serial impoundment of rivers (Williams and Burgess 1999), has fragmented populations (Dakin et al. 2015; Sammons and Early 2015) and facilitated the invasion of previously allopatric congeners following authorized and unauthorized fish stockings. These non-native congeners have negatively affected Shoal Bass populations through introgressive hybridization (Alvarez et al. 2015; Dakin et al. 2015; Tringali et al. 2015c) and possible interspecific competition (Sammons 2012; Goclowski et al. 2013).

To reverse declining trends, agencies have been actively managing and restoring Shoal Bass populations. Where Shoal Bass populations are robust enough to support sport fisheries, management tools such as daily creel and size limits have generally been effective at conserving stocks (e.g., lower Flint River, Georgia [Ingram and Kilpatrick 2015] and Chipola River, Florida [Woodside et al. 2015]). In other areas, stocking has been used to restore or supplement Shoal Bass populations. For example, from 1972 to present, the Georgia Department of Natural Resources (GADNR) has stocked Shoal Bass fingerlings in the lower Flint River between lakes Blackshear and Worth to supplement natural recruitment, which may be hindered by hydropeaking operations below Lake Blackshear. Broodstock for these fingerlings came from farther downstream within the same river basin, and year class contribution of stocked fish has varied in recent years from 14-51% (T. Ingram, GADNR, personal communication). From 2003 to 2008, the National Park Service (NPS) and GADNR stocked Shoal Bass fingerlings in a 19-km reach of the upper Chattahoochee River below Morgan Falls Dam to re-establish a population depleted by the cold water releases of an upstream dam (Long and Martin 2008; Porta and Long 2015). Similar to the Flint River stocking, broodstock came from farther downstream within the same river basin (the middle Chattahoochee River below North Highlands Reservoir), and stocked individuals contributed up to 62% to the adult population (Porta and Long 2015). Broodstock from the Chattahoochee River below North Highlands Reservoir was also employed in 2008 for a restorative stocking of fingerlings in tributaries to the Chattahoochee River situated in eastern Alabama (Halawakee, Osanippa, and Wacoochee creeks), with recovery rates varying from 4-8% (Sammons and Maceina 2009). More recently, restorative stockings in the Chattahoochee River below Morgan Falls Dam (2011-present), as well as in eastern Alabama tributaries (2009-2010), were conducted with broodstock from a different river drainage, the lower Flint River (P. O'Rouke, GADNR, personal communication; S. Rider, Alabama Department of Conservation and Natural Resources, personal communication). Despite the varied success of these efforts, stocking to sustain and restore Shoal Bass populations remains a relevant management tool, highlighting the need to evaluate genetic structure that might exist across the species' range.

Whether Shoal Bass should be managed at the species-, drainage-, or population-level remains to be addressed, affecting decisions regarding stocking protocols like broodstock source location. Within the confines of natural metapopulation structure, it remains unclear whether Shoal Bass populations were historically panmictic with little population structure across their range or relatively sedentary, accruing appreciable local population genetic structure. Recent movement studies suggest that Shoal Bass in the Chattahoochee River basin are relatively sedentary (Stormer and Maceina 2009; Sammons and Early 2015), whereas Shoal Bass in the Flint River basin have been described as potamodromous, moving long distances (≤ 200 km; Sammons 2015) to spawn in large aggregations at a few particular locations (Ingram et al. 2013; Taylor and Peterson 2015). Additional evidence suggests that marked differences in population demographics exist among populations across their native range. In the Chattahoochee River basin, Shoal Bass are long-lived (up to 14 years), slow-growing, and experience low annual mortality (20%; Porta and Long 2015; *see* Chapter 4). Conversely, Shoal Bass in the Flint River basin grow rapidly and exhibit higher total annual mortality (49%; Ingram and Kilpatrick 2015). In the Chipola River, Florida, Shoal Bass have shorter lifespans (up to 10 years) and also experience higher annual mortality (57%; Woodside et al. 2015). Because these basins differ in their natural habitats and their degree of anthropogenic alteration, it is unclear if these observed differences in movement and demographics represent natural adaptions, clinal variation, or responses to recent habitat alteration. A better understanding of existing population structure and genetic variation is imperative for guiding future management efforts to ensure existing genetic diversity and evolutionary potential is conserved.

A growing paradigm in fisheries management is conservation of genetically distinct populations to maintain genetic variation in a species (Hallerman 2003). Maintaining genetic variation requires the conservation of the genetic diversity both within and among populations (Meffe 1986). Such measures safeguard against the loss of advantageous local adaptations and also maintain a species' capacity to evolve in response a changing environment (Reisenbichler 1997). Maintaining genetic diversity and adaptive fitness is an especially acute issue for threatened or imperiled species that may already be experiencing a loss of genetic diversity because of small population sizes, inbreeding depression, and genetic drift (Meffe 1986). When gene flow between genetically dissimilar populations is artificially increased to ameliorate inbreeding depression, such as through stocking, outbreeding depression may result. This phenomenon is caused by the introgression of maladapted genes or the disruption of coadapted genomes (Lynch 1991) and results in a loss of fitness and increased susceptibility to disease (Hallerman 2003; Goldberg et al. 2005). Because stocking practices for Shoal Bass are still in their early stages, consideration of existing population structure in future stocking efforts can inform management to prevent inbreeding and outbreeding depression and further conservation of the species.

With these concerns in mind, our objective was to characterize hierarchical population structure of Shoal Bass within their native range. For each population identified, we investigated potential population structuring mechanisms by summarizing genetic diversity measures and examining relatedness and inbreeding levels, which also provided insights into conservation-genetic status. We also quantified hierarchical genetic variation and differentiation of Shoal Bass among all sample sites. Results can inform range-wide management and conservation strategies for Shoal Bass by providing a population-genetic framework for management units, which could help inform management efforts (e.g., stocking and reintroduction efforts) towards conserving existing genetic diversity within the species.

Methods

Study area and sampling.— A collaborative sampling effort was undertaken by a group of resource managers and research scientists affiliated with GADNR, Auburn University, University of Georgia, Oklahoma State University, and the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWRI). To encompass potential genetic variation within Shoal Bass, samples from a subset of rivers and streams that spanned the species' extant native range were obtained. Shoal Bass were targeted using a variety of methods (e.g., electrofishing and hook-and-line angling), and fin-clips were stored in individually labeled vials of 95% non-denatured ethanol. Samples were collected at individual localities that spanned approximately 100 m to 5 km in stream length, and geographic coordinates were taken near the center of each. Localities were grouped into a number of "sites" based on their geographic proximity to other localities and potential barriers to gene flow (e.g., dams) between localities. Within each site, samples were ordered from upstream-to-downstream based on their sampling locality and, within a given locality, samples were also sorted by sampling year (oldest-to-newest).

Molecular analyses.— We used 16 di-nucleotide microsatellite DNA markers developed to amplify *Micropterus* (*Msaf 05, 06, 08, 09, 10, 12, 13, 17, 22, 24, 25, 27, 28, 29, 31,* and *32*; Seyoum et al. 2013) to discern species composition, explore population structure, and quantify genetic differentiation. We isolated genomic DNA from fin-clip tissue samples using the Puregene DNA Purification Kit (Gentra Systems). Six multiplex polymerase chain reactions (PCR) were used to amplify microsatellites, with the following parameters used for all loci: 94°C for 2 min, 35 denaturation cycles of 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 10 min. Capillary electrophoresis was performed using a 3130 XL Genetic Analyzer on PCR products containing 13 µL of formamide (denaturation for 4 min at 95°C) and Genescan ROX 500 size standard (Applied Biosystems). Determination of allele length variants was performed in GeneMapper v. 4 (Applied Biosystems). Some putative Shoal Bass genotypes contained ≥ 1 locus that was not scored (~24 %), but all were retained for analysis because removing genotypes could reduce ability to discern population signals.

Reference samples.— Reference genotypes for congener taxa existed, but reference genotypes for Shoal Bass had to be determined from our sample dataset using a genetic clustering analysis. Reference genotypes for the following six congener taxa relevant to the ACF Basin were obtained from established sources and provided by the FWRI: Largemouth Bass x Florida Bass *M. floridanus* intergrades, Alabama Bass *M. henshalli*, Spotted Bass, Choctaw Bass *M. sp. cf. punctulatus*, Smallmouth Bass *M. dolomieu,* and Bartram's Bass *M. sp. cf. cataractae* (Taylor and Peterson 2014; Freeman et al. 2015; Tringali et al. 2015a). By including all reference congener genotypes and all putative Shoal Bass genotypes in this preliminary clustering analysis, we determined how reference congener taxa and genetic clusters aligned and which Shoal Bass genotypes would provide suitable references for range-wide comparisons. Briefly, we used Program STRUCTURE (Pritchard et al. 2000) and the approaches detailed in the following sections to identify the optimal number of genetic clusters (*K*) within this combined dataset. We performed five independent Program STRUCTURE runs that did not incorporate any *a priori* grouping information for each *K* ranging 1-15. We then estimated the optimal *K* value using the maximum value obtained from a suite of four supervised estimators (MedMeaK, MaxMeaK, MedMedK, and MaxMedK) that disregard 'spurious clusters' that fail to obtain a mean or median membership coefficient threshold of ≥ 0.50 within at least one given site or population (Puechmaille 2016). From the optimal *K* clustering assignment, we selected a number of Shoal Bass reference genotypes representative of the entire sampled range that had individual genomic proportion (*q*-value) assignments of $q \geq 0.98$ to Shoal Bass-affiliated clusters. Studies that employed similar markers and methodologies to assess hybridization reported thresholds for pure Shoal bass at $q \geq 0.980$ in the Chipola River (Tringali et al. 2015c) and $q > 0.982$ in the lower Flint River (Alvarez et al. 2015).

Taxonomic screening.— The reference genotypes for Shoal Bass and six congener taxa were used to perform a formal taxonomic assignment to identify 'pure' Shoal Bass genotypes, wherein each putative Shoal Bass genotype was proportionally assigned (i.e., *q*-values) to each taxa based on the allelic frequencies of reference genotypes. To complete the taxonomic assignment, we employed a Bayesian clustering approach in Program STRUCTURE v. 2.3.4 (Pritchard et al. 2000) and accessory programs. Program STRUCTURE proportionally assigns individual genotypes to a given number of genetic clusters (*K*) based on linkage equilibrium and conformance to Hardy-Weinberg equilibrium (Pritchard et al. 2000).

In Program STRUCTURE, we assumed the admixture ancestry model and independent allele frequencies, and we used a burn-in of 20,000 and 200,000 Markov chain Monte Carlo (MCMC) repetitions for each run. The 'PopFlag' option was employed so that genomic proportions for putative Shoal Bass genotypes were estimated using the allele frequencies from the reference genotypes of each taxon. We ran 20 independent runs at the optimal *K* value identified in the previous analysis used to identify Shoal Bass reference samples. We input those 20 runs into Program STRUCTURE HARVESTER web v. 0.6.94 (Earl and vonHoldt 2012) to obtain input files for Program CLUMPP v. 1.1.2, which provided optimal alignment of independent STRUCTURE runs with cluster matching and permutation (Jakobsson and Rosenberg 2007). Final *q*-value assignment in Program CLUMPP was obtained using the *G*' pairwise matrix similarity statistic and the 'LargeKGreedy' algorithm for 1,000 randomly sequenced runs. Program CLUMPP was used to obtain optimal alignment of independent runs with the settings used previously. To determine the threshold for 'pure' Shoal Bass individuals, we arcsine transformed (for normality) the *q*-values for assignment of reference Shoal Bass genotypes to the Shoal Bass cluster, calculated 95% confidence intervals for the transformed probabilities, and used the resulting back-transformed lower tail as a classification threshold for 'pure' Shoal Bass within our taxonomic assignment (Littrell et al. 2007).

Population structure.— After choosing reference genotypes and screening all samples to identify 'pure' Shoal Bass, we assessed hierarchical genetic structure using a similar Bayesian clustering approach implemented in Program STRUCTURE (Pritchard et al. 2000). For all STRUCTURE runs, we assumed the admixture ancestry model and correlated allele frequencies, and we used a burn-in of 20,000 and 200,000 MCMC repetitions for each run. We estimated *K* within each hierarchical level of genetic structure using the maximum value of four supervised estimators (MedMeaK, MaxMeaK, MedMedK, and MaxMedK) that disregard 'spurious clusters' that fail to obtain a mean or median membership coefficient threshold of ≥ 0.50 for at least one site (Puechmaille 2016).

We proceeded in a hierarchical fashion, first estimating the optimal *K* value at the uppermost hierarchical level across all sites. At this level, uneven sampling across sites could reduce power to detect structure at sites with smaller sample sizes and emphasize differences within sites that have larger sample sizes (Puechmaille 2016), potentially underestimating *K*. To reduce the effect of uneven sampling, we used supervised estimators that accurately identified hierarchical structure in unevenly sampled datasets (Puechmaille 2016). Additionally, we analyzed a dataset of approximately equal numbers of individuals that contained a maximum of 20 randomly selected genotypes per site. For each dataset, we ran 10 independent runs of *K* = 1-15 without any *a priori* sample site information. The dataset producing the largest *K* value was then adopted to explore the secondary level of hierarchical genetic structure among sites that had a mean membership coefficient ≥ 0.50 to a given cluster. Other sites were considered mixtures of the clusters identified at the uppermost level and were not explored further. To explore the secondary level of hierarchical genetic structure, we conducted 10 independent runs of *K* = 1-5 for each combination of sites dominated by a given genetic cluster. If no structure was detected, we also performed the analysis using sample site as prior information ('LocPrior' option), which allows STRUCTURE models to more easily detect existing structure among sites despite weak population signals that may not be detected using standard models (Pritchard et al. 2000). Final q-value assignments were obtained in Program CLUMPP using settings detailed previously. For each hierarchical level's optimal K, we recorded the mean and SD of the log probability $L(K)$, which is a measure of model fit.

Structuring mechanisms.– Because STRUCTURE models assume linkage equilibrium and Hardy-Weinberg equilibrium within populations (Pritchard et al. 2000), differences in homozygosity, allelic diversity, private alleles, and mating patterns (i.e., relatedness and inbreeding levels) can provide insights into structuring mechanisms. In each population or subpopulation identified, we reported the number of private alleles and the mean number of alleles (A), effective number of alleles (A_e), expected heterozygosity (H_e), and observed heterozygosity (H_o) as calculated in Program GENALEX v. 6.502 (Peakall and Smouse 2006). We also calculated the mean pairwise relatedness (r) and mean inbreeding coefficient (F) within each population and subpopulation. These metrics were calculated in Program COANCESTRY (Wang 2011) using 1,000 bootstraps and 100 control samples to estimate r with the TrioML estimator (Wang 2007) and F with the Ritland estimator (Ritland 1996). We also tested for differences in mean r and F between subpopulations contained within a higher-level population using 1,000 bootstraps to generate 95% C.I.'s used to determine significance at $P \leq 0.05$ (Wang 2011). We visualized proportional structure of relatedness in all identified populations using the following general kinship categories: 0.0% = unrelated;

0.1-6.2% = second (or greater) cousins; 6.3-12.5% = first cousins or half-niece/nephew; 12.6-25.0% = grandchild or niece/nephew; 25.1-50.0% = approaching child or sibling; and 50.1-100.0% = inbred.

Genetic differentiation.— We performed an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in Program ARLEQUIN v. 3.5.1.3 (Excoffier and Lischer 2010) to estimate the amount of genetic variation present at the following hierarchical levels: within sites, among sites within major river drainages, and among major river drainages. We used the locus-by-locus AMOVA methodology to produce a global AMOVA from a weighted average over all loci. We also computed pairwise fixation index values (F_{ST}; Wright 1951; Weir and Cockerham 1984), which provide a commonly used measure of genetic differentiation between sites. Negative F_{ST} values were interpreted as no differentiation ($F_{ST} = 0$). Tests of significance for the global AMOVA and pairwise F_{ST} values were calculated based on 10,000 permutations at $P \leq 0.05$.

Results

From 2005 to 2015, our collaborative efforts produced 829 putative Shoal Bass genotypes from 13 sites that spanned the extant range of the species (Table 1; Fig. 1). Two sites contained ≥ 150 individuals: the lower Flint River below Lake Blackshear (site 9) and the Chipola River (site 13). All other sites were represented by 17-77 individuals each.

Reference samples.— The six congener taxa included in our analyses were represented by approximately 40-60 reference genotypes each, although Bartram's Bass was represented by only 15 samples (Table 2). Among the six congener reference taxa and all putative Shoal Bass genotypes, the optimal number of clusters was $K = 10$, wherein the six congener taxa were each represented by distinct genetic clusters and putative Shoal Bass genotypes contained four additional, unique genetic clusters. The four Shoal Bass-affiliated clusters identified were likely an artifact of the large sample size of putative Shoal Bass genotypes rather than taxon-level differences (Puechmaille 2016); thus, we grouped the four Shoal Bass clusters into one, from which the $q \geq 0.98$ selection criteria was applied to attain 60 reference Shoal Bass genotypes (mean $q = 0.992$; range: 0.980-0.994).

Taxonomic screening.— The formal taxonomic assignment using the six congener reference taxa plus the Shoal Bass references was conducted at $K = 7$ (we considered the four Shoal Bass-affiliated clusters to represent one taxa, plus six other distinct clusters that aligned with congener taxa). The resulting classification threshold for pure Shoal Bass was at $q \geq 0.988$. At this threshold, 644 pure Shoal Bass genotypes were used to explore hierarchical population structure and genetic variation, with individual sites containing a range of 13-183 fish (Fig. 2).

Population structure.— At the uppermost hierarchical level, the complete dataset of pure Shoal Bass genotypes (N = 644) was estimated to contain $K = 5$ clusters, whereas the subsampled dataset of evenly-represented genotypes was estimated to contain $K = 4$ clusters. The complete dataset at $K = 5$ provided an assignment with clusters representing the Chattahoochee and Chestatee rivers above Lake Lanier (sites 1 and 2); Little Uchee Creek (site 5); the upper Flint River and Big Lazer Creek (sites 6-8); the lower Flint River (sites 9 and 10); and the Chipola River (site 13; Fig. 3a). Sites 3, 4, 11, and 12 were areas of mixture with no clear assignment to any cluster (mean membership coefficients < 0.50). The subsampled dataset at $K = 4$ revealed a distinct cluster in Little Uchee Creek (site 5), another among upper Flint River sites and Big Lazer Creek (sites 6-8), and all other sites were a mixture of two other clusters. Because the complete dataset produced the largest K value, it was adopted to explore the secondary level of hierarchical genetic structure.

At the secondary hierarchical level, substructure was evident in three groups (Fig. 3b). Substructure was detected ($K = 2$) within the Chipola River (site 13) without prior information; however, the substructure recovered did not correspond with spatial position within the river, but rather sampling year (2007-09 vs. 2010). Additional substructure ($K = 2$) was also recovered between the upper Chattahoochee River (site 1) and Chestatee River (site 2), as well as between Big Lazer Creek (site 7) and mainstem upper Flint River sites (sites 6 and 8), although only when site information was used as prior information during model runs. No additional structure ($K = 1$) was recovered in Little Uchee Creek (site 5) or in the lower Flint River sites (sites 9 and 10), regardless of whether site information was used as prior information. We did not investigate genetic structure beyond the second hierarchical level, as population signals were much weaker at the secondary level and, in many instances, subpopulation boundaries corresponded with sampling sites (i.e., smallest spatial grain). Mean estimates of $L(K)$ indicated best fit at the uppermost hierarchical level ($K = 5$) and less fit in groupings among the secondary hierarchical level (Table 3).

Structuring mechanisms.– No large deviations from Hardy-Weinberg equilibrium occurred, as cluster-specific H_o did not deviate $\geq |0.031|$ from H_e (Table 4). At the uppermost hierarchical level, allelic diversity was relatively low in the Little Uchee Creek (A_e = 1.378) and Chipola River (A_e = 1.958) clusters. At the secondary level, the Chestatee River cluster also had relatively low allelic diversity (A_e = 1.995). Heterozygosity was markedly low (H_o = 0.199) in the Little Uchee Creek cluster. In contrast, the upper Flint River cluster had some of the highest measures of allelic diversity (A_e = 2.396) and heterozygosity (H_o = 0.370). The Chipola River population had the highest number of private alleles, with 4 in the 2007-09 sample years and an additional 4 in the 2010 sample. Mean pairwise relatedness was highest in Little Uchee Creek (r = 0.162), followed by the 2010 sub-grouping in the Chipola River (r = 0.138), which also had the highest mean inbreeding coefficient (F = 0.131; Table 5). Tests for significant differences in mean r and F between subpopulations nested within an upper-level population revealed one significant difference – pairwise relatedness between the upper Chattahoochee River and the Chestatee River (r = 0.082 vs. 0.107, respectively). Proportional structure of pairwise relatedness was generally similar across all sites, with a few notable exceptions: Little Uchee Creek had a relatively high percentage of pairwise relationships indicative of inbreeding (18%, compared to approximately 1% elsewhere), Big Lazer Creek had a relatively high percentage of pairwise relationships approximating first cousins (23%, compared to 10-15% elsewhere), and the 2010 grouping in the Chipola River had a relatively low percentage of pairwise relationships that were unrelated (31%, compared to approximately 50% elsewhere; Fig. 4).

Genetic differentiation.— The global AMOVA indicated that a significant majority (87.4%; $P < 0.0001$) of genetic variation was located within sites (Table 6). Significant variation was also attributable to differences among sites within river drainages (8.9%; $P < 0.0001$), and a small, but significant, remainder of variation was attributable to differences among river drainages (3.6%; $P = 0.021$). Pairwise F_{ST} values indicated that the following sites were significantly ($P \leq 0.05$) differentiated from all other sites: upper Chattahoochee River (site 1), Chestatee River (2), Big Creek (3), Little Uchee Creek (5), Big Lazer Creek (7), and Chipola River (13; Table 7). The Little Uchee Creek (5) population consistently had the highest pairwise F_{ST} values, ranging from 0.20 to 0.36. The Chipola River (13) population also had high pairwise F_{ST} values, ranging from 0.07 to 0.19.

Discussion

Recent efforts to manage and conserve black basses have increasingly focused on understanding genetic diversity within species (Shaw 2015). Our results demonstrate that considerable, and previously undocumented, genetic structure and diversity exists within the Shoal Bass across its native range. These results not only provide insight into the ecology of the species and the conservation-genetic status of populations, but also identify population boundaries that can be used to inform future management and restoration efforts for Shoal Bass.

The population structure uncovered at the uppermost hierarchical level provides insight into broad-scale Shoal Bass metapopulation structure, although results are likely influenced by natural landscape features and recent anthropogenic alterations. Population structure at this level generally coincided with at physical barriers to movement (e.g., waterfalls or dams) or areas of suboptimal habitat (e.g., impoundments or riverine areas devoid of shoals) that have restricted dispersal to a degree in which population boundaries are maintained. For example, the population above Lake Lanier within the upper Chattahoochee and Chestatee rivers appears to be isolated from downstream populations by Lake Lanier (*see also* Dakin et al. 2015). The Little Uchee Creek population is likely isolated by at least one 4-m tall waterfall (Stormer and Maceina 2009) and presumably suboptimal, slow-moving habitats that occur in Little Uchee Creek as it flows south of the Fall Line and meets the mainstem Chattahoochee River. Similarly, the Fall Line, a high-gradient transition zone between the Piedmont and Southeastern Plains ecoregions, appears to serve as a natural structuring mechanism between the upper and lower Flint River populations, although Lake Blackshear and stocking activities downstream could have confounded the observed differences in genetic structure. The Chipola River population appears to be isolated from other populations by Dead Lake, a swampy natural lake situated between shoal habitats in the Chipola River and the larger Apalachicola River (Tanner 1966). Despite the appreciable population structure, individual proportional assignments suggest there has been some degree of genetic connectivity among most mainstem populations, which attests to the dispersal capability of the species (*see* Sammons 2015). However, our results may not accurately reflect present-day connectivity because of time lags associated with genetic approaches (Epps and Keyghobadi 2015); thus, populations with recently-imposed barriers to connectivity may become increasingly differentiated in the future.

At the secondary level of population structure, finer-scale structuring mechanisms were evident that also provided insight into the conservation status of these populations. For example, the Little Uchee Creek population had relatively high measures of genetic differentiation, low measures of genetic diversity, and a high proportion of inbred individuals. Lack of genetic connectivity, along with the effects of genetic drift in a small population, could explain these results. In fact, since our sampling of Little Uchee Creek in 2007, the Shoal Bass population now appears to be functionally eliminated (Sammons and Maceina 2009). In the naturally-isolated Chipola River population, we discovered temporal population structure that was likely caused by familial influences linked to recruitment variation. The 2010 Chipola River sample displayed increased relatedness measures and approximately double the amount of inbreeding from previous years' samples. Poor recruitment and year-class failures have been documented in the Chipola River population in association with increased spring and summer discharge (Woodside et al. 2015). The 2010 sample was dominated by the 2008 year-class, one of the first year-classes following a prolonged span of unfavorable discharge conditions from 2000-2006 (Woodside et al. 2015). Overall, these results suggest that increased inbreeding may result from extreme recruitment variability in isolated Shoal Bass populations (e.g., Big Creek; *see* Chapter 4), although further investigation of this hypothesis is warranted.

Our study has obvious implications for the scale at which management could affect conservation genetics within and among Shoal Bass populations. Population-genetic diversity serves as a "diversified portfolio" to maintain overall stability and adaptability of a species (Hallerman 2003; Schindler et al. 2010), which is particularly pertinent for species of conservation concern like Shoal Bass (Meffe 1986). The large amount of within-site genetic variation obtained in our study mirrors that observed in similar species, such as riverine and lacustrine populations of Smallmouth Bass (Borden and Stepien 2006; Hallerman et al. 2015), but management at such a fine spatial scale is typically impractical. Conversely, conducting supplemental stocking and other management activities at a scale that only considers three main river drainages (Chattahoochee, Flint, and Apalachicola) would potentially only maintain 3.6% of existing variation. We identified five distinct genetic populations of Shoal Bass at the uppermost hierarchical level, and some appreciable substructure below that level, which could be used to define relevant management units (Barthel et al. 2010; Hallerman et al. 2015), both among populations (i.e., upper-level hierarchical structure) and within populations (i.e., secondary-level structure). Similarly, measures of genetic differentiation identified populations harboring unique diversity. For example, the Chipola River population had high levels of genetic differentiation when compared to other populations in the Chattahoochee and Flint Rivers, highlighting the relative distinctiveness of that population. A more thorough sampling of genotypes across the native range of the species, especially in isolated tributaries, could identify additional population boundaries relevant to management.

Because stocking efforts are ongoing and likely to continue to supplement and restore Shoal Bass populations, our results could be useful in refining those strategies. Some Shoal Bass populations are fragmented and vulnerable to the effects of low genetic diversity and inbreeding depression (e.g., Big Creek; Dakin et al. 2015). Other populations have seemingly suffered functional extirpations, including the Little Uchee Creek population in the years following our genetic sampling (Stormer and Maceina 2008; Sammons and Maceina 2009). Introgressive hybridization with non-native congener species like Spotted Bass, Smallmouth Bass, and Alabama Bass appears to be a widespread threat to the genetic integrity of the Shoal Bass throughout its native range (Alvarez et al. 2015; Dakin et al. 2015; Tringali et al. 2015c). Stocking efforts could serve to supplement dwindling populations, restore extirpated areas of the species' range, and swamp gene pools with native alleles to combat introgression of non-native alleles. For example, supplemental stocking to restore native Guadalupe Bass *M. treculii* in Texas streams reduced introgression with non-native Smallmouth Bass without severely depressing existing genetic diversity (Bean et al. 2013; Fleming et al. 2015; *but see* Littrell et al. 2007). Similar efforts to restore or supplement Shoal Bass populations may be warranted and hold promise as management and conservation tool, but the source of broodstock would be important given the results of this study.

Despite the potential utility of stocking, stocking-related activities that disregard existing population boundaries could result in loss of genetic diversity and distinctiveness. For example, non-significant pairwise F_{ST} values typically occurred between sites situated in close geographic proximity; however, comparisons between the Chattahoochee River below Morgan Falls Dam (site 4) and some upper Flint River (sites 6 and 8) and lower Flint River sites (9-12) represented disparate regions of the range with no significant genetic differentiation – a result that may be related to past stocking activities. In 2005, stocking below Morgan Falls Dam used broodstock from within the river basin, and these fish had q-value assignments similar to other upper Chattahoochee River sites. However, the majority of fish in the 2015 sample had q-value assignments resembling lower Flint River fish, which had recently been used as a broodstock source below Morgan Falls Dam. Using broodstock from a different population to supplement a target population can result in a loss of genetic diversity and local adaptations (Rhymer and Simberloff 1996). Furthermore, the effects of artificially mixing genetically distinct populations can lead to outbreeding depression and loss of fitness (Hallerman 2003). For example, Largemouth Bass crossed from two distinct populations exhibited increased susceptibility to disease than either non-outcrossed native population (Goldberg et al. 2005). Planning broodstock collections, translocations, and stocking efforts within genetic management units could better safeguard the existing genetic diversity within and among populations while also avoiding potential outbreeding depression.

At present, management agencies and stakeholders are devising a range-wide management plan for Shoal Bass, complete with benchmark goals for conservation. Results of this study could be incorporated into that plan similar to what was done for Florida Bass (Barthel et al. 2010; Porak et al. 2015) and Neosho Smallmouth Bass *M. dolomieu velox* (Taylor et al. 2016). General guidelines, such as identifying potential broodstock sources, screening brood fish for non-native alleles, hatchery propagation, and similar stocking-related protocols that have the potential to affect populations across jurisdictional boundaries could all be considered, as well as potential trade-offs associated with inbreeding depression and outbreeding depression. Expanded sampling and population-genetic research would provide more detailed delineations relevant to future management efforts, and could also allow for landscape-genetic approaches to quantify the effects of anthropogenic barriers on gene flow. Until such research is completed, the results presented herein provide novel insights into the population structure of the Shoal Bass that could help serve as a blueprint for management within a population-genetic framework.

References

Alvarez, A. C., D. L. Peterson, A. T. Taylor, M. D. Tringali, and B. L. Barthel. 2015. Distribution and amount of hybridization between Shoal Bass and the invasive Spotted Bass in the lower Flint River, Georgia. Pages 503–521 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Barthel, B. L., D. J. Lutz-Carrillo, K. E. Norberg, W. F. Porak, M. D. Tringali, T. W. Kassler, W. E. Johnson, A. M. Readel, R. A. Krause, and D. P. Philipp. 2010. Genetic relationships among populations of Florida Bass. Transactions of the American Fisheries Society 139(6):1615–1641.

Bean, P. T., D. J. Lutz-Carrillo, and T. H. Bonner. 2013. Rangewide survey of the introgressive status of Guadalupe Bass: implications for conservation and management. Transactions of the American Fisheries Society 142(3):681–689.

Birdsong, T., D. Krause, J. Leitner, J. M. Long, S. Robinson, and S. Sammons. 2010. A business plan for the conservation of native black bass species in the southeastern U.S. National Fish and Wildlife Foundation, Washington, D.C.

Borden, C. W., and C. A. Stepien. 2006. Discordant population genetic structuring of Smallmouth Bass, *Micropterus dolomieu* Lacepéde, in Lake Erie based on mitochondrial DNA sequences and nuclear DNA microsatellites. Journal of Great Lakes Research 32(2):242–257.

Dakin, E. E., B. A. Porter, B. J. Freeman, and J. M. Long. 2015. Hybridization threatens Shoal Bass populations in the upper Chattahoochee River Basin. Pages 491–501 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

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

Epps, C. W., and N. Keyghobadi. 2015. Landscape genetics in a changing world: disentangling historical and contemporary influences and inferring change. Molecular Ecology 24(24):6021–6040.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3):564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131(2):479–491.

