Utility of Single Nucleotide Polymorphism (SNP) Resources for Black Bass (*Micropterus* spp.) Conservation and Management

by

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Abstract

The black basses (*Micropterus* spp.) are a genus of apex predators and important game fishes in North American freshwater ecosystems. Efforts to improve recreational bass fisheries have led to the widespread stocking of black bass species, often facilitating introgressive hybridization between endemic and non-native species. Phenotypic differentiation of black bass species and their hybrids is notoriously unreliable. Molecular tools are needed to rapidly and accurately assess bass populations, whether they are intensively managed in a reservoir or the target for conservation in un-impacted streams. My thesis describes the development and application of practical tools to better integrate molecular analyses with black bass conservation and management. Following a review of pertinent literature in **Chapter I**, in **Chapter II** I detail the development, validation, and field-testing of a methodology to collect bass DNA through buccal swabbing. This method is simple, robust, and cost-effective, allowing angler involvement in genetic sample collection from bass populations otherwise difficult to obtain. In **Chapter III**, I utilize recently developed diagnostic black bass single nucleotide polymorphism (SNP) marker panels to provide one of the first genetic analyses of black bass populations in the Altamaha River Basin (ARB). My results, from over 500 individuals, shed light on the status of introduced Spotted Bass (*M. punctulatus*) in the basin, hybridization patterns of introduced Shoal Bass (*M. cataractae*), and provide an important revision to the accepted intergrade status of native Largemouth Bass in the drainage. I also provide evidence pointing to the presence of a genetically distinct bass in the ARB Coastal Plain, deserving of closer scrutiny in the future.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

DNA	Deoxyribonucleic acid
ADCNR	Alabama Department of Conservation and Natural Resources
FLMB	Florida Bass *(Micropterus floridanus)*
GADNR	Georgia Department of Natural Resources
LMB	Largemouth bass *(Micropterus salmoides)*
mtDNA	Mitochondrial DNA
ARB	Altamaha River Basin
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism

Chapter I: Literature Review

Overview

The black basses (*Micropterus spp.*) are a genus of popular game fish endemic to North America east of the Rocky Mountains (Near et al. 2003). Black bass species are ecologically important members of freshwater systems because they are carnivorous top-level predators (Etnier and Starnes 1993). At the top of the food chain black bass prey upon a number of fish species, and, therefore, help to maintain the health and viability of the ecosystem (Olsen and Young 2003). In addition to their ecological value, black bass are of high economic importance due to their popularity with recreational anglers. More than 30,000 competitive angling events occur annually in the United States, with 80% of those events targeting black bass species (Schramm et al. 1991). As a result of their popularity in sport fishing, black bass species have been widely stocked outside of their native ranges in an attempt to enhance recreational fishing opportunities and expand populations of bass. Due to this stocking, black basses can now be found throughout North America and on other continents including South America, Africa, Europe, and Asia (Casal 2006; Loppnow et al. 2013; Ellender and Weyl 2014).

Black basses have few reproductive barriers to hybridization relative to other groups of fish (Koppelman 2015). Habitat alteration and the widespread stocking of non-native black bass species, have served to accelerate this tendency. Interspecific hybridization and introgression can ultimately affect the genetic identity of endemic species and alter endemic biodiversity (Mallet 2005; Tanaka 2007). Adverse effects following introductions include lowered fitness, disruption of local adaptation, and eventual genetic extirpation or extinction of species with limited geographic ranges (Rhymer and Simberloff 1996; Randi 2008). Since black bass species are notoriously hard to phenotypically identify, molecular marker tools are needed to assess

hybridization, delineate species, and maintain broodstock purity (in the case of managed, stocked fisheries) (Taylor et al. 2018). In this thesis, I describe the development and application of new genetic techniques and resources to better manage and conserve black bass diversity. In the following introduction, I review existing knowledge of a) black bass species and their ecology; b) conservation concerns for black bass species and impacts of hybridization; and c) molecular marker tools and approaches for black basses.

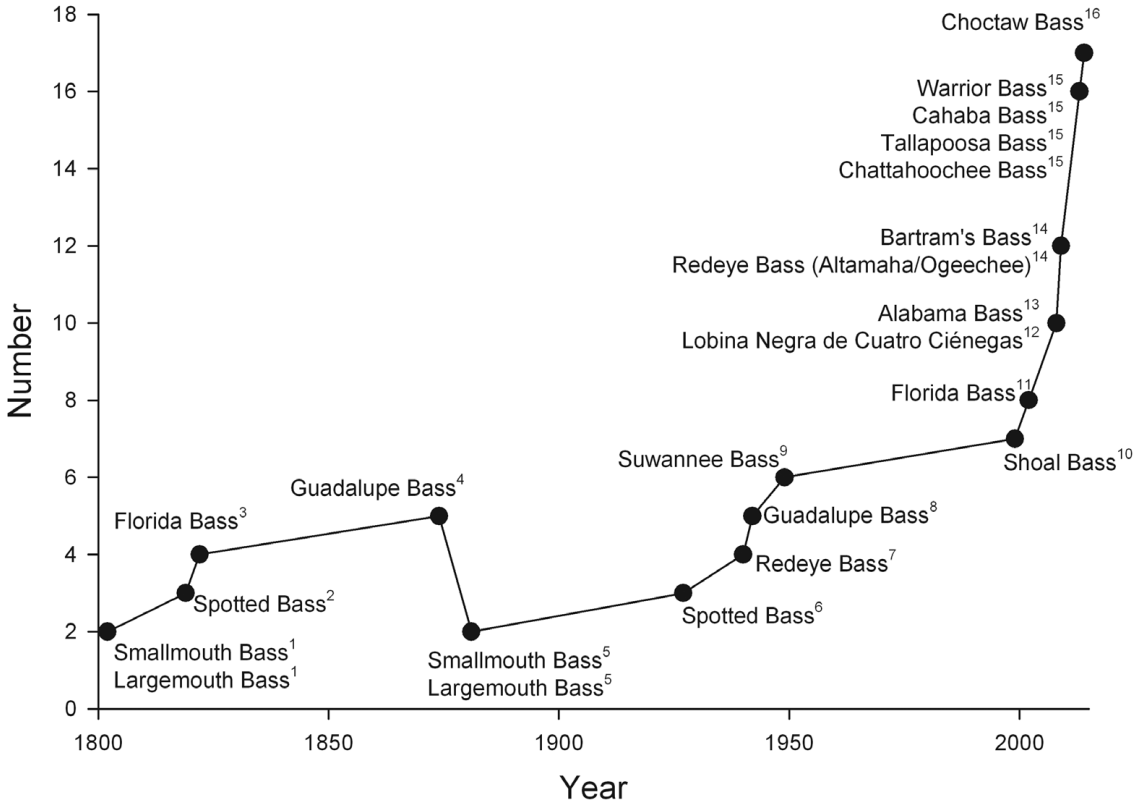
I. Black Basses

a) Ecology of Black Basses

Black basses inhabit a diverse array of freshwater ecosystems from small ponds and streams to large lakes and rivers (Koppelman and Garrett 2002). During the early stages of development, black bass fry primarily feed on zooplankton and insect larvae present in the littoral zone (Gilliam 1982; Keast and Eadie 1985). Within the first year of development, black bass typically undergo a niche shift and begin feeding on small young-of-year fish such as Bluegill (*Lepomis macrochirus*) (Olson 1996). Once adults, black bass migrate to the limnetic zone and become top-level predators by preying upon a number of fish species. Consequently, due to their role as apex predators they have a large effect on trophic cascades (Carpenter and Kitchell 1996). Black bass can determine the size and species composition of the planktivorous fish assemblage through their prey selection (Tonn and Magnuson 1982). The selected planktivores then determine the composition of zooplankton, which, in turn, regulate the composition and density of phytoplankton in the system (Brooks and Dodson 1965; Sommer 1989).

b) Black Bass Species

Currently, in the genus *Micropterus* there are 14 recognized species: Largemouth Bass (*Micropterus salmoides*) (Lacepede 1802), Smallmouth Bass (*Micropterus dolomieu*) (Lacepede 1802), Spotted Bass (*Micropterus punctulatus*) (Rafinesque 1819), Florida Bass (*Micropterus floridanus*) (Lesueur 1822), Guadalupe Bass (*Micropterus treculii*) (Vaillant and Bocourt 1874), Alabama Bass (*Micropterus henshalli*) (Hubbs and Bailey 1940), Redeye Bass (*Micropterus coosae*) (Hubbs and Bailey 1940; now Coosa Bass per Baker et al. 2013), Suwannee Bass (*Micropterus notius*) (Bailey and Hubbs 1949), Shoal Bass (*Micropterus cataractae*) (Williams and Burgess 1999), Cahaba Bass (*Micropterus cahabae*) (Baker et al. 2013), Tallapoosa Bass (*Micropterus tallapoosae*) (Baker et al. 2013), Warrior Bass (*Micropterus warriorensis*) (Baker et al. 2013), Chattahoochee Bass (*Micropterus chattahoochae*) (Baker et al. 2013), and Choctaw Bass (*Micropterus haiaka*) (Tringali et al. 2015). Additional *Micropterus* subspecies that are recognized, but still provisional are the Cuatro Ciénegas Bass (*Micropterus sp. cf. salmoides*) (Jelks et al 2008), Bartram's Bass (*M. sp. cf. coosae*) (Straight et al. 2009), and Altamaha Bass (*M. sp. cf. coosae*) (Straight et al. 2009). A chronology of the current recognized black bass species is presented in Figure 1. The native geographical range descriptions of the black bass species is presented in Figure 2.



[1]Lacepèdé 1802; [2]Rafinesque 1819; [3]LaSueur 1822; [4]Vaillant and Bocourt 1874; [5]Henshall 1881; [6]Hubbs 1927; [7]Hubbs and Bailey 1940; [8]Hubbs and Bailey 1942; [9]Bailey and Hubbs 1949; [10]Williams and Burgess 1999; [11]Kassler et al. 2002; [12]Jelks et al. 2008; [13]Baker et al. 2008; [14]Staight et al. 2009; [15]Baker et al. 2013; [16]Tringali et al. This volume

Figure 1 Chronology of the current recognized black bass species over time and a reflection of how the scientific community recognized the various species at the time. The superscripts denote the source used to recognize the species (adapted from Long et al. 2015).

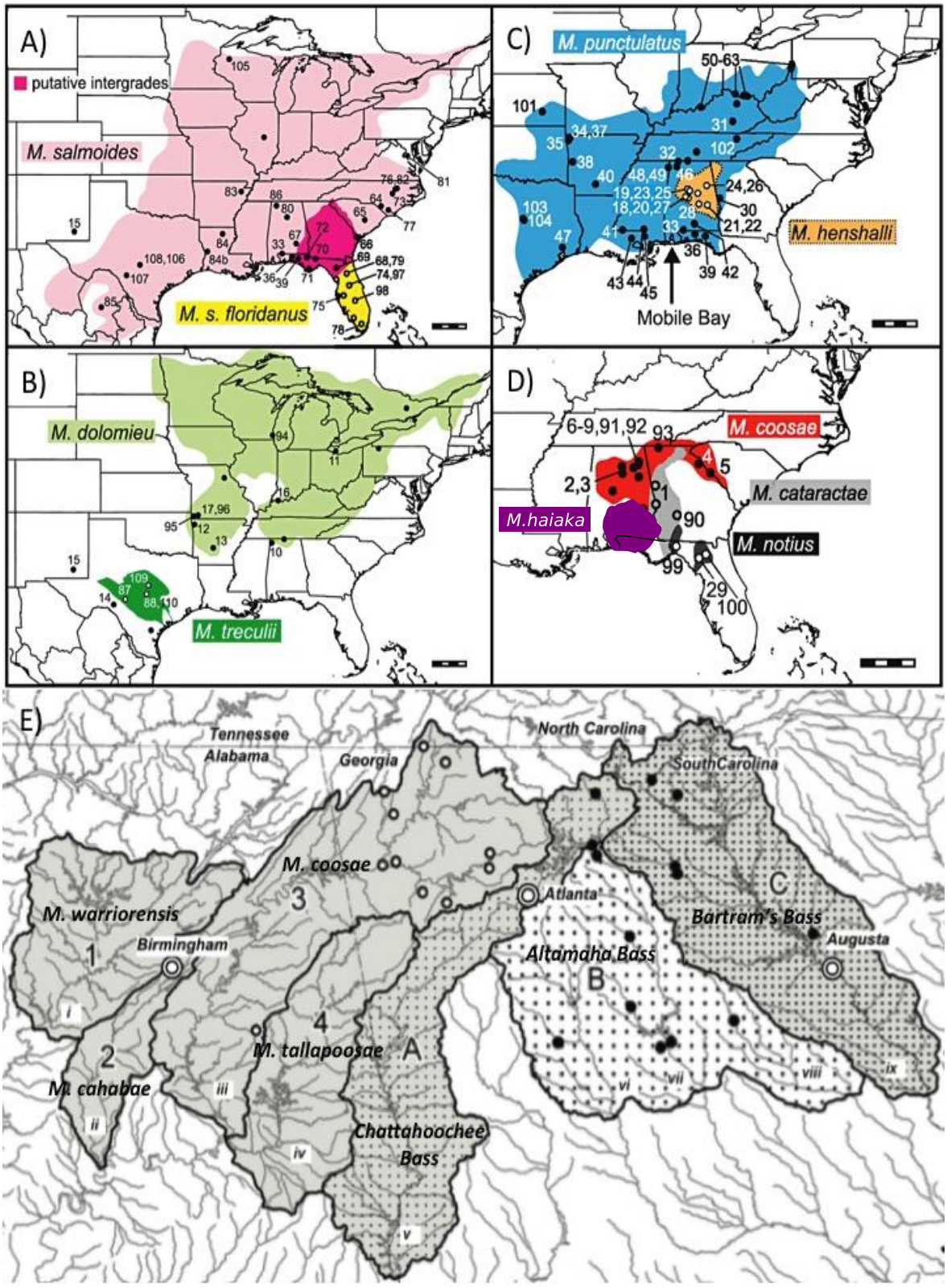


Figure 2 Native geographical range descriptions of **A)** *M. salmoides* (pink), *M. floridanus* (yellow), and the putative integrade (dark pink); **B)** *M. dolomieu* (light green) and *M. treculii* (green); **C)** *M. punctulatus* (blue) and *M. henshalli* (orange); **D)** *M. coosae* (red), *M. cataractae* (grey), *M. haiaka* (purple), and *M. notius* (black) (Adapted from Bagley et al. 2011; Tringali et al. 2015); and **E) 1.** *M. warriorensis*, 2. *M. cahabae*, 3. *M. coosae*, 4. *M. tallapoosae*, A. Chattahoochee Bass, B. Altamaha Bass, and C. Bartram's Bass (Adapated from Freeman et al. 2015)

The most widely distributed species within *Micropterus* are the Largemouth Bass, Spotted Bass, and Smallmouth Bass. Within its native range, the Largemouth Bass is composed of two species: the Northern Largemouth Bass and the Florida Bass. The native range of the Florida Bass includes peninsular Florida, while the range of the Northern Largemouth Bass extends through the North American corridor between northeast Mexico and southeast Canada (Figure 2A; MacCrimmon and Robbins 1975). Due to secondary contact, the two species have naturally hybridized and coexist in introgressed populations, originally limited to the Southeast U.S. (Philipp et al. 1983). In addition to natural hybridization, Florida Bass have been extensively stocked outside of their native range due to their superior growth compared to their northern counterpart (Chew 1975; Addison and Spencer 1971). These stockings have further facilitated hybridization among these species, which has greatly expanded the intergrade zone in the Southeastern U.S. and beyond (Philipp et al. 1983).

The next species occupying a large range within the United States is the Smallmouth Bass. Smallmouth Bass are endemic to the Ohio, Tennessee, upper Mississippi, and Saint Lawrence-Great Lakes systems (Figure 2B; Scott and Crossman 1973). Two subspecies were described including the Northern Smallmouth Bass (*M. d. dolomieu*) and Neosho Smallmouth Bass (*M. d. velox*) (Hubbs and Bailey 1940). Smallmouth Bass have naturally expanded their native range through drainage dispersal, but extensive expansion has occurred through intentional and unintentional stockings (Robbins and MacCrimmon 1974; Borden and Krebs 2009). Invasive Smallmouth Bass have been documented to reduce native small-bodied fish abundance and diversity through predation, and to outcompete other piscivorous game fish (Jackson 2002).

Another widely distributed species the Spotted Bass, which is native to the central and lower Mississippi River basin in addition to the Ohio River basin (Figure 2C; Page and Burr 1991). Three subspecies of the Spotted Bass have been described including: the Northern Spotted Bass (*M. p. punctulatus*), Alabama Spotted Bass (*M. p. henshalli*), and the currently invalidated Wichita Spotted Bass (*M. p. wichitae*) (Bailey and Hubbs 1940; Cofer 1995; Warren 2009). Recently, due to morphological and genetic variation the Alabama subspecies was elevated as a new black bass species, the Alabama Bass (Baker et al. 2008). Alabama Bass are endemic to the Mobile River basin of Alabama, Georgia, and Mississippi (Figure 2C; Bailey and Hubbs 1940). The range of Alabama Bass has been expanded through illegal introductions by anglers into the Savannah River, Hiwassee River, Tennessee River, and the Chattahoochee River basins (Pierce and Van Den Avyle 1997; Barwick et al. 2006; Moyer et al. 2014).

Redeye Bass are native to the Mobile basin above the Fall Line, and the headwaters of the Savannah, Altamaha, and Chattahoochee River systems (Figure 2D; Page and Burr 1991; Mettee et al. 1996). Stockings have increased the range of the Redeye Bass, primarily in streams of North Carolina, Tennessee, and California (Koppelman and Garrett 2002). There has been significant genetic divergence found among Redeye Bass populations throughout their native range (Birdsong et al. 2015). Recently, due to morphological and mitochondrial DNA differences, Baker et al. (2013) described four new species: Cahaba Bass, restricted to the Cahaba River system; Tallapoosa Bass, restricted to the Tallapoosa River system; Warrior Bass, from the Black Warrior River system; and Chattahoochee Bass, from the Chattahoochee River system (Figure 2E). Coosa Bass, restricted to the Coosa River system retained the original nomenclature of *M. coosae*. In addition, there are two putative Redeye subspecies that have been recently described but are still under review, Bartram's Bass and Altamaha Bass. Above the Fall

Line, Bartram's Bass are endemic to the Savannah River basin and Altamaha Bass are found in the Ocmulgee, Oconee, and Ogeechee River basins in Georgia (Freeman et al. 2015). Genetic analyses currently have provided conflicting answers as to whether these last two subspecies are phylogenetically related to Redeye Bass (as traditionally assumed) or the Shoal Bass (Freeman et al. 2015).

Shoal Bass are native to the Chattahoochee and Flint River systems in Alabama and Georgia, in addition to the Apalachicola and Chipola River systems in Florida (Williams and Burgess 1999). Shoal Bass are fluvial specialists and require fast-flowing riverine conditions with coarse bed sediments (Sammons et al. 2015). Shoal Bass were assigned a conservation status of high concern in Alabama, Georgia, and Florida (Deacon et al. 1979; Williams et al. 1989). Threats to Shoal Bass populations include anthropogenic introductions of non-native species and habitat loss or degradation (Taylor and Peterson 2014; Sammons et al. 2015). As part of an effort to conserve Shoal Bass populations, the native black bass initiative (NBBI) was developed to provide regional conservation strategies, objectives, and targets to restore populations of endemic black bass species (Birdsong et al. 2015).

The remaining black bass species have more restricted ranges including: Suwannee Bass, Guadalupe Bass, and Choctaw Bass. Suwannee Bass have the smallest range among black bass species encompassing only 8,500 km^2 (Bonvechio et al. 2010). Suwannee Bass are present throughout the Suwannee, Ichetucknee, Santa Fe, Wacissa, St. Marks, and Wakulla Rivers in Florida, in addition to the Alapaha, Withlacoochee, and Ochlockonee Rivers in Florida and Georgia (Figure 2D; Nagid et al. 2010). Guadalupe Bass are endemic to Central Texas including the San Antonio, Guadalupe, Colorado, and Brazos river systems (Figure 2B; Hubbs 1957). The most recently described species, Choctaw Bass, are endemic to the coastal river systems in the

Florida panhandle and Alabama (Figure 2D). They have been identified in the Choctawhatchee, Yellow, Blackwater, Escambia-Conecuh, and Perdido River systems in the Florida panhandle (Tringali et al. 2015). Due to the restricted ranges of these black bass species, they warrant conservation concern and require proper management strategies in order to protect their populations and genetic integrity (Birdsong et al. 2015).

c) Economic Value of Black Bass

Recreational angling has evolved into a multibillion-dollar industry that attracts millions of anglers each year. In 2016, fishing-related expenditures reached a total of $46.1 billion and attracted over 35 million anglers (USDI 2016). An important aspect of recreational angling is organized competitive sport fishing, which targets specific species for rewards (Schramm et al. 1991). Competitive angling events in the United States have increased from 18,303 in 2000 to the most recent estimate of 32,321 in 2005 (Kerr and Kamke 2003; Schramm and Hunt 2007). These tournaments have a significant impact on the local economy in the communities surrounding the events due to purchases at hotels, gas stations, restaurants, and fishing shops (Driscoll et al. 2012). In addition, tournaments enhance the management of valuable fisheries by promoting catch-and-release angling, recruiting new anglers, and receiving angler input on issues such as fish length limits (Weathers et al. 2000).

Black basses have solidified their spot as the most popular angled species in the United States due to their aggressive feeding behavior and ability to reach large overall sizes. In a ratio of 10 to 1, black bass represent the majority of freshwater tournament fishing in the United States when compared to other species (Duttweiler 1985). In 2011, there were a recorded 27.1 million anglers freshwater fishing, and over 10.6 million of the anglers were targeting black bass

species (USDI 2011). While all of the species in *Micropterus* are targeted in sport fishing, three of the most popular angled species are the Largemouth Bass, Smallmouth Bass, and Spotted Bass. Despite the limited range of the remaining species, recently there has been interest in capturing these specimens for sport fishing (i.e. Georgia Bass Slam; Redeye Slam). These programs were developed to harness angler's ability to catch different species of black bass in order to promote interest in the conservation and management of black bass species in their native environments.

II. Conservation Concerns

Threats to black bass biodiversity include habitat degradation and alteration, environmental pollution, competition, and hybridization with non-native congeners. While some of the species occupy large geographic ranges (e.g. Largemouth Bass, Smallmouth Bass, and Spotted Bass), the remaining species are most threatened by changes to their habitats. One of the greatest threats for *Micropterus* species with restricted ranges is habitat degradation and alteration since they are habitat specialists (Koppelman and Garrett 2002). Changes in stream flow, addition of impoundments, and urban development have been common documented reasons for population declines for restricted black bass species (Ramsey 1976; Edwards 1978; Koppelman and Garrett 2002). For example, dams in the Chattahoochee River have restricted Shoal Bass to only small reaches of the river, which limited their effective population size and genetic diversity within the system (Williams and Burgess 1999; Dakin et al. 2007; Sammons and Maceina 2009). As urbanization continues to expand exponentially, many native populations of black bass near large cities are negatively impacted. For example, there have been declines in Guadalupe Bass populations near fast-growing regions of Texas due to changes in hydrologic

flow, habitat degradation, and loss of watershed connectivity (Hurst et al. 1975; Edwards 1980). In addition, Redeye Bass populations throughout the upper and middle portions of the Savannah River are threatened by urbanization and land-use changes (Birdsong et al. 2015).

Another important conservation concern for rare black species is hybridization with non-native introduced species. The introduction of non-native bass species to enhance recreational angling opportunities and populations of bass has been a common practice in the southeastern United States (Baker et al. 2013). Stocking programs have been largely conducted by state and federal management agencies, but there have been a number of documented cases of unauthorized black bass introductions by anglers (Robbins and MacCrimmon 1974; Jackson 2002). Non-native introductions have artificially accelerated the rate of hybridization and introgression within *Micropterus*, which can negatively impact endemic species and alter biodiversity (Jackson 2002; Mallet 2005; Tanaka 2007).

Black bass hybridization has been well documented, involving nearly all species-pairs (Edwards 1979; Philipp et al. 1983; Whitmore 1983; Maciena et al. 1988; Morizot et al. 1991; Dunham et al. 1992; Gilliland 1992; Koppelman 1994; Forshage and Fries 1995; Gelwick et al. 1995; Pierce and Van Den Avye 1997; Pipas and Bulow 1998; Barwick et al. 2006; Alvarez et al. 2015; Barthel et al. 2015; Tringali et al. 2015). Allendorf et al. (2001) categorized hybrids by their origin and extent: (1) natural hybrid taxon, (2) natural introgression, (3) natural hybrid zone, (4) hybrid without introgression, (5) complete introgression, and (6) complete admixture. Hybrids within *Micropterus* tend to be types 4 and 5, with type 6 resulting in the loss of an endemic species (Koppelman 2015). Anthropogenic introductions can be especially problematic for endemic black bass species (particularly those species occupying small geographic ranges)

since they have weak reproductive barriers and lack refuge from introgression (Bangs et al. 2017).

Guadalupe Bass are confined to the state of Texas where non-native Smallmouth Bass were intentionally stocked in order to promote recreational angling (Edwards 1979). Whitmore and Butler (1982) determined that the Smallmouth Bass were hybridizing with Guadalupe Bass based on morphological and biochemical genetic analyses. The facilitated hybridization and introgression with Smallmouth Bass was one of the primary reasons that Guadalupe Bass were listed a species of special concern (Hubbs et al. 2008). For the Redeye species group, populations of Bartram's Bass that occur in the Savannah River are in danger of extirpation via hybridization with Alabama Bass, which were introduced into the system to enhance the black bass sport fishery (Barwick et al. 2006; Bangs et al. 2017). Another species of concern, Shoal Bass, are threatened by introgressive hybridization with introduced species such as Spotted Bass, Alabama Bass, and Smallmouth Bass (Alvarez et al. 2015; Dakin et al 2015; Tringali et al. 2015).

As a management action, many agencies rely on the removal of non-native species and hybrids in order to help sustain endemic populations. Unfortunately within *Micropterus*, the extent of interspecific introgression is often underestimated due to similar morphological and genetic similarities that hinder detection (Koppelman 2015). While phenotypic characteristics can be used with some degree of accuracy to identify black bass species and recent hybrids (i.e., first filial generation), this method is unable to quantify individual- and population-level introgression (Allendorf et al. 2001; Koppelman 2015). Assessing the purity, hybridization, and extent of introgression within black bass species is only possible using molecular techniques. These techniques can be beneficial for optimizing the identification of individuals, enhancing

stocks, and preserving genetic diversity (Dinesh et al. 1993; Garcia and Benzie, 1995; Tassanakajon et al. 1997, 1998).

III. Molecular Marker Tools and Approaches for Black Basses

Initially, morphometric and meristic techniques were used to differentiate between *Micropterus* species (Bailey and Hubbs 1949), but, as discussed, these methods alone are unreliable for analyzing integrade individuals and populations. This led to the development of molecular methods to assess purity and hybridization among the genus. Within this section I will review existing knowledge on molecular marker tools for black bass species including: a) allozyme markers; b) Mitochondrial DNA (mtDNA) markers; c) Random Amplified Polymorphic DNA (RAPD) markers; d) microsatellites; e) Single Nucleotide Polymorphism (SNP) markers; and f) DNA collection methods.

a) *Allozyme Markers*

One of the first genetic evaluations of the Largemouth Bass subspecies involved the use of allozymes (Philipp et al. 1983). The study evaluated Largemouth Bass from 90 populations across the United States and assessed genetic variation at 28 different enzyme loci; 16 of the 28 enzyme loci were found to be polymorphic at two loci, isocitrate dehydrase (Idh-B) and aspartate aminotransferase (Aat-B). The loci were used to determine the contributions of each subspecies to a gene pool, which led to the discovery that the integrade zone was much larger than previously proposed. Subsequently, Williamson et al. (1986) conducted a study validating the markers discovered by Philipp et al. (1983). In addition, they identified five more polymorphic markers that could be used to distinguish the subspecies. Aside from Largemouth Bass, allozyme

loci have also been used to examine introgressive hybridization in other *Micropterus* species. Whitmore (1983) used diagnostic allozyme loci to detect introgressive hybridization of Guadalupe Bass with non-native Smallmouth Bass in Texas. In Tennessee, allozyme loci were used to determine that introduced Redeye Bass were hybridized with native Smallmouth Bass (Turner et al. 1991). In addition, native populations of Smallmouth Bass in central Missouri were invaded by Spotted Bass and allozyme patterns revealed high hybrid proportions (Koppelman 1994). Pierce and Van Den Avyle (1997) used three allozyme loci to distinguish Spotted Bass, Smallmouth Bass, and their hybrids in Georgia and Alabama. Although alloyzmes can provide important genetic information on populations, the low number of diagnostic loci makes this method unreliable for assessing contributions to integrade individuals (Li et al. 2015).

b) Mitochondrial DNA (mtDNA) Analysis

Due to the advancement of technology in the 1990's, there was a shift from enzyme-based markers to DNA-based markers, including mitochondrial DNA (mtDNA) markers. mtDNA markers are valued for its uses in genetic analyses since it is highly stable, contains hundreds of copies, and does not recombinant (Richard and Paques 2000). Studies on black bass species using mtDNA sequences have been used to determine purity, construct phylogenetic trees, and monitor hybridization between species. One of the first DNA-based marker studies on black bass species was conducted using restriction fragment length polymorphism (RFLP) analysis of mtDNA to examine variation between the Largemouth Bass subspecies (Nedbal and Philipp 1994). The study found strong differentiation between the subspecies and that RFLP analysis provided better resolution than protein electrophoresis. In order to facilitate the identification of mtDNA in the Largemouth Bass subspecies, Bremer et al. (1998) developed a

rapid assay using PCR to amplify the mtDNA and a restriction enzyme digest to identify RFLP. Following that study, Kassler et al. (2002) used mtDNA sequence RFLP polymorphisms to determine that the two Largemouth Bass subspecies were distinct enough to be elevated to a species status. The study also evaluated the genetic relationships of the *Micropterus* taxa that were recognized at the time. RFLP analysis of mtDNA has also been used in phylogenetic studies to measure genetic characteristics and distances among black bass species (Johnson et al. 2001). Some of the drawbacks of RFLP analysis of mtDNA are that it can be costly, labor intensive, and takes up to a week to obtain results (Williams et al. 1998).

Aside from RFLP analysis, examples of mtDNA sequences commonly used in black bass studies are cytochrome b (cytb) and NADH dehydrogenase subunit 2 (ND2). These sequences have been used to examine the genetic structure of populations, construct phylogenetic trees, and monitor hybridization between species. Near et al. (2003) used a phylogenetic analysis of gene sequences from cytb and ND2 to estimate the ages of speciation events and rates of diversification in black basses. Freeman et al. (2015) used 20 diagnostic characteristics found in mtDNA ND2 gene sequences to distinguish members within the Shoal Bass clade from other black bass species. The genetic structure of Smallmouth Bass populations in the Arkansas River basin (Coughlin et al. 2003) and Lake Erie (Borden and Stepien 2006) were analyzed using mtDNA sequences and microsatellites. In addition, mtDNA markers have been used to investigate hybridization between species such as: Spotted Bass and Smallmouth Bass (Avise et al. 1997); Guadalupe Bass and Largemouth Bass (Near et al. 2003, 2005); Redeye Bass and Alabama Bass (Barwick et al. 2006); and Bartram's Bass with Alabama Bass and Smallmouth Bass (Leitner 2015). Recently, mtDNA sequences were used to separate Redeye Bass into five black bass species (Baker et al. 2013).

While mtDNA markers can provide better resolution than enzyme-based markers and is able to distinguish geographic populations, it is only maternally inherited and is unreliable for studying rates of hybridization between the species (Hurst and Jiggins 2005). Recently, there has been a rising concern in the incongruence between mtDNA and nuclear data used in species delineation (Chong et al. 2016). Common incongruences have been found in gene trees generated using mtDNA data compared to gene trees constructed using nuclear data (Sota and Vogler 2001; Wiens et al. 2010). These incongruences can stem from reasons such as gene duplication, hybridization between lineages, and incomplete lineage sorting (Doyle 1992; Degnan and Rosenberg 2009; Hobolth et al. 2011). In many instances, mtDNA and nDNA markers have been analyzed together to provide informative and congruent phylogenetic reconstructions of closely related species or populations (Reed and Sperling 1999; Caterino et al. 2001; Rubinoff and Sperling 2002).

c) Random Amplified Polymorphic DNA (RAPD)

The next DNA-based marker investigated for its uses in delineating species was random amplified polymorphic DNA (RAPD). RAPDs are generated using PCR to amplify genomic DNA with single primers of an arbitrary nucleotide sequence (Williams et al. 1990). This analysis can provide a quick and efficient screen for polymorphism at a high number of loci. In addition, it can be used to examine genetic variation without prior knowledge of the genome or genetic sequences of the species being investigated (Welsh and McClelland 1990; Williams et al. 1990; Fischer et al. 2000; Klinbunga et al. 2000). Williams et al. (1998) discovered 15 DNA markers using RAPD analysis that could be used to identify the Largemouth Bass subspecies and their intergrades. The increase in fixed loci or species-specific markers increased the ability to

detect hybridization and introgression (Campton 1987). Another advantage of the RAPD technique is noninvasive tissue sampling. Instead of sacrificing a fish for liver or muscle samples, tissue could be collected by fin clipping or gill arch puncture (Williams et al. 1998). One of the main disadvantages of using RAPD markers is that they are dominant, which results in a loci being scored based upon band presence or absence. The score may be interpreted incorrectly because whether or not band absence is from a lack of amplification or from problems with DNA quality cannot be distinguished (Kumari and Thakur 2014). Another disadvantage found in studies was that there was a lack of repeatability and inconsistencies found between lab members (Weeden et al. 1992; Penner et al. 1993; Skroch and Nienhuis 1995; Williams et al. 1998).

d) Microsatellites

Microsatellite markers offer an alternative to RFLP and RAPD since they are co-dominant and do not use maternally inherited DNA. Microsatellites are nucleotide tandem repeats located in the non-coding region of the genome (Hannan 2018). They offer a higher level of polymorphism and larger number of loci, which makes them ideal for analyzing hybrid populations (Jeffreys et al. 1994). The sampling method for microsatellites is minimally invasive and nonlethal, requiring only a fin clip as a source of DNA. Lutz-Carrillo et al. (2006; 2008) examined 11 and 52 microsatellite loci for DNA variation in Largemouth Bass. The microsatellite markers discovered were able to detect introgression within the sampled individuals and provided more accurate estimates of admixture proportions. Unfortunately, the methods used predated capillary gel electrophoresis making the markers unsuitable for PCR multiplexing due to various annealing temperatures and cycles (Seyoum et al. 2013). To

streamline the process, Seyoum et al. (2013) used a PCR-based isolation of microsatellite arrays (PIMA) to isolate 18 microsatellite loci for Largemouth Bass. The markers were cross amplified in seven other *Micropterus* species, which could provide use in monitoring hybridization, but there were no fixed allelic differences found between taxa (Seyoum et al. 2013). Malloy et al. (2000) isolated and characterized microsatellite loci for Smallmouth Bass and cross-amplified the markers in Spotted Bass. Microsatellite markers have been commonly used to monitor hybridization among *Micropterus* species such as: Smallmouth Bass and Guadalupe Bass (Littrell et al. 2007); and Shoal Bass with Alabama Bass, Smallmouth Bass, and Spotted Bass (Alvarez et al. 2015; Dakin et al. 2015; Tringali et al. 2015). While investigating Shoal Bass hybridization in the Chipola River, Florida, Tringali et al. (2015) discovered the Choctaw Bass using 17 microsatellite loci. Some of the drawbacks of using microsatellite markers are the higher cost and longer time frame associated with assays since they require several multiplexes as well as their restriction to non-coding regions of the genome.

e) Single Nucleotide Polymorphism (SNP) Markers

Recent advances in high throughput sequencing technology have provided fast and cost-effective methods to generate sequencing data (Stupar and Springer 2006; Hudson 2008). One of these methods is single nucleotide polymorphism (SNP) genotyping. SNPs are the most common form of genetic variation in individuals and are distributed throughout coding and non-coding regions of the genome (Hinds 2005; Danzmann et al. 2016). This holds importance when examining which traits are under selection during introgressive hybridization (Fitzpatrick et al. 2009; Shen et al. 2012). In addition, they are valued for their ease of multiplexing and low genotyping error rate for high throughput analyses (Slate et al. 2009; Pritchard et al. 2012). Li et

al. (2015) utilized RNA-sequencing (RNA-seq) to develop transcriptomes for Florida Bass and Largemouth Bass, and identified SNPs with fixed allelic differences between the species. From these SNPs, they developed a subset of 25 into a diagnostic multiplex assay to assess Largemouth Bass purity and hybridization. Recently, Zhao et al. (2018) validated 38 additional SNP markers using the same methods and reference samples as Li et al. (2015). These SNP panels have been proven to be powerful tools for assessing purity and hybridization for hatchery and wild Largemouth Bass specimens.

While these resources can be used for Florida Bass and Largemouth Bass, more recent research has focused on developing SNP markers for other *Micropterus* species that are most threatened by anthropogenic introductions. Thongda et al. (2018, unpublished data) developed species-diagnostic SNP markers for black basses through initial genotype-by-sequencing (GBS), followed by validation in additional samples using two panels of 64 SNPs. The panels have been tested on more than 1,500 black bass, and have clearly delineated a majority of the species and their hybrids. The benefits of these panels are that they are cost effective, have a quick turnaround (~1 day), and are highly informative. The two panels developed can be a useful tool for black bass conservation and management, as discussed in Chapter III.

f) DNA Collection Methods

An important aspect of conducting genetic analyses in fish is acquiring a source of high quality DNA, which can be supplied from blood, liver, muscle, fin clips, barbels, mucosal cells, or buccal cells. The advancement in technology for genetic markers created a shift in genetic sampling techniques from destructive sampling, which involved sacrificing the fish, to nondestructive and noninvasive techniques. Before the development of the PCR, destructive

sampling was commonly used to acquire tissue samples for allozyme and mtDNA genetic analyses (Allendorf 2017). Nondestructive and less invasive techniques became more prevalent since many of the analyses involved valuable broodstock or endangered species. Whitmore et al. (1992) developed a minimally invasive protocol of extracting mtDNA from epithelial tissue on fish scales. They were able to sequence amplified segments from Largemouth Bass and Channel Catfish (*Ictalurus punctatus*), which revealed diagnostic allelic variations between the two species (Whitmore et al 1992).

A shift to DNA-based markers opened the door to one of the most common genetic collection techniques used in fishes, fin clipping. Fin clipping has been frequently used in RAPD, microsatellite, and SNP genetic analyses (Williams et al. 1998; Wasko et al. 2003; Lutz-Carrillo et al. 2006, 2008; Baird et al. 2008; Li et al. 2015). The benefits of this sampling method are that it is minimally invasive and the fin clips can be stored long-term in ethanol for repeated extractions. Conversely, this method does require laboratory materials such as scissors, forceps, and sterile vials for the ethanol. For fisheries biologist, this sampling method can become time consuming when attempting to sample large populations in the field. Studies on the effects of fin clipping have been conducted on hatchery fish (Armstrong 1949; Shetter 1951, 1952), fish in a lake system (Phinny and Mathews 1969; Nicola and Cordone, 1973; Mears and Hatch 1976), and streams (Saunders and Allen 1967; Weber and Wahle 1969). Most of these studies found no significant effect of fin clipping on fish, but some studies did find that the removal of fins impacted survival and growth (Saunders and Allen 1967; Weber and Wahle 1969; Nicola and Cordone 1973).