Fleming, P. B., G. P. Garrett, and N. G. Smith. 2015. Reducing hybridization and introgression in wild populations of Guadalupe Bass through supplemental stocking. Pages 537–547 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Freeman, B. J., A. T. Taylor, K. J. Oswald, J. Wares, M. C. Freeman, J. M. Quattro, and J. K. Leitner. 2015. Shoal Basses: a clade of cryptic identity. Pages 449–466 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Goclowski, M. R., A. J. Kaeser, and S. M. Sammons. 2013. Movement and habitat differentiation among adult Shoal Bass, Largemouth Bass, and Spotted Bass in the upper Flint River, Georgia. North American Journal of Fisheries Management 33(1):56–70.

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

Hallerman, E. M., editor. 2003. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Hallerman, E. M., N. Johnson, and D. Dutton. 2015. Implications of microsatellite DNA variation upon management of Virginia Smallmouth Bass populations. Pages 615–633 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Ingram, T. R., and J. M. Kilpatrick. 2015. Assessment of the Shoal Bass population in the lower Flint River, Georgia. Pages 157–168 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Ingram, T. R., J. E. Tannehill, and S. P. Young. 2013. Post-release survival and behavior of adult Shoal Bass in the Flint River, Georgia. North American Journal of Fisheries Management 33(4):717–722.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. W. Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33(8):372–407.

Johnston, C. E., and R. A. Kennon. 2007. Habitat use of the Shoal Bass, *Micropterus cataractae*, in an Alabama Stream. Journal of Freshwater Ecology 22(3):493–498.

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

Long, J. M., and C. Martin. 2008. The evolution of fisheries management of the upper Chattahoochee River in response to changing water management. Pages 21–36 *in* M. S. Allen, S. M. Sammons, and M. J. Maceina, editors. Balancing fisheries management and water uses for impounded river systems. American Fisheries Society, Symposium 62, Bethesda, Maryland.

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

Meffe, G. K. 1986. Conservation genetics and the management of endangered fishes. Fisheries 11(1):14–23.

Peakall, R., and P. E. Smouse. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288–295.

Porak, W. F., B. L. Barthel, R. B. Stout, M. D. Tringali, M. Matthews, W. F. Pouder, and D. P. Philipp. 2015. The development of a genetic conservation program for the Florida Bass in its native range. Pages 585–600 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Porta, M. J., and J. M. Long. 2015. Evaluation of a five-year Shoal Bass conservation-stocking program in the upper Chattahoochee River, Georgia. Pages 169–180 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945–959.

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

Reisenbichler, R. R. 1997. Genetic factors contributing to declines of anadromous salmonids in the Pacific Northwest. Pages 223–244 *in* D. J. Stouder, P. A. Bisson, and R. J. Naiman, editors. Pacific Salmon & their Ecosystems. Springer US.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27:83–109.

Ritland, K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genetics Research 67(2):175–185.

Sammons, S. M. 2012. Diets of juvenile and sub-adult size classes of three *Micropterus spp.* in the Flint River, Georgia: potential for trophic competition. Southeastern Naturalist 11(3):387–404.

Sammons, S. M. 2015. First evidence of potadromy and partial migration in black basses: Shoal Bass *Micropterus cataractae* (Actinopterygii, Centrarchidae) in the Upper Flint River, USA. Hydrobiologia 751(1):135–146.

Sammons, S. M., and L. A. Early. 2015. Movement and habitat use of Shoal Bass in a regulated portion of the Chattahoochee River, Alabama-Georgia, USA. Pages 249–261 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Sammons, S. M., and M. J. Maceina. 2009. Conservation status of Shoal Bass in Alabama: distribution, abundance, stocking efficacy, and possible effects of sympatric congeneric black bass in selected tributaries of the Chattahoochee River, Alabama. Alabama Division of Wildlife and Freshwater Fisheries, Final Report, Montgomery, Alabama.

Sammons, S. M., K. L. Woodside, and C. J. Paxton. 2015. Shoal Bass *Micropterus cataractae* Williams & Burgess, 1999. Pages 75–81 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Schindler, D. E., R. Hilborn, B. Chasco, C. P. Boatright, T. P. Quinn, L. A. Rogers, and M. S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465(7298):609–612.

Seyoum, S., B. L. Barthel, M. D. Tringali, M. C. Davis, S. L. Schmitt, P. S. Bellotti, and W. F. Porak. 2013. Isolation and characterization of eighteen microsatellite loci for the Largemouth Bass, *Micropterus salmoides*, and cross amplification in congeneric species. Conservation Genetics Resources 5(3):697–701.

Shaw, A. C. 2015. Black bass diversity and conservation: an overview. Pages 3–8 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

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

Stormer, D. G., and M. J. Maceina. 2009. Habitat use, home range, and movement of Shoal Bass in Alabama. North American Journal of Fisheries Management 29(3):604–613.

Tanner, W. F. 1966. Late Cenozoic history and coastal morphology of the Apalachicola River region, western Florida. Pages 83–97 Deltas in Their Geologic Framework. Houston Geological Society.

Taylor, A. T., J. M. Long, M. R. Schwemm, M. D. Tringali, and S. K. Brewer. 2016. Identification of Neosho Smallmouth Bass (*Micropterus dolomieu velox*) stocks for possible introduction into Grand Lake, Oklahoma. U.S. Fish and Wildlife Service Cooperator Science Series, 121–2016.

Taylor, A. T., and D. L. Peterson. 2014. Shoal bass life history and threats: a synthesis of current knowledge of a *Micropterus* species. Reviews in Fish Biology and Fisheries 24(1):159–167.

Taylor, A. T., and D. L. Peterson. 2015. Movement, homing, and fates of fluvial-specialist Shoal Bass following translocation into an impoundment. Southeastern Naturalist 14(3):425–437.

Tringali, M. D., B. L. Barthel, S. Seyoum, and J. R. Knight. 2015a. The Choctaw Bass: an undescribed species of *Micropterus* in the Gulf Coastal Plain Rivers of Florida. Pages 421–448 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. 2015b. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Tringali, M. D., P. A. Strickland, R. A. Krause, S. Seyoum, B. L. Barthel, A. Alvarez, and C. Puchulutegui. 2015c. Pages 523–536 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Conservation status of Shoal Bass in the Chipola River, Florida: the threat of hybridization with native and nonnative congeners. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Wang, J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetics Research 89(3):135–153.

Wang, J. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11(1):141–145.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6):1358–1370.

Williams, J. D., and G. H. Burgess. 1999. A new species of bass, *Micropterus cataractae* (Teleostei: Centrarchidae), from the Apalachicola River basin in Alabama, Florida, and Georgia. University of Florida.

Woodside, K. L., C. J. Paxton, and N. C. Kierl. 2015. Stock assessment of Shoal Bass in the Chipola River, Florida. Pages 139–155 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323–354.

Table 1. Sample collection data for analysis of Shoal Bass genetic structure across their native range, including site number (see Fig. 1), location description, major river drainage (“CHA” = Chattahoochee River, “FLI” = Flint River, “APA” = Apalachicola River), collection years, number (*N*) of putative Shoal Bass *Micropterus cataractae* collected, and number (*N*) of genetically ‘pure’ Shoal Bass following taxonomic screening.

Site	location	drainage	collection years	putative *N*	pure *N*
1	Upper Chattahoochee River	CHA	2005, 2013	69	59
2	Chestatee River	CHA	2013	42	32
3	Big Creek	CHA	2005, 2015	77	23
4	Chattahoochee River below Morgan Falls Dam	CHA	2005, 2015	42	19
5	Little Uchee Creek	CHA	2008	22	22
6	Upper Flint River above Fall Line	FLI	2009, 2011	32	24
7	Big Lazer Creek	FLI	2013	18	16
8	Upper Flint River below Fall Line	FLI	2011	17	13
9	Lower Flint River below Lake Blackshear	FLI	2009 - 2011	159	137
10	Lower Flint River below Lake Worth	FLI	2009 - 2011	28	24
11	Lower Flint River	FLI	2009, 2011	50	35
12	Ichawaynochaway Creek	FLI	2010 - 2011	64	57
13	Chipola River	APA	2007 - 2010	209	183
		Totals:	**2005 - 2015**	**829**	**644**

Table 2. Common name, scientific name, and number of individuals included as reference genotypes for the taxonomic screening of microsatellite DNA genotypes for analysis of Shoal Bass genetic structure across their native range.

Common name	**scientific name**	***N***
Largemouth Bass x Florida Bass intergrades	*Micropterus salmoides* x *M. floridanus*	62
Alabama Bass	*M. henshalli*	63
Spotted Bass	*M. punctulatus*	69
Choctaw Bass*	*M. haiaka*	56
Smallmouth Bass	*M. dolomieu*	41
Bartram's Bass**	*M. sp. cf. cataractae*	15
Shoal Bass	*M. cataractae*	60
		Total: 366

*Choctaw Bass has been recommended for species recognition (Tringali et al. 2015a)

**Bartram’s Bass has been recommended for species recognition (Freeman et al. 2015)

Table 3. Bayesian clustering model specifications and fit statistics used to investigate hierarchical Shoal Bass genetic structure across their native range, including hierarchical level of genetic structure (level), genetic cluster (site numbers from Table 1), estimated number of clusters (*K*), whether or not site locations were used as model priors (LocPrior), mean estimated log probability of the data (*L*(*K*)), and standard deviation of each mean estimate (SD).

Level	genetic cluster (site numbers)	*K*	runs	LocPrior	mean estimated L(*K*)	SD
1	All	5	10	No	-13617.26	911.89
2	1 and 2	2	10	Yes	-1853.21	4.05
2	5	1	10	No	-211.03	0.29
2	6, 7, and 8	2	10	Yes	-1286.00	26.89
2	9 and 10	1	10	No	-3237.38	0.24
2	13 (by year)	2	10	No	-3238.13	39.65

Table 4. Measures of genetic diversity within Shoal Bass populations identified based on analysis of 16 microsatellite DNA loci. Levels indicate the hierarchy of genetic population structure, wherein level 1 is the uppermost hierarchical level and level 2 is secondary structure nested within level 1. Site numbers corresponding to locations in the study map (Fig. 1) are given in parentheses after site names. Measures of genetic diversity include the mean across all loci for expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (A), and effective number of alleles (A_e). We also reported the number of private alleles ($A_{private}$) and sample size of genotypes (N).

[Level] cluster name (site number)	H_e	H_o	A	A_e	$A_{private}$	N
[1] Above Lake Lanier (1 and 2)	0.324	0.320	3.625	2.223	3	91
[2] Upper Chattahoochee River (1)	0.316	0.319	3.500	2.206	2	59
[2] Chestatee River (2)	0.324	0.324	2.875	1.995	1	32
[1] Little Uchee Creek (5)	0.189	0.199	1.625	1.378	1	22
[1] Upper Flint (6, 7, and 8)	0.364	0.334	4.313	2.419	3	53
[2] Upper Flint Mainstem (6 and 8)	0.370	0.345	3.938	2.396	2	37
[2] Big Lazer Creek (7)	0.332	0.309	3.063	2.165	1	16
[1] Lower Flint (9 and 10)	0.309	0.313	4.375	2.308	3	161
[1] Chipola River (13)	0.327	0.310	3.313	1.958	8	183
[2] Chipola River, years 2007-09	0.322	0.309	2.875	1.844	4	123
[2] Chipola River, year 2010	0.326	0.319	2.813	1.861	4	41
[2] Chipola River, year unknown	0.311	0.292	2.188	1.804	0	19
[1] Mixed (3, 4, 11, and 12)	0.324	0.323	4.500	2.345	1	134

Table 5. Mean pairwise relatedness (*r*) and mean inbreeding coefficient (*F*) measures within Shoal Bass populations identified based on analysis of 16 microsatellite DNA loci. Levels indicate the hierarchy of genetic population structure, wherein level 1 is the uppermost hierarchical level and level 2 is secondary structure nested within level 1. Site numbers corresponding to locations in the study map (Fig. 1) are given in parentheses after site names. Sample sizes (*N*) differed because *r* is calculated with pairwise groupings of individuals, whereas *F* is calculated using the number of individuals. We also tested for significant differences ($P \leq 0.05$) in mean *r* and *F* between subpopulations [Level 2] contained within a higher-level population [Level 1]. Significant comparisons are in **bold**.

	r			***F***		
[Level] cluster name (site number)	**est.**	**SD**	***N***	**est.**	**SD**	***N***
[1] Above Lake Lanier (1 and 2)	0.078	0.129	4095	0.000	0.141	91
[2] Upper Chattahoochee River (1)	**0.082**	0.127	1711	-0.007	0.151	59
[2] Chestatee River (2)	**0.107**	0.163	496	0.013	0.118	32
[1] Little Uchee Creek (5)	0.162	0.220	231	-0.068	0.266	22
[1] Upper Flint (6, 7, and 8)	0.060	0.103	1378	0.030	0.109	53
[2] Upper Flint Mainstem (6 and 8)	0.071	0.111	666	0.027	0.113	37
[2] Big Lazer Creek (7)	0.087	0.120	120	0.038	0.100	16
[1] Lower Flint (9 and 10)	0.078	0.130	12880	-0.010	0.101	161
[1] Chipola River (13)	0.115	0.164	13366	0.078	0.459	164
[2] Chipola River, years 2007-09	0.137	0.179	7503	0.061	0.361	123
[2] Chipola River, year 2010	0.138	0.170	820	0.131	0.668	41

Table 6. Hierarchical analysis of molecular variation (AMOVA) for Shoal Bass genotyped with 16 microsatellite DNA loci. Thirteen sample sites spanned the native range of the species and were grouped into three major river drainages – the Chattahoochee, Flint, and Apalachicola drainages (Table 1). The variation (%) describes the amount of genetic variation contained within each hierarchical level, and *P*-values ≤ 0.05 were considered significant.

Source of variation	**sums of squares**	**variation (%)**	***P*-value**
Among major river drainages	191.53	3.6	0.02
Among sites within major river drainages	200.56	8.9	< 0.01
Within sites	3177.47	87.4	< 0.01
Total	**3569.56**	**100.0**	

Table 7. Shoal Bass were sampled at 13 sites (see Table 1 for site names) that spanned the species' native range, and samples were genotyped with 16 microsatellite DNA loci. Pairwise fixation index (F_{ST}; Wright 1951) values as calculated by Weir and Cockerham (1984) are reported below, which quantify genetic differentiation between Shoal Bass sampled at each site. Values in **bold** were considered significant at $P \leq 0.05$. Negative values are artifacts of calculation methods and were interpreted as $F_{ST} = 0$.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13
1	-												
2	**0.03384**	-											
3	**0.02320**	**0.06987**	-										
4	**0.02192**	**0.01638**	**0.03870**	-									
5	**0.30018**	**0.31559**	**0.35637**	**0.29983**	-								
6	**0.03198**	**0.05069**	**0.03631**	0.01246	**0.30621**	-							
7	**0.04290**	**0.03967**	**0.06998**	**0.02994**	**0.28072**	**0.04112**	-						
8	**0.03024**	**0.02511**	**0.04092**	-0.00627	**0.28492**	0.00278	**0.02918**	-					
9	**0.04094**	**0.05044**	**0.03205**	0.00798	**0.26841**	**0.02312**	**0.05114**	0.00447	-				
10	**0.03925**	**0.06033**	**0.02136**	0.01147	**0.31081**	**0.02619**	**0.06781**	0.01653	0.00359	-			
11	**0.05256**	**0.06244**	**0.06678**	0.01213	**0.27974**	**0.01536**	**0.04756**	0.01207	**0.01778**	**0.01960**	-		
12	**0.03618**	**0.04757**	**0.04677**	0.00143	**0.28500**	**0.01513**	**0.04821**	0.00488	**0.01072**	0.00814	0.00672	-	
13	**0.12232**	**0.12416**	**0.13356**	**0.10779**	**0.19874**	**0.12395**	**0.07330**	**0.08866**	**0.13851**	**0.14254**	**0.12035**	**0.13370**	-

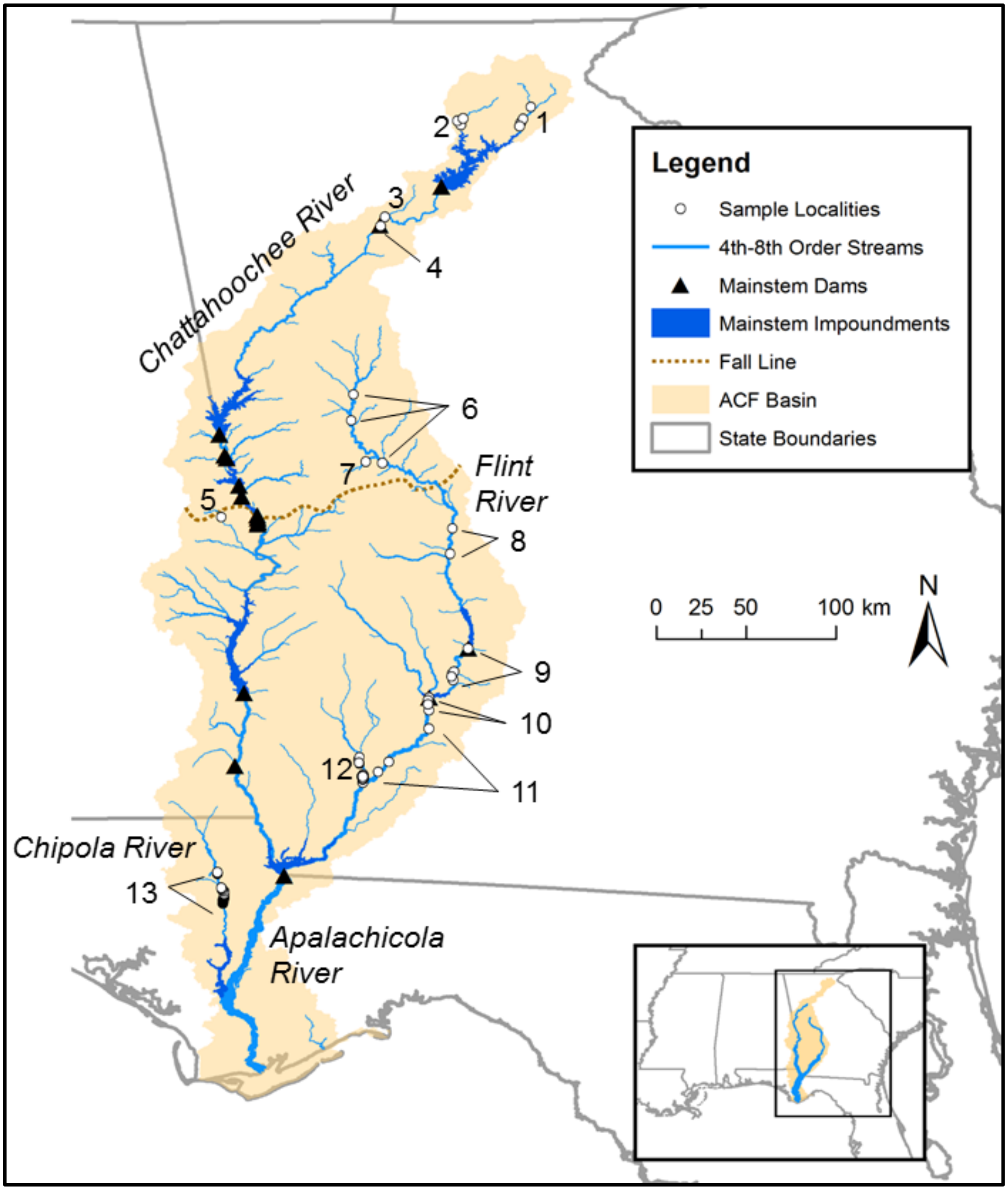

Figure 1. Collection localities for Shoal Bass *Micropterus cataractae* genetic samples genotyped with 16 microsatellite DNA loci. Samples were used to characterize genetic population structure across the species' native range of the Apalachicola-Chattahoochee-Flint Basin. Site numbers correspond to locality data in Table 1.

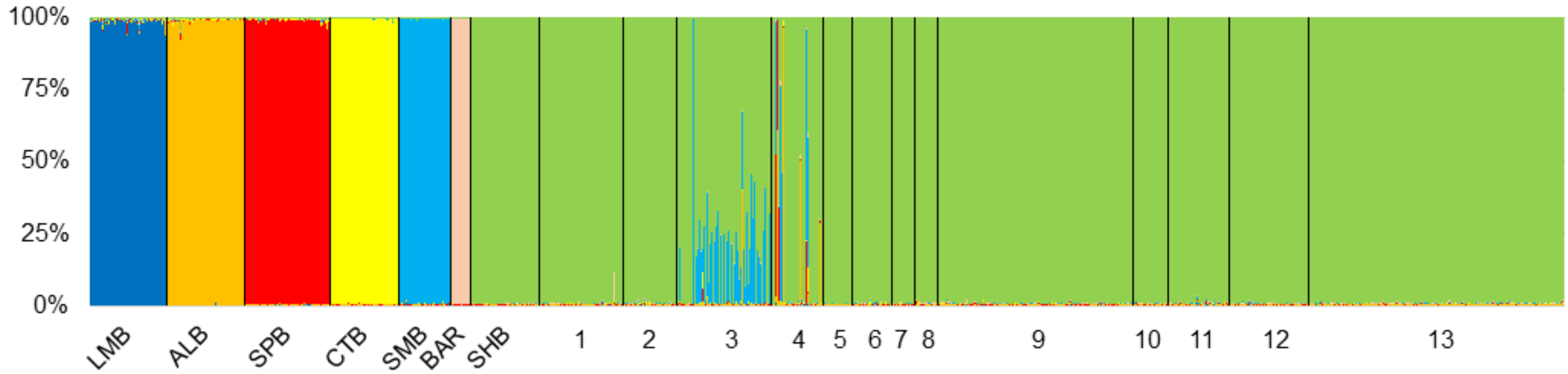

Figure 2. Taxonomic assignment of 829 putative Shoal Bass collected from 13 sites (see Table 1) and genotyped with 16 microsatellite DNA loci. Assignments were made using the allele frequencies of reference genotypes for seven *Micropterus* taxa ("LMB" = Largemouth Bass x Florida Bass intergrades; "ALB" = Alabama Bass; "SPB" = Spotted Bass; "CTB" = Choctaw Bass; "SMB" = Smallmouth Bass; "BAR" = Bartram's Bass; "SHB" = Shoal Bass). Colors represent distinct genetic clusters, and each individual's proportional assignment to those clusters is represented within a single vertical bar.

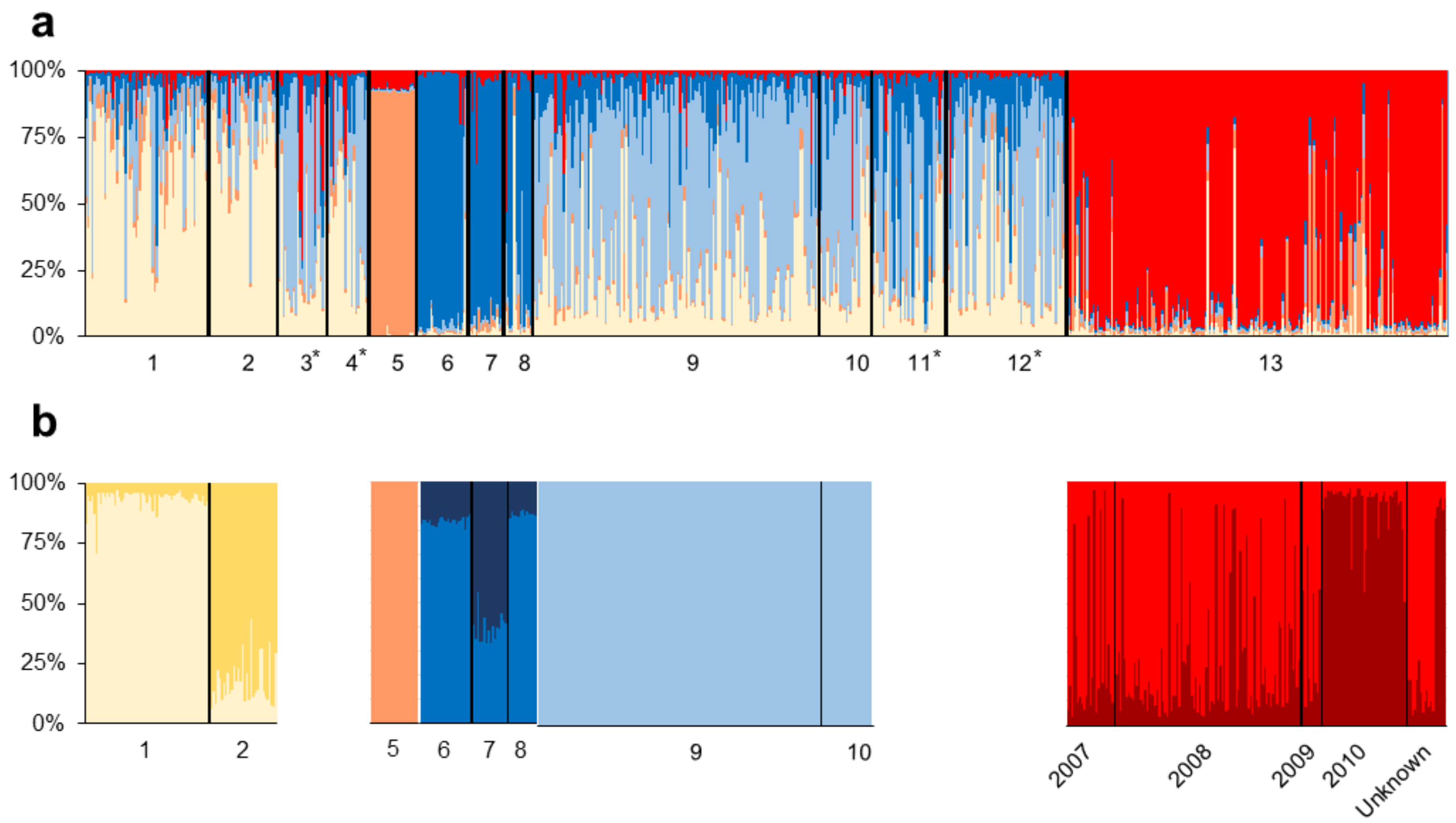

Figure 3. Hierarchical population structure within 644 pure Shoal Bass collected from 13 sites across the species' range (see Table 1) and genotyped with 16 microsatellite DNA loci. The uppermost level of hierarchical structure contained five genetic clusters (**a**). Structure at the secondary level was also apparent in some populations (**b**). Sites at the uppermost level that did not have a mean membership coefficient ≥ 0.50 to a given cluster (*) were considered mixtures of multiple clusters and not investigated for lower-level structure. Colors represent distinct genetic clusters, and each individual's proportional assignment to those clusters is represented within a single vertical bar.

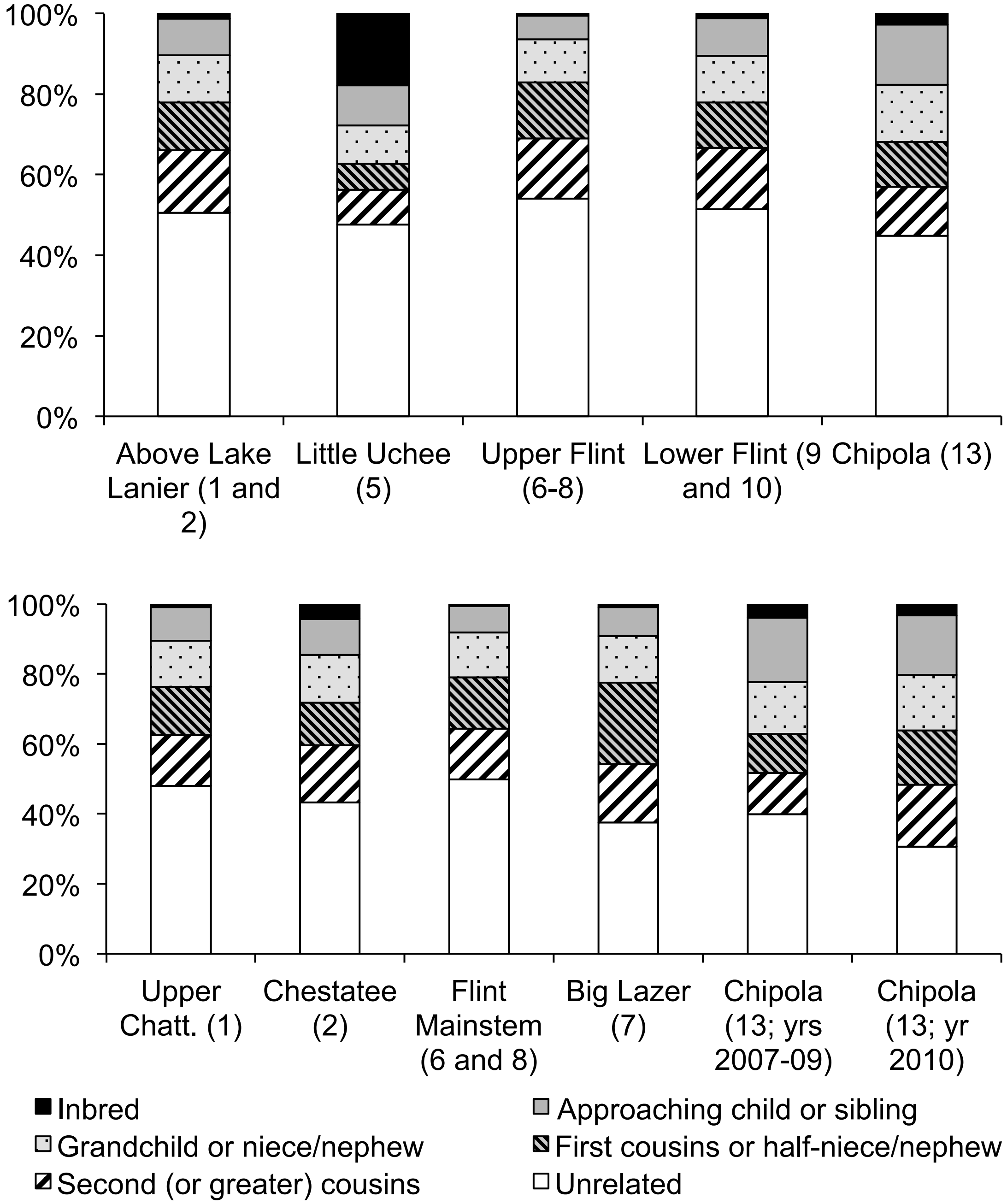

Figure 4. Proportional structure of pairwise relatedness (*r*) in identified genetic clusters (site #'s in parentheses correspond to Table 1) of Shoal Bass across their native range at (a) the uppermost hierarchical level of genetic structure and (b) the secondary level of genetic structure. General kinship categories that correspond to the following ranges in *r* values: 50.1-100.0% = inbred; 25.1-50.0% = approaching child or sibling; 12.6-25.0% = grandchild or niece/nephew; 6.3-12.5% = first cousins or half-niece/nephew; 0.1-6.2% = second (or greater) cousins; and 0.0% = unrelated.

CHAPTER III

CONSERVATION-GENETIC INFLUENCES OF IMPOUNDMENTS ON NATIVE FLUVIAL BLACK BASSES INHABITING UPSTREAM TRIBUTARIES

Abstract

The widespread construction of impoundments within fluvial systems has resulted in alteration and loss of fluvial habitats, posing a significant conservation threat to native fluvial fishes. In contrast, non-native black bass (genus *Micropterus*) species are often introduced into impoundments to increase sportfishing opportunities. Impoundments and non-native black bass fisheries may pose conservation threats to native, fluvial black bass species inhabiting upstream tributaries. We tested conservation-genetic hypotheses regarding the role of impoundments in facilitating non-native invasion and introgression, as well as fragmenting native populations, in two impounded systems of the southeastern U.S. Each impounded system – Lake Sidney Lanier, Georgia, and Tenkiller Ferry Lake, Oklahoma –featured a native, fluvial black bass and an introduced non-native congener. Results from both case studies revealed that non-native invasion and introgression into upstream native populations generally increased with proximity to impoundment, likely because impoundments produce non-native propagule pressure. Whether impoundments

fragmented native populations was somewhat unclear, and time lags in the responses of the genetic measures could have influenced our ability to detect the effects of fragmentation if it was occurring. Overall, this study provides insights into the dynamics of non-native invasion and introgression, as well as the status of native populations in each tributary. These results can help inform management practices that best conserve existing genetic diversity within and among native populations in impounded systems.