Alternatively, buccal swabbing is a non-invasive sampling method that requires no training, inexpensive materials, and minimal handling time of the fish. While common in

terrestrial species, to this date, there have been no studies of buccal swabbing in Largemouth Bass. Previous research on buccal swabbing in sunfish (*Lepomis*), involved the development a field sampling method to collect DNA suitable for PCR amplification and polymorphic analysis (Smalley and Campanella, 2005). This sampling method is rapid, simple, and particularly amenable to large sample sizes. Livia et al. (2006) described a DNA sampling method to sample and store high-quality DNA from body mucus and buccal cells in Northern Pike (*Esox lucius*) and Brown Trout (*Salmo trutta*). The method developed was simple, cost effective, and the samples could be stored for years at room temperature. Another study on Zebrafish (*Danio rerio*) and Three-spined Sticklebacks (*Gasterosteus aculeatus*) compared DNA samples from fin clipping and skin swabbing techniques (Breacker et al. 2017). They found that fin clip extractions produced higher concentrations of DNA, but that swab extractions consistently produced sufficient DNA concentrations suitable for PCR amplification.

g) Angler-Driven Sampling

Successful citizen science projects have been developed collecting DNA samples from tarpon (Guindon 2015), wolves (Granroth-Wilding et al. 2017), and grizzly bears (Sorensen et al. 2017). Guindon et al. (2015) reported that anglers throughout the coastal southeastern United States sent in a total of 24,572 DNA samples from tarpon. The project transitioned from fin clips to gape scrapes, skin cells sampled from the outer jaw. They found that the benefits of using citizen scientists are that they were able to sample fish statewide and collect DNA samples on fish that are difficult for biologists to catch in great numbers. Fin clipping, as discussed above, can have drawbacks for angler-driven sampling, as most anglers do not have ethanol readily

available and standardizing fin clip size and location can be problematic. Additionally, fin clips (while usually found to be harmless) can be perceived by the public to be unduly injurious.

Currently, most of the genetic sampling for black bass species is conducted by state and federal agencies using electrofishing methods. Recent genotyping of Largemouth Bass in Lake Guntersville, Alabama has revealed that trophy-sized largemouth bass (>2268g) targeted by tournament anglers represent a genotype and size class that significantly differs from Largemouth Bass sampled by electrofishing. Tournament specimens were found to have significantly higher Florida Bass alleles and heterozygosity (Gowan 2015). Due to the relatively rare nature of these specimens, angler-driven sampling is beneficial because biologists may not be able to easily collect fish using electrofishing methods. In addition, angler-driven genetic sampling can be used to help monitor purity and hybridization of rare black bass species with restricted ranges and help raise public awareness about current issues regarding black bass conservation.

IV. Chapter Overviews

Chapter Two

Here, I investigated the use of buccal swabbing of Largemouth Bass as a method to obtain a DNA sample and developed a protocol that could be employed by anglers. To do so, I determined the most favorable swab duration and location on Largemouth Bass with regard to the amount of DNA extracted, DNA purity, and the SNP genotyping accuracy of this method compared to fin clipping. In addition, I evaluated swab kit storage parameters such as: storage duration, storage temperature, and adverse conditions. To roll out an angler-driven component, I developed an angler-driven genetic sampling program, which supplied anglers with a swab kit

that can be used to sample DNA from trophy Largemouth Bass and sent back to our lab for SNP analysis. Aside from trophy bass, the protocol established can be used for other important specimens such as brood fish and threatened or endangered species.

Chapter Three

I also explored the use of species-specific diagnostic SNP markers on black bass species in the Altamaha River Basin (ARB), including the Altamaha, Oconee, and Ocmulgee rivers. The Georgia Department of Natural Resources (GADNR) has dedicated an immense amount of effort into managing black bass populations across the state of Georgia. In this study, I analyzed black bass samples collected by the GADNR, which were comprised of Largemouth Bass, Spotted Bass, and Shoal Bass samples. The samples were extracted for DNA and genotyped using two panels of 64 SNP markers developed by Thongda et al. (2018, unpublished data). The goal of this study was to analyze patterns of purity and hybridization in the ARB to assist the GADNR in making informed decisions when it comes to the conservation and management of black bass species.

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Chapter II: Development of a Swab Protocol for an Angler-Driven Program to Promote the Genetic Assessment of Black Bass Populations

Abstract

Black basses (*Micropterus* spp.) represent some of the most highly sought after game fishes in North America. Efforts to improve recreational bass fisheries have led to the widespread stocking of black bass species, often facilitating introgressive hybridization between endemic and non-native species. Currently, most genetic sampling aimed at monitoring black bass populations is conducted using fin clips stored in ethanol. In order to expand the collection of DNA samples to hard to obtain specimens or subpopulations, I have established an angler-driven protocol that includes a minimally invasive buccal swab kit and room temperature storage within a breathable sleeve. Here, I tested duration of swabbing (3, 5, 10 seconds), swab location (tongue, cheek), holding temperature (23°C, 35°C), storage variability (21-54°C), and storage duration (1 week, 1 month, 4 months) in order to determine the best methodology for downstream DNA extraction and SNP genotyping on black bass. I also developed a rapid and inexpensive DNA extraction method to be used on buccal swab and fin clip samples. The results from this study indicate minimal to no effect of swab location, swab duration, storage temperature, or storage duration on DNA concentration, DNA purity, and SNP genotyping accuracy when compared to fin clip DNA. I present here a field-tested swab sampling protocol suitable for applications that require engaging the angling public in genetic sample collection.

Introduction

Black basses (*Micropterus spp.*) encompass a large, and growing, genus of fishes endemic to North America (Near et al. 2003). Their popularity in recreational fishing and their widespread stocking over the last 100 years, has led many to overlook their importance to freshwater ecosystems and their surprising diversity (Birdsong et al. 2015). More careful study, utilizing molecular and meristic measures, over the last decade has increased our appreciation for micropterid species with restricted geographic ranges and differing life histories when compared to the well-studied Largemouth Bass (Long et al. 2015). Many of these same species are of increasing conservation concern due to habitat degradation and non-native introductions (Littrell 2007; Bangs et al. 2017; Taylor 2017), but are also increasingly recognized as desirable game fishes by recreational anglers.

Recreational fishing continues to be driven by Largemouth Bass, with tournament fishing growing in popularity and economic importance (Chen et al. 2003). State conservation/fisheries agencies in the southern U.S., responding to requests from the angling public, often stock Florida Bass (*Micropterus floridanus*) in public reservoirs, in an attempt to enhance trophy bass productivity (Maceina and Murphy 1992). The resulting populations contain native Northern Largemouth Bass (*Micropterus salmoides*) or intergrade bass, stocked Florida Bass, F1s, and backcrosses. Agencies need to assess the relative impacts of each of these groups of fish on angler success in order to evaluate the costs and benefits of stocking programs, which necessitates genetic analyses (Seyoum et al. 2013; Li et al. 2015). Previous research by our group has indicated that standardized electrofishing surveys may be biased toward smaller fish with a higher percentage of Largemouth Bass alleles (Gowan 2015). Conversely, tournament anglers

appear to be successfully targeting numerically smaller populations of larger fish with higher Florida Bass contributions in order to increase bag weights.

Involving anglers in genetic sample collection from black basses would, therefore, allow greater access to species that live in hard-to-sample habitats (e.g. Redeye Bass, Shoal Bass) as well as capturing a more representative sample of Largemouth Bass (Florida and Northern) for management assessments. The involvement of the angling public necessitates closer consideration of sampling methodologies. Fin clipping is by far the most common method employed by biologists in the field (Ryba et al. 2008; Sanderson et al. 2009; Peterson and Lutz-Carrillo 2017; Stepien et al. 2017), however, it has several drawbacks when extended to the public. First, the technique may be viewed as causing pain, disturbance, or stress. Second, an angler must be supplied with vials containing alcohol and given specifications on the size of sample to be obtained, fin location(s), proper rinsing of tools (which may also need to be supplied), and proper storage and shipment of vials. Instructions and collection supplies need to remain close at hand with the angler for an indefinite period until a specimen is encountered. By contrast, the general public is increasingly familiar and comfortable with cheek swab techniques used for obtaining human genetic samples (either for medical testing or ancestry-type research; Richards et al. 1993; Meulenbelt et al. 1996; Rogers et al. 2007). The technique is minimally invasive, rapid, and causes no pain. In fish, relatively few studies have examined buccal swabbing (Smalley and Campanella 2005; Campanella and Smalley 2006; Livia et al. 2006; Reid et al. 2012; Colussi et al. 2017).

The aim of my study was to develop and optimize a buccal swabbing protocol for Largemouth Bass, including consideration of swabbing location, duration, and storage parameters, to ultimately enable angler involvement in genetic sampling of black basses.

Materials and Methods

Sample Collection

Initial evaluation of commercially available swabs in 2015 included the Puritan Cap-Shure (Guilford, ME), EpiCentre Catch All (Madison, WI—now discontinued), Omni Swab (Sigma Aldrich, St. Louis, MO), and Puritan Histobrush (Guilford, ME). Highest DNA yields were consistently obtained with the Histobrush (Gowan 2015, unpublished data). The Histobrush was therefore selected for studies described below.

In order to test for differences in results depending on swab duration and location, Largemouth Bass (n= 72) were collected from Lee County Lake, Alabama using a boat-mounted electrofisher in August 2016. Largemouth Bass were sampled intermittently throughout the day and stored in a live well. Individual Largemouth Bass were swabbed using a sterile Puritan Histobrush on the tongue or cheek for 3 (n= 12), 5 (n= 12), and 10 (n= 12) seconds and the swab samples were stored in a Fitzco DryPak Swab Sleeve (Fitzco, Minneapolis, MN). In addition, small pelvic fin clips were obtained from each individual and stored in sterile vials filled with 95% ethanol. The samples were brought back to the lab and stored for one week at room temperature prior to DNA extraction, DNA quantification, and SNP genotyping.

To test for differences in storage temperature, a separate set of Largemouth Bass (n = 25) were collected using similar methods described above from a private pond in Auburn, AL in October 2016. Individual Largemouth Bass were swabbed with two separate Puritan Histobrushes on the tongue for 10 s and were again stored in Fitzco DryPaks. One set of the swabs (n= 25) was stored at room temperature (23°C), while the second set (n= 25) was stored in a New Brunswick Innova 4000 incubator at 35°C for one week. Small pelvic fin clips were taken

from each individual and were stored in sterile vials filled with 95% ethanol. After a week, the swab samples and fin clips were extracted for DNA and SNP genotyped.

To understand the effect of storage duration at room temperature, Largemouth Bass (n= 60) were netted from tanks at American Sport Fish Hatchery, Montgomery, AL in July 2017. Individual Largemouth Bass were swabbed on the tongue for 10 s using a Histobrush and the swabs were stored in Fitzco DryPaks. One set of swabs (n= 20) was stored at room temperature for one week, the second set (n= 20) was stored for 1 month, and the third set (n= 20) was stored for 4 months prior to DNA extraction and SNP genotyping. In addition, small pelvic fin clips were obtained from each individual and stored in 95% ethanol at room temperature for one week prior to DNA extraction and SNP genotyping.

To test the effectiveness of storing swab samples in a varying, humid temperature environment, Largemouth Bass (n=34) were netted from tanks at the Aquatic Animal Health Research Unit, Auburn, Alabama in August 2017. Individual Largemouth Bass were swabbed for 10 s using a Histobrush and the swabs were stored in Fitzco DryPaks. Small pelvic fin clip samples were taken from each individual and stored in 95% ethanol. The swab samples were stored on a car dashboard for one week in August 2017. The temperature was recorded hourly using a HOBO Water Temperature Pro v2 Data Logger. After one week, the swab and fin clip samples were extracted for DNA and SNP genotyped.

DNA Extraction & SNP Genotyping

DNA samples from fin clips and swabs were extracted using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) following the manufacturer's protocol. DNA concentrations (ng/ul) and DNA purity ratios (260/230, 260/280) were estimated using a NanoDrop ND-2000

UV-VIS Spectrophotometer. The DNA samples were then SNP genotyped using a 38-plex Florida/Northern Largemouth Bass (FLNB) panels (Zhao et al 2018) on the Agena MassARRAY iPLEX platform following the manufacturer's protocol (Agena Bioscience® Inc., San Diego, CA).

To prepare for SNP genotyping, each DNA sample was diluted in a 96-well plate to the desired concentration of 20 ng/µl using high performance liquid chromatography (HPLC) grade water. Using a multichannel pipette, 2 µl of the diluted DNA samples was placed onto a new 96-well plate and prepared for three rounds of polymerase chain reactions (PCR). The first PCR amplifies a specific fragment of genomic DNA using designed primers and the iPLEX Gold Reagent Kit according to the manufacturer's protocol (Gabriel et al. 2009). The conditions of the first PCR included the following parameters: pre-denature at 94°C for 2 min, 45 cycles of denaturation of 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The second PCR utilized shrimp alkaline phosphatase (SAP) in order to remove remaining, nonincorperated dNTPS from amplification products. The conditions for the second PCR included: enzyme activation at 37°C for 40 min, and enzyme degradation at 85°C for 5 min. The last PCR was the primer extension, designed to extend the primer by one mass-modified nucleotide depending on the allele and assay design (Gabriel et al. 2009). The conditions of the third PCR included: pre-denature at 94°C for 30 s, 45 cycles of denaturation at 94°C for 5 s, annealing at 52°C for 5 s, extension at 80°C for 5 s, and final extension at 72°C for 5 min.

Once the plate finished the PCR processes, 41 µl of HPLC grade water was added to each well using a multi-channel pipette. Then, SpectroCLEAN resin was added to the wells to remove salts such as sodium, potassium, and magnesium ions. The plate was rotated at 360° for 20 min

and then spun down in a centrifuge at 2000 g for 5 min. The Agena MassARRAY Nanodispenser was used to transfer the samples from the plate on to a silica chip using a capillary action of slotted pins and contact dispersing for nanovolumes (Gabriel et al. 2009). Once the transfer was complete, the chip was placed into the MassARRAY compact mass spectrometer. Each sample was shot with a laser under vacuum by the matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) method. The SNP genotypes were called in the SEQUENOM SYSTEM TYPER 4.0 Analysis software. A final genotype was called and placed into a category based on the significance of the allele (e.g. conservative, moderate, aggressive, user call). All individuals had call rates >90% (>35/38 SNPs). A schematic representation of the SNP genotype reaction including the amplification, SAP treatment, iPLEX reaction, and the MALDI-TOF mass spectrometry analysis is presented in **Figure 3**.

DNA Digestion Protocol

In order to reduce the time and cost of DNA extractions, a new protocol was developed using a digestion of sodium hydroxide (NaOH) and hydrochloric acid (HCl). To test this protocol, swabs (n=100) and fin clips (n=100) were extracted using the digestion mix. Fin clips that were stored in 95% ethanol were subsampled for DNA extraction. The subsamples were dried using Kim wipes and placed in sterile 2 mL centrifuge tubes. For the swab samples, the end of the Histobrush was cut off and placed in the 2 mL centrifuge tubes for extraction. 100 µl of NaOH was added to each sample, the tubes were vortexed, and placed in an incubator at 95°C for 30 min to 1 hr. Once the fin clips were fully broken down, the samples were placed on ice for 5 min. After, 100 µl of HCl was added to each sample, the tubes were vortexed, and placed in a centrifuge at 8,000 rpm for 1 min. Once finished, the DNA concentrations (ng/ul) and purity

ratios (260/230, 260/280) were estimated for each sample using the NanoDrop ND-2000 UV-VIS Spectrophotometer. The samples were sequenced on the 38-plex FLNB SNP panels using the Agena MassARRAY analyzer, as previously described (Zhao et al. 2018).

Genotype Analysis

The final Largemouth Bass genotypes were analyzed in RStudio (version 1.0.136) and compared to a reference genotype of a pure Florida Bass (FLMB) and a pure Northern Largemouth Bass (NLMB) (Table 1; Zhao et al. 2018). For each genotype, the FLMB allele frequency, the NLMB allele frequency, heterozygous (HE) allele frequency, and homozygous allele frequency were computed using the following formulas:

FLMB Allele Frequency =((FLMB SNPs)+(1/2 * HE SNPs)/ Total Scored SNPs)

NLMB Allele Frequency =((NLMB SNPs)+(1/2 * HE SNPs)/ Total Scored SNPs)

Heterozygous Allele Frequency =((HE SNPs)/ Total Scored SNPs)

Homozygous Allele Frequency =((FLMB SNPs + NLMB SNPs)/ Total Scored SNPs)

The frequencies were used to calculate the percentages of FLMB, NLMB, heterozygosity, and homozygosity for each individual by multiplying the frequencies by 100%.

Comparison of Swab and Fin Clip Genotypes

In order to confirm that the swab sampling method was producing the same genotype results as the fin clip sampling method, the individual genotypes of fin clips were compared allele by allele to the same individual's swab genotypes. The comparison between the genotypes produced a percentage of genotype match, 100% indicating that there were no differences among

the genotyped markers with both DNA sources. The genotype match percentages for each sample were calculated in RStudio.

Statistical Analysis

For swab duration and location, an analysis of variance (ANOVA) was performed in RStudio to test for significant differences between each combination of swab duration and location for DNA concentration (ng/µl) and genotype match (%). A p-value cut-off of less than 0.05 was required to reject the null hypothesis and infer significance. Following the ANOVA, a Tukey HSD was performed to determine which swab duration and location combinations were different. For swab temperature testing, a Welch two-sample t-test in RStudio was performed to test for a significant difference between storing at room temperature and storing in an incubator at 35°C for DNA concentration and genotype match. For swab storage testing, an ANOVA was performed in RStudio to test for significant differences between storage for one week, one month, and four months for DNA concentration and genotype match. Following the ANOVA, a Tukey HSD was performed to analyze the differences among the storage durations.

Angler Testing

To test results obtained from angler-collected swabs, kits (n=150) were distributed by land and boat to anglers at Lake Eufaula, Alabama and Lake Martin, Alabama. Swab kits (**Figure 4**) were composed of a Histobrush, DryPak, instruction card, and prepaid envelope to send the swab kit back to the Auburn University Aquatic Genetics and Genomics Laboratory for analysis. Anglers were instructed to swab a Largemouth Bass that weighed 1,814g or more. The swab kits were either collected from the anglers or sent directly by the angler to the lab after use.

Upon receipt, the samples were extracted for DNA, measured for DNA concentration, and SNP genotyped. For the swab packs, I analyzed the SNP call rate as the percentage of alleles that were called during the genotyping process. 38 out of 38 SNPs indicates a 100% SNP call rate.

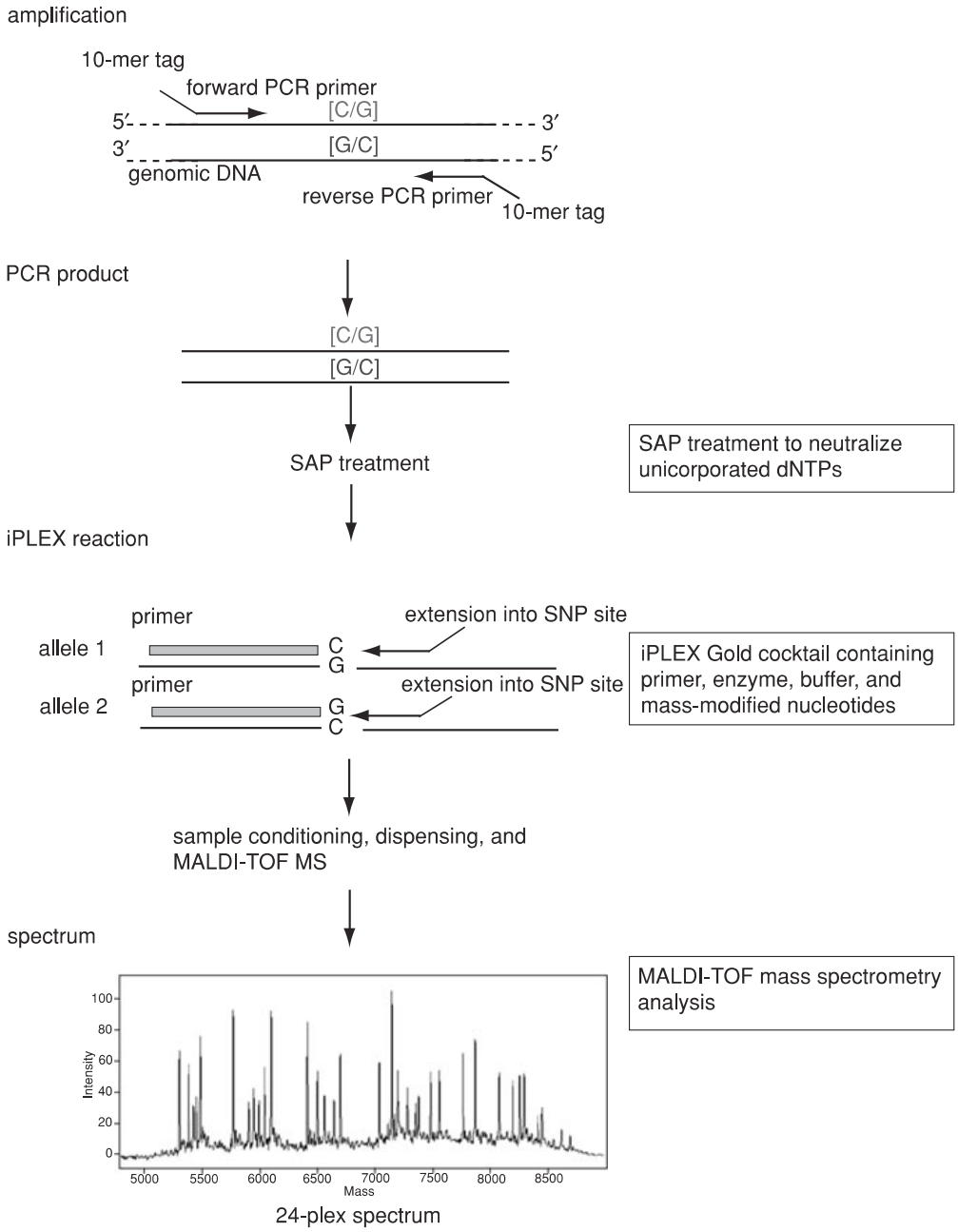

Figure 3 Schematic of the SNP genotype reaction including: amplification, SAP treatment, iPLEX reaction, and the MALDI-TOF mass spectrometry analysis (Adapted from Gabriel et al. 2009).

Table 1 List of the 38 SNP markers with fixed allelic differences used to determine purity and hybridization of FLMB and NLMB. The reference genotypes displayed are those of a pure FLMB (*M. floridanus*), pure NLMB (*M. salmoides*), and pure F1 Hybrid.

Markers	**FLMB**	**NLMB**	**F1**
X2FLContig12388	T	A	TA
X2FLContig124	T	C	TC
X2FLContig132	G	A	GA
X2FLContig18667	G	A	GA
X2FLContig19961	T	C	TC
X2FLContig2242	T	C	TC
X2FLContig2279	T	G	TG
X2FLContig2283	T	G	TG
X2FLContig2861	A	G	AG
X2FLContig288	A	T	AT
X2FLContig31979	T	A	TA
X2FLContig3379	G	A	GA
X2FLContig36172	T	C	TC
X2FLContig4936	C	T	CT
X2FLContig5713	A	G	AG
X2FLContig692	C	T	CT
X2FLContig9758	G	T	GT
X2FLContig987	G	A	GA
X2NBContig8717	T	C	TC
FLContig11272	T	C	TC
FLContig1595	T	C	TC
FLContig16665	A	C	AC
FLContig17151	A	T	AT
FLContig1811	C	A	CA
FLContig1826	T	A	TA
FLContig298	A	G	AG
FLContig21621	G	A	GA
FLContig21676	G	A	GA
FLContig21917	C	T	CT
FLContig238	G	T	GT
FLContig23633	C	T	CT
FLContig2635	A	G	AG
FLContig3296	T	G	TG
FLContig3616	A	G	AG
FLContig4773	C	T	CT
FLContig4919	T	G	TG
FLContig6127	C	G	CG
NBContig12358	G	A	GA
Fixed Alleles	**38**	**38**	**38**

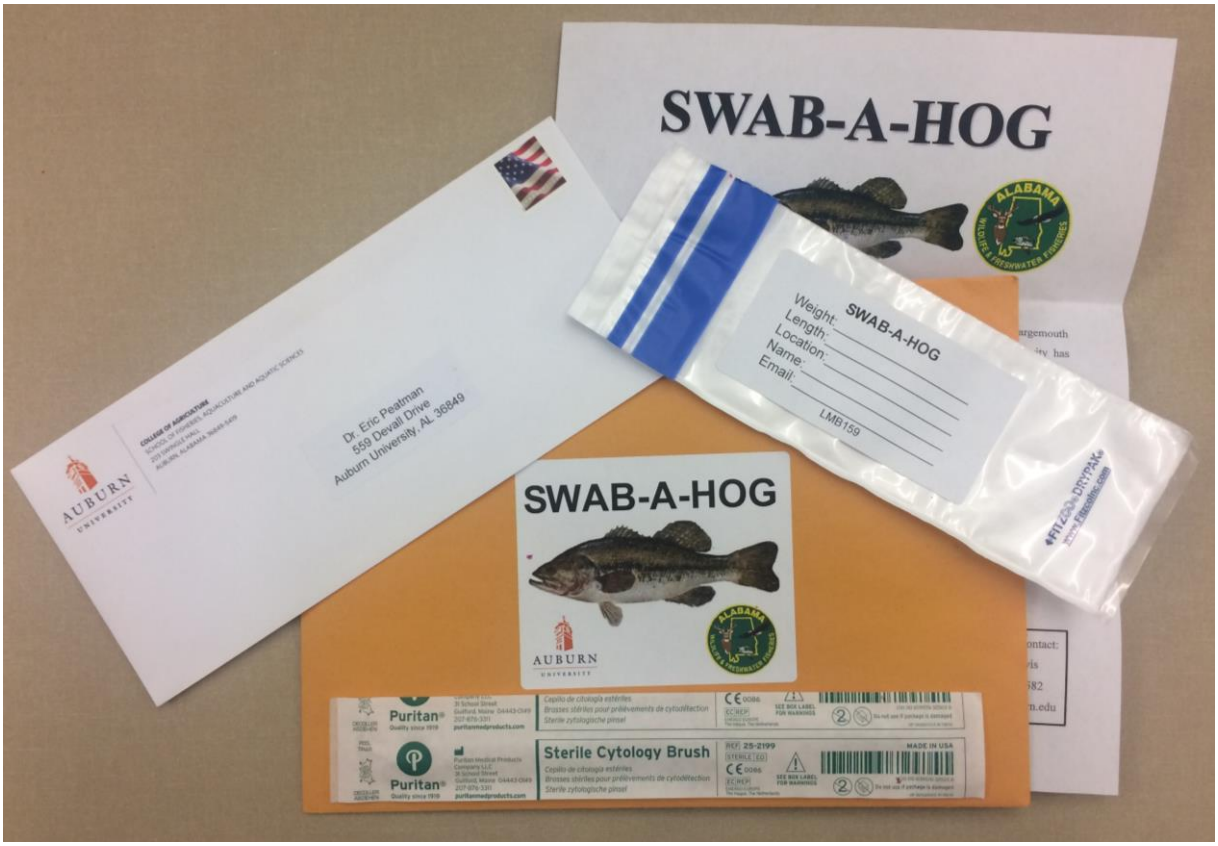

Figure 4 Swab pack composed of a Puritan Histobrush, Fitzco Drypak, instruction card, and prepaid envelope.

Results

Swab Location and Duration Analysis

We first analyzed the effect of swab location and swab duration, comparing DNA yield and SNP genotyping results from swabbing either the tongue or the cheek of the Largemouth Bass. The analysis of swab duration on the tongue, the summary of means (±SD) for DNA concentration, DNA purity ratios (260/280, 260/230), and genotype match percentages are presented in **Table 2**. There was a significant difference ($p < 0.05$) found between DNA concentration for 10 s swabs compared to 3 and 5 s swabs. For the DNA purity ratios, a 260/280 ratio of ~1.8 and a 260/230 ratio of ~2.0-2.2 is generally accepted as "pure" DNA. Significantly different purity ratios may indicate the presence of protein, phenol or other contaminants (Desjardins & Conklin 2010). For the 260/280 and 260/230 purity ratios, there were no significant differences between 3, 5, and 10 s swabs. Also, the 260/230 ratios were slightly higher than what is considered pure, but typically a low 260/230 ratio is characteristic of a contamination. We compared genotypes obtained from the swabs with that obtained from the matched fin clip ("genotype match" percentage). For genotype match, there were no significant differences found between any of the durations on the tongue, indicating that any of the swab durations on the tongue can provide adequate material for DNA extraction and SNP genotyping.

The analysis of swab duration on the cheek, the summary of means (±SD) for DNA concentration, DNA purity ratios, and genotype match percentages are also presented in **Table 2**. For DNA concentration, there was an increase in DNA yield with increasing duration, but there was no significant difference found between the treatments. For the DNA purity ratios, there was a significant difference in the 260/230 ratios between 3 s swabs when compared to 5 s and 10 s

swabs ($p < 0.05$). For the genotype match percentages, there were no significant differences found, but there was a larger error rate observed in the 3 s cheek swabs (1.32%).

Table 2 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280, 260/230) and genotype match between swabs and fin clips (%) from 3, 5, 10 second swabs on the tongue and cheek. Superscripts of letters denote significant differences ($p < 0.05$) within each swab location.

Swab Location	Swab Duration (seconds)	N	DNA Concentration (ng/µl)	260/280	260/230	Genotype Match (%)
Tongue	3	12	58.72 ± 44.36[a]	2.02 ± 0.07[a]	2.48 ± 0.49[a]	99.34 ± 1.19[a]
	5	12	63.32 ± 36.38[a]	2.01 ± 0.06[a]	2.78 ± 0.38[a]	99.78 ± 0.76[a]
	10	12	106.13 ± 37.48[b]	2.05 ± 0.03[a]	2.47 ± 0.11[a]	99.56 ± 1.02[a]
Cheek	3	12	69.24 ± 39.35[a]	2.04 ± 0.05[a]	2.59 ± 0.32[b]	98.68 ± 2.38[a]
	5	12	74.94 ± 22.32[a]	2.06 ± 0.03[a]	2.27 ± 0.17[a]	99.12 ± 1.71[a]
	10	12	101.32 ± 90.65[a]	2.03 ± 0.05[a]	2.17 ± 0.26[a]	99.34 ± 1.19[a]

In an overall comparison of the tongue (n= 36) to the cheek (n= 36), there were no significant differences for DNA concentration and the 260/280 DNA purity ratios. There was a significant difference ($p < 0.05$) found for the 260/230 DNA purity ratios with an overall average of 2.38 ± 0.30 for the cheek compared to an average of 2.58 ± 0.38 for the tongue. For genotype match percentages, the overall average for the cheek was 99.05% ± 1.80 and the tongue was 99.56% ± 0.99. The small error rate that was observed (<1%) was a result of the SEQUENOM SYSTEM TYPER 4.0 Analysis software, which calls the SNP genotypes and places them into a category based on the significance of the allele (e.g. conservative, moderate, aggressive, user call). The observed error rate was typically a result of a homozygous allele "T" being categorized as a heterozygous allele "AT" or vice versa (**Table 3**). Instead of this resulting from a problem in DNA quality or contamination since most of the alleles were categorized as "conservative", it

stemmed from the software improperly labeling an allele (**Figure 5**). Fortunately, this problem can be corrected manually in the software, but, for the purposes of this study, the alleles were left unchanged to observe any problems during the genotyping process for swab samples.

Table 3 Genotype results from six markers for a FLMB and NLMB to compare the results of an individual fin clip and swab genotype results. For marker FLContig17151, the allele is heterozygous "AT" for the fin clip, but is a homozygous "T" for the swab. This resulted in a small genotyping error rate (<1%) due to the Genotyper software that categorizes the alleles.

Genotype Results	FLContig 16665	FLContig 17151	FLContig 1811	FLContig 1826	FLContig 298	FLContig 21621
FLMB Genotype	A	A	C	T	A	G
NLMB Genotype	C	T	A	A	G	A
Fin Clip Genotype	CA	AT	C	TA	A	AG
Swab Genotype	CA	T	C	TA	A	AG

Results of this portion of the study revealed little differences between tongue and cheek locations; therefore the tongue was selected for the rest of the study because the cheek is close to the gills, which could be irritated during DNA sampling of small fish specimens. For swab duration, 10 s was used to optimize DNA concentrations for extraction and SNP genotyping. However, it should be noted, our results indicate that the 3 and 5 s duration should also provide sufficient cell harvest, adequate DNA, and accurate genotyping.

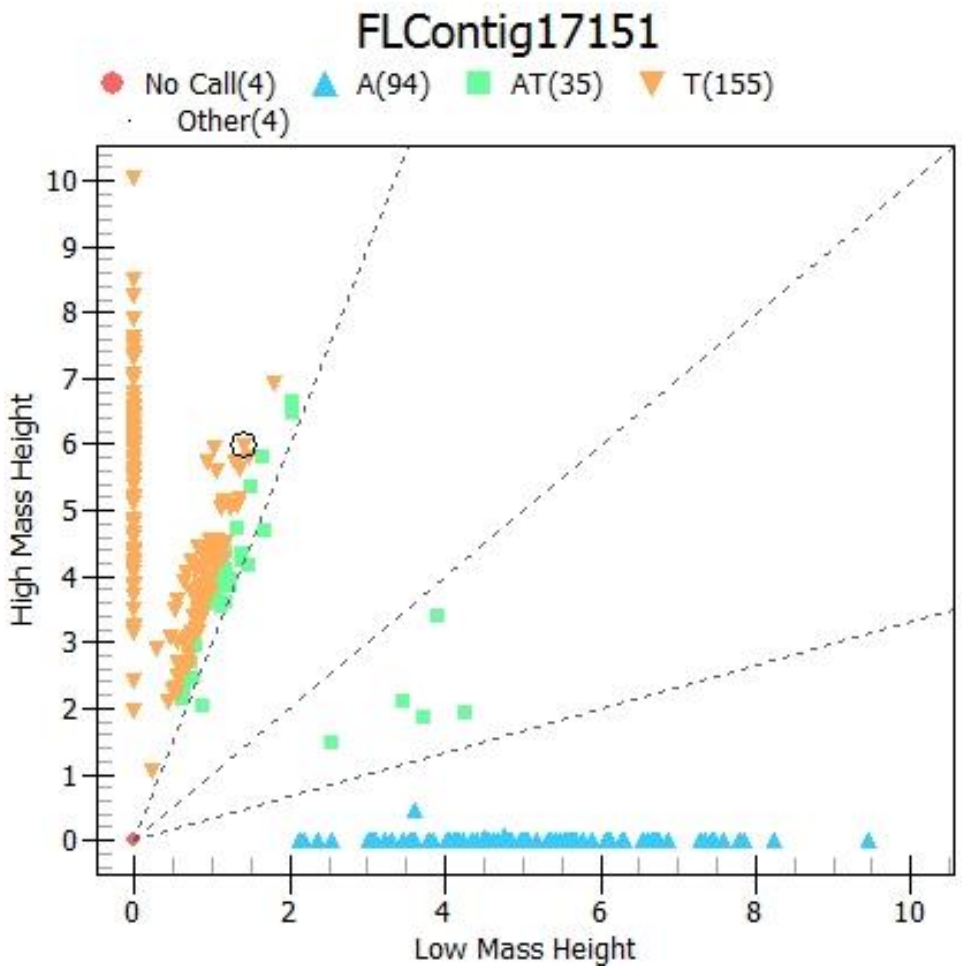

Figure 5 Call cluster plot for marker FLContig17151, which clusters the alleles ("A", "AT", "T") based on high and low mass height. The plot shows close differentiation between "AT" and "T" alleles, which can impact the genotype results.

Swab Storage Temperature Analysis

We next asked whether storage of the swab samples (inside the DryPak) at an elevated temperature would impact DNA degradation and eventual genotyping success. Given angler involvement, our method needed to be robust in situations where samples may not be immediately returned to the lab. Samples were obtained by swabbing the tongue for 10 s. The summary of means (±SD) for DNA concentrations, DNA purity ratios, and genotype match for swab storage at room temperature and in an incubator at 35°C for 1 wk are presented in **Table 4**. There was no significant difference between swab storage at room temperature and storage at 35°C in terms of DNA concentration, purity, or genotype match to fin clip samples, indicating that the swab samples could tolerate high temperatures prior to extraction and genotyping.

Table 4 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280; 260/230), and genotype match (%) from swabs stored at room temperature and in an incubator for one week. Means were similar between temperatures for each variable.

Storage Temperature	N	DNA Concentration (ng/µl)	260/280	260/230	Genotype Match (%)
Room Temp. (23°C)	25	142.25 ± 21.66	1.75 ± 0.25	1.87 ± 0.40	99.36 ± 1.15
Incubator (35°C)	25	135.80 ± 25.61	1.85 ± 0.17	1.94 ± 0.32	99.36 ± 1.15

Swab Storage Duration Analysis

We next examined whether longer storage durations, as potentially encountered during storage in the lab, would impact eventual extraction and genotyping success and accuracy. The summary of means (±SD) for DNA concentration, purity ratios, and genotype match are presented in **Table 5.** Storage duration at room temperature had no significant impact on DNA concentration, purity, or genotype match. Genotype match did trend downward for 4 mo samples (98.95 vs. >99% for shorter storage). However, the elevated error resulted from genotyper calls that failed to differentiate a single homozygous vs. heterozygous allele.

Table 5 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280; 260/230), and genotype match (%) from swabs stored at room temperature for one week, one month, and four months. Means were similar between storage duration for each variable.

Storage Duration	N	DNA Concentration (ng/µl)	260/280	260/230	Genotype Match (%)
1 week	20	139.48 ± 60.95	2.02 ± 0.15	2.27 ± 0.16	99.61 ± 0.96
1 month	20	171.72 ± 79.82	2.04 ± 0.03	2.15 ± 0.22	99.03 ± 1.57
4 months	20	162.19 ± 68.94	2.05 ± 0.05	2.32 ± 0.11	98.95 ± 1.52

Swab Storage High/Variable Temperature Analysis

We also simulated a scenario where a swab was left in an angler's vehicle prior to being mailed back to the lab. We were interested whether swabs could tolerate this humid/variable environment. Swabs were left in a vehicle on the dashboard for one week in summer prior being returned to the lab for extraction. The summary of means for the DNA concentration, purity ratios, and genotype match percentages are presented in **Table 6**. Temperatures were recorded daily using a HOBO data logger (**Figure 6**), also placed on the dashboard, with observed temperatures ranging from 21°C to 56°C. After one week, DNA concentration and purity were sufficient and genotype match rate high, indicating that swab samples are highly tolerant of storage in harsh and variable environments.