Introduction

Freshwater fishes are experiencing unprecedented imperilment across North America. Approximately 39% of described species are considered imperiled or extinct, and extinction rates are approximately 877 times greater than natural background rates (Jelks et al. 2008; Burkhead 2012). Anthropogenic activities have acutely altered fluvial habitats, resulting in the widespread imperilment of fish species inhabiting North America's rivers and streams (Allan and Flecker 1993). Among the most pertinent threats to fluvial fishes are widespread alteration and loss of habitat, fragmentation of populations, and negative interactions with non-native species (Allan and Flecker 1993; Jelks et al. 2008). Addressing how fluvial species respond to these threats can help inform natural resource management and conservation of biodiversity.

The construction of dams and impoundments is one of the most profound alterations to fluvial habitats, not only causing localized loss of fluvial habitats, but also affecting fishes in downstream and upstream reaches. About half of the world's large river systems are affected by damming (Nilsson 2005), and the downstream effects of dams are well documented. Dams interrupt downstream movement of nutrients and sediment, alter natural temperature and flow regimes, and create physical barriers to fish movement (Ward and Stanford 1995; Porto et al. 1999; Poff et al. 2007). In response to these alterations, fluvial fish assemblages downstream of dams often suffer loss of sensitive species and become dominated by tolerant species (Poff and Zimmerman 2010). Impoundments in lower reaches of fluvial systems can also affect fishes inhabiting upstream tributaries (Pringle 1997). For species that do not typically inhabit lentic habitats, impoundments can restrict gene flow among populations inhabiting impoundment tributaries, resulting in increased risk of inbreeding and local extinction (Hudman and Gido 2013; Fluker et al. 2014). Impoundments can also serve as sources for upstream invasion of facultative species that can dwell in impoundments and fluvial systems (Pringle 1997); for example, impoundments serve as 'stepping-stones' for the invasion of non-natives into interconnected fluvial systems (Johnson et al. 2008). The widespread dispersal of species originating from downstream impoundment (Ruhr 1957), coupled with losses of native fluvial species upstream of impoundments (Winston et al. 1991), can result in homogenized or otherwise altered fish communities in upstream tributaries (Taylor et al. 2001; Herbert et al. 2003; Guenther and Spacie 2006).

To increase sportfishing opportunities in impoundments, natural resource managers have historically stocked non-native sportfish species like black bass (genus *Micropterus*) that can persist in lentic habitats. In the U.S., black bass species typically stocked to create impoundment fisheries include the Largemouth Bass (*M. salmoides*), Florida Bass (*M. floridanus*), Spotted Bass (*M. punctulatus*), Alabama Bass (*M. henshalli*), and Northern Smallmouth Bass (*M. dolomieu dolomieu*). Black bass are among the most popular sportfish in the U.S., and anglers pursuing these species contribute significantly to a multi-billion dollar sportfishing industry (Long et al. 2015). Despite the many perceived benefits these non-native impoundment fisheries create, the introduction of species outside of their native ranges poses potential harm to fish species native to recipient drainages – a consequence not typically considered during the extensive stocking of black bass that began as early as the mid-1800's (Jackson 2002; Long et al. 2015). Non-native black bass species are generally tolerant of both lentic and fluvial habitats; thus, when stocked in impoundments, these species have potential to invade interconnected fluvial habitats and further threaten native fishes (*see* Marchetti et al. 2004). For example, Largemouth Bass is one of the 100 worst invasive species on the planet (www.iucngisd.org). The Largemouth Bass has been introduced widely throughout the world because of its notoriety as a sportfish, but negatively affects native fishes through predation (Jackson 2002).

Over the past several decades, researchers, anglers, and managers have shown increased interest in the conservation of native black bass taxa (Birdsong et al. 2010; Tringali et al. 2015a). These taxa – including Shoal Bass (*M. cataractae*), Neosho Smallmouth Bass (*M. dolomieu velox*), Guadalupe Bass (*M. treculii*) and Bartram's Bass (*M. sp. cf. cataractae*) – are typically endemic to one or a few river drainages of the southeastern U.S. and are more specialized in their use of fluvial habitats than congener species that support impoundment fisheries (Birdsong et al. 2010; Tringali et al. 2015a). Many of these fluvial black bass taxa face similar conservation threats as those driving imperilment of fluvial fishes across North America: alteration and loss of habitat, fragmentation of populations, and negative interactions with non-native congener species

(Allan and Flecker 1993; Birdsong et al. 2010). For example, native taxa are often involved in extensive introgressive hybridization with non-native congeners, or replaced by non-natives altogether – a problem that appears most common in impounded systems (Pierce and Avyle 1997; Barwick et al. 2006; Stormer and Maceina 2008; Koppelman 2015).

As impoundments have been constructed across the southeastern U.S., the lentic habitats created favored non-native black bass fisheries at the expense of fluvial habitats and the native black basses that inhabit them (Long et al. 2015). Beyond this initial loss of fluvial habitats when impoundments are inundated, impoundments created over a half-century ago may still impose a number of conservation-genetic threats to native, fluvial black bass populations. High abundance of non-native congeners in impoundments may create non-native propagule pressure that encourages upstream invasion and hybridization with native species inhabiting upstream tributaries (Ricciardi et al. 2010). Impounded habitats may also create movement barriers for fluvial black bass species, as they do for other fluvial fish species (Hudman and Gido 2013; Fluker et al. 2014), leaving populations in upstream tributaries fragmented from one another. Hybridization with non-natives or fragmentation could alone be cause for conservation concern for native fluvial black bass; however, a combination of both factors could exacerbate the effects of hybridization (Ricciardi et al. 2010), leading to loss of native species.

These conservation-genetic concerns regarding the role of impoundments on fluvial black bass species can be examined through a series of hypotheses, which we test in two case studies of impounded systems of the southeastern U.S. One, we hypothesize that increased relative abundance of non-native congeners and the amount of hybridization with native taxa would occur in interconnected tributaries in relation to proximity to the impoundment. Two, we hypothesize that increased population structure and differentiation would be evident between impoundment tributaries and the degree of differentiation would exceed levels found in tributaries with no impoundment interposed. And three, we hypothesize that introduced populations form a panmictic source of non-natives that exhibit founder effects of decreased genetic diversity and heterozygosity compared to native species. *Case Study I* was conducted on Lake Sidney Lanier (hereafter, "Lake Lanier"), Georgia, and involved native Shoal Bass and non-native Alabama Bass. *Case Study II* was performed on Tenkiller Ferry Lake (hereafter, "Lake Tenkiller"), Oklahoma, and included native Neosho Smallmouth Bass and a non-native strain of Northern Smallmouth Bass. Hereafter, we cover each case study separately, detailing the study area, study species, sample collection, genotyping, conservation-genetic analyses, and results. We then consider the results of both case studies, as well as other relevant literature, in an overall discussion of the role impoundments may play in the decline of native, fluvial black bass species.

Case Study I: Lake Lanier, Georgia

I. Methods

Study area and species.– Lake Lanier is situated within the upper Chattahoochee River basin in Georgia (Fig. 1). Completed in 1956, Lake Lanier has a surface area of 150 km^2 and impounds two major tributaries, the Chattahoochee and Chestatee rivers. The Chattahoochee River is a fifth-order tributary draining approximately 970 km^2 as it enters the northeast portion of Lake Lanier, whereas the Chestatee River enters the lake's northwest corner as a fourth-order stream that drains approximately 600 km^2. The most direct route between the two river interfaces spans approximately 65 km of impounded habitat within Lake Lanier. Shoal Bass, a fluvial-specialist species native to the basin, inhabits the high-gradient shoal habitats that exist in both tributary rivers, but do not typically occupy or persist in impounded habitats (Williams and Burgess 1999; Sammons and Early 2015; Taylor and Peterson 2015). In contrast, the non-native Alabama Bass was first documented in the Chestatee River near Lake Lanier in 1970, likely originating from unauthorized angler introduction(s), and the species now supports a popular impoundment fishery (Williams and Burgess 1999; Baker et al. 2008; Rider and Maceina 2015). Alabama Bass in Lake Lanier currently support a popular sport fishery that probably motivated additional unauthorized introductions of the species into neighboring drainages (Pierce and Avyle 1997; Leitner et al. 2015). A previous study suggested that Shoal Bass in the Chattahoochee River upstream of Lake Lanier were relatively pure of non-native alleles (Dakin et al. 2015), but introgression between Shoal Bass and other non-native congeners like Spotted Bass and Smallmouth Bass currently threatens Shoal Bass conservation throughout large portions of their native range (Alvarez et al. 2015; Dakin et al. 2015; Tringali et al. 2015b).

Sample collection.– To collect samples for genotyping, we used a jet-drive boat electrofisher to sample black bass at a series of four sites in each river that spanned upstream from each river's interface with Lake Lanier. Each sample site encompassed approximately 350 m of stream length, sampling effort was standardized to 15 min of pedal time at each site, and sample numbers represented raw catch of each species at each site. Most sample sites were situated in or near shoal habitats, but surrounding riffle, run, and pool habitats were also sampled. We collected fin-clip tissue samples from Shoal Bass in May 2013 and from Alabama Bass in May 2014, using coloration and morphological characteristics to identify each species in the field (Taylor and Peterson 2014).

Genotyping.– We genotyped samples with 16 di-nucleotide microsatellite DNA markers developed to amplify in black bass species (*Msaf 05, 06, 08, 09, 10, 12, 13, 17, 22, 24, 25, 27, 28, 29, 31,* and *32*; Seyoum et al. 2013). Genomic DNA was isolated from fin-clips using the Puregene DNA Purification Kit (Gentra Systems). We amplified microsatellites using six multiplex polymerase chain reactions (PCR). We used the following PCR amplification parameters for all loci: 94°C for 2 min, 35 denaturation cycles of 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 10 min. We performed capillary electrophoresis with a 3130 XL Genetic Analyzer using PCR products that contained 13 μL of formamide (denaturation for 4 min at 95°C) and Genescan ROX 500 size standard (Applied Biosystems). We determined length of allele variants in GeneMapper v. 4 (Applied Biosystems). Prior to data analyses, we screened for duplicate genotypes using the multilocus matching function in Program GENALEX v. 6.502 (Peakall and Smouse 2006) and retained only the first collection of any duplicated genotype.

Data analyses.– To examine the abundance non-natives and amount of hybridization with native taxa in impoundment tributaries, we first used a Bayesian clustering algorithm to screen collected genotypes against other congener taxa and identify pure and hybrid individuals of each taxon of interest. We obtained reference genotypes for each case study and used Program STRUCTURE v. 2.3.4 and accessory programs to proportionally assign individual genotypes (*q*-values) to a given number of genetic clusters (*K*) based on linkage equilibrium and conformance to Hardy-Weinberg equilibrium (Pritchard et al. 2000). We used genotypes of seven reference taxa that included Shoal Bass, Alabama Bass, and five other congener taxa relevant to the study basin ($K = 7$; *see* Chapter 2). In Program STRUCTURE, we employed the 'PopFlag' option to assign collected genotypes proportionally to taxa based solely on the allelic frequencies of reference taxa genotypes (Pritchard et al. 2000). We assumed the admixture ancestry model and independent allele frequencies for model runs, and we used a burn-in of 20,000 and 200,000 Markov chain Monte Carlo (MCMC) repetitions for each run. We then ran 20 independent runs at the optimal *K* value previously identified for the reference taxa dataset. STRUCTURE runs were input into Program STRUCTURE HARVESTER web v. 0.6.94 (Earl and vonHoldt 2012) to obtain input files for Program CLUMPP v. 1.1.2, which provided optimal alignment of independent STRUCTURE runs using cluster matching and permutation (Jakobsson and Rosenberg 2007). We obtained final *q*-value assignment in Program CLUMPP using the *G*' pairwise matrix similarity statistic and the 'LargeKGreedy' (for $K > 4$) or 'Greedy' (for $K \leq 4$) algorithms for 1,000 randomly sequenced runs. Thresholds for 'pure' individuals of the four taxa of interest were calculated following Littrell et al. (2007). We removed any genotypes assigned to any taxa other than Shoal Bass or Alabama Bass prior to any further analyses. To visualize any spatial trends in introgression, we plotted the overall genomic proportions of individuals collected at each sample site (Q_i; introgression index), as well as the proportion of individuals classified as hybrids at each site (*H*; hybridity index).

Hybridization categories generally followed Dakin et al. (2015): 'pure' species met the *q*-value thresholds attained from the methods of Littrell et al. (2007), 'backcrosses' had assignments between $q = 0.7500$ and the 'pure' threshold assignment to a respective taxa, and all remaining individuals were considered first filial generation (F1) or later-generation hybrids.

To examine the hypotheses of increased population structure and genetic differentiation in native fluvial taxa inhabiting impoundment tributaries and panmictic populations of non-natives in the same areas, we characterized population structure and genetic differentiation within the 'pure' genotypes belonging to each taxa of interest. Pure genotypes were organized by tributary, wherein individual sites were ordered from nearest impoundment to farthest upstream. Within Program STRUCTURE, we ran 10 independent, exploratory runs each of $K = 1\text{-}5$ that assumed the admixture model and correlated allele frequencies, and each run had a burn-in of 20,000 and 200,000 MCMC repetitions. We estimated the optimal *K* within each dataset using the maximum value of four supervised estimators that disregard 'spurious clusters' that fail to obtain a mean or median membership coefficient threshold of ≥ 0.50 for at least one sample location (Puechmaille 2016). If no population structure was detected, we allowed Program STRUCTURE to use tributary of origin as prior information ('LocPrior' option), which confers improved detection of weak population signals that may otherwise go undetected by conventional model parameterization (Pritchard et al. 2000). Once the optimal *K* value was identified, 20 independent STRUCTURE runs were input into Program CLUMPP and final *q*-value assignments were obtained using the settings detailed previously. To quantify genetic differentiation of each taxa across the study area, we performed a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992). We conducted a locus-by-locus AMOVA in Program ARLEQUIN v. 3.5.1.3 (Excoffier and Lischer 2010) to produce a global AMOVA as a weighted average over all loci. Within the AMOVA, we estimated the amount of genetic variation present at the following three hierarchical levels, along with their associated *F*-statistic values that indicate the amount of differentiation at each level: within sample sites (F_{ST}), among sample sites within a tributary (F_{SC}), and among tributaries (F_{CT}). Tests of significance for AMOVA results were calculated based on 10,000 permutations at $P \leq 0.05$. Any negative values obtained were considered artifacts of calculation methods and interpreted as zero values.

To examine whether non-native black bass populations are experiencing founder effects, and to provide context to genetic structure and differentiation results, we also explored potential structuring mechanisms within the pure taxa of interest by tributary system. We reported the number of private alleles ($A_{private}$), and the mean across all loci for number of alleles (A), effective number of alleles (A_e), expected heterozygosity (H_e), and observed heterozygosity (H_o) as calculated in Program GENALEX v. 6.502 (Peakall and Smouse 2006). We also calculated mean pairwise relatedness (r) and mean inbreeding coefficient (F) within each tributary using Program COANCESTRY (Wang 2011). Specifically, we used 1,000 bootstraps and 100 control samples to estimate r with the TrioML estimator (Wang 2007) and F with the Ritland estimator (Ritland 1996). We also tested for differences in mean r and F between tributaries using 1,000 bootstraps to generate 95% C.I.'s used to determine significance at $P \leq 0.05$ (Wang 2011). These results provide insights into the model assumptions of Program STRUCTURE (i.e., linkage equilibrium and Hardy-Weinberg equilibrium), and also provide a baseline characterization of the conservation-genetic status of fish inhabiting each tributary.

I. Results

Samples were genotyped from four sites in each tributary river, with sample sites spanning 6-24 river-kilometers (rkm) upstream of Lake Lanier in the Chattahoochee River and from 3-15 rkm upstream in the Chestatee River (Table 1; Fig. 1). Roughly the same number of putative Shoal Bass samples were obtained in in the Chattahoochee and Chestatee rivers ($N = 50$ and 45, respectively), but more Alabama Bass were sampled in the Chestatee River ($N = 63$) than in the Chattahoochee River ($N = 36$). In both rivers, sites closer to Lake Lanier had increased relative abundance of putative Alabama Bass and sites farther upstream had higher relative abundance of Shoal Bass.

Field identification of putative Shoal Bass and Alabama Bass aligned well with taxonomic assignment, with only one putative Shoal Bass from the Chattahoochee River (site #1) being assigned a majority proportion to the Alabama Bass cluster (Fig. 2). The genomic proportion threshold for pure Shoal Bass was $q \geq 0.9880$ and $q \geq 0.9674$ for pure Alabama Bass, which resulted in 71 pure Shoal Bass and 65 pure Alabama Bass genotypes (Table 1). Prior to evaluating genomic proportions and hybridization categories by site, we removed 2 putative Shoal Bass genotypes and 4 putative Alabama Bass genotypes that were assigned $q \geq 0.0500$ to black bass taxa not of interest in our study (see Appendix I). Alabama Bass dominated the overall genomic proportions of sample sites closest to Lake Lanier in both rivers ($Q_i = 0.850$ in Chattahoochee River and

(Q_i = 0.980 in Chestatee River), whereas Shoal Bass genomic proportions gradually became dominant with increasing distance upstream from Lake Lanier in both rivers (Fig. 3). Chestatee River sites also had higher genomic proportions of Alabama Bass than corresponding Chattahoochee river sites. In regards to hybridization categories, sites closer to Lake Lanier in both rivers were dominated by pure Alabama Bass and backcrossed Alabama Bass, whereas pure Shoal Bass and backcrossed Shoal Bass became dominant at upstream sites (Fig. 4). In the Chattahoochee River, the proportion of hybrid individuals decreased from H = 0.474 to 0.100 moving upstream, whereas the proportion of hybrid individuals in the Chestatee River had a less-pronounced directional trend (range in H = 0.160 to 0.313).

Population structure (K = 2) was detected for both native Shoal Bass and non-native Alabama Bass when tributary was used as prior information in Program STRUCTURE, suggesting population structure existed in both species but was relatively weak (Pritchard et al. 2000). Population structure in Shoal Bass aligned with tributary system, with Chattahoochee River genotypes having an average assignment of q = 0.9426 to one cluster and in the Chestatee River an average assignment of q = 0.6561 to a second genetic cluster (Fig. 5a). Population structure in non-native Alabama Bass also aligned with tributary system, with fish from the Chattahoochee River having an average assignment of q = 0.7533 to one genetic cluster and in the Chestatee River averaging q = 0.7946 to a different cluster (Fig. 5b). AMOVA results were similar for both species, with a small (approximately 4%), but statistically significant, percentage of genetic variation occurring among tributaries (Table 2). The remainder of genetic variation (> 96%) in both species occurred within sites.

Shoal Bass and Alabama Bass had similar levels of heterozygosity in both tributaries, with no appreciable deviations from Hardy-Weinberg expectations (Table 3). Across all sites, non-native Alabama Bass had slightly lower allelic diversity ($A_e = 1.761$) compared to Shoal Bass ($A_e = 2.059$). Shoal Bass inhabiting the Chattahoochee River had more private alleles compared to the Chestatee River (10 versus 4), whereas Alabama Bass had more private alleles in the Chestatee River (11 versus 3). For Shoal Bass, mean pairwise relatedness was significantly higher in the Chattahoochee River ($r = 0.087$) compared to the Chestatee River ($r = 0.018$), but mean inbreeding coefficients (F) were approximately zero for Shoal Bass in both rivers (Table 4). Mean pairwise relatedness was higher in Alabama Bass than Shoal Bass, but did not significantly differ between rivers. The inbreeding coefficient was noticeably higher in Alabama Bass in the Chattahoochee River ($F = 0.106$) than in the Chestatee River ($F = 0.012$), but not significantly different.

Case Study II: Lake Tenkiller, Oklahoma

II. Methods

Study area and species.– Lake Tenkiller is situated within the Illinois River basin of Arkansas and Oklahoma (Fig. 6). Lake Tenkiller was completed in 1953 and has a surface area of 52 km^2. This impoundment is fed by two major tributary streams, the sixth-order Illinois River that drains approximately 2,529 km^2 and the fifth-order Baron Fork that drains 896 km^2 prior to its confluence with the Illinois River, situated just upstream of Lake Tenkiller. A smaller, fourth-order tributary, Caney Creek, drains 238 km^2 prior to emptying into Lake Tenkiller east of the Illinois River confluence. Approximately 15 km of impounded habitat is situated between the interfaces of the Illinois River and Caney Creek. Neosho Smallmouth Bass are native to all three tributaries to Lake Tenkiller (Hubbs and Bailey 1940; Stark and Echelle 1998); however, Neosho Smallmouth Bass do not generally support fisheries in impoundments constructed within their native range (Stark and Echelle 1998). As a result, anglers have favored the stocking of non-native Northern Smallmouth Bass into impoundments. In 1991 and 1992, the Oklahoma Department of Wildlife Conservation (ODWC) stocked Lake Tenkiller with Tennessee lake strain Smallmouth Bass fingerlings from Percy Priest Lake, TN broodstock (Malloy 2001; Boxrucker et al. 2004). A post-stocking survey in 1999 revealed that Smallmouth Bass in Lake Tenkiller had 85-90% non-native alleles and that no non-native alleles were detected upstream in the Baron Fork (Malloy 2001). Despite this result, researchers have warned that non-native alleles could quickly infiltrate native Neosho Smallmouth Bass populations inhabiting interconnected fluvial habitats (Stark and Echelle 1998; Malloy 2001).

Sample collection.– We sampled Smallmouth Bass for genotyping in May-September of 2014 and 2015. We sampled the Illinois River, Baron Fork, and Caney Creek at several sites spanning upstream from each system's interface. An additional sample was taken near the dam of Lake Tenkiller. Sampling was conducted with boat- and backpack- mounted electrofishing units and supplemented by angling in areas where electrofishing was impractical, and sample sites usually spanned approximately 300 m of stream. Because we were unable to reliably differentiate the two Smallmouth Bass forms in the field, we fin-clipped all fish identified as Smallmouth Bass.

Genotyping.– Samples were genotyped using seven di-nucleotide microsatellite DNA markers developed to amplify in black bass species (*Mdo*03, Malloy et al. 2000; *Msaf* 01, 05, 06, 14, 17, and 29, Seyoum et al. 2013). Genomic DNA was isolated from fin-clips using the DNeasy Blood and Tissue Kit (Qiagen Corp.). We amplified microsatellites using two multiplex polymerase chain reactions (PCR), and used the following PCR amplification parameters for all loci: 95°C for 15 min, 35 cycles of 94°C for 30 s, 58°C for 90 s, 72°C for 90 s, and 72°C for 10 min. We performed capillary electrophoresis with an ABI 3730 Genetic Analyzer with a performed on solutions containing 1 μL of PCR products (diluted 1:100), 9 μL formamide (Applied Biosystems, Inc.), and Genescan ROX 500 size standard (Applied Biosystems, Inc.). Length of allele variants was determined in GeneMapper v. 5 (Applied Biosystems). Prior to data analyses, we screened for duplicate genotypes using the multilocus matching function in Program GENALEX and retained only the first collection of any duplicated genotype.

Data analyses.– For comparative purposes, data analyses for this case study followed those performed in *Case Study I*. For taxonomic assignment of our sample dataset in Program STRUCTURE, we used genotypes of four reference taxa that included Neosho Smallmouth Bass, Tennessee lake strain Smallmouth Bass, sympatric Spotted Bass, and a genetically-distinct hatchery stock of Smallmouth Bass that may also have been introduced into the study area ($K = 4$; Taylor et al. 2016). In the calculation of the AMOVA, the site in Lake Tenkiller was incorporated at the "tributary" level. To further investigate site-level genetic differentiation in Neosho Smallmouth Bass inhabiting the three tributary streams, we also calculated pairwise fixation index values (F_{ST}; Wright 1951; Weir and Cockerham 1984). Tests of significance for F_{ST} results were calculated based on 10,000 permutations at $P \leq 0.05$, and any negative values obtained were considered artifacts of calculation methods and interpreted as zero values.

II. Results

Fish identified as Smallmouth Bass were collected from 12 sites in the Lake Tenkiller study area: 1 site situated near Lake Tenkiller's dam, 3 sites spanning 54 rkm upstream of the lake in the Illinois River, 4 sites spanning 19 rkm upstream in the Baron Fork, and 4 sites spanning 13 rkm upstream in Caney Creek (Table 5; Fig. 6). In general, 20-30 individuals were collected at each site in Lake Tenkiller, the Illinois River, and Caney Creek. A lower sample size of approximately 12 genotypes were taken from Baron Fork sites because this tributary was not directly fragmented from the Illinois River by Lake Tenkiller, but hybridization levels and natural population structure in this tributary were still of interest. A larger sample size of 47 was taken at the uppermost site on the Illinois River to represent a longer sampling reach (approximately 7.5 rkm; between Round Hollow and Peavine public access areas).

Based on taxonomic assignment of collected genotypes, Smallmouth Bass collected in Lake Tenkiller were assigned predominately to Tennessee lake strain, whereas collections from tributary streams were predominately assigned to Neosho Smallmouth Bass (Fig. 7). The genomic proportion threshold for pure Neosho Smallmouth Bass was $q \geq 0.9455$ and $q \geq 0.8936$for pure Tennessee lake strain, which resulted in 143 pure Neosho Smallmouth Bass and 22 pure Tennessee Lake Strain genotypes across all sample sites (Table 5). Reference Tennessee lake strain genotypes were assigned up to $q = 0.1690$ to the distinct hatchery stock of Smallmouth Bass, suggesting the two groups shared some alleles; thus, we removed any sampled genotypes assigned $q \geq 0.0500$ to Spotted Bass or $q \geq 0.1000$ to the distinct hatchery stock of Smallmouth Bass prior to assessing any trends in introgression or hybridization categories. In total, 26 genotypes were removed: 9 from Lake Tenkiller, 15 from Illinois River, 1 from Baron Fork, and 1 from Caney Creek (see Appendix II). By sample site, overall genomic proportions for Tennessee lake strain Smallmouth Bass were highest at the Lake Tenkiller site ($Q_i = 0.957$) and lowest at the upstream sites in Baron Fork (site #8) and Caney Creek (site #'s 11 and 12; $Q_i < 0.010$; Fig. 8). Overall genomic proportions of Tennessee lake strain Smallmouth Bass were highest at sites closer to Lake Tenkiller in all three tributaries – only in the Illinois River did Tennessee lake strain proportions remain relatively high ($Q_i = 0.180$) at the farthest upstream sites. The proportion of hybrid individuals (H) was > 0.500 in all Illinois River sites, varied between 0.083-0.273 in Baron Fork sites, and decreased with upstream distance from the lake in Caney Creek from 0.348 to 0.042 (Fig. 9). Pure Tennessee lake strain Smallmouth Bass and their backcrosses were recovered only in Lake Tenkiller and Illinois River sites, whereas hybrids in the Baron Fork and Caney Creek represented F1 or Neosho backcrosses.

Population structure was evident within the pure genotypes of both native Neosho Smallmouth Bass, but was not recovered within non-native Tennessee lake strain Smallmouth Bass. Program STRUCTURE detected weak population structure (i.e., only when using tributary of origin as prior information; Pritchard et al. 2000) within Neosho Smallmouth Bass ($K = 2$), but not between Tennessee lake strain fish captured in Lake

Tenkiller versus the Illinois River. Neosho Smallmouth Bass in Illinois River sites were assigned an average genomic proportion of q = 0.7003 to one cluster, whereas Caney Creek was assigned an average q = 0.8257 to a different cluster (Fig. 10). Baron Fork sites had an intermediate population assignment, sharing a slight majority of its assignment with the genetic cluster affiliated with Caney Creek (average q = 0.5925). AMOVA results revealed that 2.2% of genetic variation – a small, but statistically significant amount – was contained among tributaries for Neosho Smallmouth Bass (Table 6). AMOVA results indicated that no discernable variation was contained between Tennessee lake strain fish from Lake Tenkiller and the Illinois River. A small, non-significant amount (9.3%) of genetic variation was contained among sites within these two systems. A significant majority of genetic variation was contained within sites for both Smallmouth Bass forms. Site-level pairwise F_{ST} comparisons also displayed the greatest genetic differentiation among Neosho Smallmouth Bass between sites in the Illinois River and Caney Creek (up to F_{ST} = 0.134), although the uppermost site in the Baron Fork also had significant differentiation from Caney Creek sites (up to F_{ST} = 0.060; Table 7).

Potential structuring mechanisms were summarized by tributary, including Lake Tenkiller as its own site, for each species. Observed heterozygosity was higher in non-native Tennessee than in Neosho Smallmouth Bass, but observed heterozygosity in Tennessee lake strain fish was slightly lower than expected values (Table 8). Tennessee lake strain fish also had a higher overall mean effective number of alleles (A_e = 4.772) than did native Neosho Smallmouth Bass (A_e = 3.979). Of the three tributary systems containing Neosho Smallmouth Bass, the Illinois River had the highest mean effective number of alleles ($A_e = 4.772$) and number of private alleles (13). Mean pairwise relatedness (r) and inbreeding coefficients (F) were generally similar between native and non-native Smallmouth Bass forms (Table 9). Small, but statistically significant, differences in r existed among Neosho Smallmouth Bass inhabiting each of the three tributaries. Neosho Smallmouth Bass in the Baron Fork had a significantly lower F (approximately zero) compared to the Illinois River and Caney Creek, which were statistically similar to one another. There were no statistically significant differences in r or F between Tennessee lake strain fish sampled from Lake Tenkiller versus Illinois River sites, although r was approximately double at Illinois River sites ($r = 0.121$).

Overall Discussion

Overall, our results provide novel insights into how impoundments and the non-native black bass species introduced into them, can jeopardize the conservation of native fluvial black basses inhabiting upstream tributaries. Impoundments appear to generate non-native propagule pressure that facilitates invasion and subsequent introgression into native populations inhabiting upstream tributaries. A number of ecological mechanisms likely influence the patterns and trajectories of the introgression observed, so the eventual outcome of introgression remains unclear at this time. Investigations into whether impoundments impose population structure on native populations were somewhat inconclusive, perhaps because of time lags affiliated with the genetic approaches employed in our study. Conversely, population structure evident in one introduced population (i.e., Alabama Bass in Lake Lanier) revealed that non-natives invading upstream habitats do not necessarily originate from one panmictic population. Results from both case studies highlight the conservation challenges that impoundments and associated non-native congener fisheries impose on conservation of native black bass populations.