Table 6 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280, 260/230) and genotype match (%) from swabs (n=34) stored in a car for one week with temperatures ranging from 21-56 °C.

Swab Location	Temperature	N	DNA Concentration (ng/µl)	260/280	260/230	Genotype Match (%)
Car	21-56°C	34	107.25 ± 25.68	2.04 ± 0.07	2.59 ± 0.32	99.65 ± 1.18

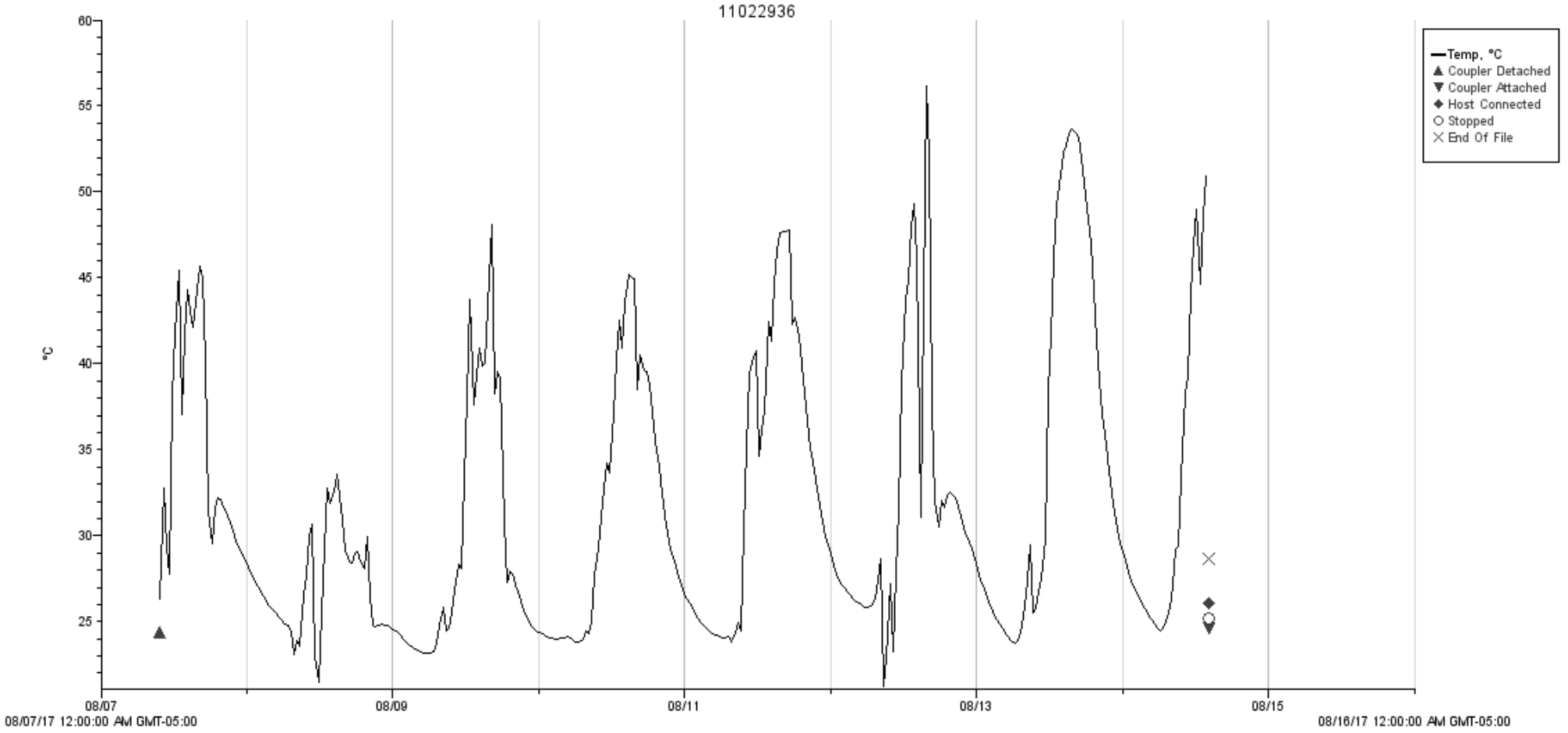

Figure 6 Graph of temperatures (°C) recorded daily from the HOBO Water Temperature Pro v2 Data Logger during the week that swab samples (n=34) were stored on a vehicle dashboard.

Angler Distribution Testing

To verify that similar results could be obtained from angler-collected swab samples, swab packs were distributed to anglers at Lake Martin, Alabama and Lake Eufaula, Alabama. Swabs were either collected subsequently in person or sent by the angler via standard mail to the lab for analysis. The summary of means (±SD) for DNA concentration, DNA purity ratio, and the SNP call rate are presented in **Table 7**. Most samples were sent to the lab or collected within one to two weeks after DNA sampling and all 56 samples showed high DNA concentrations with pure DNA ratios. The SNP call rate was very high (99.82%) from the samples indicating that the general angling public had no issues utilizing the swab protocol effectively.

Table 7 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280; 260/230), and SNP call rate (%) from swabs collected from anglers at Lake Martin and Lake Eufaula in Alabama.

DNA Collector	N	DNA Concentration (ng/µl)	260/280	260/230	SNP Call Rate (%)
Anglers	56	143.86 ± 53.28	2.08 ± 0.12	2.43 ± 0.25	99.82 ± 1.26

Performance with Crude DNA Extraction

DNA extractions can represent a significant cost component of genotyping, particularly if commercial kits (e.g. Qiagen) are used. In order to minimize costs of an angler-driven genetics program, it is desirable to develop least-cost protocols. Accordingly, we examined whether a low cost crude DNA extraction using NaOH/HCl could be adapted for both fin clips and swabs. The summary of means (±SD) for DNA concentration and DNA purity ratios from fin clip (n=100) and swab samples (n=100) that were extracted using the simplified digestion protocol (see Methods) are presented in **Table 8**. While the DNA purity ratios for the crude extraction method were lower than those typically obtained with the Qiagen DNeasy kit (compare with **Table 2**, for example), this method provided sufficient quantities of DNA for successful SNP genotyping.

Table 8 Summary of means (±SD) for DNA concentration (ng/ul), DNA purity ratios (260/280; 260/230), and SNP call rate (%) from swabs and fin clips extracted using the digestion protocol.

DNA Source	N	DNA Concentration (ng/µl)	260/280	260/230	SNP Call Rate (%)
Swabs	100	132.55 ± 35.41	1.58 ± 0.11	0.50 ± 0.04	99.81 ± 0.26
Fin Clips	100	213.92 ± 32.28	1.43 ± 0.08	0.54 ± 0.06	99.43 ± 0.57

Discussion

The goal of this study was to examine the utility and accuracy of buccal swabs as a method for DNA sampling in black basses. I sought to develop a protocol that would lend itself to angler-driven genetic sampling, necessitating a method that was simple, affordable, and robust. Offering anglers an alternative to the use of ethanol as a preservative was of partial interest, given issues associated with distribution, storage, and shipping of alcohols by the general public. This differs from other fish-based buccal swabbing protocols that recommend immediate storage in ethanol (e.g. Smalley and Campanella 2005; Colussi et al. 2017)

There was minimal effect of swab location (tongue vs. cheek), swabbing duration (3-10 s), storage temperature, or storage duration on DNA concentration, DNA purity, and genotyping accuracy/concordance with fin clip DNA. Importantly, sufficient DNA and accurate genotypes were also obtained from swabs extracted with a rapid, minimal cost digestion method. Depending on extraction method, 100-200 ul of DNA (~120 ng/ul concentration) was consistently obtained, allowing for a given swab sample to be genotyped 50-100 times using our SNP markers. Use of short amplicon SNPs rather than traditional microsatellites likely increased tolerance of degradation and increased genotyping success (Senge et al. 2011; von Thaden et al. 2017).

Buccal swabbing is well suited to angler collection of black basses, given that these fish are often held and controlled by their lip, leaving their mouth open and accessible for sampling. An angler can easily hold the bass with one hand and swab the tongue with other before releasing the bass back into the water. By contrast, fin clipping is difficult and cumbersome for a solo angler (or biologist), as the fish must be controlled while samples are obtained, which often requires two people. Vials of ethanol must also be kept nearby, where it can be easily spilled or

contaminated by an uncontrolled bass. Although most of the results from this study were based on 10 s of swabbing on the tongue, results indicate that shorter durations and off-target swabbing (cheek) had little impact on genotyping success and accuracy. This flexibility is critical when dealing with the angling public. Indeed, the results from angler-derived swab samples demonstrated that a variety of anglers in different scenarios (bank fishing, boats, etc) could provide an adequate DNA samples.

Beyond obtaining sufficient DNA for genotyping, the effect of various storage procedures for swab samples was of interest, particularly elevated temperatures or long wait periods between sampling and extraction. Anglers cannot be expected to deliver samples to the lab immediately, nor to follow complicated drying or preservation steps. However, drying a swab sample prior to storage is the most critical step, as humid conditions can quickly alter cells, lead to bacterial and fungal contamination, and degrade DNA (Colussi et al. 2017). My study successfully employed the Fitzco DryPak sleeve as a sterile, breathable storage solution for the collected swabs. The DryPak has a two-way breathable material that allows the moist swab to quickly dry within the sleeve. Given that these sleeves are light and contain no liquid, anglers can easily place the swab/sleeve in an envelope and return to a lab via standard mail. More importantly, this study demonstrated that if angler delayed sending the sample, for example leaving the sample in a tackle box or vehicle, sufficient DNA could still be obtained. Furthermore, samples do not need to be immediately chilled, transferred to ethanol, or extracted after being received in the laboratory. Rather, they can remain within the DryPak at room temperature prior to processing for up to 4 months.

Overall, the protocol developed in this study allows anglers to easily collect genetic samples, alone in challenging field conditions, to carry with him or her a light swab pack (sterile

swab/sleeve/return envelope), to sample a specimen by swabbing the tongue, place the swab into the Drypak sleeve, and to return the sample either by mail or to a pre-determined collection box, all without undue haste or concern regarding temperature or humidity conditions. Swabs can then be extracted with a crude digestion method that reduces time and assay cost relative to commercial kits. Cost savings accrued from the digestion extraction more than compensate for the higher cost of swab/sleeve (~$0.80) when compared to a tube with ethanol (~$0.50) needed for a fin clip. However, for an angler-directed program, there would be significant additional costs associated with providing sampling tools as well as higher shipping costs if a fin clip (in ethanol) was utilized.

In conclusion, I present here a field-tested swab sampling protocol suitable for application to the angling public. While the focus here was confined to Largemouth Bass (including Florida Bass and intergrades), recent work in our lab indicates its utility for other black basses as well. The protocol as described is currently being used successfully in an ongoing angler-driven survey of trophy bass genetics on Lake Eufaula, Alabama as well as by private pond owners.

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Chapter III: Evaluation of Black Bass Purity and Hybridization in the Altamaha River Basin using Species-Diagnostic SNP markers

Abstract

Species within the genus *Micropterus* are top-level predators and some of the most highly sought-after game fishes in North American freshwater ecosystems. Within the last decade, biologists have recognized that the genus is home to a startling diversity of additional range-restricted species and strains. Many of these are currently imperiled by habitat degradation and introgression with introduced, non-native species. Protecting endemic diversity first requires accurate assessment of black bass species composition and the ability to differentiate pure and hybridized individuals. Accordingly, in the current study, I utilized recently developed black bass SNP marker panels to characterize black bass populations in the main stems of the Ocmulgee, Oconee, and Altamaha Rivers, to better understand their purity and hybridization patterns. The results, from over 500 individuals, shed light on the status of Spotted Bass (*M. punctulatus*) in the basin, hybridization patterns of introduced Shoal Bass (*M. cataractae*), and provide an important revision to the accepted intergrade status of Largemouth Bass in the drainage. I also provide evidence pointing to the presence of a genetically distinct black bass in the ARB Coastal Plain, deserving of closer scrutiny in the future.

Introduction

Black basses (*Micropterus* spp.) represent ecologically important members of freshwater ecosystems throughout North America. As apex predators, black bass can determine the abundance and diversity of small-bodied fish species, and indirectly regulate the composition and density of zooplankton and phytoplankton assemblages (Brooks and Dodson 1965; Sommer 1989; Carpenter and Kitchell 1996). In addition to their ecological value, black bass are the most popular angled species and attract millions of anglers each year (USDI 2011). Recently, competitive bass fishing has evolved into a multibillion-dollar industry with more than 24,000 competitive angling events occurring annually in the United States (Schramm et al. 1991). Black bass tournaments have a significant impact on the local economy in the communities surrounding the events due to purchases at hotels, gas stations, restaurants, and fishing shops (Driscoll et al. 2012). Due to their ecological and economic value, there has been a considerable amount of effort dedicated to the conservation and management of black bass species.

Important conservation concerns for black bass species include habitat loss or alteration, environmental pollution, competition, and introgressive hybridization with non-native congeners. While some of the black bass species occupy large geographic ranges (e.g. Largemouth Bass (*M. salmoides*), Smallmouth Bass (*M. dolomieu*), and Spotted Bass (*M. punctulatus*)), the remaining species are most threatened by changes to their habitats. A rising concern for black bass with restricted ranges is non-native introductions and subsequent introgressive hybridization between non-native and endemic species (Koppelman 2015). Due to the widespread stocking of non-native species and intrinsically weak reproductive barriers, the rate of interspecific hybridization within *Micropterus* has been artificially accelerated (Birdsong et al. 2015). Introgressive hybridization can negatively impact endemic species through the disruption of local adaptions,

promotion of lowered fitness, and eventual genetic extirpation or extinction (Rhymer and Simberloff 1996; Randi 2008). Thus, molecular markers tools have become necessary components of native black bass conservation plans, to delineate species and assess the extent of hybridization and introgression.

Molecular markers such as allozymes, mitochondrial DNA (mtDNA), microsatellites, and single nucleotide polymorphism markers (SNP) have been important tools in black bass conservation and management (Philipp et al. 1983; Kassler et al. 2002; Seyoum et al. 2013; Freeman et al. 2015; Li et al. 2015; Tringali et al. 2015). Recent advances in high throughput sequencing technology have provided fast and cost-effective methods to generate sequencing data (Stupar & Springer 2006; Hudson 2008). Available sequence data has allowed for SNP genotyping, valued for its low cost, ease of multiplexing, and low genotyping error rate for high throughput analyses (Slate et al. 2009; Pritchard et al. 2012). Li et al. (2015) and Zhao et al. (2018) developed highly informative SNP marker panels for Florida Bass (*Micropterus floridanus*) and Northern Largemouth Bass (*Micropterus salmoides*). While these panels have been powerful tools for Largemouth Bass, recent work has focused on developing SNP markers for additional black bass species, many of which are threatened by introgressive hybridization. Thongda et al. (2018, unpublished data) developed species-diagnostic SNP markers for black bass species using genotype-by-sequencing (GBS), followed by validation using two panels of 64 SNPs. These panels have been tested on >1500 black bass individuals, and have been shown to be useful tools for delineating species and assessing hybridization between species.

The Altamaha River basin (ARB) is the third largest contributor of freshwater to the Atlantic Ocean on North America's eastern shore. The Oconee and Ocmulgee Rivers begin in the Piedmont region of the state of Georgia, over the "Fall Line," and into the Coastal Plain,

where they converge to form the Altamaha River which then flows unimpeded for 137 miles to the southern Atlantic Ocean (the longest free free-flowing river system on the East Coast of the US). The basin is also known for having produced the world record largemouth bass, angled from an oxbow lake off the Ocmulgee River near Lumber City, Georgia in 1932. However, knowledge of black bass diversity and genetic composition in the ARB is remarkably limited, particularly below the Fall Line. In regards to the best characterized of black bass species, the Largemouth Bass, the ARB has been lumped into the intergrade zone since the seminal work of Bailey and Hubbs (1949) in which they found seven specimens to be intermediate in meristic characteristics between *M. salmoides* and *M. floridanus*. Philipp et al. (1983), in the first broad biochemical evaluation of Bailey and Hubbs' classifications, failed to sample the ARB in the Coastal Plain, beginning a pattern that has continued until today.

The goal of the current study was to utilize black bass SNP marker panels to characterize black bass populations in the main stems of the Ocmulgee, Oconee, and Altamaha Rivers, to better understand their purity and hybridization patterns. The results, from over 500 individuals, shed light on the status of introduced Spotted Bass (*M. punctulatus*) in the basin, hybridization patterns of introduced Shoal Bass (*M. cataractae*), and provide an important revision to the accepted intergrade status of ARB Largemouth Bass. I also provide evidence pointing to the presence of a genetically distinct bass in the ARB Coastal Plain, deserving of closer scrutiny in the future.

Material and Methods

Sample Collection

The Georgia Department of Natural Resources (GADNR) collected a total of 581 black bass specimens in 2016 and 2017 from multiple locations within the ARB including: the Altamaha River (n=86), Ocmulgee River (n=352), and Oconee River (n=143) (**Figure 7**). The biologists sampled for 1 h at fixed sites using a boat-mounted electrofisher and collected all black bass encountered within that 1 h. Specimens were identified by phenotypic characteristics (**Table 9**). Based upon phenotypic characteristics, the samples were composed of Largemouth Bass (n=455), Shoal Bass (n=60), and Spotted Bass (n=66). Each specimen was measured for total length (TL, mm), weight (g), and small fin clip samples were taken and preserved in vials with 95% ethanol. Fin clip samples were sent to the Auburn University Aquatic Genetics and Genomics Laboratory and stored at room temperature prior to DNA extraction and SNP genotyping. Details of the sampling locations, phenotypic identifications, lengths (mm), and weights (g) for each black bass sample are presented in **Appendix I**.

DNA Extraction & SNP Genotyping

DNA samples from fin clips were extracted using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) following the manufacturer's protocol. DNA concentrations (ng/ul) and DNA purity ratios (260/230, 260/280) were estimated using a NanoDrop ND-2000 UV-VIS Spectrophotometer. The DNA samples were then SNP genotyped on the Agena MassARRAY iPLEX platform following the manufacturer's protocol (Agena Bioscience® Inc., San Diego, CA). Largemouth Bass samples were sequenced using the 38-plex Florida/Northern Largemouth Bass (FLNB) marker panel (Zhao et al. 2018) to determine purity and hybridization between

Florida Bass and Northern Largemouth Bass. Largemouth Bass, Spotted Bass, and Shoal Bass samples were sequenced using a 64-plex black bass panel (Thongda et al. 2018, unpublished data) to determine purity and hybridization among Spotted Bass, Shoal Bass, Smallmouth Bass, Largemouth Bass, Alabama Bass, and Redeye Bass.

To prepare for SNP genotyping, each DNA sample was diluted in a 96-well plate to the desired concentration of 20 ng/µl using high performance liquid chromatography (HPLC) grade water. Using a multichannel pipette, 2 µl of the diluted DNA samples was placed onto a new 96-well plate and prepared for three rounds of polymerase chain reactions (PCR). The first PCR amplifies a specific fragment of genomic DNA using designed primers and the iPLEX Gold Reagent Kit according to the manufacturer's protocol (Gabriel et al. 2009). The conditions of the first PCR included the following parameters: pre-denature at 94°C for 2 min, 45 cycles of denaturation of 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The second PCR utilized shrimp alkaline phosphatase (SAP) in order to remove remaining, nonincorperated dNTPS from amplification products. The conditions for the second PCR included: enzyme activation at 37°C for 40 min, and enzyme degradation at 85°C for 5 min. The last PCR was the primer extension, designed to extend the primer by one mass-modified nucleotide depending on the allele and assay design. The conditions of the third PCR included: pre-denature at 94°C for 30 s, 45 cycles of denaturation at 94°C for 5 s, annealing at 52°C for 5 s, extension at 80°C for 5 s, and final extension at 72°C for 5 min.

Once the plate finished the PCR processes, 41 µl of HPLC grade water was added to each well using a multi-channel pipette. Then, SpectroCLEAN resin was added to the wells to remove salts such as sodium, potassium, and magnesium ions. The plate was rotated at 360° for 20 min and then spun down in a centrifuge at 2000 g for 5 min. The Agena MassARRAY Nanodispenser

was used to transfer the samples from the plate on to a silica chip using a capillary action of slotted pins and contact dispersing for nanovolumes (Gabriel et al. 2009). Once the transfer was complete, the chip was placed into the MassARRAY compact mass spectrometer. Each sample was shot with a laser under vacuum by the matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) method. The SNP genotypes were called in the SEQUENOM SYSTEM TYPER 4.0 Analysis software. A final genotype was called and placed into a category based on the significance of the allele (e.g. conservative, moderate, aggressive, user call). All individuals had call rates >90% (>35/38 SNPs; >58/64 SNPs).

Data Analysis- Largemouth Bass Samples

The final 38-plex Largemouth Bass genotypes were analyzed in RStudio (version 1.0.136) and compared to a reference genotype of a pure Florida Bass (FLMB) and a pure Northern Largemouth Bass (NLMB) (Zhao et al. 2018). For each genotype, the FLMB allele frequency, the NLMB allele frequency, heterozygous (HE) allele frequency, and homozygous allele frequency were computed using the following formulas:

FLMB Allele Frequency= ((FLMB SNPs)+(1/2 * HE SNPs)/ Total Scored SNPs)

NLMB Allele Frequency= ((NLMB SNPs)+(1/2 * HE SNPs)/ Total Scored SNPs)

Heterozygous Allele Frequency= ((HE SNPs)/ Total Scored SNPs)

Homozygous Allele Frequency= ((FLMB SNPs + NLMB SNPs)/ Total Scored SNPs)

The frequencies were used to calculate the percentages of FLMB, NLMB, heterozygosity, and homozygosity for each individual by multiplying the frequencies by 100%. Details of the 38-plex FLNB results for the Largemouth Bass samples are presented in **Appendix II**.

Data Analysis- Black Bass Samples

The 64-plex genotype data for the ARB Largemouth Bass, Spotted Bass, and Shoal Bass samples were analyzed using STRUCTURE version 2.3.4 (Pritchard et al., 2000; 2010) to identify the species status of unknown samples or evaluate genetic purity. The genotypes of pure representatives of the initial six species: Largemouth Bass (Florida and Northern combined), Alabama Bass, Spotted Bass, Redeye Bass, Shoal Bass, and Smallmouth Bass (**Table 10**) were analyzed using STRUCTURE assuming K=6. All initial 200 samples of each species had membership coefficients (Q-value) >0.95 in STRUCTURE analysis. These genotypes were utilized as putative reference genotypes for resolving the taxonomic status of the black bass samples from the ARB. Due to the presence of Redeye Bass alleles in the ARB, we reanalyzed black bass samples with Redeye Bass alleles using STRUCTURE and included the drainage appropriate form, the Altamaha Bass (*M. sp. cf. coosae*; **Table 10).** STRUCTURE analysis was performed using the admixture model with a burn-in of 10,000 iterations followed by 100,000 repetitions of Markov chain Monte Carlo (MCMC) simulation (Thongda et al. 2018, unpublished data). The STRUCTURE results yielded the individual genomic proportion values for each individual (Q-value). According to previous approaches, individuals were assigned with membership coefficients of ≥0.90 to a single species ("pure"), individuals with ~0.75-0.90 coefficients as backcrossed, and <0.75 as F_1 or later-generation hybrid (Dakin et al. 2015).

Statistical Analysis

Significant differences in FLMB allele percentages and heterozygosity between river systems and sampling locations were assessed with an analysis of variance (ANOVA) in RStudio (version 1.0.136). A p-value cut-off of less than 0.05 was used to reject the null hypothesis and

infer significance. Significant comparisons were further examined using a Tukey HSD to identify sampling locations and river systems where differences occurred.

Table 9 Black bass species identified by phenotypic characteristics and the number of individuals (N) sampled from the Altamaha, Ocmulgee, and Oconee Rivers in 2016 and 2017.

	Species	**River**	**N**
1	Florida Bass/ Northern Largemouth Bass (*M. floridanus/ M. salmoides*)	Altamaha	86
		Ocmulgee	226
		Oconee	143
2	Spotted Bass (*M. punctulatus*)	Ocmulgee	66
3	Shoal Bass (*M. cataractae*)	Ocmulgee	60
		Total	**581**

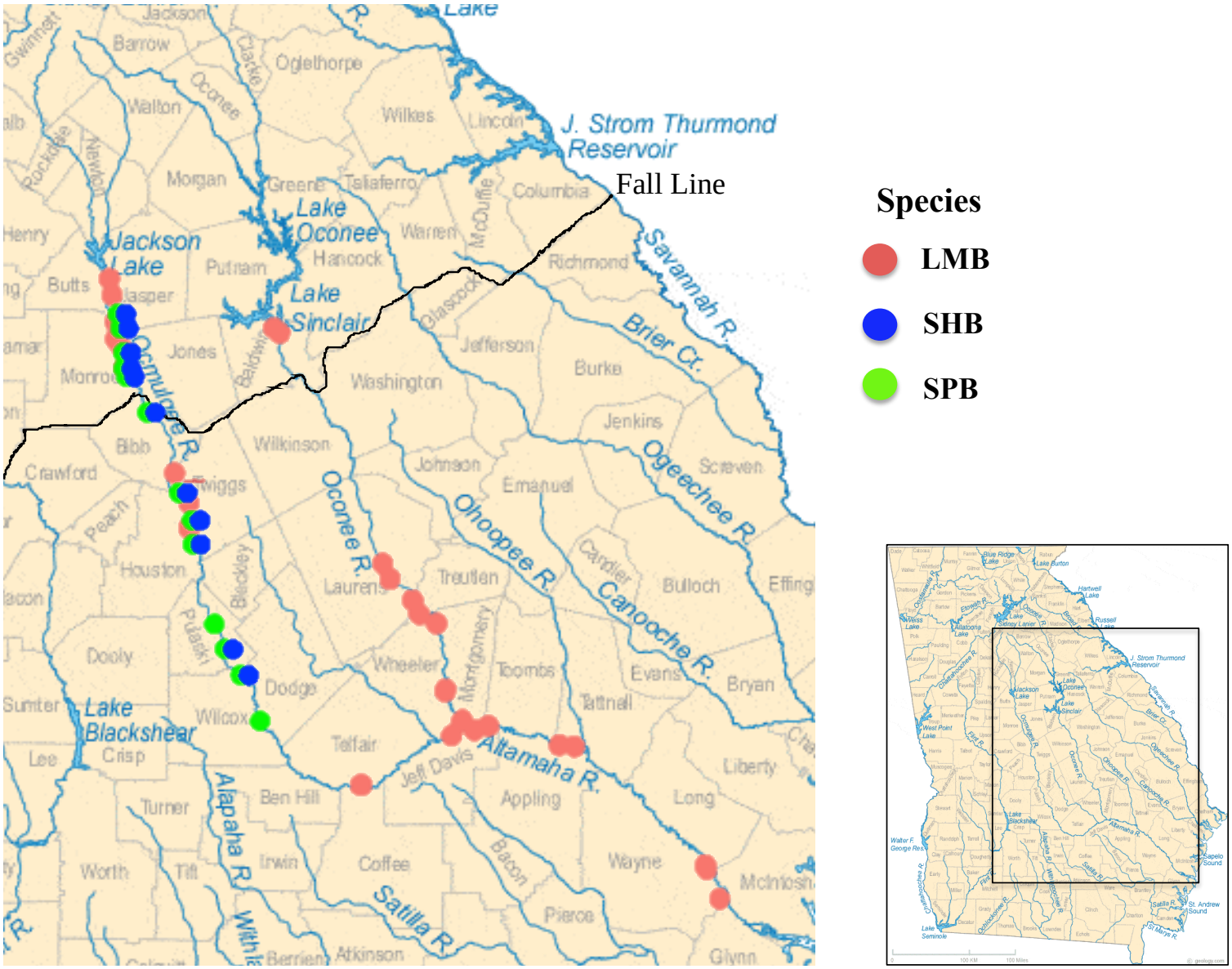

Figure 7 Sampling locations of Largemouth Bass (n=455), Shoal Bass (n=60), and Spotted Bass (n=66) on the Altamaha, Oconee, and Ocmulgee Rivers.

Table 10 The 64-plex markers panel for Alabama Bass (ALB), Largemouth Bass (LMB), Shoal Bass (SHB), Smallmouth Bass (SMB), Spotted Bass (SPB), Redeye Bass (CSB), and Altamaha Bass (ALTB). A single letter, "G" represents a homozygous GG genotype. The slash (/) indicates polymorphic markers found in a particular species (Thongda et al. 2018, unpublished data).

	Markers	ALB	LMB	SHB	SMB	SPB	CSB	ALTB
1	MpRETP16597T	G	G	G	G	T	T	T
2	MpRETP29431T	C	C	C	C	C/T	T	T
3	MpRETP30468C	G	G	G	G	C	C	C
4	MpRETP5605T	C	C	C	C	C/T	C/T	T
5	MpRETP8149T	C	C	C	C	T	T	T
6	MpSHTP18868C	T	T	C	T	C	T	T
7	RETP3652A	T	T	T	T	T	A	A
8	RETP4454T	C	C	C	C	C	T	C
9	RETP4714A	T	T	T	T	T	A	T
10	RETP5033T	A	A	A	A	A	T	T
11	RETP5103T	C	C	C	C	C	T	T
12	RETP5317T	C	C	C	C	C	T	T
13	RETP5475A	G	G	G	G	G	A	G
14	RETP7076G	T	T	T	T	T	G	T
15	RETP7594T	C	C	C	C	C	T	T
16	ShTP20328A	G	G	A	G	G	G	G
17	SMShTP1235G	A	A	G/A	G	A	A	A
18	SMShTP5873G	A	A	G	G/A	A	A	A
19	SPMhRETP1192T	T	A	A	A	A	T	T
20	SPMhRETP12009T	T	A	A	A	A	T	T
21	SPMhRETP18151G	G	A	A	A	A	G	G
22	SPMhRETP18863T	C/T	C	C	C	C	T	C
23	SPMhRETP21822A	A	G	G	G	G	A	A
24	SPMhRETP2910G	G	C	C	C	C	G	G
25	SPMhRETP6389C	C/T	T	T	T	T	C	C
26	SPMhRETP7161A	A	G	G	G	G	A	A
27	SPMhRETP7458C	C	T	T	T	C/T	C	C
28	SPMhRETP8383G	G	C	C	C	C	G	G
29	MpRETP17639A	A/G	G	G	G	A	A	A
30	MpRETP9363A	T	T	T	T	A/T	A	A
31	MpSMBTP16142A	C	C	C	A	A	C	C
32	RETP10556A	G	G	G	G	G	A	G
33	RETP13743C	C/G	G	G	G	G	C	C
34	RETP3097A	G	G	G	G	G	A	A
35	RETP4504A	C	C	C	C	C	A	A
36	RETP4592A	C	C	C	C	C	C/A	C
37	RETP4763G	A	A	A	A/G	A	G	G
38	RETP4967G	C	C	C	C	C	G	G
39	RETP5089T	G	G	G	G	G	T	G
40	RETP5172C	A	A	A	A	A	C	C
41	RETP5306A	T	T	T	T	T	A	A
42	RETP5360T	C	C	C	C	C	T	C
43	ShTP6906C	T	T	C	T	T	T	T
44	ShTP9651T	C	C	T	C	C	C	C
45	ShTP9781T	C	C	T	C	C	C	C
46	SMRETP23527A	G	G	G	A	G	A	G
47	SMRETP25045A	G	G	G	A	G	A	A
48	SMRETP6977C	A	A	A	C	A	C	C
49	SMShTP13277T	C	C	T	T	C	C	C
50	SMShTP21440A	G	G	A	A	G	G	G
51	SMShTP5127T	A	A	T	T	T/A	A	A
52	SMShTP7448C	A	A	C	C	A	A	A
53	SPMhRETP11109C	C/T	T	T	T	T	C	T
54	SPMhRETP1486CG	CG	G	G	G	G	CG	CG
55	SPMhRETP15885T	T	G	G	G	G	T	T
56	SPMhRETP18435T	T	C	C	C	C	T	T
57	SPMhRETP24673A	A	G	G	G	G	G/A	G
58	SPMhRETP26317T	T	C	C	C	C	T	T
59	SPMhRETP28164T	T	G	G	G	G	G/T	G
60	SPMhRETP3437T	T	C	C	C	C	T	T
61	SPMhRETP4105A	A	C	C	C	C	A	C
62	SPMhRETP6034T	T	C	C	C	C	T	T
63	SPMhRETP8469A	A	G	G	G	G	A	A
64	SPMhRETP8660C	C	T	T	T	T	C	C
	Fixed Alleles	59	64	63	62	59	60	64

Results

Largemouth Bass Samples

Altamaha River

In 2017, the GADNR collected a total of 86 black bass samples from the Altamaha River that were phenotypically identified as Largemouth Bass. The average TL was 288 mm and the average weight was 410 g. After DNA extraction and SNP genotyping using the 38-plex FLNB panel, the FLMB, NLMB, and heterozygous allele frequencies and percentages were computed for each individual. The overall average FLMB allele percentage was 95.23%, ranging from 73.68%-100% FLMB. The average heterozygous allele percentage was 7.24%, with the highest individual being 42.11% heterozygous. FLMB individuals with membership coefficients ≥95% were designated as a pure FLMB. Out of the 86 samples, there were a total of 42 individuals with FLMB allele percentages ≥95%, indicating a high proportion of pure FLMB within the Altamaha River.

The details of the sampling locations and the FLMB genetic results from each location are presented in **Table 11**. In addition, there were 10 samples collected from the Altamaha River with a novel genotypic signature on the 38-plex FLNB panels (discussed in a later section). To our knowledge, this is the largest genetic assessment of Largemouth Bass in the Altamaha River to-date.

Table 11 Sampling locations, numbers of individuals sampled in each location (N), number of pure FLMB, mean FLMB allele percentage (±SD), and mean heterozygous allele percentage (±SD) of LMB sampled on the Altamaha River, Georgia.

Location	Latitude	Longitude	N	Pure FLMB	FLMB%	Heterozygous %
Altamaha	31.437082	-81.610819	10	6	95.77 ± 1.97	6.87 ± 2.92
	31.527692	-81.658362	20	12	95.72 ± 2.45	6.97 ± 3.65
	31.539978	-81.663094	10	3	94.74 ± 2.15	7.37 ± 4.44
	31.901442	-82.141395	6	6	97.37 ± 1.18	4.38 ± 2.15
	31.905851	-82.195989	9	6	96.78 ± 1.87	5.26 ± 2.94
	31.951484	-82.506691	13	6	93.52 ± 6.30	9.72 ± 10.33
	31.963876	-82.454308	8	3	93.35 ± 4.54	8.53 ± 6.41
		Total	**76**	**42**	**95.23 ± 3.60**	**7.24 ± 5.59**

Oconee River

In 2017, a total of 83 black bass samples were collected from the Oconee River below the Fall Line and phenotypically identified as Largemouth Bass. The average TL was 325 mm and the average weight was 629 g. After genotyping, the average FLMB allele percentage was 92.50%, ranging from 67.11%-100% FLMB. The average heterozygous allele percentage was 11.48%. Out of the 83 samples, a total of 35 individuals were scored as pure FLMB. In addition, there was one sample that had a novel genetic signature similar to the samples found in the Altamaha River.

In 2016, a total of 60 black bass samples were collected from Lake Sinclair on the Oconee River above the Fall Line. The average FLMB allele percentage from the samples was 73.35% and the average heterozygous allele percentage was 34.52%. An overview of the genetic results from the Oconee River samples collected above and below the Fall Line in 2016 and 2017 is presented in **Table 12**. There was a significant difference ($p < 0.0001$) in FLMB and heterozygous allele percentages between samples collected above the Fall Line in Lake Sinclair compared to samples collected below the Fall Line on the Oconee River.

Table 12 Sampling locations, number of individuals sampled in each location (N), number of pure FLMB, mean FLMB allele percentage (±SD), and mean heterozygous allele percentage (±SD) of LMB sampled below the Fall Line (BFL) and above the Fall Line (AFL) on the Oconee River. Asterisk denotes significance ($p < 0.0001$).

Location	Latitude	Longitude	N	Pure FLMB	FLMB%	Heterozygous %
BFL	31.981720	-82.551251	8	3	95.07 ± 2.31	8.55 ± 3.65
	32.068641	-82.613544	11	9	96.77 ± 1.70	5.02 ± 3.98
	32.075346	-82.610978	13	8	95.55 ± 2.49	6.07 ± 2.49
	32.275183	-82.644092	5	2	96.05 ± 3.22	6.58 ± 4.56
	32.303273	-82.707319	5	2	92.37 ± 5.46	14.21 ± 11.41
	32.346647	-82.732044	10	3	88.89 ± 9.14	13.80 ± 7.66
	32.409344	-82.814521	10	3	90.92 ± 7.56	15.00 ± 10.96
	32.457086	-82.840895	20	5	89.08 ± 8.31	17.11 ± 12.36
		Total	**82**	**35**	**92.50 ± 6.78**	**11.48 ± 9.53**
AFL	33.151803	-83.216958	29	0	72.83 ± 5.57	33.92 ± 5.56
	33.167180	-83.239103	31	0	73.83 ± 5.26	34.04 ± 8.06
		Total	**60**	**0**	**73.35 ± 5.39***	**34.52 ± 6.93***

Ocmulgee River

In 2017 from the Ocmulgee River, a total of 107 black bass were sampled and phenotypically identified as Largemouth Bass. Overall, the average TL was 337 mm and average weight was 814 g. The average FLMB allele percentage was 86.98% and the average heterozygous percentage was 24.01%. Out of the 107 samples, 80 individuals were collected below the Fall Line and the remaining 27 individuals collected above the Fall Line. Below the Fall Line, genotyping indicated a total of 17 pure FLMB whereas 3 pure FLMB were identified above the Fall Line.

Six of the samples from the Ocmulgee River had a genetic signature reflective of a species other than Largemouth Bass. The samples were therefore also run on the 64-plex black bass panels and analyzed in STRUCTURE to determine their genetic identity. The STRUCTURE results indicated that one of the individuals was a pure Shoal Bass

(LMBOMR103; Q=0.97), four were pure Alabama Bass (LMBOMR112, 113, 117, 154; Q> 0.90), and there was one black bass hybrid (LMBOMR176). Interestingly, the black bass hybrid was comprised of Alabama Bass (Q=0.42), Redeye Bass (Q=0.25), Shoal Bass (Q=0.19), and Spotted Bass (Q=0.13) (**Appendix III**). Given the presence of Redeye Bass alleles, I subsequently reanalyzed the results with the drainage appropriate form, the Altamaha Bass. Results indicated that this individual was an Alabama Bass hybridized with an Altamaha Bass/Shoal Bass hybrid (**Appendix IV**). Additionally, among the phenotypically identified LMB, I identified eight fish with unknown genetic signatures in the lower Ocmulgee River samples matching those previously identified in the Altamaha and Oconee Rivers.