The stocking of non-native black bass species into impoundments facilitates the invasion of non-natives into upstream fluvial habitats, as well as a rapid onset of hybridization and subsequent introgression of non-native alleles into native black bass gene pools. Results from both case studies suggest that hybridization has progressed quickly in 10-15 years since previous assessments. In 2005, 22 putative Shoal Bass were sampled in the Chattahoochee River upstream of Lake Lanier and 20 (91%) were considered pure Shoal Bass, with only one F1 hybrid (4.5%) and one backcrossed individual (4.5%; Dakin et al. 2015). Our collections from 2013-2014 in the Chattahoochee and Chestatee rivers illustrate that F1 hybrids and backcrossed individuals of either species now compose 13-47% of individuals at a given site. In fact, our Lake Lanier case study likely underestimated hybridization rates because we only genotyped individuals that had the phenotypic traits associated with 'pure' individuals from either species despite encountering some individuals that appeared to be hybrids. In Lake Tenkiller, an assessment 7-8 years post-stocking of Tennessee lake strain fingerlings revealed 85-90% non-native alleles in Lake Tenkiller and did not detect non-native alleles upstream in the Baron Fork (Malloy 2001). Approximately 16 years later, our Lake Tenkiller sample was estimated to contain 96% non-native Tennessee Lake strain alleles. Furthermore, the proportion of hybrid individuals (F1 hybrids and backcrosses) was > 0.50 at all Illinois River sampling sites and varied from 0.04-0.35 at sites in Baron

Fork and Caney Creek. Our results align well with other investigations of the spread of non-native black bass from impoundments. In 2004, a survey of native Bartram's Bass and non-native Alabama Bass in the Savannah River basin, detected non-natives and hybrids at a few tributary sites nearest impoundments; however, only 5-6 years later, non-natives and their hybrids had quickly spread upstream and into many other tributaries (Leitner et al. 2015). Interestingly, no non-natives or hybrids were detected in tributaries connecting directly to mainstem rivers (Leitner et al. 2015), suggesting that impounded habitats were facilitating the initial invasion of non-natives and their hybrids into tributaries directly entering impoundments. Similarly, non-native Florida Bass stocked into Texas impoundments have infiltrated native Largemouth Bass populations inhabiting upstream tributaries, with non-native haplotypes detected up to 80 km upstream of source impoundments (Ray et al. 2012). Overall, this body of evidence suggests that the introduction of non-native black basses into impoundments creates propagule pressure (*see* Ricciardi et al. 2010), which facilitates upstream invasion into fluvial habitats and encourages hybridization with native black basses.

The invasion and introgression of non-native alleles was fairly widespread but non-uniform in both case studies, providing insight into the spatial and environmental factors that oppose non-native propagule pressure originating from downstream impoundments. Both case studies revealed a spatial trend wherein the highest proportions of pure non-natives and non-native introgression were at sites closer to impoundment. Perhaps the artificial and transitory habitats created in river-impoundment interfaces differentially favor non-native congeners over native species (*see* Buckmeier et al. 2014). Conversely, maybe natives are more abundant and better adapted to upstream fluvial habitats, thus providing non-natives less opportunity for successful invasion (*see* Bennett et al. 2010). In the Lake Tenkiller case study, small stream size (e.g., Caney Creek) appeared to favor pure Neosho Smallmouth Bass over non-native Tennessee lake strain fish and their hybrids. Although the proximate ecological mechanisms behind this trend remain uninvestigated, a reasonable hypothesis is that Neosho Smallmouth Bass are better adapted to the range of environmental conditions within these smaller streams than the Tennessee lake strain, which originated from fish presumably adapted to life in the much larger Tennessee River system. Similar spatial and environmental factors, like elevation, water temperature, and migration barriers have been found to counteract propagule pressure of non-native fish invasion and hybridization in other fishes (Bennett et al. 2010). In contrast, Alabama Bass in the Lake Lanier tributaries do not appear to be experiencing the same degree of ecological pressures that inhibit upstream invasion, suggesting the specific biology and life-history requirements of non-native species influences the success and eventual extent of invasion (*see* Marchetti et al. 2004). Gaining a better understanding of the ecological mechanisms that influence non-native black bass invasion and introgression with native forms would help managers predict which native populations are most threatened by non-native invasion and introgression so that conservation actions could be prioritized accordingly.

Our results suggest that the conservation of native taxa in our two case studies is threatened by hybridization; however, the speed at which hybridization might progress and the eventual outcome of the native taxa in each study is uncertain at this time. In general, the speed at which native species are lost to extinction by hybridization increases when: 1) reproductive barriers are weak between taxa; 2) the native species is relatively rare or low in abundance; and 3) the non-native species gains a competitive advantage over the native species (Wolf et al. 2001). Natural reproductive barriers between black bass species are weakened or altogether compromised when non-native congeners are introduced into the range of native species (Koppelman 2015), indicating the potential for extinction via hybridization exists in such scenarios. Whether the propagule pressured applied by non-native abundance in downstream impoundments will increase, remain steady, or decrease over time is an important consideration, as increased pressure could overwhelm any opposing ecological mechanisms that favor natives (Bennett et al. 2010). Furthermore, environmental variability associated with fluvial habitats (i.e., dynamic streamflow and disturbances) may periodically cause poor recruitment in native fluvial populations (Brewer and Long 2015; *see* Chapter 4), which could lower abundance of natives and lead to increased invasion success by non-natives. Although direct competition between black bass species is largely speculative, native fluvial species are generally more specialized in their habitat use and diets than their non-native counterparts (Sammons 2012; Goclowski et al. 2013; Shaw 2015). Native specialist species may be able to maintain a stronghold over non-native species in unaltered habitats, but alteration of habitats could erode selective pressures that favor natives against non-native invasion and introgression. For example, presence of native Brook Trout (*Salvelinus fontinalis*) in the eastern U.S. is less affected by non-native Brown Trout (*Salmo trutta*) in natural habitat conditions than in degraded habitats (Wagner et al. 2013). Hybridization itself can also influence the speed and eventual outcome of hybridization events by affecting the fitness of hybrid individuals. Hybrids experiencing reduced fitness (i.e., outbreeding depression) could be at a disadvantage in terms of reproductive output, favoring the maintenance of species boundaries; however, hybrids that experience no reduction in fitness could completely swamp or displace the native species (Wolf et al. 2001). Other black bass hybrids experience increased vulnerability to disease beyond the F1 generation (Goldberg et al. 2005), suggesting that outbreeding depression may slow or prevent complete genetic swamping of native taxa. However, continued monitoring of non-native invasion and hybridization rates is warranted to monitor the spread of non-native alleles and detect any increasing trends in hybridization rates.

Concurrent fragmentation of fluvial taxa and non-native propagule pressure could exacerbate the effects of hybridization (Ricciardi et al. 2010), leading to genetic swamping or complete species replacement; however, our investigation into whether impoundments fragment native fluvial black bass populations inhabiting upstream tributaries was somewhat inconclusive. In the Lake Lanier case study, Shoal Bass in the Chattahoochee and Chestatee rivers had weak population structure and genetic differentiation that was comparable to levels found in the unimpounded upper Flint River basin between mainstem river sites and a tributary, Big Lazer Creek (*see* Chapter 2). In the Lake Tenkiller case study, differences in genetic structure and differentiation occurred between the Illinois River and Baron Fork, which are not fragmented by impoundment. Although levels of differentiation between Illinois River and Caney Creek were slightly greater, it is unclear how much differentiation is attributed to natural population structure versus the potential fragmentation imposed by intervening impoundment. Time lags in the response of genetic measures to underlying fragmentation could help explain our results. A prevalent issue in field of landscape genetics, time lags can be influenced by the genetic markers used, the genetic response considered, and the biology of the study organism (Epps and Keyghobadi 2015). Although the microsatellite markers employed in our case studies typically mutate on the order of decades, measures of differentiation like F_{ST} are often better indicators of historical, rather than contemporary, connectivity (Epps and Keyghobadi 2015). Additionally, species with higher dispersal capabilities, more connected population structures, and longer generation times will generally have increased lag time (e.g., Shoal Bass; *see* Chapter 2). For example, several studies have found that minnow and darter species are fragmented by impoundments using methods similar to those we employed (Hudman and Gido 2013; Fluker et al. 2014). If native fluvial black bass populations are fragmented by impoundment but time lags have prevented a clear detection of these effects in our case studies, native populations not only face increased severity of effects from non-native invasions and hybridization (Sakai et al. 2001), but also face increased threat of inbreeding depression and local extinction (Frankham 1995; Jager et al. 2001).

Non-native congener population structure within impoundments revealed that non-native populations are not necessarily panmictic, and genetic diversity of introduced populations can be comparable to that of native populations. From our two case studies, it appears that strength of non-native population structure may depend on location(s) of initial establishment. In Lake Tenkiller, Tennessee lake strain Smallmouth Bass were rare in standardized sampling until about a decade post-stocking (early 2000's), wherein they became increasingly common in the lower end of the lake (i.e., near the dam) by 2010 (Josh Johnston, ODWC, personal communication). From 2010 until present, lake strain Smallmouth Bass have been encountered farther north within the lake each year, perhaps because of changes in lake habitat over time (Josh Johnston, ODWC, personal communication). As such, this population likely had a gradual expansion northward towards tributary interfaces, resulting in no evidence of genetic structure between fish sampled in the impoundment versus in the Illinois River. In Lake Lanier, however, population structure was evident within non-native Alabama Bass. The first record of Alabama Bass in the Lake Lanier area was recorded in the Chestatee River in 1970 (Williams and Burgess 1999), likely the result of an unauthorized angler introduction (Baker et al. 2008). If fish were initially introduced into the Chestatee River arm of the impoundment, our results support that some fish from this original introduction became founders of an extensive main-lake population that eventually entered and colonized the Chattahoochee River. Alabama Bass in the Chattahoochee River had a decreased number of private alleles, an increased inbreeding coefficient, and lower levels of introgression into upstream sites that could be indicative of a more recent invasion period. Alternatively, these results could also be explained by multiple introductions of Alabama Bass, but we know of no documentation to support this hypothesis. We also hypothesized that stocked populations would likely suffer from founder effects like increased homozygosity and decreased allelic diversity because of a presumably low effective size of breeders. Surprisingly, non-native congener heterozygosity allelic diversity measures were comparable, or exceeded, levels observed in native populations of fluvial black bass species. These genetic details of non-native black bass populations are relevant to native black bass conservation because they highlight that non-native propagule pressure could be spatially and temporally variable depending on the dynamics of population expansion within impoundments, and that introduced non-native populations do not necessarily suffer any adverse effects from decreased genetic diversity.

Although the eventual outcomes for the native black bass populations in our case studies are difficult to predict, our results reveal existing consequences to the conservation of native black bass species and native genetic diversity. Hybridization can disrupt coadapted gene complexes that could render the negative effects of outbreeding depression in native populations, including decreased fitness and increased susceptibility to disease (Goldberg et al. 2005). Furthermore, maintaining overall adaptability of native species by conserving genetic diversity within and among populations – a particularly important conservation goal for imperiled species (Meffe 1986) – appears to be an increasingly difficult task. For example, the Lake Tenkiller case study coupled with results from Taylor et al. (2016) suggest that larger rivers harbor the highest genetic diversities for Neosho Smallmouth Bass and are also most likely to be infiltrated by non-native Tennessee lake strain alleles. In contrast, populations in smaller streams may experience less introgression but have relatively low genetic diversity as well. Overall, impoundments and invading non-native congeners pose a very real threat towards the loss of genetically ‘pure’ native species or complete species replacement by non-native congeners and the loss of genetic diversity within native species. In fact, some of these mechanisms may have contributed to the replacement of native Shoal Bass with non-native Spotted Bass in several tributaries to impoundments in the Chattahoochee River basin (Stormer and Maceina 2008).

Hybridization among sympatric black bass species is a natural phenomenon, but increased hybridization rates resulting from habitat alteration and introduction of non-native congeners is unnatural (Koppelman 2015). Habitat alteration and ensuing hybridization is often the final push toward extinction for many native fluvial fishes (Allan and Flecker 1993) – in the case of native black bass conservation, virtually no management actions can be taken to ensure the long-term conservation of natives once non-natives are established in impoundments. Efforts to educate managers and anglers on the potential negative effects of non-native species, along with the enforcement of strict invasive species laws, could help curb and prevent future introductions. Otherwise, stocking programs designed to swamp tributary populations with native genetics could temporarily alleviate any hybridization and inbreeding concerns (Fleming et al. 2015), but non-native propagule pressure originating from downstream impoundments will likely outlast short-term stocking efforts (*sensu* Leitner et al. 2015). Identifying genetic refuge populations above movement barriers (i.e., lowhead dams) or creating artificial refuge populations may also be prudent depending on the range-wide conservation status of the species. Future monitoring efforts are warranted for both study areas to provide an improved understanding of the ecological mechanisms regulating the extent and severity of introgression and to determine whether genetic time lags may have disguised the role of impoundments in fragmenting native populations. Until such efforts are completed, these case studies present a case for managers and anglers alike to consider the long-term effects of impoundments and non-native black bass introductions on the conservation of native, fluvial black basses.

References

Allan, J. D., and A. S. Flecker. 1993. Biodiversity conservation in running waters. BioScience 43(1):32–43.

Alvarez, A. C., D. L. Peterson, A. T. Taylor, M. D. Tringali, and B. L. Barthel. 2015. Distribution and amount of hybridization between Shoal Bass and the invasive Spotted Bass in the lower Flint River, Georgia. Pages 503–521 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Baker, W. H., C. E. Johnston, and G. W. Folkerts. 2008. The Alabama Bass, *Micropterus henshalli* (Teleostei: Centrarchidae), from the Mobile River Basin. Zootaxa 1861:57–67.

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

Bennett, S. N., J. R. Olson, J. L. Kershner, and P. Corbett. 2010. Propagule pressure and stream characteristics influence introgression: Cutthroat and Rainbow Trout in British Columbia. Ecological Applications 20(1):263–277.

Birdsong, T., D. Krause, J. Leitner, J. M. Long, S. Robinson, and S. Sammons. 2010. A business plan for the conservation of native black bass species in the southeastern U.S. National Fish and Wildlife Foundation, Washington, D.C.

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

Brewer, S. K., and J. M. Long. 2015. Biology and ecology of Neosho Smallmouth Bass and the genetically distinct Ouachita lineage. Pages 281–295 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Buckmeier, D. L., N. G. Smith, B. P. Fleming, and K. A. Bodine. 2014. Intra-annual variation in river–reservoir interface fish assemblages: implications for fish conservation and management in regulated rivers. River Research and Applications 30(6):780–790.

Burkhead, N. M. 2012. Extinction rates in North American freshwater fishes, 1900–2010. BioScience 62(9):798–808.

Dakin, E. E., B. A. Porter, B. J. Freeman, and J. M. Long. 2015. Hybridization threatens Shoal Bass populations in the upper Chattahoochee River Basin. Pages 491–501 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

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

Epps, C. W., and N. Keyghobadi. 2015. Landscape genetics in a changing world: disentangling historical and contemporary influences and inferring change. Molecular Ecology 24(24):6021–6040.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10(3):564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131(2):479–491.

Fleming, P. B., G. P. Garrett, and N. G. Smith. 2015. Reducing hybridization and introgression in wild populations of Guadalupe Bass through supplemental stocking. Pages 537–547 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Fluker, B. L., B. R. Kuhajda, and P. M. Harris. 2014. The effects of riverine impoundment on genetic structure and gene flow in two stream fishes in the Mobile River basin. Freshwater Biology 59(3):526–543.

Frankham, R. 1995. Conservation genetics. Annual Review of Genetics 29(1):305–327.

Goclowski, M. R., A. J. Kaeser, and S. M. Sammons. 2013. Movement and habitat differentiation among adult Shoal Bass, Largemouth Bass, and Spotted Bass in the upper Flint River, Georgia. North American Journal of Fisheries Management 33(1):56–70.

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

Guenther, C. B., and A. Spacie. 2006. Changes in fish assemblage structure upstream of impoundments within the upper Wabash River basin, Indiana. Transactions of the American Fisheries Society 135(3):570–583.

Herbert, M. E., F. P. Gelwick, and W. L. Montgomery. 2003. Spatial variation of headwater fish assemblages explained by hydrologic variability and upstream effects of impoundment. Copeia 2003(2):273–284.

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

Hudman, S. P., and K. B. Gido. 2013. Multi-scale effects of impoundments on genetic structure of Creek Chub (*Semotilus atromaculatus*) in the Kansas River basin. Freshwater Biology 58(2):441–453.

Jackson, D. A. 2002. Ecological effects of *Micropterus* introductions: the dark side of black bass. Pages 221–232 *in* David P. Philipp and Ridgway, M.S., editors. Black bass: ecology, conservation, and management. American Fisheries Society Symposium 31, Bethesda, Maryland.

Jager, H. I., J. A. Chandler, K. B. Lepla, and W. V. Winkle. 2001. A theoretical study of river fragmentation by dams and its effects on White Sturgeon populations. Environmental Biology of Fishes 60(4):347–361.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. W. Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33(8):372–407.

Johnson, P. T., J. D. Olden, and M. J. Vander Zanden. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. Frontiers in Ecology and the Environment 6(7):357–363.

Koppelman, J. B. 2015. Black bass hybrids: a natural phenomenon in an unnatural world. Pages 467–479 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Leitner, J. K., K. J. Oswald, M. Bangs, D. Rankin, and J. M. Quattro. 2015. Hybridization between native Bartram's Bass and two introduced species in Savannah drainage systems. Pages 481–490 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

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

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

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

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

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

Meffe, G. K. 1986. Conservation genetics and the management of endangered fishes. Fisheries 11(1):14–23.

Nilsson, C. 2005. Fragmentation and flow regulation of the world's large river systems. Science 308(5720):405–408.

Peakall, R., and P. E. Smouse. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288–295.

Pierce, P. C., and M. J. V. D. Avyle. 1997. Hybridization between introduced Spotted Bass and Smallmouth Bass in reservoirs. Transactions of the American Fisheries Society 126(6):939–947.

Poff, N. L., J. D. Olden, D. M. Merritt, and D. M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Sciences 104(14):5732–5737.

Poff, N. L., and J. K. H. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55(1):194–205.

Porto, L. M., R. L. McLaughlin, and D. L. G. Noakes. 1999. Low-head barrier dams restrict the movements of fishes in two Lake Ontario streams. North American Journal of Fisheries Management 19(4):1028–1036.

Pringle, C. M. 1997. Exploring how disturbance is transmitted upstream: going against the flow. Journal of the North American Benthological Society 16(2):425–438.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945–959.

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

Ray, J. W., M. Husemann, R. S. King, and P. D. Danley. 2012. Genetic analysis reveals dispersal of Florida Bass haplotypes from reservoirs to rivers in Central Texas. Transactions of the American Fisheries Society 141(5):1269–1273.

Ricciardi, A., L. A. Jones, Å. M. Kestrup, and J. M. Ward. 2010. Expanding the propagule pressure concept to understand the impact of biological invasions. Pages 225–235 *in* D. M. Richardson, editor. Fifty Years of Invasion Ecology. Wiley-Blackwell.

Rider, S. J., and M. J. Maceina. 2015. Alabama Bass *Micropterus henshalli* Hubbs & Bailey, 1940. Pages 83–91 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Ritland, K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. Genetics Research 67(2):175–185.

Ruhr, C. E. 1957. Effect of stream impoundment in Tennessee on the fish populations of tributary streams. Transactions of the American Fisheries Society 86(1):144–157.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32:305–332.

Sammons, S. M. 2012. Diets of juvenile and sub-adult size classes of three *Micropterus spp.* in the Flint River, Georgia: potential for trophic competition. Southeastern Naturalist 11(3):387–404.

Sammons, S. M., and L. A. Early. 2015. Movement and habitat use of Shoal Bass in a regulated portion of the Chattahoochee River, Alabama-Georgia, USA. Pages 249–261 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Seyoum, S., B. L. Barthel, M. D. Tringali, M. C. Davis, S. L. Schmitt, P. S. Bellotti, and W. F. Porak. 2013. Isolation and characterization of eighteen microsatellite loci for the Largemouth Bass, *Micropterus salmoides*, and cross amplification in congeneric species. Conservation Genetics Resources 5(3):697–701.

Shaw, A. C. 2015. Black bass diversity and conservation: an overview. Pages 3–8 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Stark, W. J., and A. A. Echelle. 1998. Genetic structure and systematics of Smallmouth Bass, with emphasis on Interior Highlands populations. Transactions of the American Fisheries Society 127(3):393–416.

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

Taylor, A. T., J. M. Long, M. R. Schwemm, M. D. Tringali, and S. K. Brewer. 2016. Identification of Neosho Smallmouth Bass (*Micropterus dolomieu velox*) stocks for possible introduction into Grand Lake, Oklahoma. U.S. Fish and Wildlife Service Cooperator Science Series, 121–2016.

Taylor, A. T., and D. L. Peterson. 2014. Shoal bass life history and threats: a synthesis of current knowledge of a *Micropterus* species. Reviews in Fish Biology and Fisheries 24(1):159–167.

Taylor, A. T., and D. L. Peterson. 2015. Movement, homing, and fates of fluvial-specialist Shoal Bass following translocation into an impoundment. Southeastern Naturalist 14(3):425–437.

Taylor, C. A., J. H. Knouft, and T. M. Hiland. 2001. Consequences of stream impoundment on fish communities in a small North American drainage. Regulated Rivers: Research & Management 17(6):687–698.

Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. 2015a. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Tringali, M. D., P. A. Strickland, R. A. Krause, S. Seyoum, B. L. Barthel, A. Alvarez, and C. Puchulutegui. 2015b. Conservation status of Shoal Bass in the Chipola River, Florida: the threat of hybridization with native and nonnative congeners. Pages 523–536 *in* M. D. Tringali, J. M. Long, T. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82.

Wagner, T., J. T. Deweber, J. Detar, and J. A. Sweka. 2013. Landscape-scale evaluation of asymmetric interactions between Brown Trout and Brook Trout using two-species occupancy models. Transactions of the American Fisheries Society 142(2):353–361.

Wang, J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetics Research 89(3):135–153.

Wang, J. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources 11(1):141–145.

Ward, J. V., and J. A. Stanford. 1995. The serial discontinuity concept: extending the model to floodplain rivers. Regulated Rivers: Research & Management 10(2–4):159–168.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6):1358–1370.

Williams, J. D., and G. H. Burgess. 1999. A new species of bass, *Micropterus cataractae* (Teleostei: Centrarchidae), from the Apalachicola River basin in Alabama, Florida, and Georgia. University of Florida.

Winston, M. R., C. M. Taylor, and J. Pigg. 1991. Upstream extirpation of four minnow species due to damming of a prairie stream. Transactions of the American Fisheries Society 120(1):98–105.

Wolf, D. E., N. Takebayashi, and L. H. Rieseberg. 2001. Predicting the risk of extinction through hybridization. Conservation Biology 15(4):1039–1053.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323–354.

Table 1. Sample site information for a study to assess the influence of Lake Lanier, GA, on hybridization between, and population structure within, native Shoal Bass (*Micropterus cataractae*) and non-native Alabama Bass (*M. henshalli*). Table includes sample site numbers (corresponding to Fig. 1), site names ("D.S." = downstream of), geospatial coordinates in decimal degrees, years that collections were made, approximate elevation of each site (m), approximate number of river-kilometers (rkm) of each site from Lake Lanier, the number of putative Shoal Bass and Alabama Bass collected at each site, and the number of taxonomically-screened 'pure' species at each site.

							Shoal Bass		**Alabama Bass**	
Site #	**site name**	**latitude**	**longitude**	**collection year(s)**	**elevation (m)**	**rkm from lake**	**putative *N***	**pure *N***	**putative *N***	**pure *N***
				Chattahoochee River						
1	Flat Rock	34.466399	-83.686461	2013, 2014	330	6.4	3	1	17	9
2	Bull Shoals	34.482440	-83.680216	2013, 2014	330	8.8	24	18	16	10
3	Crow Island	34.503651	-83.666475	2013, 2014	334	11.7	10	9	0	0
4	Buck Shoals	34.563347	-83.628713	2013, 2014	362	24.0	13	12	3	1
							50	**40**	**36**	**20**
				Chestatee River						
5	Big Rock	34.458609	-83.966767	2013, 2014	328	3.0	0	0	23	16
6	Canoe Launch	34.471844	-83.979555	2013, 2014	330	5.8	6	3	28	19
7	Horseshoe Bend	34.492659	-83.997084	2013, 2014	333	9.3	18	15	7	6
8	D.S. Hwy 60 bridge	34.504223	-83.968851	2013, 2014	338	14.6	21	13	5	4
							45	**31**	**63**	**45**

Table 2. A hierarchical analysis of molecular variance (AMOVA) for native Shoal Bass (*Micropterus cataractae*) and non-native Alabama Bass (*M. henshalli*) inhabiting the Chattahoochee and Chestatee rivers, both of which are tributaries to Lake Lanier, GA. Fish were genotyped using 16 microsatellite DNA loci. The percentage of molecular variation is partitioned into the following three hierarchical levels (largest to smallest): among tributaries, among sites within tributaries, and within sites. Corresponding *F*-statistics describe the amount of genetic differentiation at each level. Asterisks (*) indicate statistically significant ($P \leq 0.05$) amounts of molecular variation.

	Shoal Bass				**Alabama Bass**			
Source of variation	**sums of squares**	**variation (%)**	***P*-value**	***F*-statistic**	**sums of squares**	**variation (%)**	***P*-value**	***F*-statistic**
Among tributaries	8.55	3.6	* < 0.01	F_{CT} = 0.036	7.91	3.7	* 0.01	F_{CT} = 0.037
Among sites within tributaries	10.78	-0.9	0.87	F_{SC} = -0.009	12.87	0.1	0.35	F_{SC} = 0.001
Within sites	343.89	97.3	* 0.04	F_{ST} = 0.028	311.71	96.3	* < 0.01	F_{ST} = 0.037
Total	**363.22**	**100.0**			**332.48**	**100.0**		

Table 3. A summary of population-genetic structuring mechanisms for native Shoal Bass (*Micropterus cataractae*) and non-native Alabama Bass (*M. henshalli*) inhabiting the Chattahoochee and Chestatee rivers, both of which are tributaries to Lake Lanier, GA. The following measures are averaged across 16 microsatellite DNA loci: expected heterozygosity (H_e), and observed heterozygosity (H_o), mean number of alleles (A), and effective number of alleles (A_e). We also reported the number of private alleles ($A_{private}$) by river.

Site	H_e	H_o	A	A_e	$A_{private}$	N
Shoal Bass						
All Chattahoochee River sites (1-4)	0.313	0.312	3.313	2.140	10	40
All Chestatee River sites (5-8)	0.323	0.323	2.938	1.979	4	31
Combined	0.318	0.317	3.125	2.059	--	71
Alabama Bass						
All Chattahoochee River sites (1-4)	0.335	0.335	2.688	1.771	3	20
All Chestatee River sites (5-8)	0.312	0.337	3.188	1.752	11	45
Combined	0.323	0.336	2.938	1.761	--	65

Table 4. A summary of mean (and standard deviation; SD) pairwise relatedness (*r*) and mean (SD) inbreeding coefficient (*F*) for native Shoal Bass (*Micropterus cataractae*) and non-native Alabama Bass (*M. henshalli*) inhabiting the Chattahoochee and Chestatee rivers, both of which are tributaries to Lake Lanier, GA. The following measures were calculated from fish genotyped with 16 microsatellite DNA loci. Sample sizes (*N*) are different because *r* is calculated from pairwise comparisons of individual genotypes, whereas *F* is calculated from individual genotypes directly. **Bolded** lowercase letters indicate statistically significant ($P \leq 0.05$) differences in *r* or *F* between tributary river systems for a particular species.

	r			***F***		
Site	***r* est.**	**SD**	***N***	***F* est.**	**SD**	***N***
	Shoal Bass					
All Chattahoochee River sites (1-4)	0.087 **a**	0.131	780	-0.020	0.096	40
All Chestatee River sites (5-8)	0.018 **b**	0.167	465	0.014	0.113	31
Combined	0.081	0.136	2485	-0.005	0.105	71
	Alabama Bass					
All Chattahoochee River sites (1-4)	0.104	0.137	190	0.106	0.373	20
All Chestatee River sites (5-8)	0.126	0.177	990	0.012	0.305	45
Combined	0.103	0.158	2080	0.041	0.330	65

Table 5. Sample site information for a study to assess the influence of Lake Tenkiller, OK, on hybridization between, and population structure within, native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (*M. d. dolomieu*). Table includes sample site numbers (corresponding to Fig. 1), site names ("D.S." = downstream, "U.S." = upstream), geospatial coordinates in decimal degrees, years that collections were made, approximate elevation of each site (m), approximate number of river-kilometers (rkm) of each site from Lake Tenkiller, the number of putative Smallmouth Bass ("SMB") collected at each site, and the number of taxonomically-screened 'pure' of each species sampled at each site.

Site #	**site name**	**latitude**	**longitude**	**collection year(s)**	**elevation (m)**	**rkm from lake**	**SMB field collection *N***	**pure Neosho *N***	**pure lake strain *N***
				Lake Tenkiller					
1	Lake Tenkiller	35.59885	-95.044454	2014	192	N/A	**32**	**0**	**16**
				Illinois River					
2	U.S. of interface	35.842261	-94.920055	2015	194	1.3	26	7	2
3	Riverside Park	35.922055	-94.923975	2015	203	12.7	22	5	4
4	Round Hollow to Peavine	36.09421	-94.830422	2015	240	54.0	47	17	0
							95	**29**	**6**
				Baron Fork					
5	Welling Rd bridge	35.870224	-94.896924	2015	200	5.3	11	8	0
6	U.S. Wall Trip Br confl.	35.894349	-94.863118	2015	208	10.8	12	11	0
7	West of N 4580 Rd	35.912631	-94.846221	2015	217	15.1	12	8	0
8	U.S. Hwy 51 bridge	35.936556	-94.827673	2015	220	19.1	12	11	0
							47	**38**	**0**
				Caney Creek					
9	D.S. S 581 Rd access	35.793278	-94.846425	2015	200	1.8	24	15	0
10	U.S. S 581 Rd access	35.798125	-94.840462	2015	210	2.8	29	22	0
11	Bidding Crk confl.	35.841145	-94.789427	2015	229	10.9	17	16	0
12	N 4630 Rd crossing	35.841508	-94.77270	2015	237	13.3	24	23	0
							94	**76**	**0**

Table 6. A hierarchical analysis of molecular variance (AMOVA) for native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (*M. d. dolomieu*) inhabiting several tributaries to Lake Tenkiller, OK, including the Illinois River, Baron Fork, and Caney Creek. Fish were genotyped using 7 microsatellite DNA loci. The percentage of molecular variation is partitioned into the following three hierarchical levels (largest to smallest): among tributaries, among sites within tributaries, and within sites. Since Tennessee lake strain fish were captured from a sample in the lake, this site was incorporate at the tributary-level in these analyses. Corresponding *F*-statistics describe the amount of genetic differentiation at each level. Asterisks (*) indicate statistically significant ($P \leq 0.05$) amounts of molecular variation. Negative values are artifacts of calculation methods and were interpreted as zero.

	Neosho Smallmouth Bass				**Tennessee lake strain Smallmouth Bass**			
Source of variation	**sums of squares**	**variation (%)**	***P*-value**	***F*-statistic**	**sums of squares**	**variation (%)**	***P*-value**	***F*-statistic**
Among tributaries	12.94	2.2	* < 0.01	F_{CT} = 0.022	5.05	-2.9	0.72	F_{CT} = -0.029
Among sites within tributaries	19.65	0.7	0.06	F_{SC} = 0.007	4.29	9.3	0.06	F_{SC} = 0.090
Within sites	568.40	97.1	* < 0.01	F_{ST} = 0.029	109.72	93.6	* < 0.01	F_{ST} = 0.064
Total	**600.99**	**100.0**			**119.07**	**100.0**		

Table 7. Pairwise fixation index (F_{ST}; Wright 1951) values as calculated by Weir and Cockerham (1984), which quantify genetic differentiation, are reported for Neosho Smallmouth Bass genotyped with 7 microsatellite DNA loci. Site numbers (see Table 5 for site descriptions) represent the Illinois River (2-4), Baron Fork (5-8), and Caney Creek (9-12), all of which are tributaries to Lake Tenkiller, OK. Values in **bold** were considered significant at $P \leq 0.05$. Negative values are artifacts of calculation methods and were interpreted as $F_{ST} = 0$.