In 2016 samplings, a total of 59 phenotypic Largemouth Bass samples were collected from Lake Juliette, above the Fall Line. Additionally, 60 samples from the Ocmulgee River below the Fall Line were phenotypically identified as Largemouth Bass. The average FLMB allele percentage on Lake Juliette was 73.96%, with an average heterozygous percentage of 34.15%. The Ocmulgee River samples collected below the Fall Line had an average FLMB allele percentage of 93.35% and an average heterozygous allele percentage of 9.56%. Of the 59 samples, 26 were scored as pure FLMB. The combined 2016 and 2017 Largemouth Bass genetic results and sampling information from the Ocmulgee River is presented in **Table 13**. There was a significant difference ($p < 0.0001$) in FLMB allele and heterozygous percentages between samples collected above the Fall Line when compared to samples below the Fall Line on the Ocmulgee River.

Table 13 Sampling locations, number of individuals sampled in each location (N), number of pure FLMB, mean FLMB allele percentage (±SD), and mean heterozygous allele percentage (±SD) of LMB sampled below the Fall Line (BFL) and above the Fall Line (AFL) on the Ocmulgee River, Georgia. Asterisk denotes significance ($p < 0.0001$).

Location	Latitude	Longitude	N	Pure FLMB	FLMB%	Heterozygous %
BFL- 2016	NA	NA	60	26	93.35 ± 3.77	9.56 ± 7.28
BFL- 2017	31.7835	-82.9166	8	3	91.45 ± 6.56	14.47 ± 9.44
	31.7845	-82.9223	8	6	95.72 ± 1.17	7.24 ± 1.22
	31.9353	-82.5894	8	6	96.01 ± 3.76	6.67 ± 4.82
	32.5611	-83.5466	7	2	92.67 ± 4.01	13.16 ± 8.18
	32.5648	-83.5484	24	7	91.12 ± 9.35	12.98 ± 9.03
	32.6393	-83.5487	6	2	94.96 ± 1.54	5.70 ± 5.11
	32.7296	-83.5993	6	0	86.84 ± 15.68	14.91 ± 10.61
		Total	**127**	**52**	**92.87 ± 6.32**	**10.44 ± 7.82**
AFL-2016	33.0474	-83.7863	59	0	73.96 ± 5.05	34.15 ± 7.53
AFL-2017	33.1094	-83.7954	6	2	89.47 ± 8.29	17.29 ± 14.88
	33.1346	-83.8159	6	0	75.22 ± 4.95	34.65 ± 8.39
	33.1862	-83.8174	3	0	68.42 ± 6.58	35.09 ± 18.67
	33.2655	-83.8279	5	0	70.00 ± 7.11	38.95 ± 14.22
	33.3136	-83.8381	6	1	77.19 ± 11.75	28.95 ± 14.89
		Total	**85**	**3**	**75.12 ± 7.52***	**32.76 ± 10.68***

ARB Largemouth Bass

Combining results from 2016 and 2017, GADNR collected a total of 455 black bass phenotypically identified as Largemouth Bass from the Altamaha (n=86), Oconee (n=143), and Ocmulgee (n=226) rivers. Upon genetic analysis using the 38-plex FLNB panels, it was determined that there were 76 Largemouth Bass collected from the Altamaha River, 142 from the Oconee River, and 212 from the Ocmulgee River. There is clear differentiation between Largemouth Bass populations above the Fall Line compared to those below the Fall Line in the Ocmulgee and Oconee Rivers. The average FLMB allele percentage from each sampling location on the ARB is presented in **Figure 8**, and the average heterozygous allele percentage from each location is presented in **Figure 9**. Largemouth Bass samples collected below the Fall Line (n=285) had more FLMB alleles ($p < 0.05$) and less heterozygosity ($p < 0.05$) compared to samples collected above the Fall Line (n=145).

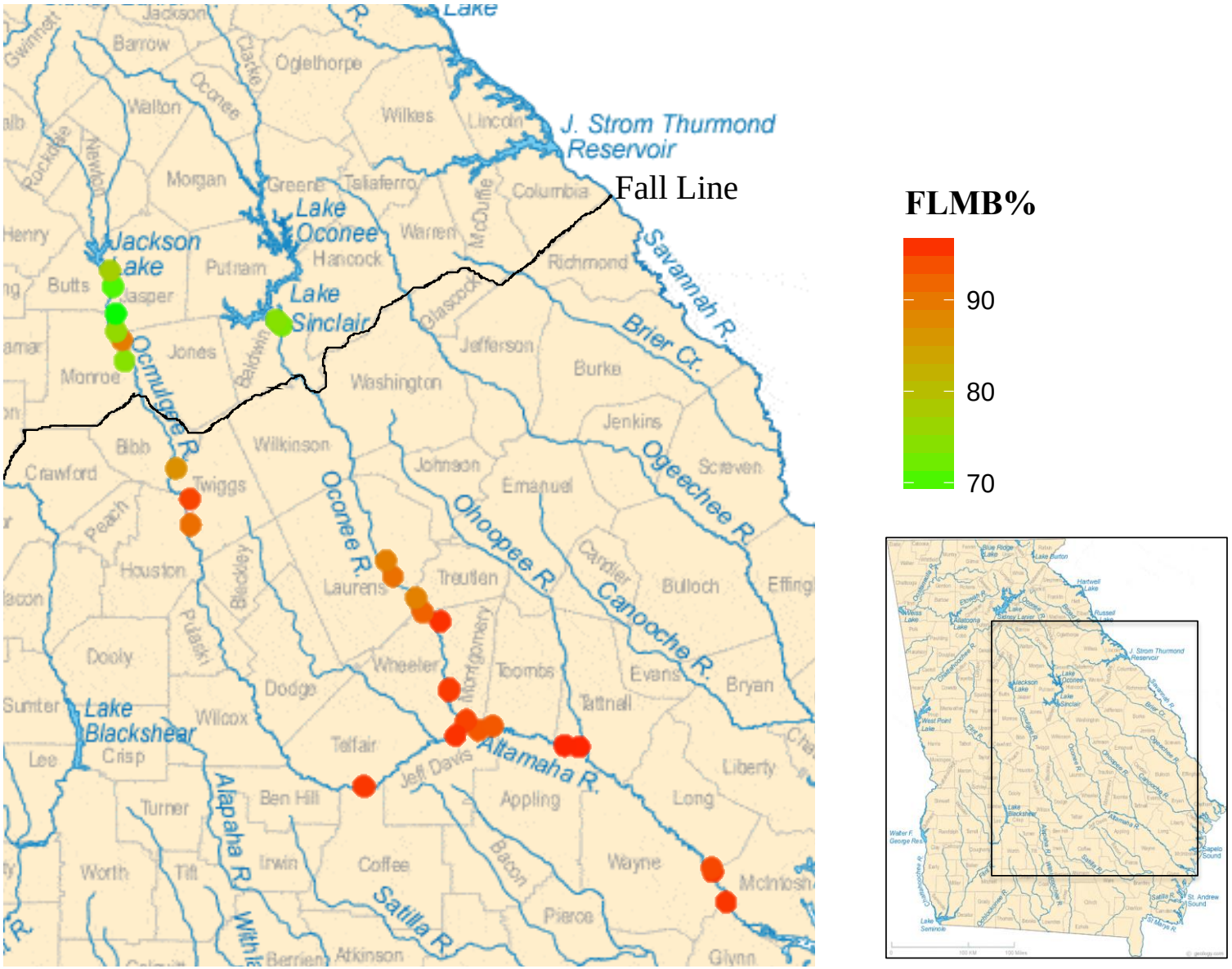

Figure 8 Average FLMB allele percentages in each of the sampling locations on the Altamaha, Oconee, and Ocmulgee Rivers.

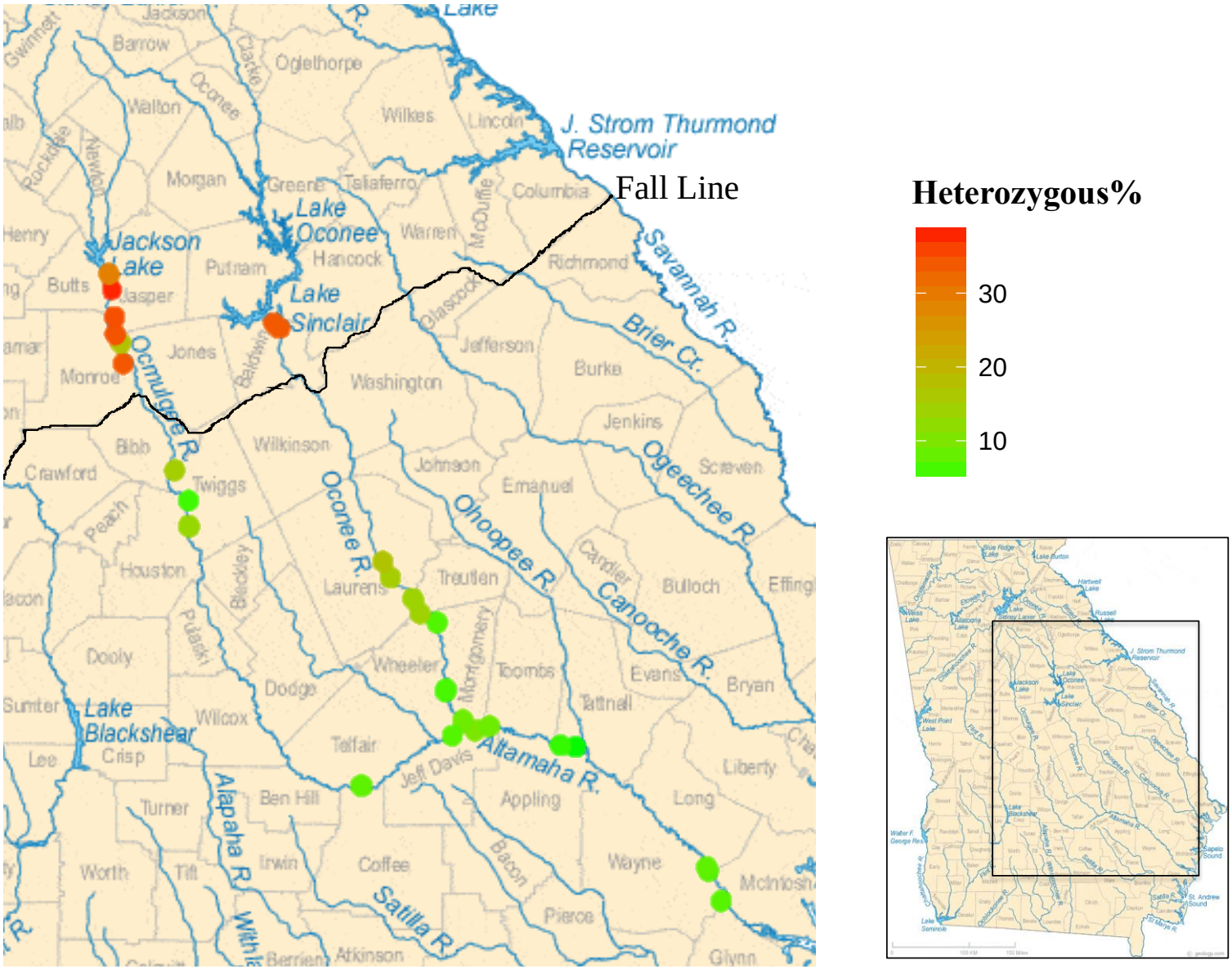

Figure 9 Average heterozygous allele percentages in each of the sampling locations on the Altamaha, Oconee, and Ocmulgee Rivers.

Spotted Bass Samples

Ocmulgee River

In 2017, GADNR collected a total of 66 black bass samples phenotypically identified as Spotted Bass in the Ocmulgee River. Of the 66 samples, 30 individuals were collected below the Fall Line and 36 individuals collected above the Fall Line. The overall average TL was 297 mm and the average weight was 400 g. The samples were genotyped using the 64-plex black bass panels and analyzed within STRUCTURE to determine the individual's respective species groups, identify hybrids, and reveal misidentified samples. The STRUCTURE results of the Spotted Bass samples from the Ocmulgee River are presented in **Appendix III**. The samples were predominately Alabama Bass with an average Q-value of 0.96. Out of the 66 samples, 60 were identified as pure Alabama Bass ($Q \geq 0.90$). The details of the sampling locations and STRUCTURE results are presented in **Table 14**.

The remaining individuals (n=6) were identified as black bass hybrids or another pure black bass species. Hybrids with Redeye Bass allele inclusion were reanalyzed in STRUCTURE with Altamaha Bass (**Table 15**). The new analysis identified one of the individuals (SPBOMR056), sampled from above the Fall Line, as a pure Altamaha Bass (Q=0.97). Out of the five remaining hybrid individuals, three were identified as Alabama Bass and Shoal Bass hybrids (SPBOMR003, 030, 052). One of individuals (SPBOMR020) was a first filial (F1) generation hybrid of Alabama Bass and Altamaha Bass. The remaining individual (SPBOMR009) was an Alabama Bass and Smallmouth Bass hybrid.

Table 14 Sampling locations, number of individuals sampled in each location (N), number of pure ALB, and the number of hybrids sampled from below the Fall Line (BFL) and above the Fall Line (AFL) on the Ocmulgee River, Georgia.

Location	Latitude	Longitude	N	Pure ALB	Hybrid
BFL	31.9801	-83.2843	1	1	
	32.1316	-83.3598	12	10	2
	32.2167	-83.4206	1	1	
	32.2994	-83.4626	2	2	
	32.5611	-83.5466	2	1	1
	32.6393	-83.5487	6	6	
	32.7296	-83.5993	6	6	
AFL	32.9912	-83.7235	6	6	
	33.1075	-83.8046	4	4	
	33.1346	-83.8159	7	6	1
	33.1862	-83.8174	7	5	2
	33.2655	-83.8279	6	6	
	33.3136	-83.8381	6	6	
		Total	**66**	**60**	**6**

Table 15 Sample ID and STRUCTURE analysis results (Q-value) for black bass hybrids and pure black bass species that were phenotypically identified as Spotted Bass on the Ocmulgee River, Georgia (OCM) above the Fall Line (AFL) and below the Fall Line (BFL). The highlighted Q-values are genomic proportions ≥ 0.05.

Sample ID	Location	ALB	ALTB	LMB	SHB	SMB	SPB	Species
SPBOMR003	OCM-BFL	0.73	0.00	0.00	0.27	0.00	0.00	ALB x SHB
SPBOMR009	OCM-BFL	0.88	0.01	0.00	0.04	0.05	0.02	ALB x SMB
SPBOMR030	OCM-BFL	0.49	0.00	0.00	0.50	0.00	0.00	ALB x SHB
SPBOMR056	OCM-AFL	0.00	0.97	0.01	0.00	0.00	0.01	ALTB
SPBOMR020	OCM-AFL	0.56	0.44	0.00	0.00	0.00	0.00	ALB x ALTB
SPBOMR052	OCM-AFL	0.81	0.00	0.00	0.18	0.01	0.00	ALB x SHB

Shoal Bass Samples

Ocmulgee River

Shoal Bass specimens (n=60) were collected from the Ocmulgee River above and below the Fall Line. The average TL of was 306 mm and the average weight was 526 g. The samples were genotyped on the 64-plex black bass panels and analyzed within STRUCTURE to determine their genetic identity and assess hybridization between Shoal Bass and introduced or native black bass species (**Appendix III)**. The details of the sampling location and STRUCTURE results are presented in **Table 16**. The overall average Shoal Bass Q-value was 0.90. Out of the 60 samples, a total of 48 individuals could be regarded as pure Shoal Bass ($Q \geq 0.90$). One of the samples (SHBOMR018) was incorrectly identified as a Shoal Bass and was instead a pure Alabama Bass (Q=0.97). The remaining individuals (n=10) were hybrids of various black bass species proportions. Shoal Bass samples with Redeye Bass allele inclusion were reanalyzed in STRUCTURE with Altamaha Bass, as described previously (**Table 17**).

The majority of the black bass hybrids (n=7) were collected on the Ocmulgee River above the Fall Line, and the remaining individuals were collected on the river below the Fall Line (n=3). The hybrid individuals collected below the Fall Line consisted of a Shoal Bass and Altamaha Bass hybrid (SHBOMR001), Shoal Bass and Alabama Bass hybrid (SHBOMR008), and a Shoal Bass, Alabama Bass, and Smallmouth Bass hybrid (SHBOMR030). Above the Fall Line, one of the individuals (SHBOMR012) was an F1 of a Shoal Bass (Q=0.50) and Alabama Bass (Q=0.49). Three of the hybrid individuals (SHBOMR015, 017, 019) were collected at the same location, and were all determined to be Alabama Bass, Altamaha Bass, and Shoal Bass hybrids of various proportions. Two individuals (SHBOMR023, 061) were Shoal Bass and

Altamaha Bass hybrids, and the remaining individual (SHBOMR026) was a Shoal Bass and Smallmouth Bass hybrid.

Table 16 Sampling locations, number of individuals sampled in each location (N), number of pure SHB, and the number of hybrids sampled from below the Fall Line (BFL) and above the Fall Line (AFL) on the Ocmulgee River, Georgia.

Location	Latitude	Longitude	N	Pure SHB	Hybrid
BFL	32.1315	-83.3598	4	2	1
	32.2167	-83.4206	6	5	1
	32.5611	-83.5466	2	2	
	32.6393	-83.5487	6	6	
	32.7296	-83.5993	6	5	1
AFL	32.9912	-83.7235	7	5	2
	33.1075	-83.8046	3	2	1
	33.1346	-83.8159	7	3	4
	33.1862	-83.8174	6	6	
	33.2655	-83.8279	6	6	
	33.3136	-83.8381	6	6	
		Total	59	48	10

Table 17 Sample ID and STRUCTURE analysis results (Q-value) for black bass hybrids and pure black bass species that were phenotypically identified as Shoal Bass on the Ocmulgee River, Georgia (OCM) above the Fall Line (AFL) and below the Fall Line (BFL). The highlighted Q-values are genomic proportions ≥ 0.05.

Sample ID	Origin	ALB	ALTB	LMB	SHB	SMB	SPB	Species
SHBOMR001	OCM-BFL	0.00	0.38	0.00	0.59	0.01	0.01	SHB x ALTB
SHBOMR008	OCM-BFL	0.33	0.01	0.01	0.65	0.00	0.00	SHB x ALB
SHBOMR030	OCM-BFL	0.23	0.01	0.02	0.68	0.06	0.00	SHB x ALB x SMB
SHBOMR012	OCM-AFL	0.49	0.00	0.00	0.50	0.00	0.00	SHB x ALB
SHBOMR015	OCM-AFL	0.71	0.06	0.00	0.22	0.00	0.00	ALB x SHB x ALTB
SHBOMR017	OCM-AFL	0.67	0.24	0.00	0.08	0.00	0.00	ALB x ALTB x SHB
SHBOMR019	OCM-AFL	0.54	0.30	0.00	0.15	0.00	0.00	ALB x ALTB x SHB
SHBOMR018	OCM-AFL	0.98	0.00	0.01	0.01	0.01	0.01	ALB
SHBOMR023	OCM-AFL	0.00	0.20	0.01	0.79	0.00	0.01	SHB x ALTB
SHBOMR026	OCM-AFL	0.00	0.00	0.00	0.84	0.12	0.03	SHB x SMB
SHBOMR061	OCM-AFL	0.00	0.17	0.01	0.81	0.00	0.00	SHB x ALTB

Cryptic Black Bass Form in the ARB

During the course of the genetic survey of Largemouth Bass in the Altamaha River Drainage, a novel genetic signature was found among samples phenotypically identified as LMB in the Altamaha (n=10), Ocmulgee (n=8), and Oconee (n=1) rivers (**Figure 10**). Details of the sampling locations and results from the 38-plex FLNB results are presented in **Appendix V**. There were a total of 10 individuals collected from the Altamaha with high homozygosity (≥96-100%), but a hybrid pattern of diagnostic Northern and Florida allele usage. The individuals were 60-63% FLMB and 37-40% NLMB, with five of the samples exhibiting a limited amount of heterozygosity (~3-5%). These low levels of heterozygosity exclude the possibility that these bass are intergrade Largemouth, as heterozygosity in intergrade bass on this panel averages around 50%. By contrast, a true FLMB x NLMB F1 will be scored at 100% heterozygous (Li et al. 2015; Zhao et al. 2018). The 10 samples were also run on the 64-plex black bass panels and analyzed using STRUCTURE to ensure that they were not another pure black bass species that has already been described. The results from STRUCTURE indicated that the samples are designated as pure Largemouth Bass species ($Q \geq 0.95$), but as indicated from the 38-plex FLMB panels they are neither a pure FLMB or NLMB, nor an intergrade Largemouth Bass. These results suggest that these 10 specimens are a potential novel cryptic black bass form in the ARB.