Site #	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2	--										
3	**0.0401**	--									
4	0.0066	0.0056	--								
5	-0.0136	0.0324	-0.0019	--							
6	0.0076	**0.0616**	0.0102	-0.0159	--						
7	0.0050	0.0376	0.0045	-0.0093	0.0119	--					
8	0.0234	**0.0395**	0.0017	-0.0077	-0.0060	0.0025	--				
9	-0.0123	**0.0686**	**0.0274**	-0.0070	0.0017	**0.0279**	0.0124	--			
10	0.0167	**0.0627**	**0.0267**	0.0004	-0.0025	**0.0276**	0.0090	0.0005	--		
11	0.0122	**0.1067**	**0.0575**	0.0133	0.0096	**0.0527**	**0.0435**	0.0064	0.0094	--	
12	**0.0351**	**0.1341**	**0.0675**	**0.0253**	0.0137	**0.0604**	**0.0532**	**0.0329**	0.0090	0.0062	--

Table 8. A summary of population-genetic structuring mechanisms for native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (*M. d. dolomieu*) inhabiting several tributaries to Lake Tenkiller, OK. The following measures are averaged across 7 microsatellite DNA loci: expected heterozygosity (H_e), and observed heterozygosity (H_o), mean number of alleles (A), and effective number of alleles (A_e). We also reported the number of private alleles ($A_{private}$) by tributary.

Site	H_e	H_o	A	A_e	$A_{private}$	N
Neosho Smallmouth Bass						
All Illinois River sites (2-4)	0.679	0.650	10.571	4.565	13	29
All Baron Fork sites (5-8)	0.613	0.639	9.571	3.748	6	38
All Caney Creek sites (9-12)	0.553	0.543	10.429	3.623	12	76
Combined	0.615	0.611	10.190	3.979	--	143
Tennessee lake strain Smallmouth Bass						
Lake Tenkiller site (1)	0.809	0.764	7.571	5.128	22	16
All Illinois River sites (2-4)	0.829	0.786	5.571	4.417	8	6
Combined	0.819	0.775	6.571	4.772	--	22

Table 9. A summary of mean (and standard deviation; SD) pairwise relatedness (*r*) and mean (SD) inbreeding coefficient (*F*) for native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (*M. d. dolomieu*) inhabiting several tributaries to Lake Tenkiller, OK. The following measures were calculated from fish genotyped with 7 microsatellite DNA loci. Sample sizes (*N*) are different because *r* is calculated from all pairwise comparisons of individual genotypes, whereas *F* is calculated from individual genotypes directly. **Bolded** lowercase letters indicate statistically significant ($P \leq 0.05$) differences in *r* or *F* between tributaries for a particular species.

	r			***F***		
Site	***r* est.**	**SD**	***N***	***F* est.**	**SD**	***N***
	Neosho Smallmouth Bass					
All Illinois River sites (2-4)	0.041 **a**	0.081	406	0.069 **d**	0.229	29
All Baron Fork sites (5-8)	0.063 **b**	0.107	703	-0.020 **e**	0.040	38
All Caney Creek sites (9-12)	0.088 **c**	0.140	2850	0.011 **d**	0.086	76
Combined	0.060	0.109	10153	0.014	0.126	143
	Tennessee lake strain Smallmouth Bass					
Lake Tenkiller site (1)	0.068	0.109	120	0.010	0.110	16
All Illinois River sites (2-4)	0.121	0.205	15	-0.008	0.057	6
Combined	0.053	0.105	231	0.005	0.099	22

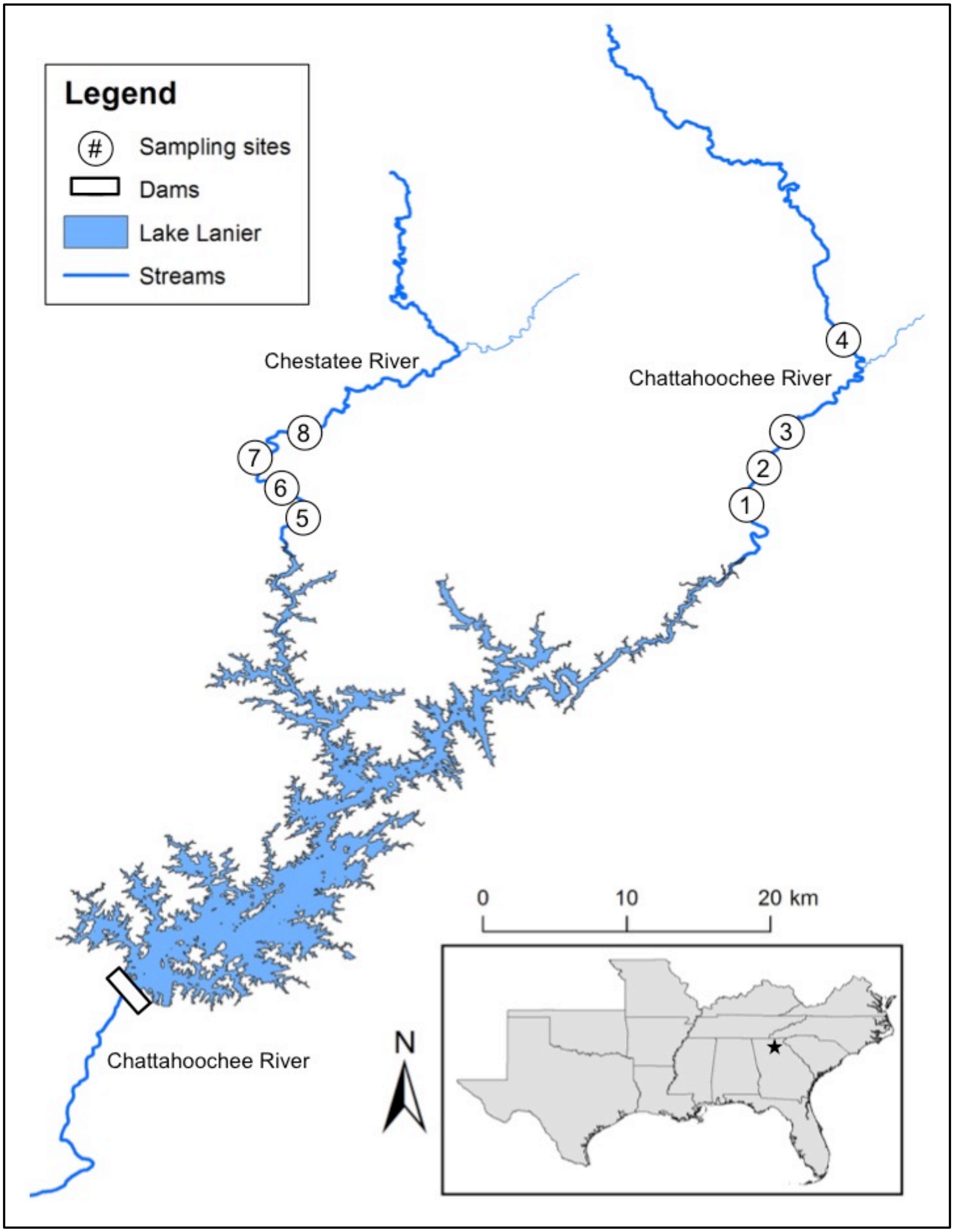

Figure 1. Lake Sidney Lanier, GA, study area and sample sites (see Table 1 for site descriptions) in the Chattahoochee and Chestatee rivers used to address the potential role of impoundments on hybridization between, and population structure within, native Shoal Bass (*Micropterus cataractae*) and non-native Alabama Bass (*M. henshalli*).

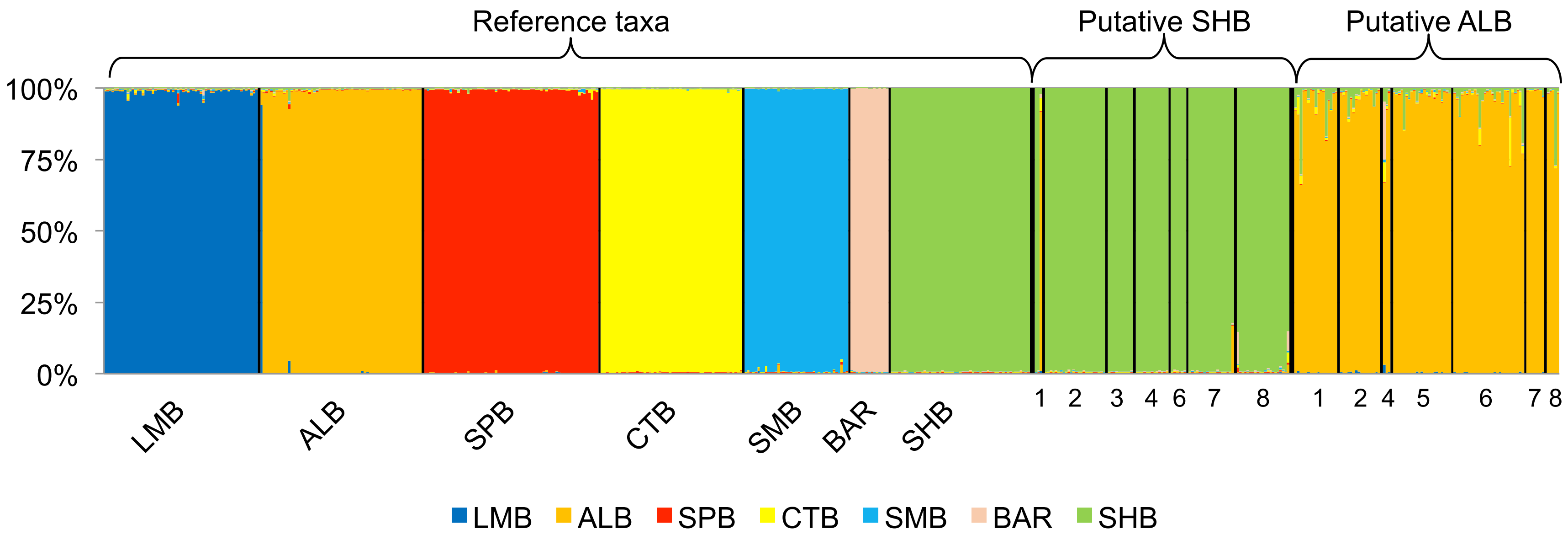

Figure 2. Taxonomic assignment of 95 putative Shoal Bass (*Micropterus cataractae*) and 99 putative Alabama Bass (*M. henshalli*) collected from the Chattahoochee and Chestatee rivers (numbers correspond with sample sites in Table 1), which were genotyped with 16 microsatellite DNA loci. Assignments were made using the allele frequencies of reference taxa genotypes for seven *Micropterus* taxa ("LMB" = Largemouth Bass x Florida Bass intergrades; "ALB" = Alabama Bass; "SPB" = Spotted Bass; "CTB" = Choctaw Bass; "SMB" = Smallmouth Bass; "BAR" = Bartram's Bass; "SHB" = Shoal Bass). Colors represent distinct genetic clusters, and each individual's proportional assignment to those clusters is represented within a single vertical bar.

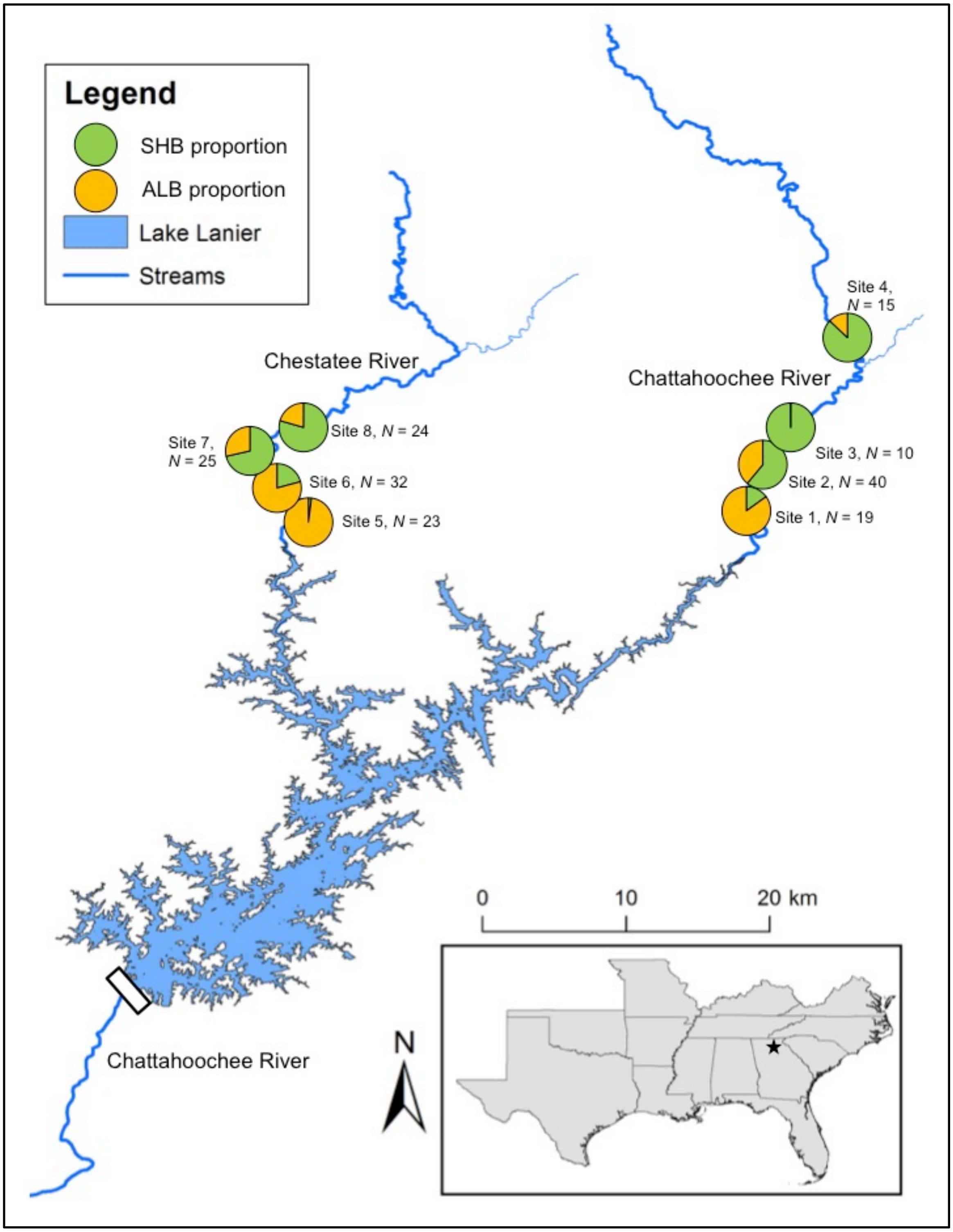

Figure 3. Genomic proportions of native Shoal Bass ("SHB"; *Micropterus cataractae*) and non-native Alabama Bass ("ALB"; *M. henshalli*) at a number of sample sites extending upstream from Lake Sidney Lanier, GA, into the Chattahoochee and Chestatee rivers. Site numbers (see Table 1) and sample sizes (*N*) are reported for each sample site.

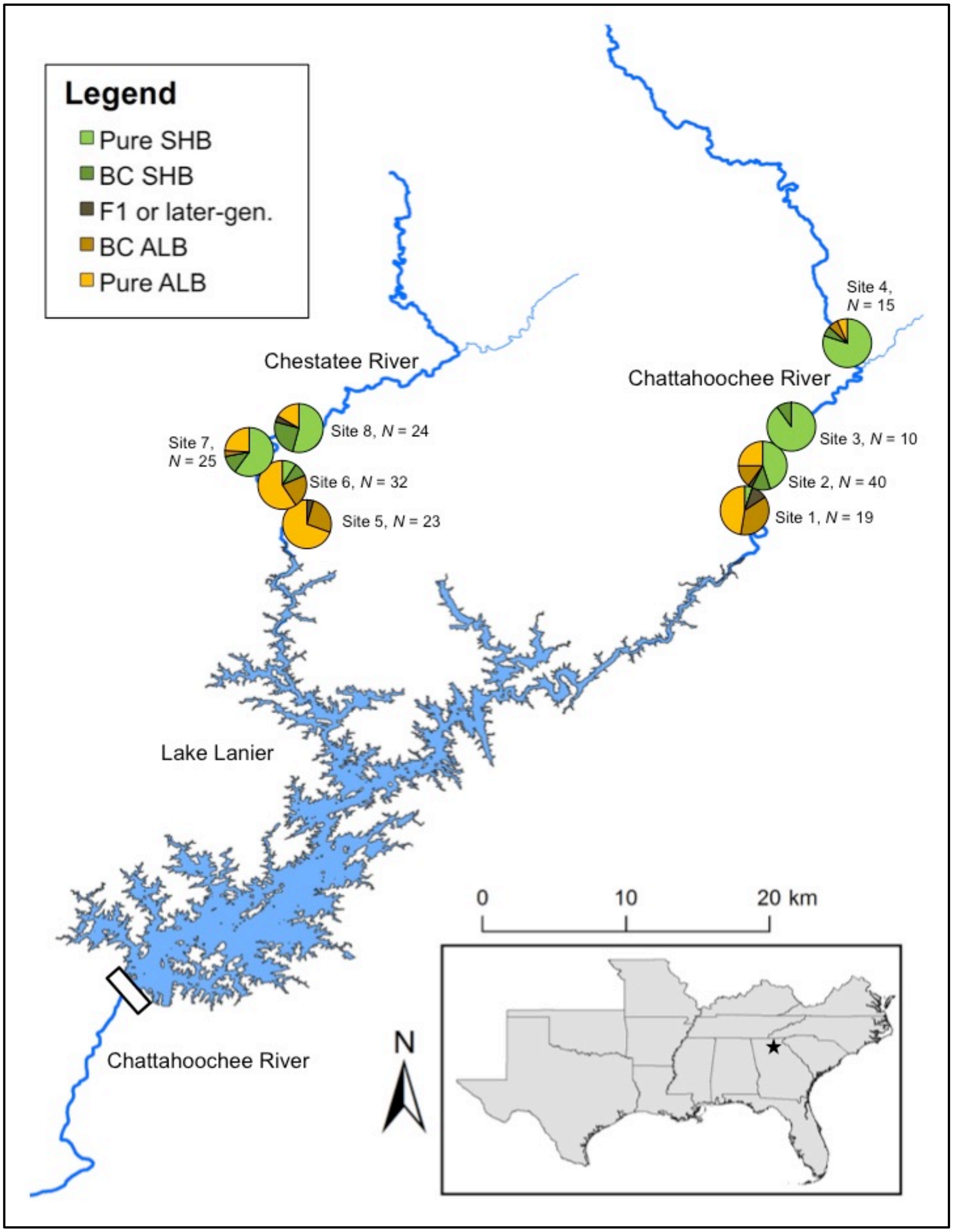

Figure 4. Proportions of individuals belonging to five hybridization categories at sample sites in the Chattahoochee and Chestatee rivers upstream of Lake Sidney Lanier, GA. Colors in the figure legend correspond to pure, native Shoal Bass (*Micropterus cataractae*; "SHB"), individuals backcrossed towards Shoal Bass ("BC SHB"), F1 or later-generation hybrids between Shoal Bass and non-native Alabama Bass (*M. henshalli*), backcrossed Alabama Bass ("BC ALB"), and pure Alabama Bass ("ALB").

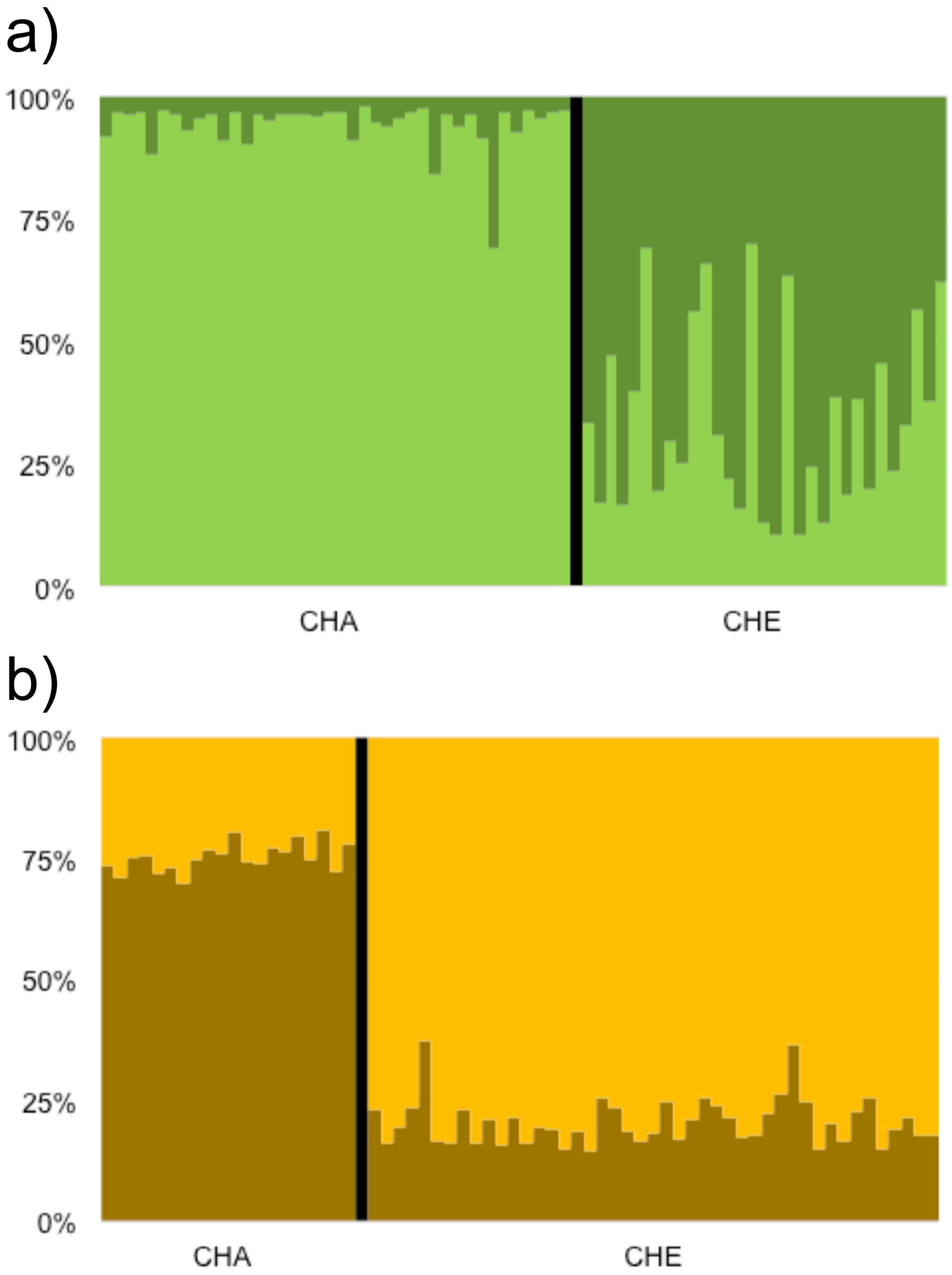

Figure 5. Population structure assignments to pure genotypes of (a) native Shoal Bass (*Micropterus cataractae*) and (b) non-native Alabama Bass (*M. henshalli*) within two tributaries of Lake Sidney Lanier, GA – the Chattahoochee River ("CHA") and the Chestatee River ("CHE"). Samples were genotyped with 16 microsatellite DNA markers and population structure ($K = 2$) in both species was estimated in Program STRUCTURE, using tributary of origin as prior information in the Bayesian clustering algorithm. Individual genotypes are ordered from downstream sites (left) to upstream sites (right) within each tributary.

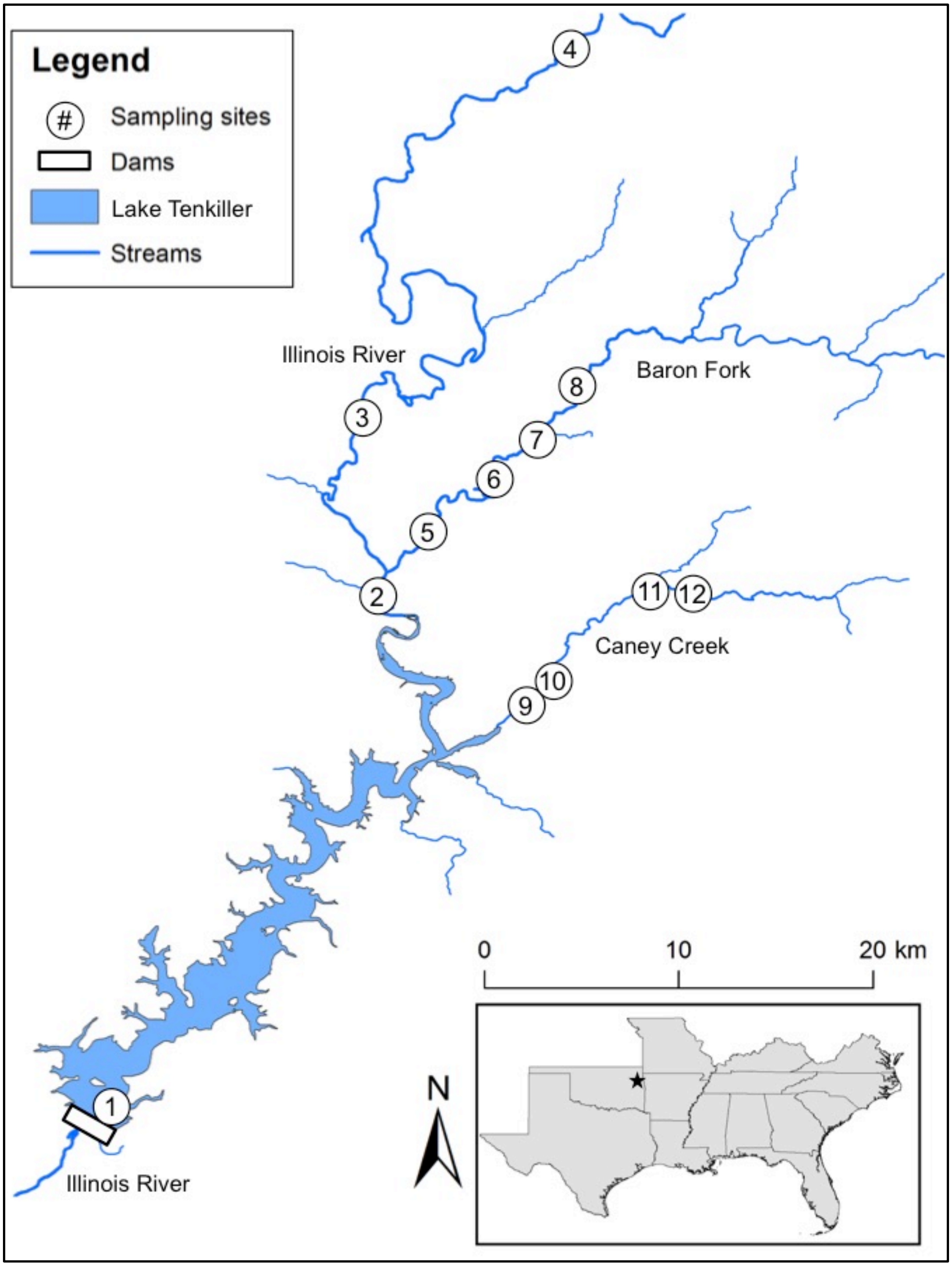

Figure 6. Tenkiller Ferry Lake, OK, study area and sample sites (see Table 5 for site descriptions) in the lake, the Illinois River, the Baron Fork, and Caney Creek that were used to address the potential role of impoundments on hybridization between, and population structure within, native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (*M. d. dolomieu*).

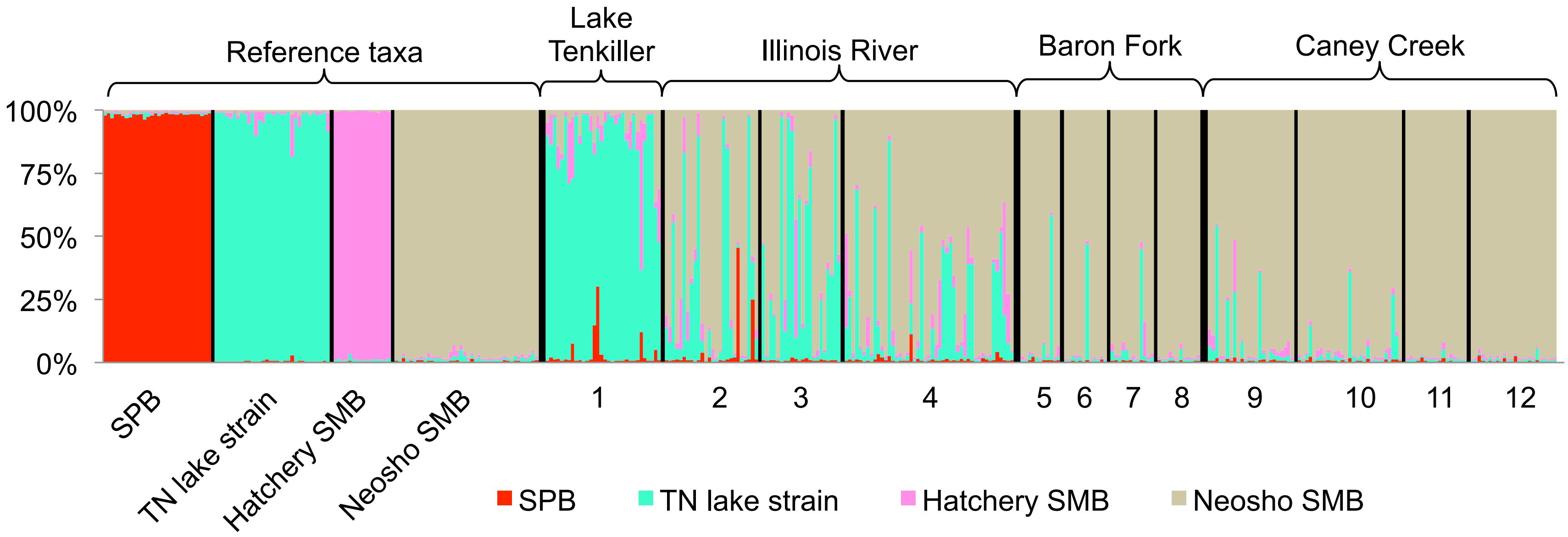

Figure 7. Taxonomic assignment of 268 individuals identified as Smallmouth Bass (*Micropterus dolomieu*) from Lake Tenkiller, OK, and three tributary streams: the Illinois River, Baron Fork, and Caney Creek (numbers correspond with sample sites in Table 5). Individuals were genotyped with 7 microsatellite DNA loci, and assignments were made using the allele frequencies of reference taxa genotypes of four genetically distinct *Micropterus* groups ("SPB" = Spotted Bass [*M. punctulatus*]; "TN lake strain" = Tennessee lake strain Smallmouth Bass [*M. dolomieu dolomieu*]; "Hatchery SMB" = a genetically distinct hatchery form of Smallmouth Bass [*M. dolomieu dolomieu*]; and "Neosho SMB" = Neosho Smallmouth Bass [*M. dolomieu velox*]). Colors represent distinct genetic clusters, and each individual's proportional assignment to those clusters is represented within a single vertical bar.

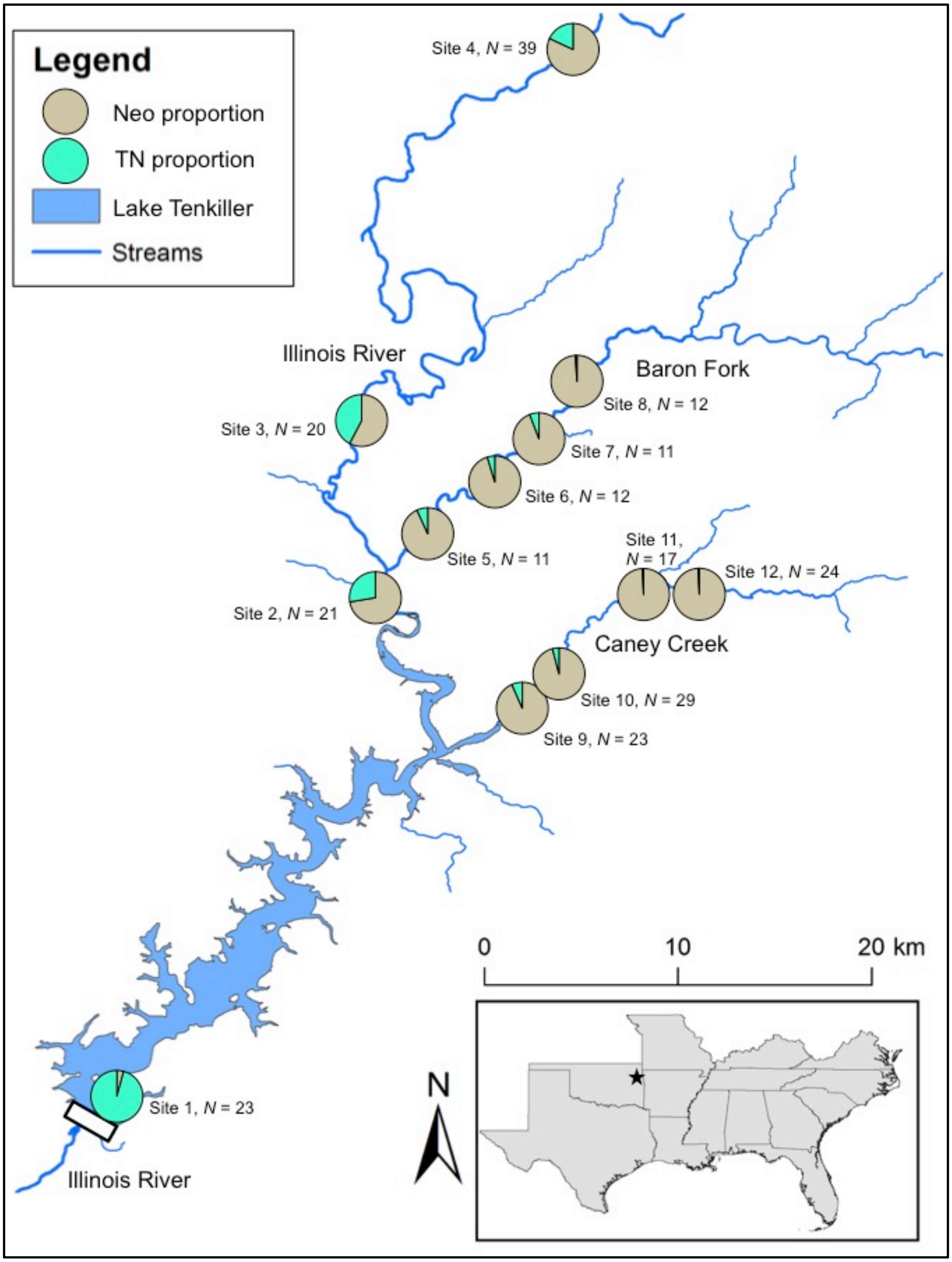

Figure 8. Genomic proportions of native Neosho Smallmouth Bass ("Neo"; *Micropterus dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass ("TN"; *M. d. dolomieu*) at a number of sample sites within, and extending into three upstream tributaries of, Lake Tenkiller, OK. Site numbers (see Table 5) and sample sizes (*N*) are reported for each sample site.

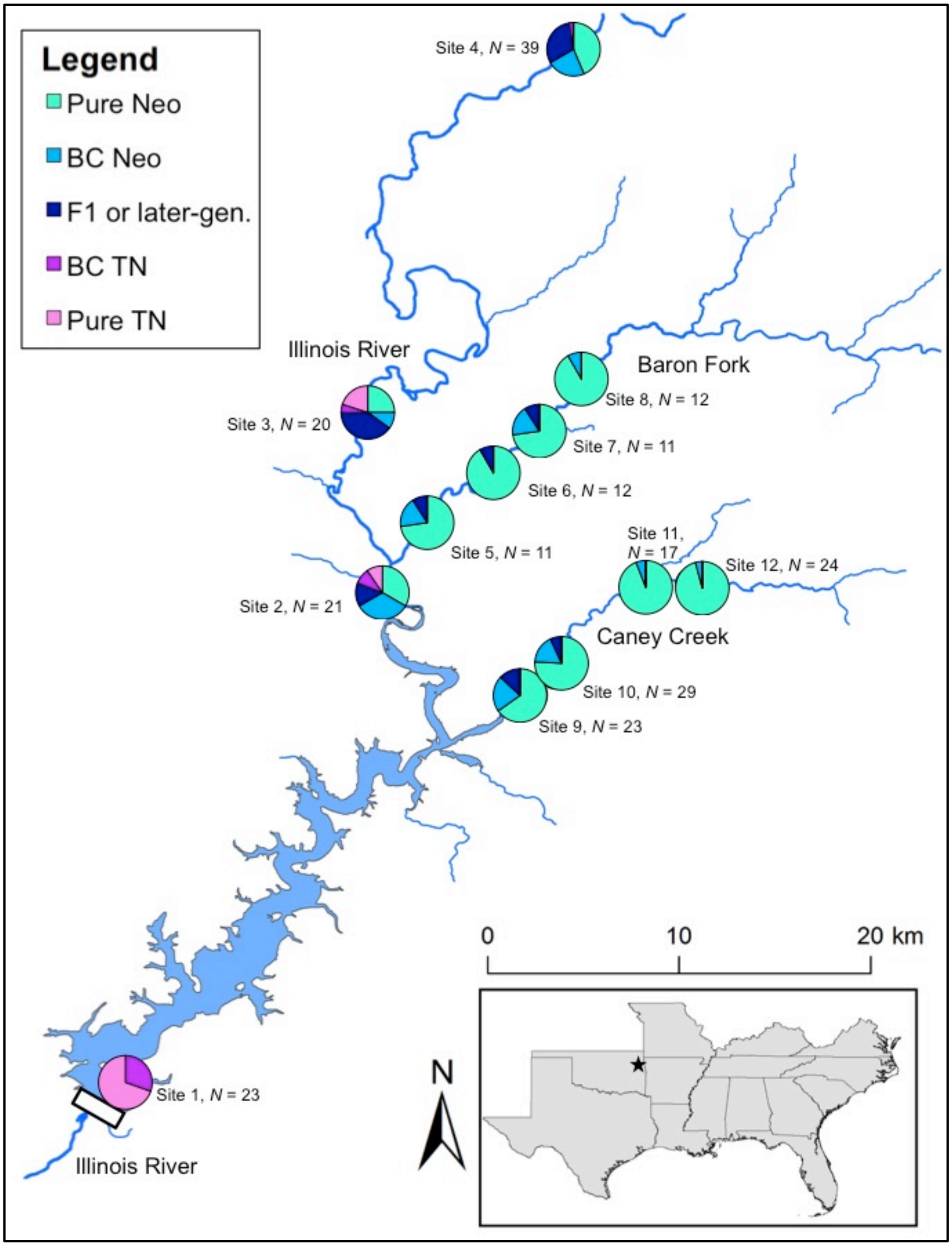

Figure 9. Proportions of individuals belonging to five hybridization categories at sample sites within, and in upstream tributaries of, Lake Tenkiller, OK. Colors in the figure legend correspond to pure, native Neosho Smallmouth Bass (*Micropterus dolomieu velox*; "Neo"), individuals backcrossed towards Neosho Smallmouth Bass ("BC Neo"), F1 or later-generation hybrids between Neosho Smallmouth Bass and non-native Tennessee lake strain Smallmouth Bass (*M. dolomieu dolomieu*), backcrossed Tennessee lake strain ("BC TN"), and pure Tennessee lake strain Smallmouth Bass ("TN").

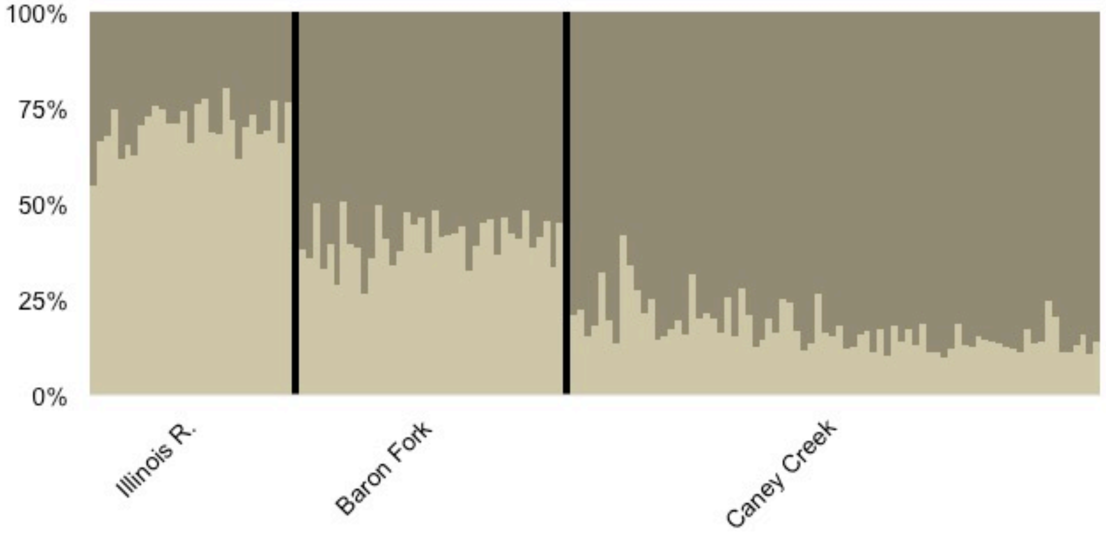

Figure 10. Population structure assignments to pure genotypes of native Neosho Smallmouth Bass (*Micropterus dolomieu velox*) within three tributaries of Lake Tenkiller, OK. Samples were genotyped with 7 microsatellite DNA markers and population structure (*K* = 2) in both species was estimated in Program STRUCTURE, using tributary of origin as prior information in the Bayesian clustering algorithm. Individual genotypes are ordered from downstream sites (left) to upstream sites (right) within each tributary.

Appendix I. Putative Shoal Bass (“SHB”; *Micropterus cataractae*) and Alabama Bass (“ALB”; *M. henshalli*) that were assigned ≥ 5% to a different taxonomic group based on a genetic clustering analysis of fish genotyped with 16 microsatellite DNA markers. Fish were sampled from the Chattahoochee (“CHA”) and Chestatee (“CHE”) rivers (see Table 1 for site numbers) that feed Lake Sidney Lanier, GA. Assignments were made using the allele frequencies of reference taxa genotypes for seven *Micropterus* taxa (“LMB” = Largemouth Bass x Florida Bass intergrades; “ALB” = Alabama Bass; “SPB” = Spotted Bass; “CTB” = Choctaw Bass; “SMB” = Smallmouth Bass; “BAR” = Bartram’s Bass; “SHB” = Shoal Bass). Bolded proportional assignments are indicative of values that surpassed the ≥ 5% threshold.

				Individual genomic proportion (*q*) assignment						
Count	**site #**	**tributary**	**field ID**	**LMB**	**ALB**	**SPB**	**CTB**	**SMB**	**BAR**	**SHB**
1	1	CHA	ALB	0.0091	0.8952	0.0020	**0.0635**	0.0011	0.0048	0.0243
2	4	CHA	ALB	0.0311	0.6355	0.0029	**0.0693**	0.0091	**0.2038**	0.0484
3	6	CHE	ALB	0.0030	0.7949	0.0013	**0.0566**	0.0010	0.0054	0.1379
4	6	CHE	ALB	0.0040	0.7204	0.0019	**0.1702**	0.0010	0.0038	0.0985
5	8	CHE	SHB	0.0010	0.0040	0.0168	0.0095	0.0021	**0.1133**	0.8534
6	8	CHE	SHB	0.0020	0.0291	0.0086	0.0359	0.0039	**0.0685**	0.8519

Note: proportional assignments to Choctaw Bass and Bartram’s Bass do not necessarily indicate these taxa are present in the study area. These assignments are more likely indicative of hybridization with native Chattahoochee Bass (*M. chattahoochae*) or Redeye Bass (*M. coosae*) – both of which are relatives to Bartram’s Bass (Freeman et al. 2015) and have been documented in the study area (A. Taylor, unpublished data).

Appendix II. Putative Smallmouth Bass (“SMB”; *Micropterus dolomieu*) that were assigned ≥ 5% to sympatric Spotted Bass (“SPB”; *M. punctulatus*) or ≥ 10% to a genetically distinct hatchery form of Smallmouth Bass (“Hatchery SMB”). These individuals were removed because they were not of direct interest to this study of native Neosho Smallmouth Bass (“Neosho SMB”; *M. dolomieu velox*) and non-native Tennessee lake strain Smallmouth Bass (“TN lake strain”; *M. dolomieu dolomieu*) in Lake Tenkiller, OK, and surrounding tributaries (“TNKR” = within Lake Tenkiller proper; “ILL” = Illinois River; “BF” = Baron Fork; “CC” = Caney Creek). Bolded proportional assignments are indicative of values that surpassed the thresholds for removal.

				Individual genomic proportion (*q*) assignment			
Count	**site #**	**tributary**	**field ID**	**SPB**	**TN lake strain**	**Hatchery SMB**	**Neosho SMB**
1	1	TNKR	SMB	0.0202	0.8427	**0.1177**	0.0194
2	1	TNKR	SMB	0.0099	0.6972	**0.2448**	0.0481
3	1	TNKR	SMB	**0.0755**	0.6538	0.2407	0.0300
4	1	TNKR	SMB	**0.1474**	0.6778	0.0439	0.1310
5	1	TNKR	SMB	**0.3003**	0.6262	0.0531	0.0205
6	1	TNKR	SMB	0.0073	0.8387	**0.1116**	0.0424
7	1	TNKR	SMB	**0.1208**	0.2443	0.5949	0.0401
8	1	TNKR	SMB	**0.0508**	0.5593	0.0246	0.3653
9	1	TNKR	SMB	0.0106	0.4668	**0.2107**	0.3120
10	2	ILL	SMB	0.0102	0.0433	**0.1990**	0.7475
11	2	ILL	SMB	0.0224	0.8085	**0.1386**	0.0304
12	2	ILL	SMB	0.0100	0.0779	**0.1160**	0.7961
13	2	ILL	SMB	**0.4559**	0.0108	0.0120	0.5213
14	2	ILL	SMB	**0.2510**	0.1481	0.0219	0.5790
15	3	ILL	SMB	0.0058	0.1166	**0.1242**	0.7534
16	3	ILL	SMB	0.0154	0.0842	**0.4662**	0.4341
17	4	ILL	SMB	0.0070	0.1324	**0.3734**	0.4873
18	4	ILL	SMB	0.0075	0.0528	**0.1184**	0.8214
19	4	ILL	SMB	**0.1126**	0.1196	0.2107	0.5571
20	4	ILL	SMB	0.0064	0.0482	**0.2706**	0.6749
21	4	ILL	SMB	0.0060	0.0174	**0.1705**	0.8061
22	4	ILL	SMB	0.0146	0.3756	**0.1467**	0.4631
23	4	ILL	SMB	0.0089	0.1795	**0.4466**	0.3649
24	4	ILL	SMB	0.0068	0.0707	**0.1935**	0.7289
25	7	BF	SMB	0.0080	0.0151	**0.1365**	0.8404
26	9	CC	SMB	0.0190	0.2596	**0.2093**	0.5121

CHAPTER IV

POPULATION DYNAMICS OF SHOAL BASS
AT THE NORTHERN EXTENT OF THEIR RANGE

Abstract

Fluvial fishes of the southeastern U.S., including several black bass (genus *Micropterus*) species, face a diverse array of conservation threats. Often, the population dynamics data needed to understand drivers of imperilment and inform management decisions are lacking. We used both raw catch data and capture-mark-recapture (CMR) methods to quantify dynamics of three Shoal Bass populations in the upper Chattahoochee River Basin (UCRB) presumed to be isolated and of conservation concern. Compared to populations situated at more southerly latitudes, UCRB Shoal Bass grew slower, had increased longevity (up to 12 yrs), and experienced lower annual mortality (18-24%). Recruitment strength was most variable in Big Creek, an urbanized and flashy system, where variability in discharge appeared to negatively influence recruitment. Abundance was estimated at a number of UCRB sites, and insights drawn from this CMR study can help guide the design of future population monitoring studies. Over-winter survival might pose a recruitment pinch-point in UCRB populations, as we

estimated low over-winter survival (8%) of an age-0 cohort in Big Creek and discovered a positive relationship between fall cumulative growing degree-days and recruitment. Overall, the results of this study underscore the need for population-specific management of Shoal Bass and can be used to inform future monitoring and conservation efforts.

Introduction

Fluvial systems in the southeastern U.S. feature diverse arrays of freshwater fishes, but habitat alteration, fragmentation, and invasion of non-native species have led to high imperilment rates in this region (Allan and Flecker 1993; Benz and Collins 1996; Jelks et al. 2008). Population dynamics data are critical to informing management and conservation of imperiled fish populations, allowing managers to pinpoint critical life-history periods, assess population trends, and establish monitoring benchmarks. Relationships between population parameters and environmental factors can also be characterized, allowing management actions to become more predictive in nature. However, obtaining reliable estimates of population parameters in fluvial systems is often difficult because of logistical constraints (e.g., habitat accessibility) and study design considerations like system openness (Gwinn et al. 2011).

Black bass (genus *Micropterus*) diversity is also concentrated within the southeastern U.S., and there is growing interest in the management and conservation of native species and other native forms that include subspecies, unique genetic lineages, and undescribed species (Birdsong et al. 2010; Tringali et al. 2015). Approximately 17 forms of black bass are currently recognized, and 10 of these forms were not recorded in scientific literature until after 2000 (Long et al. 2015). Black basses have long-supported popular sport fisheries in lakes and impoundments, but recent growth in kayak angling has increased interest in fishing for fluvial species (Long et al. 2015; Sammons et al. 2015). The status of at least 10 black bass forms is either vulnerable or unknown (Birdsong et al. 2010), and conservation threats include the effects of habitat alteration and loss, as well as hybridization and competition with non-native congeners that have been widely stocked throughout the region (Birdsong et al. 2010; Tringali et al. 2015). However, a current lack of population dynamics data at local scales hinders management and conservation progress (Birdsong et al. 2010; Taylor and Peterson 2014).

The Shoal Bass (*M. cataractae*) is a fluvial-specialist black bass species endemic to the Apalachicola-Chattahoochee-Flint Basin of Georgia, Alabama, and Florida (Williams and Burgess 1999). Shoal Bass typically inhabit medium-to-large rivers and streams that contain shoal habitats – areas characterized by high flow velocities and bedrock, boulder, and gravel substrates (Williams and Burgess 1999; Taylor and Peterson 2014). Shoal Bass spawning behavior is atypical of other black basses. Adults in the Flint River are potamodromous and undertake migrations of up to 200 km (Sammons 2015), forming large spawning aggregations in shoal complexes (Goclowski et al. 2013; Taylor and Peterson 2014). In the middle Chattahoochee River system, Shoal Bass have been documented moving into large shoal complexes of tributary systems, presumably for spawning (Sammons and Early 2015). Throughout their native range Shoal Bass appear to be suffering continual declines, primarily from habitat alteration and loss, and are considered vulnerable to extinction (Jelks et al. 2008; Taylor and Peterson 2014).

Upon describing the Shoal Bass, Williams and Burgess (1999) recommended that range-wide population assessments be conducted to inform future management and conservation efforts. Several studies have since quantified various population parameters in the upper and lower Flint River, Chipola River, and several tributaries to the middle Chattahoochee River. In these systems, Shoal Bass experience rapid growth (reaching 355 mm TL by ages 4 or 5), short lifespans (10-11 yrs max), and high annual mortality rates (40-60%; Sammons and Goclowski 2012; Ingram and Kilpatrick 2015; Woodside et al. 2015). Local abundance of Shoal Bass has been estimated at some locations, with point estimates ranging from 13-72 within shoals of Little Uchee Creek, a middle Chattahoochee River tributary (Stormer and Maceina 2008), to 103-993 in several reaches of a 29.8-km long section of the Chipola River (Woodside et al. 2015). Abundance of spawning adults aggregated in a 1.6-km long spawning shoal in the lower Flint River ranged from 87-181 (Taylor 2012). These studies help fill a previous lack of population dynamic data that has hindered population-specific management (Taylor and Peterson 2014; Sammons et al. 2015).

Despite these recent studies, relatively little is known about the populations inhabiting the northern extent of the species range within the upper Chattahoochee River Basin (UCRB). Extensive damming has led to range loss and local extirpation of Shoal Bass populations within the area, but Shoal Bass are currently known to occur in four general areas within the UCRB (Williams and Burgess 1999; Long and Martin 2008). Upstream of Lake Sidney Lanier, Shoal Bass occur in the mainstem Chattahoochee and Chestatee rivers. Downstream of Lake Lanier, coldwater releases have functionally eliminated Shoal Bass from a 77-km reach of the mainstem Chattahoochee River;

however, Shoal Bass are known to occur in Big Creek, a tributary to this reach (Long and Martin 2008). Dams severely limited gene flow among UCRB populations, and the Big Creek population may be vulnerable to inbreeding depression (Dakin et al. 2015). Near the southern extent of the UCRB, a Shoal Bass population also exists in the tailwater-influenced Chattahoochee River below Morgan Falls Dam. During recent restoration efforts below Morgan Falls Dam, researchers estimated 20% annual mortality (20-49% lower than seen in other studied populations) and a maximum age of 14 yrs (3-4 yrs older than seen in other studied populations; Porta and Long 2015). However, these parameters may not be indicative of other UCRB populations because artificially-depressed water temperatures in this reach could have influenced growth and longevity (Porta and Long 2015).

With increasing interest in Shoal Bass fisheries coinciding with a lack of information to inform management of UCRB populations, our goal was to characterize Shoal Bass population dynamics in Big Creek, as well as in the Chattahoochee and Chestatee rivers above Lake Lanier. Specifically, our objectives were to 1) model individual growth parameters over time; 2) estimate annual mortality; 3) investigate potential relationships between recruitment strength and environmental factors; 4) quantify local abundance; and 5) estimate apparent survival of age-0 and age-1 cohorts. Addressing these questions can inform future management and conservation efforts for UCRB populations, and provide a better understanding of the range-wide conservation status of Shoal Bass.

Methods

I. Study sites and sampling. – Big Creek is a fourth-order stream that has a total drainage area of approximately 250 km^2 within the rolling hills of the Piedmont ecoregion. Lower portions of Big Creek's watershed are situated within the Atlanta metropolitan area and feature increased development and impervious surfaces, contributing to increased sediment loads and flashiness of streamflow (Rose and Peters 2001; Long and Martin 2008). Shoal Bass are known to occur in a 2-km reach of Big Creek from its confluence with the Chattahoochee River upstream to Roswell Mill Dam, constructed in the 1830's (Long and Martin 2008). The lower 1-km is a slow-moving, channelized reach with silt deposits and large woody debris; however, the upper 1-km features a series of shallow shoal habitats (Graf and Plewa 2006). We sampled this upper 1-km (site #1; Table 1) with a team of 4-6 backpack electrofishers and approximately 10 netters spanning the stream's width (Fig. 1). Sampling proceeded methodically upstream to the base of Roswell Mill Dam, and effort (min) was recorded as the total on-time averaged across the number of backpack electrofishers deployed. Backpack electrofisher settings were adjusted to obtain an average output of approximately 0.40 amps. Sampling was performed in the following months, with two sampling days in close proximity in each month: May 2014 (20th and 22nd), October 2014 (17th and 18th), May 2015 (13th and 15th), and May 2016 (16th and 19th).

The Chattahoochee River begins in the mountainous Blue Ridge ecoregion, but the majority of the river's catchment is situated in the Piedmont ecoregion. As the Chattahoochee River reaches Lake Lanier, it is a fifth-order stream that drains approximately 970 km^2. The watershed is moderately forested, but poultry farming and housing developments have led to increased sediment and nutrient inputs (Zeng and Rasmussen 2005). At higher elevations, cool headwater streams and the mainstem river support seasonal trout fisheries, whereas Shoal Bass mainly occur in the lower portions of the river near Lake Lanier. Therefore, we established four 350-m long sampling sites spanning upstream from the river's interface with Lake Lanier (site #'s 2-5; Table 1). We used a jet-drive boat electrofisher to sample each site with pulsed, direct current for a total effort of 15 mins pedal time. While sampling each site, two upstream-to-downstream passes were made so that each side of the river was sampled sporadically from riverbank to mid-channel. Sites were sampled in May 2013 (5th), 2014 (27th and 29th), and 2015 (18th and 20th). Sampling in May 2016 was attempted but not completed because low discharge prevented access to sampling sites.

The Chestatee River's headwaters also originate in the mountainous Blue Ridge ecoregion, and as it proceeds through the Piedmont ecoregion, the river becomes a fourth-order stream that drains approximately 600 km^2. The Chestatee River is situated within a forested watershed with one of the lowest human population densities in the UCRB (Rose and Peters 2001). A gold rush in the 1830's and 1840's exposed the basin to heavy metal contaminants, like mercury, that are still present in sediment (Leigh 1997), but effects to Shoal Bass and other fishes are unknown. A Shoal Bass population is known from the lower reaches of the Chestatee River, so we established four sampling sites and sampled them as we did in the Chattahoochee River (site #'s 6-9; Table 1). Sampling was performed in May 2013 (29th), 2014 (19th and 21st), 2015 (12th, 14th, and 18th), and 2016 (17th and 18th).

Data collection was similar among the three study systems. Black bass of all species and sizes were collected and identified. For each Shoal Bass captured, the following data were obtained: total length (TL; mm), weight (g), and a sample of 3-5 scales from the dorsal region. All sampling events after May 2013 also incorporated a capture-mark-recapture (CMR) study design (described in *III. Data Analyses*), wherein we used passive integrated transponder (PIT) tags (Oregon RFID 8mm FDX-B glass tags in Big Creek; 12.5mm FDX-B plastic-encapsulated tags in Chattahoochee and Chestatee rivers) to provide individual identification over time. Upon capture, each fish was scanned for a PIT tag with a handheld reader (Agrident APR350), and if not already tagged and ≥ 70 mm TL, a tag was injected into the coelomic cavity immediately posterior to the pectoral fin. Similar tagging methods typically yield 80-100% post-tagging survival and 95-100% tag retention (Siepker et al. 2012; Clark 2016). Fish were released near original capture locations following data collection.

II. Age estimation. – Because the populations of interest were of conservation concern, we estimated age (in years) from non-lethal scale samples using a consensus-based method (Long et al. *in review*). Briefly, two readers independently estimated annuli count from a scale sample without consideration of capture date or fish size. If the two readers' final annuli counts matched, this was adopted as the consensus count; if not, an independent concert read provided a consensus count. Because season-at-capture can influence age estimation (i.e., annulus formation during spring months), a final consensus estimation of age was made with consideration of consensus annuli counts and capture date. The reliability of this method was assessed by 1) examining precision of age estimates; 2) using mark-recapture data to verify timing of first annulus formation and to verify annulus periodicity across all ages by comparing age estimates of recaptured fish to known times-at-large, which together can provide *in situ* validation (Campana 2001); and 3) comparing age-independent von Bertalanffy growth models to models built with estimated ages to assess reliability of age estimates to inform management (Long et al. *in review*). Briefly, the results of this unpublished study showed that precision (coefficient of variation; CV) was low (mean = 5.4%) along with accuracy (57% agreement between estimated age and expected increment formation with time at large). But, age estimates were unbiased (approximately equal amounts of over- and under-estimates of age), and differences among mean estimated length-at-age from von Bertalanffy growth models varied minimally (mean CV = 4.9%) including models that were age-independent (i.e., mark-recapture measurements of length over time). Overall, these results suggested that age estimates were reliable and useful for informing management (Long et al. *in review*).

III. Data analyses. – Fish that lacked an age estimate (i.e., regenerated scales or missing samples) were proportionally assigned an age using age-length keys constructed in the FSA package for Program R (Isermann and Knight 2005; Ogle 2015). Age-length keys were built with 25-mm TL bins, and fish were assigned an age based on keys built for each system and sampling month combination to avoid potential bias introduced by variation in growth among systems or sampling seasons (Ogle 2015). To visualize differences in catch among systems (May events only, for consistency), we plotted length-frequency with the following age categories superimposed: young age classes (ages 1 and 2), non-harvestable size adults (< 355 mm TL; ages 3-6), and harvestable size adults (≥ 355 mm TL; ages 7+).

i. Growth. – To model Shoal Bass growth in the UCRB, we used the von Bertalanffy growth function:

$$E|L|t = L_\infty \left(1 - e^{-K(t - t_0)}\right),$$

where $E|L|t$ is mean length at age t. Parameters estimated by the function include the maximum mean length (L_∞), a growth parameter (K) that measures how quickly the function approaches L_∞, and the theoretical age in years for a length of zero (t_0). We obtained parameter starting values for each system (Ford 1933; Walford 1946) and then fit nonlinear regressions for the growth function. To discern any differences in growth functions among systems, we fit eight von Bertalanffy models that allowed different combinations of model parameters to be shared among all systems or to vary by system (Ogle 2015). We performed model-ranking with Akaike's information criterion (AIC; Akaike 1973) with small-sample bias adjustment (AIC_c; Hurvich and Tsai 1989) to determine the model with the best fit to the data (lowest AIC_c score), and Akaike weights (w) quantified the relative strength of evidence for each model (Burnham and Anderson 2002). The most plausible model was used to infer differences in growth parameters among populations, and estimates of mean-length-at-age and non-parametric bootstrapped 95% confidence intervals were retrieved. We built, fit, and ranked growth function models using the FSA and 'nlstools' packages in Program R (Ogle 2015).

ii. Mortality. – We estimated annual mortality in each system using raw catch data and estimated ages. We chose this approach because our mark-recapture dataset was not sufficient to estimate age-specific survival, with few exceptions (see *v. Survival of young cohorts*). For consistency among systems, we used only May sampling events.

We performed linear regression of catch-curves using the FSA package in Program R (Ogle 2015), wherein we used weighted regression to reduce the influence of older, under-represented age classes (Maceina and Bettoli 1998). We pooled catch data by age (i.e., not year-class) in each system across sample years to dampen the potential effects of recruitment variation (Ogle 2015). We only included ages that recruited to the gear in each system by excluding ages in the ascending limbs of the catch-curves (Ogle 2015).

iii. Recruitment variation. – We quantified recruitment variation with a distinct set of weighted catch-curve regressions, wherein the residuals provided an index of year-class strength (Maceina 1997; Maceina and Bettoli 1998). We used raw catch data from May samples and, again, only included ages recruited to the gear used in each system. Regressions were performed for each system and sampling year combination, keeping track of the year-class represented by each age. In this manner, a given year-class in a system could be represented by multiple residuals derived from different sampling years. We used raw residuals for modeling environmental relationships, but used Studentized residuals to facilitate interpretation of strong (≥ 2 SD) and weak (≤ -2 SD) year-classes (Maceina 1997).

We calculated environmental variables for four biologically-relevant seasons in each year: "spring" spawning and hatching period (April-June; Taylor and Peterson 2014) ; "summer" post-hatch period (July-September; Sammons and Goclowski 2012); "fall" growth period (October-December; Woodside et al. 2015); and "over-winter" survival (January-March; Suski and Ridgway 2009). For comparability, we followed Woodside et al. (2015) in calculating seasonal hydrology variables based on mean daily discharge (m^3/s): minimum, median, average, SD, and number of days above seasonal average. Mean daily discharge values were obtained from USGS stream gauge stations immediately upstream or downstream of sampling sites that provided data through the timespan of year-classes represented in our catch (02335700 [Big Creek], 02331600 [Chattahoochee River], 0233500 [Chestatee River]). To characterize temperature conditions favorable to growth, we also included seasonal cumulative growing degree-days and average growing degree-days. We calculated growing degree-days with a base temperature of 0 ºC (Schlosser et al. 2000; Chezik et al. 2013), using daily high and low air temperature records from the NOAA National Centers for Environmental Information (USW00053863 [Big Creek]; USC00093621 [Chattahoochee and Chestatee rivers]).

We investigated possible relationships between recruitment strength and environmental factors in each system using linear models built with a limited set of variables. Continuous variables were natural-log transformed and degree-day counts were transformed by the natural log of (x+1) to meet normality assumptions prior to modeling. Pearson correlation coefficients among variables commonly exceeded $r = |0.7|$; therefore, we conducted a multivariate principal components analysis (PCA) with the transformed datasets to identify redundant linear trends among variables in each system. We conducted PCA analyses in PC-ORD v. 6 (McCune and Mefford 2011) to reduce the number of variables to be modeled. We retained the highest-loading variable to each axis (i.e., highest correlated to each axis) where the number of axes considered explained at least 10% of the variation in the dataset. Because of sample size limitations and the exploratory nature of this modeling exercise, we opted to construct univariate linear models only. We related recruitment strength (residuals) to environmental variables using linear models constructed with the ‘lm’ command in Program R at a significance level of $p \leq 0.05$. We evaluated the assumption of homoscedasticity with residual plots and the assumption that residuals are normally distributed with Q-Q plots.

iv. Abundance. – We analyzed CMR data from all sampling occasions after May 2013 using Huggins' closed-population models (Huggins 1989) in Program MARK v. 6.0 (White and Burnham 1999). Huggins' models estimate abundance (N) as a derived parameter, along with 95% confidence intervals (CI's), based on capture (p) and recapture probabilities (c) from CMR histories. We considered sampling events held within the same month to represent closed periods during which N was estimated. Sampling events within each month were usually conducted 1-2 days apart; however, equipment malfunction caused a 6-day interval between sampling events for two Chestatee River sites (#'s 8 and 9) in 2015. We parameterized models to allow p to vary over time because of heterogeneity in stream conditions and sampling crews during the closed periods.

v. Survival of young cohorts. – We used open-population CMR analysis to estimate survival (ϕ) of the 2014 year-class of Shoal Bass in Big Creek. In Program MARK (White and Burnham 1999), we used the Cormack-Jolly-Seber (CJS) model with a parameterization that allowed ϕ to vary over time and we held p constant (because of data constraints). The 2014 year-class was first encountered as age-0 fish in Fall 2014, followed by age-1 fish in Spring 2015 and age-2 fish in Spring 2016. We only included capture histories of fish encountered in one of these seasons with the corresponding correct age so as to estimate ϕ for the 2014 year-class only. Capture histories associated with individual sampling events within each season/year combination were pooled for analysis, and we manually adjusted model time intervals to correspond to the elapsed time between seasons (approximately a half-year interval followed by a full-year interval). The interval between the first two seasons provided an opportunity to quantify over-winter ϕ of young-of-year Shoal Bass, a potential pinch-point in the recruitment of black basses to age-1 (Fullerton et al. 2000; Curry et al. 2005). The interval between the last two seasons was used to estimate ϕ of fish from age-1 to age-2, which may also be less than survival of older age classes whose survival was estimated with catch-curve regressions.

Results

We caught 1,028 Shoal Bass during May sampling events, with 549 (53%) from Big Creek, 185 (18%) from the Chattahoochee River, and 294 (29%) from the Chestatee River. An additional 161 Shoal Bass from Big Creek were captured in October 2014. We estimated age for 92% of scale samples and assigned ages with age-length keys to 27 samples in Big Creek, 27 in the Chattahoochee River, and 31 in the Chestatee River. Ages ranged from 1-12 yrs in all three systems, and age-0 fish were only encountered in Big Creek in October 2014. Differences in raw catch were evident among systems, with more younger and shorter fish captured in Big Creek than in either river system (Fig. 2). Harvestable-size fish (≥ 355 mm TL) comprised 10% of Big Creek's catch compared to 18% in both river systems. Maximum TL observed was 478 mm in Big Creek, 516 mm in the Chattahoochee River, and 487 mm in the Chestatee River.

i. Growth. – von Bertalanffy growth functions were built using 388 samples from Big Creek, 185 samples from the Chattahoochee River, and 294 samples from the

Chestatee River. Model selection supported the most complex model that allowed all three parameters to differ among systems, and this model had high relative strength of evidence compared to other candidate models ($w = 0.53$; Table 2). Although 95% confidence intervals overlapped for all parameters in all three systems, the Chattahoochee River had a higher estimate of L_∞ and lower estimates of K and t_0, as well as wider confidence intervals around parameter estimates (Table 3). Despite these differences, the growth functions for each system produced similar mean-length-at-age estimates, with divergence in estimates occurring mostly at younger (0 and 1 yrs) and older (11 and 12 yrs) ages (Table 4; Fig. 3).

ii. Mortality. – Shoal Bass did not fully recruit to boat-electrofishing gear used in the Chattahoochee and Chestatee rivers until age-3, but recruited to backpack-electrofishing gear in Big Creek at age-0. Because catch-curve regressions assume mortality rates are equal across all ages included, we excluded younger age classes recruited to the gear in Big Creek because they may experience disproportionately higher mortality rates than older age classes (see Results: *v. Survival of young cohorts*). In this manner, results were more comparable across systems because mortality was estimated across same range of ages. Catch-curve estimates of annual mortality for ages 3-12 were similar among systems: 18.4% (95% CI's: 7.8-27.8%; $R^2 = 0.65$) in Big Creek, 20.8% (95% CI's: 13.2-27.7%; $R^2 = 0.81$) in the Chattahoochee River, and 23.7% (95% CI's: 13.8-32.4%; $R^2 = 0.80$) in the Chestatee River (Fig. 4).

iii. Recruitment variation. – We used ages 3-12 to perform weighted catch-curve regressions to investigate recruitment variation in each system. Studentized residuals indicated strong year-classes in 2006 and 2007 in Big Creek and in 2006 in the

Chattahoochee River, whereas weak year-classes were evident in 2004, 2009, and 2013 in Big Creek, in 2003, 2004, and 2011 in the Chattahoochee River, and in 2007 and 2008 in the Chestatee River (Fig. 5). Principal components analysis identified three axes that explained at least 10% of the variation in each system, wherein the majority of variation among environmental variables (PC-axis 1) in Big Creek was driven by summer SD of discharge (49%), compared to fall minimum discharge in the Chattahoochee (50%) and Chestatee (50%) rivers (Table 5). Two significant linear models were obtained in Big Creek, one suggesting a negative relationship between recruitment strength and summer SD of discharge ($p < 0.01$; $R^2 = 0.38$) and another indicating a positive relationship between recruitment strength and fall cumulative growing degree-days ($p = 0.04$; $R^2 = 0.15$; Table 6). We obtained a single significant model in the Chattahoochee River that suggested a positive relationship between recruitment strength and winter SD of discharge ($p = 0.04$; $R^2 = 0.16$). No significant models were obtained in the Chestatee River, likely because recruitment variation was not as pronounced as in the other systems. No violations to linear model assumptions of homoscedasticity or normality of residuals were evident among the models examined.

iv. Abundance. – Numbers of marked fish were highest in Big Creek and in Chestatee River site #9, which also had some of the highest recapture rates (Table 7; CMR capture histories are provided in Appendix 1). Mean TL of captures was usually lower than mean TL of recaptured fish because tagged fish grew between initial capture and subsequent recaptures across primary periods. We did not document movement of tagged fish among sample sites in the Chestatee River, and only one fish moved between sites in the Chattahoochee River (from site #2 to #3, ~2.4 km upstream); thus, we treated sites as independent locations instead of pooled, system-wide estimates in both rivers. We were unable to estimate *N* at a subset of sites in the Chestatee River because of a lack of recaptured fish, including site # 6 (2014, 2015, and 2016), # 7 (2014 only), and # 8 (2014 only; Table 7). Point estimates of *N* varied from 10-154 at sites in the Chattahoochee and Chestatee rivers, and low proportions of recaptured fish at some sites resulted in relatively wide confidence intervals. Point estimates of *N* in Big Creek (site # 1) ranged from 219-348, and overlapping 95% CI's indicated that estimates were similar across all sampling seasons. Abundance in Big Creek was largely driven by young fish, as 78% (316 of 408) of unique PIT-tagged individuals had max age estimates between 0-2 yrs, compared to only 9% (36 of 408) having max ages of $\geq$ 7 yrs.

v. Survival of young cohorts. – The age-0 cohort spawned in 2014 was estimated to have an over-winter survival of ϕ = 8.0% (95% CI's: 1.3-35.4%) between Fall 2014 and Spring 2015. The same cohort was estimated to have an annual survival rate of ϕ = 33.3% (95% CI's: 11.9%-64.8%) between age-1 (May 2015) and age-2 (May 2016).

Discussion

The results of this study provide baseline life history and population dynamics data that can be used to tailor management, prioritize conservation, and guide future monitoring efforts. Compared to other studied populations, Shoal Bass in the UCRB have pronounced differences in growth, longevity, and annual mortality that may warrant population-specific management strategies. Over-winter survival appears to be an important pinch-point for recruitment in UCRB populations, which may help explain observed life-history characteristics. Of the three populations investigated, the isolated population in Big Creek appears to be of greatest conservation concern because of variable recruitment and low adult abundance.

The life history differences discovered in UCRB Shoal Bass populations underscores the need for population-specific management strategies. Because fishes are ectothermic, temperature-related differences in life histories are common across latitude and elevation gradients (Coutant 1976; Conover 1992; Kennedy et al. 2003). In the UCRB, Shoal Bass grew slower (reaching 355 mm TL at age 7, compared to ages 4 or 5 elsewhere; Sammons and Goclowski 2012; Woodside et al. 2015) and attained shorter maximum lengths (516 mm TL in the Chattahoochee River compared to 561 mm TL in lower Flint River; Ingram and Kilpatrick 2015) than in populations situated in southern latitudes and lower elevations. We estimated ages up to 12 yrs in all three UCRB systems compared to rare estimates of 10-11 yrs in the Flint and Chipola rivers (Sammons and Goclowski 2012; Ingram and Kilpatrick 2015; Woodside et al. 2015), suggesting Shoal Bass also live longer in UCRB populations. Coinciding with these differences, UCRB populations also experienced noticeably lower annual mortality (18-24%) compared to similarly-derived estimates of 40-69% in the Flint and Chipola rivers (Sammons and Goclowski 2012; Ingram and Kilpatrick 2015; Woodside et al. 2015). These life history differences may warrant population-specific management strategies. For example, Shoal Bass in the UCRB must live approximately 7 years to reach harvestable size (≥ 355 mm TL) whereas fish in the lower latitude portions of their range reach this size in 4-5 years, which would differentially affect population responses to exploitation. Because fishing pressure for Shoal Bass in the UCRB is unknown, creel surveys to assess angler effort and harvest are warranted, along with an evaluation of how harvest-limit adjustments could influence these populations.

Over-winter survival could be important for recruitment in UCRB populations. The low over-winter survival of the 2014 year-class in Big Creek was similar to other black bass species at northern latitudes where winters are more severe (Fullerton et al. 2000; Curry et al. 2005), suggesting a strong forcing mechanism present in this year-class at least. Winter mortality has been documented to be important for recruitment in other black bass species, with growth-dependent effects evident (Oliver et al. 1979; Miranda and Hubbard 1994). Thus, conditions favorable to faster growth prior to the over-wintering period could be favorable for UCRB Shoal Bass recruitment (Conover 1992). In our catch-curve residual analysis, recruitment in Big Creek was positively related to cumulative growing degree-days in fall, a presumed growing period for age-0 Shoal Bass. Furthermore, recruitment in the Chattahoochee River was positively related to winter SD of discharge, which may also indicate an influence of winter temperature. Discharge in the upper Chattahoochee River during winter is generally a function of the number and magnitude of rain events, which temporarily elevate water temperatures (data available from USGS gauge 02330450). Winters with above-average water temperatures, or even brief periods of elevated water temperatures, may confer improved over-winter survival of Shoal Bass in the UCRB by temporarily relieving metabolic demands on stored energy reserves and perhaps allowing foraging activity (*see* Fullerton et al. 2000). The slower growth and increased longevity we documented in UCRB populations may reflect an adaptation to poor recruitment driven by marginal temperature conditions, a phenomenon documented in other freshwater fishes (Conover 1992; Kennedy et al. 2003).

Inconsistency in recruitment variability among systems suggests that watershed-specific factors are influential. Two previous studies provide contrasting conclusions about Shoal Bass recruitment, similar to our results. Low variation in recruitment in the upper Flint River suggests that the environment is either stable or that the environment exerts little influence (Sammons and Goclowski 2012), but variable recruitment in the Chipola River has been associated with poor year-classes related to high spring and summer discharge (Woodside et al. 2015). In our study, recruitment variation coincided with the degree of landcover alteration in each watershed – from little variation in the relatively-forested Chestatee River to high variation in the urbanized Big Creek. Negative relationships between recruitment and increased variation in summer discharge in Big Creek are likely linked to increased sedimentation and flashiness associated with watershed urbanization. Sedimentation can deteriorate spawning substrate quality (Kemp et al. 2011), and larval black bass are vulnerable to downstream displacement during high discharge events (Harvey 1987). High flow events and increased variation in discharge during spawning season have been shown to negatively influence recruitment in fluvial populations of Smallmouth Bass (Lukas and Orth 1995; Smith et al. 2005), and Largemouth Bass and Suwanee Bass (Bonvechio and Allen 2005). To date, the effect of watershed land use on Shoal Bass populations is anecdotal, but has been implicated in Shoal Bass population declines in several tributaries to the middle Chattahoochee River (Stormer and Maceina 2008). Additional studies are warranted to identify the specific pathways through which landcover characteristics influence Shoal Bass recruitment and population persistence.

Recruitment variability in Big Creek, coupled with the low number of older adults, heightens conservation concern for this isolated population (Dakin et al. 2015). In Little Uchee Creek, a similar-sized tributary of the middle Chattahoochee River, adult abundance estimates were similar to those in Big Creek prior to an apparent functional extirpation of Shoal Bass in Little Uchee Creek (Stormer and Maceina 2008). Variable recruitment, low adult abundance, and the isolated nature of Big Creek raises concerns that the population could be vulnerable to local extirpation caused by several years of poor environmental conditions, demographic stochasticity, or sudden anthropogenic disturbance. In addition to these population-dynamic concerns, the genetic integrity of the Shoal Bass population in Big Creek is threatened by introgressive hybridization with non-native congener species like Smallmouth Bass (*M. dolomieu*; Dakin et al. 2015) and Alabama Bass (*M. henshalli*; A. Taylor, unpublished data). Supplemental stocking may help bolster the Big Creek population during years of poor recruitment and alleviate genetic concerns like inbreeding depression and introgression of non-native alleles (Dakin et al. 2015; Porta and Long 2015). If stocking is implemented, brood stock source is an important consideration because life history differences similar to those documented in UCRB populations have a genetic basis in other fishes (Schultz et al. 1998; Conover et al. 2009). Stocking fish from southern latitudes may increase mortality and degrade any potential local adaptations through hybridization, as documented in Largemouth Bass (*M. salmoides*; Fullerton et al. 2000).

Our efforts to estimate local abundance provided additional population status insights that can be used to inform future population monitoring efforts. Sites with appreciable uncertainty around *N* estimates had low capture and recapture rates.

Managers interested in developing long-term monitoring protocols for Shoal Bass may wish to weigh potential tradeoffs in the effort required to collect data and the degree of accuracy and precision needed with abundance estimates to inform management. If CMR approaches are employed to monitor Shoal Bass abundance in the future, additional sampling events and increased sampling effort within each event would allow for more realistic model parameterizations and provide increased precision. CMR studies are effort-intensive and may not always be feasible, but resource managers interested in long-term monitoring of population trends may benefit from understanding how *p* varies across individual (e.g., length) and environmental (e.g., substrate, flow velocity, depth, and water temperature) factors (*for example,* Price and Peterson 2010; Mollenhauer and Brewer 2017). A less-intensive monitoring option would be to use catch-per-unit effort (CPUE) as an index of abundance, but this index may be misleading when the assumption of constant *p* is violated (Hilborn and Walters 1992; Gwinn et al. 2011). If variation in *p* was quantified across a range of sampling conditions, CPUE could be adjusted to provide a more-reliable index of abundance (Hubert and Fabrizio 2007).

The results of our study point to additional sampling-related issues that should be addressed in the future. First, improved understanding of Shoal Bass movement in the UCRB would not only provide additional insight into the ecology of these northern populations, but could also be used to refine CMR study design (e.g., sampling area size and closure assumptions; Gwinn et al. 2011). We documented only one individual moving between sample sites in either river system, so Shoal Bass in the UCRB may be more sedentary than in other portions of their range (e.g., long-distance migration to spawning sites in the Flint River; Sammons 2015), or adults may have high spawning-site fidelity to particular shoals. Second, sampling in riverine shoal habitats presented a challenge to study design. On several occasions, access to sampling sites with the jet-drive boat electrofisher was impeded because of low water levels. Innovation of sampling gears and strategies for sampling shoal habitats of large rivers would make long-term monitoring efforts more practical.

Several of the results of this study, including growth models, mortality estimates, and recruitment variation, are influenced by our age estimates. Scale-based age estimates are typically less precise than those based on otoliths and may be plagued by annuli resorbing or annuli crowding in older fish, highlighting the importance of validating annuli formation (Quist et al. 2012). The scale-based age estimates used in this study were considered to be reliable at the population-level of management (i.e., similar growth model results with age-independent estimates) and without systematic bias (Long et al. *in review*). Similar conclusions were reached with known-age Smallmouth Bass, wherein scale-based age estimates were 71% accurate but produced growth curves that corresponded well to measured mean total lengths (Heidinger and Clodfelter 1987). These results demonstrate that individual-level aging error does not necessarily constrain population-level inferences. Moreover, although we found no systematic bias in our age estimates, if annuli resorbing or crowding resulted in any undetected underestimation in age, our estimates of longevity and annual mortality would only become more differentiated from other populations (i.e., even greater increased longevity and even lower annual mortality). Unbiased inaccuracies, such as ours, produce a smoothing effect across age groups, leading to reduced magnitude of strong year classes (Campana 2001);

therefore, the potential relationships we identified between recruitment strength and environmental factors should be maintained or become stronger in absence of aging error.

As biologists begin to weigh management and conservation options (e.g., stocking) and researchers continue to gain improved understanding of the distribution and population genetics of Shoal Bass, our results underscore the need for population-specific management. Shoal Bass inhabiting the UCRB have marked life-history and population-dynamic differences compared to southerly populations, including slower growth, increased longevity, lower annual mortality, and low over-winter survival. The Big Creek population appears to be of greatest conservation concern among the three UCRB populations examined in this study. Considering the fragmented nature of UCRB populations (Dakin et al. 2015), establishment of population monitoring protocols in all three study populations is justifiable.

References

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281 *in* B. N. Petrov and F. Csaki, editors. Second international symposium on information theory. Akademiai Kiado, Budapest, Hungary.

Allan, J. D., and A. S. Flecker. 1993. Biodiversity conservation in running waters. BioScience 43(1):32–43.

Benz, G. W., and D. E. Collins, editors. 1996. Aquatic fauna in peril: the southeastern perspective. Southeast Aquatic Research Institute, Decatur, AL.

Birdsong, T., D. Krause, J. Leitner, J. M. Long, S. Robinson, and S. Sammons. 2010. A business plan for the conservation of native black bass species in the southeastern U.S. National Fish and Wildlife Foundation, Washington, D.C.

Bonvechio, T. F., and M. S. Allen. 2005. Relations between hydrological variables and year-class strength of sportfish in eight Florida waterbodies. Hydrobiologia 532:193–207.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer, New York, NY.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59:197–242.

Chezik, K. A., N. P. Lester, and P. A. Venturelli. 2013. Fish growth and degree-days I: selecting a base temperature for a within-population study. Canadian Journal of Fisheries and Aquatic Sciences 71(1):47–55.

Clark, S. R. 2016. Effects of passive integrated transponder tags on the physiology and swimming performance of a small-bodied stream fish. Transactions of the American Fisheries Society 145(6):1179–1192.

Conover, D. O. 1992. Seasonality and the scheduling of life history at different latitudes. Journal of Fish Biology 41:161–178.

Conover, D. O., T. A. Duffy, and L. A. Hice. 2009. The covariance between genetic and environmental influences across ecological gradients. Annals of the New York Academy of Sciences 1168(1):100–129.

Coutant, C. C. 1976. Thermal effects on fish ecology. Pages 891–896 *in* Encyclopedia of environmental science and engineering, volume 2. Gordon and Breach Science Publishers, New York.

Curry, R. A., S. L. Currie, S. K. Arndt, and A. T. Bielak. 2005. Winter survival of age-0 Smallmouth Bass, *Micropterus dolomieu*, in north eastern lakes. Environmental Biology of Fishes 72(2):111–122.

Dakin, E. E., B. A. Porter, B. J. Freeman, and J. M. Long. 2015. Hybridization threatens Shoal Bass populations in the upper Chattahoochee River Basin. Pages 491–501 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Ford, E. 1933. An account of the herring investigations conducted at Plymouth during the years from 1924 to 1933. Journal of the Marine Biological Association of the United Kingdom 19(1):305–384.

Fullerton, A. H., J. E. Garvey, R. A. Wright, and R. A. Stein. 2000. Overwinter growth and survival of Largemouth Bass: interactions among size, food, origin, and winter severity. Transactions of the American Fisheries Society 129(1):1–12.

Goclowski, M. R., A. J. Kaeser, and S. M. Sammons. 2013. Movement and habitat differentiation among adult Shoal Bass, Largemouth Bass, and Spotted Bass in the upper Flint River, Georgia. North American Journal of Fisheries Management 33(1):56–70.

Graf, W. L., and T. M. Plewa. 2006. Channel change in Big Creek, Chattahoochee River National Recreation Area. National Park Service, South Atlantic Cooperative Ecosystems Studies Unit, Requisition Reference No. R5028050506.

Gwinn, D. C., P. Brown, J. C. Tetzlaff, and M. S. Allen. 2011. Evaluating mark–recapture sampling designs for fish in an open riverine system. Marine and Freshwater Research 62(7):835–840.

Harvey, B. C. 1987. Susceptibility of young-of-the-year fishes to downstream displacement by flooding. Transactions of the American Fisheries Society 116(6):851–855.

Heidinger, R. C., and K. Clodfelter. 1987. Validity of the otolith for determining age and growth of Walleye, Striped Bass, and Smallmouth Bass in power plant cooling ponds. Pages 241–251 *in* R. C. Summerfelt and G. E. Hall, editors. The age and growth of fish. The Iowa State University Press, Ames.

Hilborn, R., and C. J. Walters, editors. 1992. Quantitative fisheries stock assessment - choice, dynamics and uncertainty. Chapman and Hall, New York.

Hubert, W. A., and M. C. Fabrizio. 2007. Relative abundance and catch per unit effort. Pages 279–325 *in* C. S. Guy and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, Maryland.

Huggins, R. M. 1989. On the statistical analysis of capture-recapture experiments. Biometrika 76:133–140.

Hurvich, C. M., and C. L. Tsai. 1989. Regression and time series model selection in small samples. Biometrika 76:297–307.

Ingram, T. R., and J. M. Kilpatrick. 2015. Assessment of the Shoal Bass population in the lower Flint River, Georgia. Pages 157–168 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Isermann, D. A., and C. T. Knight. 2005. A computer program for age–length keys incorporating age assignment to individual fish. North American Journal of Fisheries Management 25(3):1153–1160.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. W. Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33(8):372–407.

Kemp, P., D. Sear, A. Collins, P. Naden, and I. Jones. 2011. The impacts of fine sediment on riverine fish. Hydrological Processes 25(11):1800–1821.

Kennedy, B. M., D. P. Peterson, and K. D. Fausch. 2003. Different life histories of Brook Trout populations invading mid-elevation and high-elevation Cutthroat Trout streams in Colorado. Western North American Naturalist 63(2):215–223.

Leigh, D. S. 1997. Mercury-tainted overbank sediment from past gold mining in north Georgia, USA. Environmental Geology 30(3–4):244–251.

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

Long, J. M., C. T. Holley, and A. T. Taylor. *In review*. Evaluating reliability of scale-based age estimates to inform management of vulnerable populations of Shoal Bass *Micropterus cataractae*. Journal of Fish Biology.

Long, J. M., and C. Martin. 2008. The evolution of fisheries management of the upper Chattahoochee River in response to changing water management. Pages 21–36 *in* M. S. Allen, S. M. Sammons, and M. J. Maceina, editors. Balancing fisheries management and water uses for impounded river systems. American Fisheries Society, Symposium 62, Bethesda, Maryland.

Lukas, J. A., and D. J. Orth. 1995. Factors affecting nesting success of Smallmouth Bass in a regulated Virginia stream. Transactions of the American Fisheries Society 124:726–735.

Maceina, M. J. 1997. Simple application of using residuals from catch-curve regressions to assess year-class strength in fish. Fisheries Research 32(2):115–121.

Maceina, M. J., and P. W. Bettoli. 1998. Variation in Largemouth Bass recruitment in four mainstream impoundments of the Tennessee River. North American Journal of Fisheries Management 18(4):998–1003.

McCune, B., and M. J. Mefford. 2011. PC-ORD. Multivariate analysis of ecological data. Version 6. MjM Software, Geleneden Beach, Oregon.

Miranda, L. E., and W. D. Hubbard. 1994. Length-dependent winter survival and lipid composition of age-0 Largemouth Bass in Bay Springs Reservoir, Mississippi. Transactions of the American Fisheries Society 123(1):80–87.

Mollenhauer, R., and S. K. Brewer. 2017. Multinomial N-mixture models improve the applicability of electrofishing for developing population estimates of stream-dwelling Smallmouth Bass. North American Journal of Fisheries Management 37(1):211–224.

Ogle, D. H. 2015. Introductory fisheries analyses with R. Chapman and Hall.

Oliver, J. D., G. F. Holeton, and K. E. Chua. 1979. Overwinter mortality of fingerling Smallmouth Bass in relation to size, relative energy stores, and environmental temperature. Transactions of the American Fisheries Society 108(2):130–136.

Porta, M. J., and J. M. Long. 2015. Evaluation of a five-year Shoal Bass conservation-stocking program in the upper Chattahoochee River, Georgia. Pages 169–180 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Price, A. L., and J. T. Peterson. 2010. Estimation and modeling of electrofishing capture efficiency for fishes in wadeable warmwater streams. North American Journal of Fisheries Management 30(2):481–498.

Quist, M. C., M. A. Pegg, and D. R. DeVries. 2012. Age and growth. Pages 677–731 *in* A. V. Zale, D. L. Parrish, and T. M. Sutton, editors. Fisheries techniques, third edition. American Fisheries Society, Bethesda, Maryland.

Rose, S., and N. E. Peters. 2001. Effects of urbanization on streamflow in the Atlanta area (Georgia, USA): a comparative hydrological approach. Hydrological Processes 15(8):1441–1457.

Sammons, S. M. 2015. First evidence of potadromy and partial migration in black basses: Shoal Bass *Micropterus cataractae* (Actinopterygii, Centrarchidae) in the Upper Flint River, USA. Hydrobiologia 751(1):135–146.

Sammons, S. M., and L. A. Early. 2015. Movement and habitat use of Shoal Bass in a regulated portion of the Chattahoochee River, Alabama-Georgia, USA. Pages 249–261 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Sammons, S. M., and M. R. Goclowski. 2012. Relations between Shoal Bass and sympatric congeneric black bass species in Georgia rivers with emphasis on movement patterns, habitat use, and recruitment. Georgia Department of Natural Resources, Wildlife Resources Division, Final Report, Social Circle, GA.

Sammons, S. M., K. L. Woodside, and C. J. Paxton. 2015. Shoal Bass *Micropterus cataractae* Williams & Burgess, 1999. Pages 75–81 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Schlosser, I. J., J. D. Johnson, W. L. Knotek, and M. Lapinska. 2000. Climate variability and size-structured interactions among juvenile fish along a lake–stream gradient. Ecology 81(4):1046–1057.

Schultz, E. T., D. O. Conover, and A. Ehtisham. 1998. The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. Canadian Journal of Fisheries and Aquatic Sciences 55(5):1149–1157.

Siepker, M. J., D. S. Knuth, E. L. Ball, and J. B. Koppelman. 2012. Evaluating a novel passive integrated transponder tag in Largemouth Bass. North American Journal of Fisheries Management 32(3):528–532.

Smith, S. M., J. S. Odenkirk, and S. J. Reeser. 2005. Smallmouth Bass recruitment variability and its relation to stream discharge in three Virginia rivers. North American Journal of Fisheries Management 25: 1112–1121.

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

Suski, C. D., and M. S. Ridgway. 2009. Winter biology of centrarchid fisheries. Page *in* S. Cooke and D. P. Philipp, editors. Centrarchid fishes: diversity, biology and conservation. Blackwell Publishing, West Susex, UK.

Taylor, A. T. 2012. Status assessment of a Shoal Bass population in the lower Flint River, Georgia. MS Thesis, University of Georgia, Athens, GA.

Taylor, A. T., and D. L. Peterson. 2014. Shoal bass life history and threats: a synthesis of current knowledge of a *Micropterus* species. Reviews in Fish Biology and Fisheries 24(1):159–167.

Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. 2015. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Walford, L. A. 1946. A new graphic method of describing the growth of animals. Biological Bulletin 90(2):141–147.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(sup1):S120–S139.

Williams, J. D., and G. H. Burgess. 1999. A new species of bass, *Micropterus cataractae* (Teleostei: Centrarchidae), from the Apalachicola River basin in Alabama, Florida, and Georgia. University of Florida.

Woodside, K. L., C. J. Paxton, and N. C. Kierl. 2015. Stock assessment of Shoal Bass in the Chipola River, Florida. Pages 139–155 *in* M. D. Tringali, J. M. Long, T. W. Birdsong, and M. S. Allen, editors. Black bass diversity: multidisciplinary science for conservation. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Zeng, X., and T. C. Rasmussen. 2005. Multivariate statistical characterization of water quality in Lake Lanier, Georgia, USA. Journal of Environmental Quality 34(6):1980–1991.

Table 1. Descriptions of Shoal Bass (*M. cataractae*) sample site locations in the upper Chattahoochee River Basin, Georgia, including site name, coordinates (decimal degrees), elevation (m), their distance in river-kilometers (rkm) upstream from Lake Sidney Lanier, length of stream sampled (m), and general habitat descriptions.

Site #	site name	latitude	longitude	elevation (m)	rkm from lake	length (m)	general habitat description
				Big Creek			
1	Big Creek	34.012126	-84.360198	265	NA	1000	shoal habitat with a few pools interspersed
				Chattahoochee River			
2	Flat Rock	34.466399	-83.686461	330	6.4	350	deep pool and run with bedrock substrate
3	Bull Shoals	34.482440	-83.680216	330	8.8	350	shallow bedrock shoals and cobble riffle
4	Crow Island	34.503651	-83.666475	334	11.7	350	shallow pool and run with boulder substrate
5	Buck Shoals	34.563347	-83.628713	362	24	350	deep run with boulders between shoals
				Chestatee River			
6	Big Rock	34.458609	-83.966767	328	3	350	deep pool with bedrock and sand substrate
7	Canoe Launch	34.471844	-83.979555	330	5.8	350	shallow series of bedrock shoals
8	Horseshoe Bend	34.492659	-83.997084	333	9.3	350	shallow riffle with gravel substrates
9	Hwy. 60	34.504223	-83.968851	338	14.6	350	shallow bedrock shoal and gravel run

Table 2. Model selection results comparing von Bertalanffy growth functions describing mean-length-at-age of Shoal Bass among three upper Chattahoochee River populations: Big Creek, Chattahoochee River, and Chestatee River. Parameters of the function are maximum mean length (L_∞), a growth parameter (K), and the theoretical age in years for a length of zero (t_0).

Model	**df**	**AIC$_c$**	**Δ AIC$_c$**	**Akaike weight (*w*)**
Different K, L_∞, and t_0	10	8541.47	0.00	0.53
Different K	6	8543.11	1.64	0.23
Different L_∞	6	8544.35	2.88	0.13
Different K and L_∞	8	8546.33	4.86	0.05
Different K and t_0	8	8546.60	5.13	0.04
Different L_∞ and t_0	8	8548.36	6.89	0.02
Different t_0	6	8550.87	9.40	0.00
Same K, L_∞, and t_0	4	8567.82	26.35	0.00

Table 3. von Bertalanffy growth functions describing mean-length-at-age of Shoal Bass among three upper Chattahoochee River populations (*n* = sample size in each system). Estimates and associated non-parametric bootstrapped 95% confidence intervals (CI; L = lower and U = upper) were produced for model components including maximum mean length (L_∞), a growth parameter (K), and the theoretical age in years for a length of zero (t_0).

Parameter	est.	95% LCI	95%UCI
	Big Creek (*n*=388)		
L_∞	729.94	629.45	882.58
K	0.08	0.06	0.10
t_0	-0.83	-1.04	-0.65
	Chattahoochee River (*n*=185)		
L_∞	1576.06	736.48	3413.15
K	0.03	0.01	0.08
t_0	-1.87	-2.65	-0.84
	Chestatee River (*n*=294)		
L_∞	645.38	565.37	819.06
K	0.11	0.07	0.14
t_0	-0.53	-1.00	-0.19

Table 4. von Bertalanffy growth function estimates of mean total length (TL; mm) for each age (yrs) and associated non-parametric bootstrapped 95% confidence intervals (CI; L = lower and U = upper) for Shoal Bass in three systems of the upper Chattahoochee River basin.

	Big Creek			***Chattahoochee River***			***Chestatee River***		
Age	**TL**	**LCI**	**UCI**	**TL**	**LCI**	**UCI**	**TL**	**LCI**	**UCI**
0	47.9	40.5	55.0	77.9	46.6	97.2	36.1	14.5	58.3
1	101.5	98.1	104.9	117.9	98.3	132.2	98.9	86.4	111.9
2	150.8	147.8	154.1	156.8	144.4	167.7	155.1	148.0	162.5
3	196.3	192.1	200.6	194.8	186.4	202.8	205.6	200.6	210.6
4	238.2	233.2	243.3	231.7	225.3	238.8	250.9	245.5	256.5
5	276.8	271.5	282.2	267.6	261.9	275.4	291.5	285.5	297.6
6	312.4	307.2	317.5	302.5	296.6	310.7	327.9	322.0	333.8
7	345.2	340.4	350.3	336.5	330.0	345.2	360.6	354.5	366.4
8	375.4	369.9	381.1	369.7	361.8	378.6	390.0	383.1	396.8
9	403.3	396.2	409.9	401.9	391.6	412.0	416.3	407.6	425.9
10	428.9	419.8	437.6	433.2	418.9	445.2	439.8	428.8	452.5
11	452.6	440.6	464.0	463.8	443.5	478.3	461.0	447.2	477.8
12	474.3	459.4	488.8	493.5	466.9	511.6	480.0	462.9	501.4

Table 5. Results of principal component analysis (PCA) used to reduce the number of inter-correlated environmental variables considered in linear models of recruitment strength. The highest-loading variable from any axis explaining at least 10% of the variance in each system's dataset was included. Reported are the PCA axis number, axis eigenvalue, and the % of variation explained by each axis, along with the highest-loading variable (i.e., highest correlated to each axis) to each axis and its correlation coefficient (*r*) to the axis.

Axis	**eigenvalue**	**% of variation**	**highest loading variable**	***r***
			Big Creek	
1	13.82	49.35	Summer SD of discharge	0.95
2	5.79	20.69	Spring min. discharge	-0.81
3	3.39	12.12	Fall cumulative growing degree-days	-0.77
			Chattahoochee River	
1	14.05	50.17	Fall min. discharge	0.96
2	5.92	21.13	Winter SD of discharge	0.75
3	3.72	13.27	Summer cumulative growing degree-days	-0.73
			Chestatee River	
1	13.98	49.93	Fall min. discharge	0.96
2	5.58	19.94	Spring min. discharge	-0.75
3	3.68	13.13	Spring avg. growing degree-days	-0.90

Table 6. Results of univariate linear models relating catch-curve residuals representing Shoal Bass recruitment strength in three systems of the upper Chattahoochee River basin to a subset of environmental variables obtained from principal component analysis. Models were considered significant at $P \leq 0.05$ level (**bold**).

Variable	**estimate**	**SE**	**df**	***p***	***R*^{2}**
Big Creek					
Summer SD discharge	-0.27	0.07	1, 26	**< 0.01**	0.38
Spring min. discharge	-0.36	0.22	1, 26	0.11	0.09
Fall cumulative growing degree-days	3.49	1.60	1, 26	**0.04**	0.15
Chattahoochee River					
Fall min. discharge	-0.16	0.18	1, 23	0.39	0.03
Winter SD discharge	0.55	0.26	1, 23	**0.04**	0.16
Summer cumulative growing degree-days	3.91	3.70	1, 23	0.30	0.05
Chestatee River					
Fall min. discharge	0.17	0.16	1, 28	0.31	0.04
Spring min. discharge	0.36	0.25	1, 28	0.17	0.07
Spring avg. growing degree-days	1.16	2.49	1, 28	0.65	0.01

Table 7. Capture-mark-recapture data for Shoal Bass in three upper Chattahoochee River Basin systems, including sample site, date sampled, number of fish captured, mean total length (TL; mm) of captures, number of fish recaptured, mean TL of recaptures, as well as Huggins' closed-captures estimates of abundance (*N*) and associated 95% confidence intervals (CI; L = lower and U = upper). "N/A" indicates measure was unobtainable or inestimable given the data collected. (Table continued on following page).

Site #	**date**	**# captured**	**Mean capture TL**	**# recaptured**	**Mean recapture TL**	***N* est.**	**LCI**	**UCI**
Big Creek								
1	5/20/14	22	212	N/A	N/A	242	125	579
1	5/22/14	44	208	4	300			
1	10/17/14	74	130	5	250	253	200	347
1	10/18/14	81	116	27	142			
1	5/13/15	53	168	19	188	219	164	325
1	5/15/15	67	184	38	211			
1	5/16/16	131	161	25	260	348	276	470
1	5/19/16	69	181	35	207			
Chattahoochee River								
2	5/27/14	6	280	N/A	N/A	48	19	229
2	5/29/14	8	216	1	287			
2	5/18/15	4	200	0	N/A	12	7	53
2	5/20/15	3	282	1	210			
3	5/27/14	9	272	N/A	N/A	50	25	158
3	5/29/14	11	236	2	249			
3	5/18/15	16	274	5	283	60	37	137
3	5/20/15	15	310	4	235			
4	5/27/14	9	282	N/A	N/A	144	45	708
4	5/29/14	17	252	1	215			
4	5/18/15	9	302	1	252	90	30	439
4	5/20/15	10	277	1	464			
5	5/27/14	14	289	N/A	N/A	21	17	42
5	5/29/14	6	259	4	282			

Table 7 (continued)

				Chestatee River				
6	5/19/14	0	N/A	N/A	N/A	N/A	N/A	N/A
6	5/21/14	0	N/A	N/A	N/A			
6	5/12/15	0	N/A	N/A	N/A	N/A	N/A	N/A
6	5/14/15	0	N/A	N/A	N/A			
6	5/17/16	0	N/A	N/A	N/A	N/A	N/A	N/A
6	5/18/16	0	N/A	N/A	N/A			
7	5/19/14	9	294	N/A	N/A	N/A	N/A	N/A
7	5/21/14	7	225	0	N/A			
7	5/12/15	9	285	4	338	18	13	43
7	5/14/15	6	294	3	333			
7	5/17/16	11	299	4	315	154	47	759
7	5/18/16	14	286	4	388			
8	5/19/14	3	231	N/A	N/A	N/A	N/A	N/A
8	5/21/14	2	150	0	N/A			
8	5/12/15	2	200	0	N/A	10	6	42
8	5/18/15	5	236	1	202			
8	5/17/16	3	276	2	278	12	7	53
8	5/18/16	4	238	1	300			
9	5/19/14	23	250	N/A	N/A	83	59	146
9	5/21/14	29	251	8	296			
9	5/12/15	34	272	6	259	73	59	106
9	5/18/15	30	270	19	308			
9	5/17/16	30	299	9	348	76	59	119
9	5/18/16	28	274	17	316			

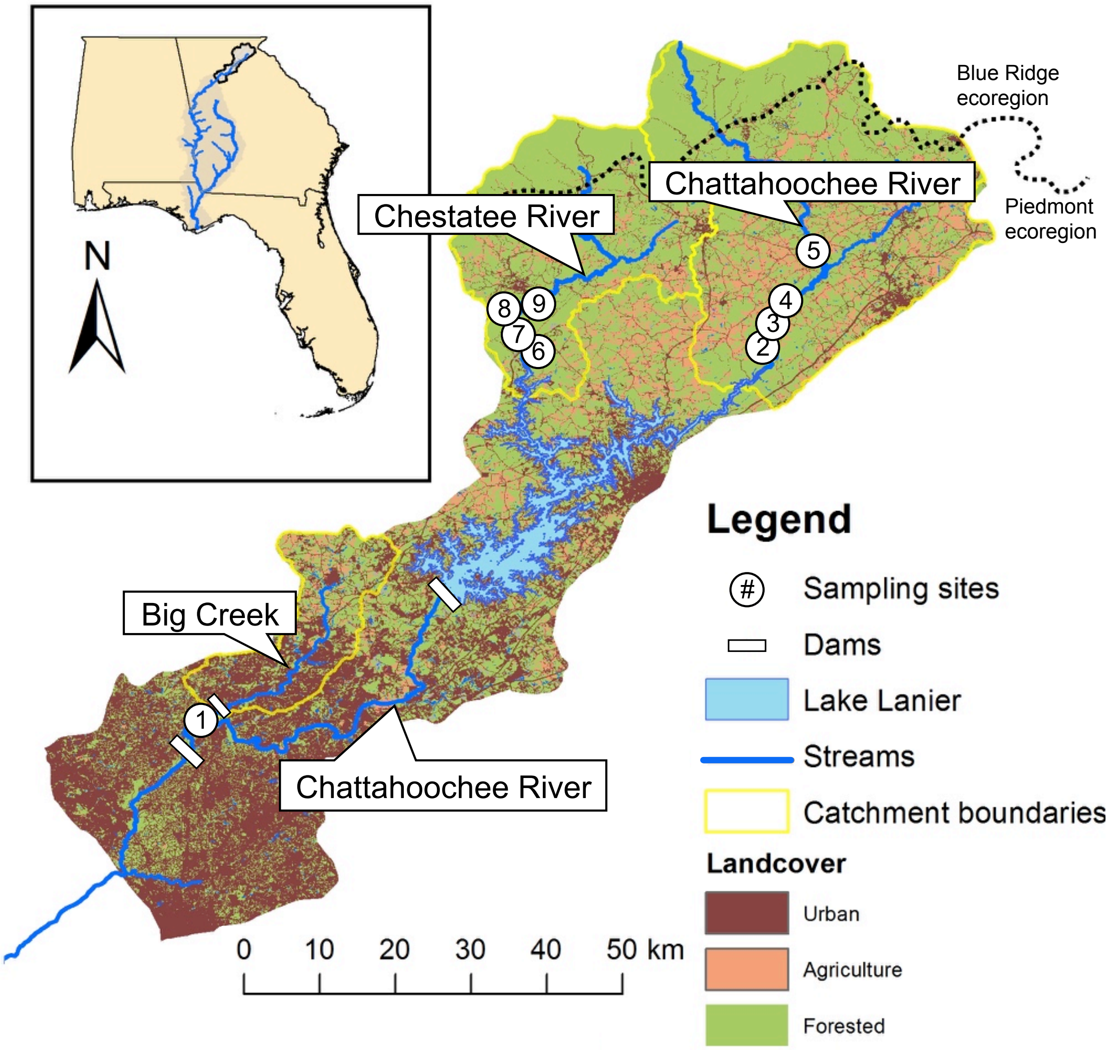

Figure 1. Study area within the upper Chattahoochee River Basin (UCRB) or northern Georgia, U.S.A. Sampling site numbers correspond with descriptions in Table 1. Both the Chattahoochee and Chestatee rivers begin in the mountainous Blue Ridge ecoregion prior to entering the rolling hills of the Piedmont ecoregion, where more agricultural activities occur. Big Creek's catchment is more urbanized because portions are situated in the Atlanta, GA, metropolitan area.

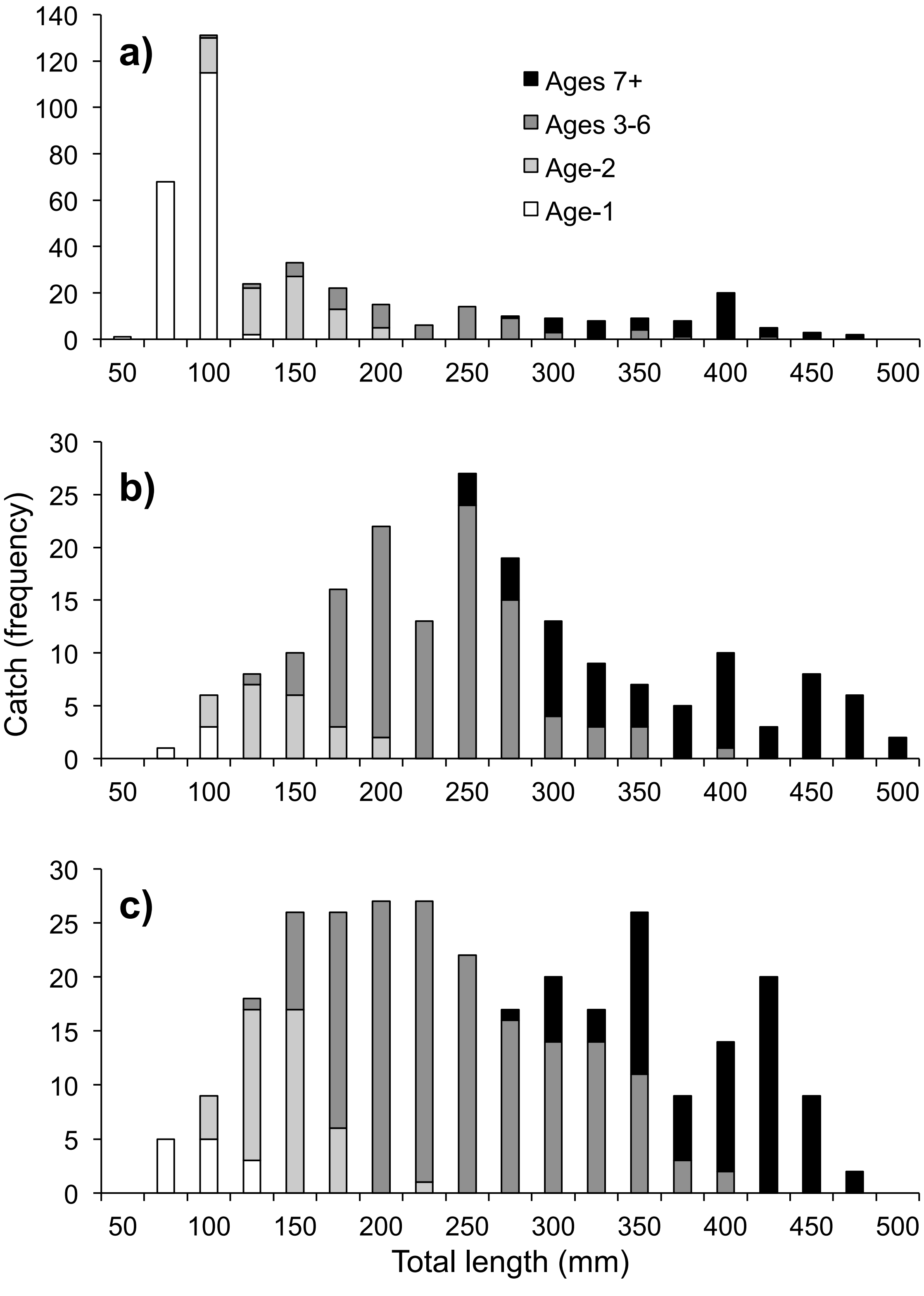

Figure 2. Length-frequency histograms, with age categories superimposed, depicting raw catch of Shoal Bass May sampling events in (a) Big Creek, (b) Chattahoochee River, and (c) Chestatee River.

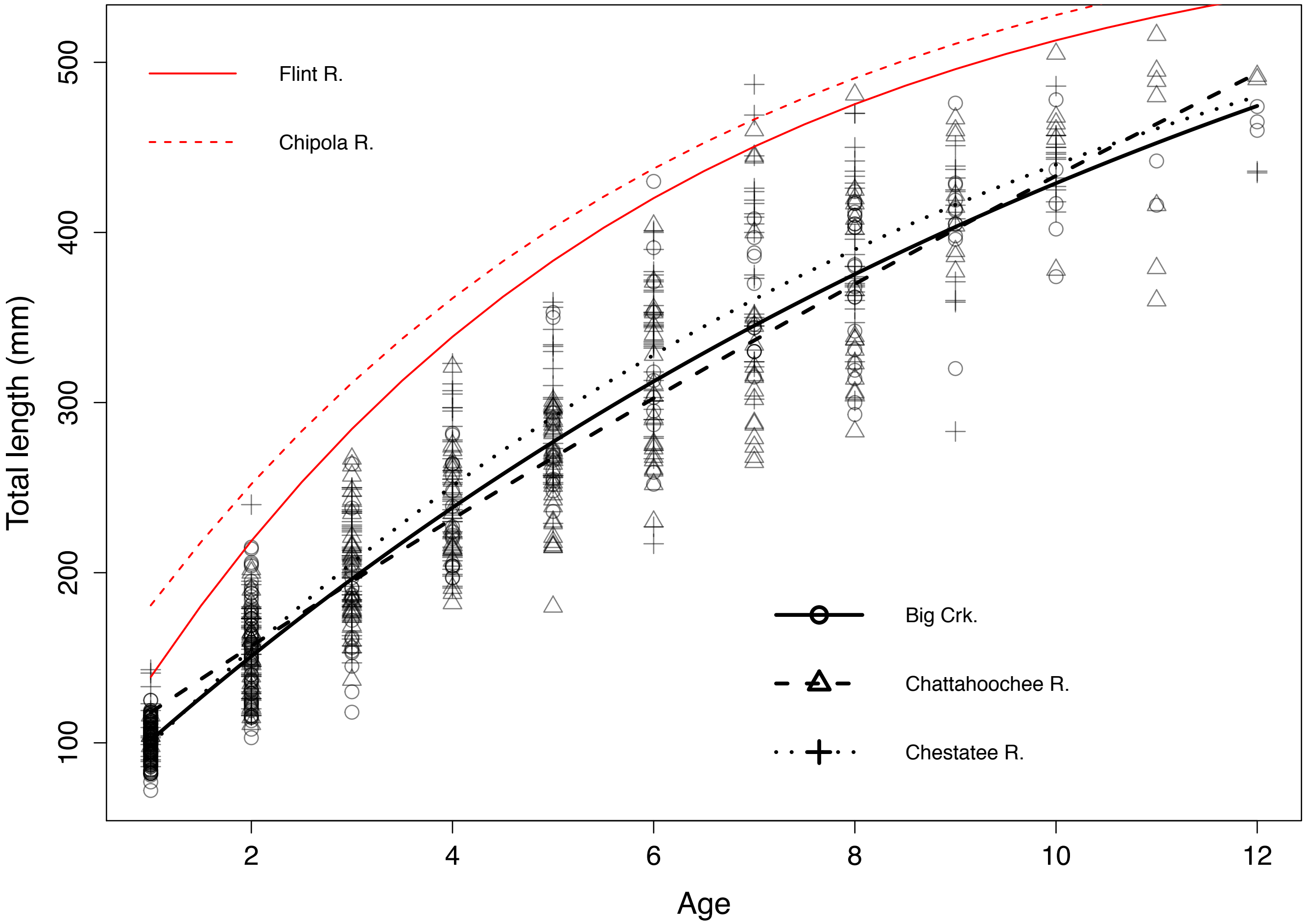

Figure 3. von Bertalanffy growth functions for Shoal Bass in three upper Chattahoochee River Basin populations (Big Creek, Chattahoochee River, and Chestatee River), including the raw data used to build each function. For comparative purposes, we included growth functions for Shoal Bass in the upper Flint River (Sammons and Goclowski 2012) and in the Chipola River (Woodside et al. 2015).

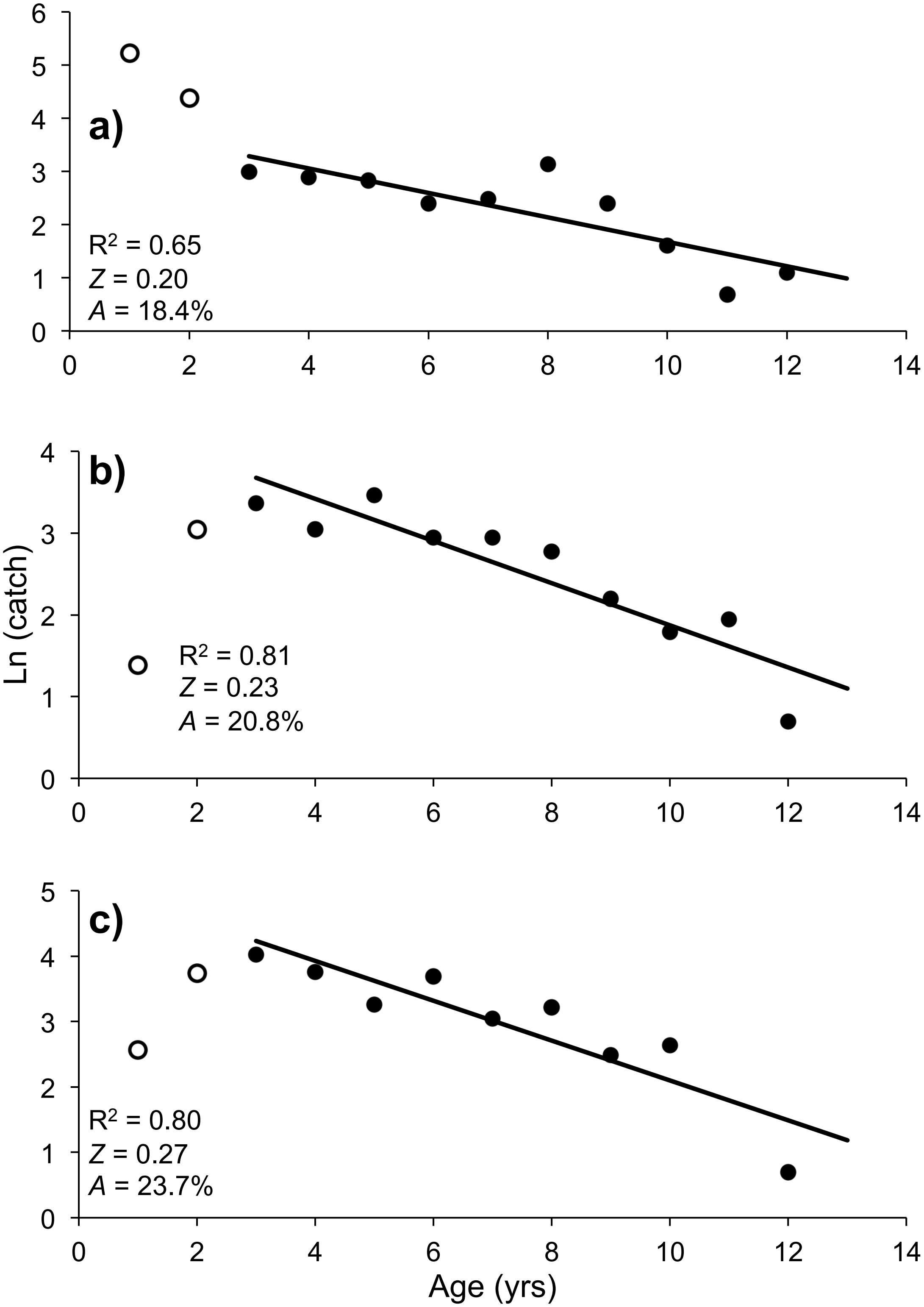

Figure 4. Catch-curve weighted regressions used to estimate annual mortality in Shoal Bass aged 3-12 in (a) Big Creek, (b) Chattahoochee River, and (c) Chestatee River. Fish aged < 3 yrs (hollow data points) were not included in regressions. R^2 measures fit to the regression line, *Z* is the instantaneous mortality rate, and *A* is annual mortality.

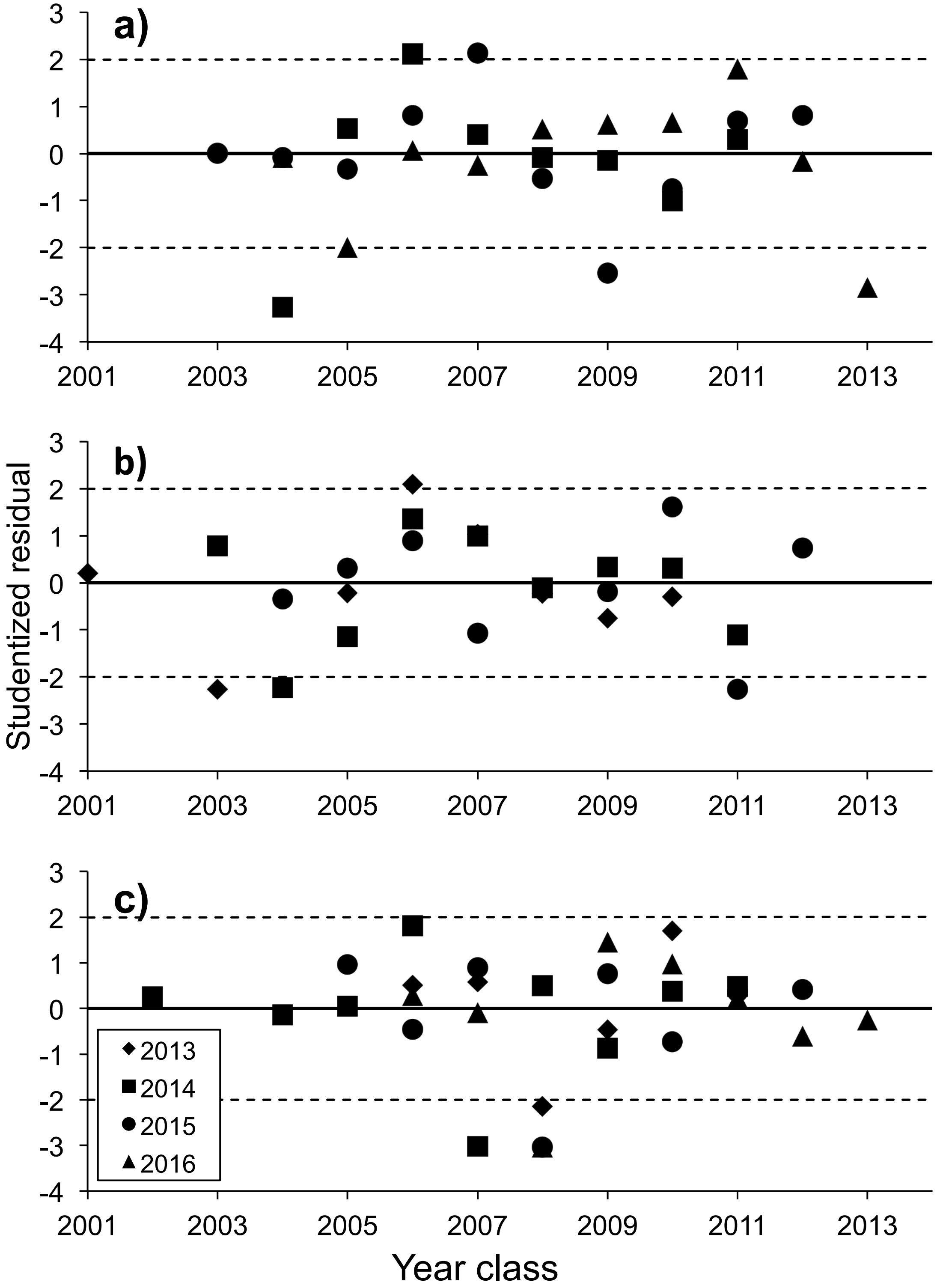

Figure 5. Studentized residuals from weighted catch-curve regressions indicating year-class strength of Shoal Bass in (a) Big Creek, (b) Chattahoochee River, and (c) Chestatee River. Studentized residuals exceeding +/- 2 SD (dashed lines) were considered strong and weak year-classes, respectively. Symbols indicate residuals calculated over different sampling years.

Appendix I. Capture-mark-recapture (CMR) histories used to build Huggins' models to estimate Shoal Bass abundance and to build Cormack-Jolly-Seber (CJS) models to estimate survival of young cohorts in Big Creek. Unique capture histories are represented by 0's and 1's (e.g., 10 represents detected on day 1 but not detected on day 2), followed by the count of individuals with that capture history. Negative counts represent the number of individuals not released back into the population (i.e., sampling mortality) following the last encounter.

<u>Huggins' model inputs:</u>

<u>Big Creek (site #1), Spring 2014:</u>

11 4;
10 18;
01 40;

<u>Big Creek (site #1), Fall 2014:</u>

11 23;
10 48;
10 -3;
01 58;

<u>Big Creek (site #1), Spring 2015:</u>

11 16;
10 36;
10 -1;
01 50;
01 -1;

<u>Big Creek (site #1), Spring 2016:</u>

11 26;
10 105;
01 43;

<u>Chattahoochee River (site #2), Spring 2014:</u>

11 1;
10 5;
01 7;

<u>Chattahoochee River (site #2), Spring 2015:</u>

11 1;
10 3;
01 2;

<u>Chattahoochee River (site #3), Spring 2014:</u>

11 2;
10 7;
01 9;

<u>Chattahoochee River (site #3), Spring 2015:</u>

11 4;
10 12;
01 11;

Chattahoochee River (site #4), Spring 2014:

11 1;
10 8;
01 15;

Chattahoochee River (site #4), Spring 2015:

11 1;
10 8;
01 9;

Chattahoochee River (site #5), Spring 2014:

11 4;
10 10;
01 2;

Chestatee River (site #7), Spring 2014:

11 0;
10 9;
01 7;

Chestatee River (site #7), Spring 2015:

11 3;
10 6;
01 3;

Chestatee River (site #7), Spring 2016:

11 1;
10 10;
01 13;

Chestatee River (site #8), Spring 2015:

11 1;
10 1;
01 4;

Chestatee River (site #8), Spring 2016:

11 1;
10 2;
01 3;

Chestatee River (site #9), Spring 2014:

11 8;
10 15;
01 21;

Chestatee River (site #9), Spring 2015:

11 14;
10 20;
01 16;

Chestatee River (site #9), Spring 2016:

11 11;
10 19;
01 17;

CJS model input:

Big Creek 2014 cohort from age-0 (Fall 2014) to age-1 (Spring 2015) to age-2 (Spring 2016):

111 2;
110 9;
101 2;
100 73;
100 -3;
011 6;
010 36;
010 -3;
001 14;

VITA

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