In the lower Ocmulgee River, specifically Telfair and Wheeler County, eight specimens were identified with similar genetic signatures to the unknown samples found in the Altamaha River. In addition, one sample with the same genotypic pattern was identified from the lower Oconee River in Wheeler County. On the 38-plex FLNB panels, the samples were 62-63% FLMB, 37-38% NLMB, and 97-100% homozygous. After genotyping on the 64-plex black bass panels, these samples were again identified as pure Largemouth Bass ($Q \geq 0.95$). Additional

molecular and morphological analyses, needed to better define the observed form, are currently underway (see **Discussion**).

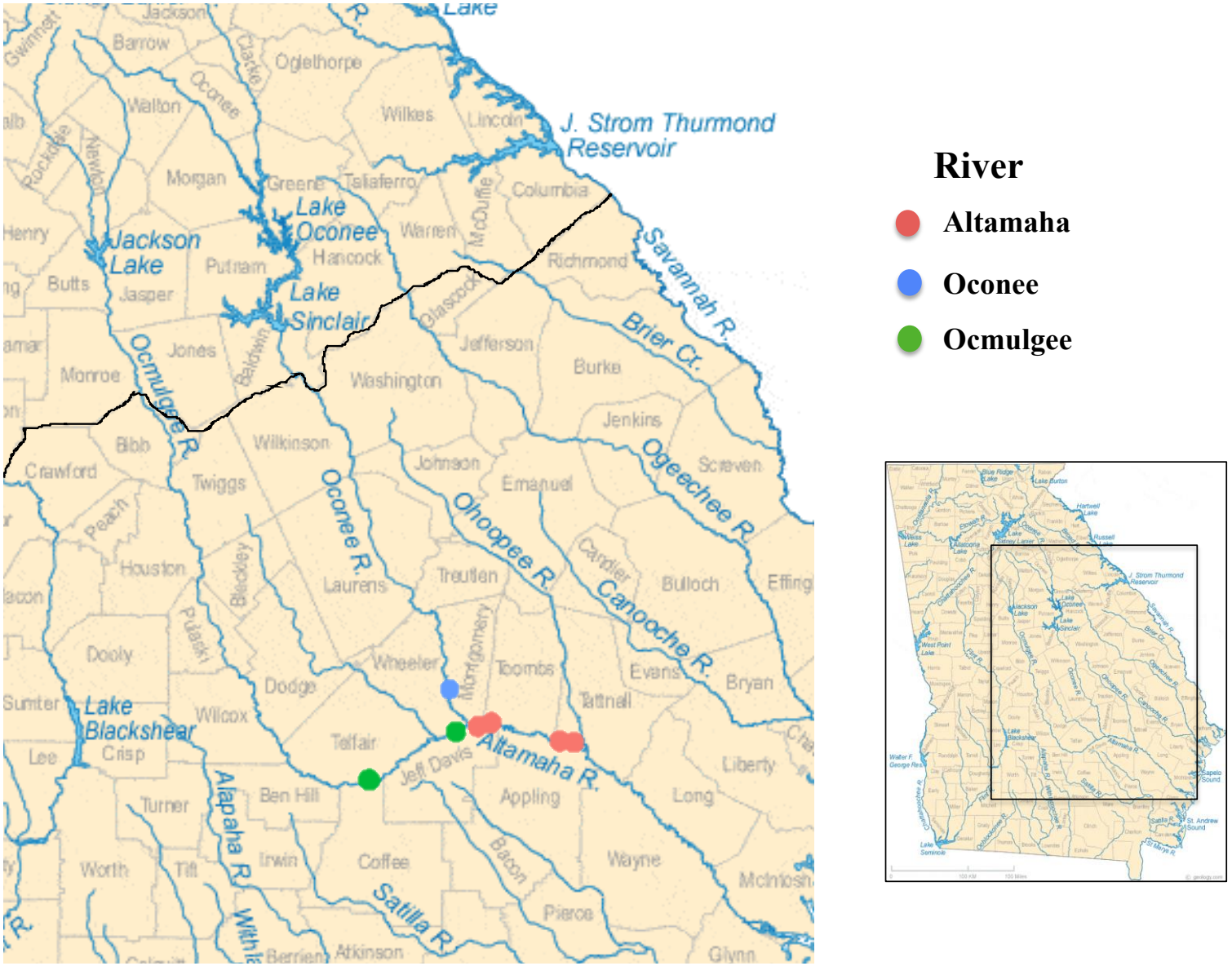

Figure 10 Sampling locations of the cryptic black bass form in the Altamaha (n=10), Oconee (n=1), and Ocmulgee (n= 8) Rivers.

Discussion

The present study utilized recently developed diagnostic SNP markers for black basses to characterize species composition and hybridization patterns in individuals sampled from the Altamaha River Basin, one of the largest freshwater drainages on the Atlantic coast of North America. Black bass populations within the drainage have received relatively little scrutiny, potentially due to its relative remoteness, lack of river access, and small number of impoundments. Our genetic results both confirm phenotypic observations (e.g. Sammons and Goclowski 2012) and differ from published reports (e.g. Bailey and Hubbs 1949), highlighting the need for closer analyses of bass populations in riverine systems using current molecular tools.

The ARB system produced the current world record Largemouth Bass from an oxbow lake off the Ocmulgee River in Telfair County, Georgia in June 1932. Based on geographically and numerically limited (n=7) sampling by Bailey and Hubbs (1949), the now mythical fish and the broader ARB has been speculatively regarded as an intergrade system ever since. Philipp et al. (1983) sampled Lake Sinclair and provided one of the few biochemical profiles of the ARB. Based on two diagnostic allozyme markers, he estimated that fish were 63.75% FLMB. However, my results paint a more interesting and complicated picture of Largemouth Bass in the drainage. As observed in Alabama (E. Peatman, Auburn University, unpublished results), the Fall Line between the Piedmont and Coastal geological regions is a major differentiating barrier for LMB. I detected significantly higher levels of FLMB alleles below the Fall Line in both the Ocmulgee and Oconee rivers. Interestingly, below the Fall Line in these rivers and the Altamaha River, a high number of pure FLMB individuals were observed, with some sample sites yielding only pure specimens. To my knowledge, the presence of pure FLMB individuals over >100 river

miles in central/south Georgia has not been reported. Although not included in the present analysis, the Satilla River LMB population has recently been found to be similarly composed of a majority of pure FLMB (E. Peatman, AU, unpublished data). The numbers of observed individuals, their large geographic range, the riverine localities, and the lack of any appreciable number of intergrade individuals within sampled fish, all argue against these fish being the result of a stocking event(s). Below the Fall Line in the ARB, Florida Bass allele percentages did not differ significantly among sample locations. Further in-depth sampling, morphological, and life history analyses are warranted to better understand and compare ARB FLMB with better-characterized, canonical Florida bass (Barthel et al. 2010).

The native range of the Spotted Bass is the central and lower Mississippi River basin, in addition to the Ohio River basin (Page and Burr 1991). Through drainage dispersal and non-native introductions, the range of the Spotted Bass has expanded extensively. In Georgia, established populations of Spotted Bass exist throughout much of the state, including the Oconee and Ocmulgee rivers. In 2005, Spotted Bass were collected from the upper Flint River where their numbers appeared to be increasing (Sammons and Goclowski 2012). Studies conducted on growth rates of Spotted Bass in the Ocmulgee River suggested that the Spotted Bass in the systems might actually be Alabama Bass (Sammons and Goclowski 2012). Alabama Bass are endemic to the Mobile River basin of Alabama, Georgia, and Mississippi (Hubbs and Bailey 1940). The range of Alabama Bass has been expanded through illegal introductions by anglers into the Savannah, Tennessee, and Chattahoochee Rivers (Pierce and Van Den Avyle 1997; Barwick et al. 2006; Moyer et al. 2014). The results from this study confirm that the populations phenotypically described as Spotted Bass in the Ocmulgee River, were predominately Alabama

Bass (~91% of collected samples). As elsewhere, the Alabama Bass is actively hybridizing with native and introduced basses including Shoal Bass and Altamaha Bass (Freeman et al. 2015).

Shoal Bass hybridization events have been well documented throughout the native range of the species (Alvarez et al. 2015; Dakin et al. 2015; Tringali et al. 2015; Taylor et al. 2018). In the ACF River basin, Shoal Bass populations have been threatened by hybridization with non-native introduced species such as Spotted Bass, Smallmouth Bass, and Alabama Bass. In addition to hybridization, these non-native species can also negatively impact Shoal Bass populations through interspecific competition since they have similar habitat preferences (Sammons and Maceina 2009). In the lower Flint River, Georgia, Alvarez et al. (2015) tested 372 black bass specimens for hybridization and reported 67 Shoal Bass and Spotted Bass hybrids. They also reported that male Shoal Bass were crossbreeding with female Spotted Bass in ~85% of the observed interactions in the Flint River. In the Chipola River, Florida, Tringali et al. (2015) found that Shoal Bass were hybridizing with the newly described Choctaw Bass (*M. sp. cf. punctulatus*), Largemouth Bass, and Spotted Bass.

In the mid 1970's, Shoal Bass from the upper Flint River were introduced into the upper Ocmulgee River below Lake Jackson by GADNR (William and Burgess 1999). Since then, Shoal Bass have spread throughout the Piedmont portions of the watershed and the population continues to expand downstream through time (Bart et al. 1994; Sammons et al. 2015). Initially, there were concerns that the introduced Shoal Bass were hybridizing with Redeye Bass. Upon further investigation, Dunham et al. (1994, unpublished data) found that hybridization events had occurred between the two species several generations ago since they did not detect F1 hybrids. They also determined that all of the polymorphic loci for Redeye Bass and Shoal Bass were at Hardy-Weinberg equilibrium. Our results confirm that introduced Shoal Bass are hybridizing

with Altamaha Bass, although 80% of Shoal Bass samples were pure individuals. These hybrids, theoretically with disrupted reproductive barriers (Bangs et al. 2017), often went on to hybridize with Alabama Bass. Further targeted study is needed to determine hybridization trends over time and whether Shoal Bass represent a threat to Altamaha Bass in the ARB going forward.

An unknown LMB form was identified over the course of genotyping phenotypic Largemouth Bass samples from the ARB. A unique genetic signature on the 38-plex and 64-plex SNP panels suggested a bass type closely related to Largemouth Bass, and yet containing a stable, non-recombinant genome with FLMB and NLMB contributions, differing from a typical intergrade individual. The signature was noted at overlapping sample locations from 2016-2018 below the Fall Line near the confluence of the Ocmulgee, Oconee, and Altamaha rivers. The genetic signature is unique in these samples when compared with over >10,000 Largemouth Bass samples from throughout the United States which have been analyzed by our lab using the 38-plex panels (Zhao et al. 2018). Intriguingly, these fish appear to co-occur in the ARB with pure FLMB, and yet no hybrids between the two have been observed over the three sample years. Mitochondrial sequencing (COI, ND2, CYTB) indicates that these individuals have a FLMB mitochondrial genome, indistinguishable at these loci from the FLMB with which they co-occur or from FLMB in Florida (unpublished result). Recent microsatellite analyses with multiple panels indicate that the novel form is genetically distinct, exhibits low levels of genetic variation overall, produces unique taxa-profiles, and carries private alleles at some loci at 100% frequency (D. Lutz-Carrillo, TPWD, personal communication). Again, no evidence of hybridization with surrounding pure FLMB was found. The pattern of alternate taxa-specific alleles and apparent reproductive isolation may indicate divergence through a phenomenon termed homoploid hybrid speciation (Feliner et al. 2017). Limited sampling combined with genotyping in 2018 has also

indicated that specimens may differ phenotypically from surrounding FLMB individuals. Efforts are currently underway to more systematically characterize the meristic and molecular features of this form as well as to better define the extent of its range and its abundance. Sampling of LMB in the adjacent Satilla and Ogeechee drainages has not identified similar individuals to-date (E. Peatman, AU, unpublished data).

In conclusion, the SNP analyses of black bass populations in the ARB offer one of the first assessments of species composition and hybridization patterns in the drainage. While my results include some notable findings (pure FLMB and a novel bass form) and substantially increase our understanding of ARB black bass populations, they also show the need for additional well-structured (and repeated) surveys on this important, yet neglected, river system.

Table 18 Respective genotypes of a ‘pure’ FLMB (yellow), ‘pure’ NLMB (green), F1 hybrid (purple), a representative intergrade LMB, and the novel form in the ARB.

FLMB	TT	TT	GG	GG	TT	TT	TT	TT	AA	AA	TT	GG	TT	CC	AA	CC	GG	GG	TT	TT
NLMB	AA	CC	AA	AA	CC	CC	GG	GG	GG	TT	AA	AA	CC	TT	GG	TT	TT	AA	CC	CC
F1	TA	TC	GA	GA	TC	TC	TG	TG	AG	AT	TA	GA	TC	CT	AG	CT	GT	GA	TC	TC
Intergrade	TA	CT	AG	GG	TT	CT	TT	TT	GG	AT	TT	AA	TT	CC	GA	CC	GT	AG	CC	CT
Novel Form	AA	CC	GG	GG	CC	CC	TT	TT	GG	TT	TT	GG	TT	CC	AA	CC	TT	GG	TT	TT

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Appendix I Sampling locations (Latitude/Longitude), sample IDs, phenotypic IDs, length (mm), and weight (g) information from the Largemouth Bass, Spotted Bass, and Shoal Bass samples collected from the Altamaha, Oconee, and Ocmulgee Rivers.

Location	ID	Phenotypic ID	Latitude	Longitude	Length (mm)	Weight (g)
Altamaha River	LMBALT001	LMB	31.96388	-82.45431	242	161
Altamaha River	LMBALT002	LMB	31.96388	-82.45431	329	440
Altamaha River	LMBALT003	LMB	31.96388	-82.45431	293	306
Altamaha River	LMBALT004	LMB	31.96388	-82.45431	389	887
Altamaha River	LMBALT005	LMB	31.96388	-82.45431	430	1050
Altamaha River	LMBALT006	LMB	31.96388	-82.45431	296	321
Altamaha River	LMBALT007	LMB	31.96388	-82.45431	270	255
Altamaha River	LMBALT008	LMB	31.96388	-82.45431	400	815
Altamaha River	LMBALT009	LMB	31.96388	-82.45431	337	559
Altamaha River	LMBALT010	LMB	31.96388	-82.45431	334	519
Altamaha River	LMBALT011	LMB	31.43708	-81.61082	165	58
Altamaha River	LMBALT012	LMB	31.43708	-81.61082	370	669
Altamaha River	LMBALT013	LMB	31.43708	-81.61082	180	78
Altamaha River	LMBALT014	LMB	31.43708	-81.61082	396	763
Altamaha River	LMBALT015	LMB	31.43708	-81.61082	322	453
Altamaha River	LMBALT016	LMB	31.43708	-81.61082	301	316
Altamaha River	LMBALT017	LMB	31.43708	-81.61082	270	239
Altamaha River	LMBALT018	LMB	31.43708	-81.61082	236	169
Altamaha River	LMBALT019	LMB	31.43708	-81.61082	386	907
Altamaha River	LMBALT020	LMB	31.43708	-81.61082	413	1044
Altamaha River	LMBALT021	LMB	31.53998	-81.66309	154	55
Altamaha River	LMBALT022	LMB	31.53998	-81.66309	380	893
Altamaha River	LMBALT023	LMB	31.53998	-81.66309	141	
Altamaha River	LMBALT024	LMB	31.53998	-81.66309	268	290
Altamaha River	LMBALT025	LMB	31.53998	-81.66309	160	50
Altamaha River	LMBALT026	LMB	31.53998	-81.66309	141	32
Altamaha River	LMBALT027	LMB	31.53998	-81.66309	173	63

Altamaha River	LMBALT028	LMB	31.53998	-81.66309	123	26
Altamaha River	LMBALT029	LMB	31.53998	-81.66309	154	47
Altamaha River	LMBALT030	LMB	31.53998	-81.66309	309	415
Altamaha River	LMBALT031	LMB	31.52769	-81.65836	448	1448
Altamaha River	LMBALT032	LMB	31.52769	-81.65836	430	1187
Altamaha River	LMBALT033	LMB	31.52769	-81.65836	273	273
Altamaha River	LMBALT034	LMB	31.52769	-81.65836	412	1071
Altamaha River	LMBALT035	LMB	31.52769	-81.65836	420	1183
Altamaha River	LMBALT036	LMB	31.52769	-81.65836	429	1238
Altamaha River	LMBALT037	LMB	31.52769	-81.65836	348	577
Altamaha River	LMBALT038	LMB	31.52769	-81.65836	195	102
Altamaha River	LMBALT039	LMB	31.52769	-81.65836	309	495
Altamaha River	LMBALT040	LMB	31.52769	-81.65836	312	453
Altamaha River	LMBALT041	LMB	31.52769	-81.65836	290	369
Altamaha River	LMBALT042	LMB	31.52769	-81.65836	352	633
Altamaha River	LMBALT043	LMB	31.52769	-81.65836	199	97
Altamaha River	LMBALT044	LMB	31.52769	-81.65836	301	347
Altamaha River	LMBALT045	LMB	31.52769	-81.65836	335	515
Altamaha River	LMBALT046	LMB	31.52769	-81.65836	357	672
Altamaha River	LMBALT047	LMB	31.52769	-81.65836	379	788
Altamaha River	LMBALT048	LMB	31.52769	-81.65836	380	840
Altamaha River	LMBALT049	LMB	31.52769	-81.65836	245	220
Altamaha River	LMBALT050	LMB	31.52769	-81.65836	275	296
Altamaha River	LMBALT051	LMB	31.90585	-82.19599	395	866
Altamaha River	LMBALT052	LMB	31.90585	-82.19599	304	321
Altamaha River	LMBALT053	LMB	31.90585	-82.19599	228	132
Altamaha River	LMBALT054	LMB	31.90585	-82.19599	409	886
Altamaha River	LMBALT055	LMB	31.90585	-82.19599	269	211
Altamaha River	LMBALT056	LMB	31.90585	-82.19599	164	48
Altamaha River	LMBALT057	LMB	31.90585	-82.19599	139	25
Altamaha River	LMBALT058	LMB	31.90585	-82.19599	274	228

Altamaha River	LMBALT059	LMB	31.90585	-82.19599	288	299
Altamaha River	LMBALT060	LMB	31.90585	-82.19599	245	113
Altamaha River	LMBALT061	LMB	31.90144	-82.14140	325	464
Altamaha River	LMBALT062	LMB	31.90144	-82.14140	421	1165
Altamaha River	LMBALT063	LMB	31.90144	-82.14140	227	135
Altamaha River	LMBALT064	LMB	31.90144	-82.14140	266	206
Altamaha River	LMBALT065	LMB	31.90144	-82.14140	242	156
Altamaha River	LMBALT066	LMB	31.90144	-82.14140	339	458
Altamaha River	LMBALT067	LMB	31.90144	-82.14140	135	25
Altamaha River	LMBALT068	LMB	31.90144	-82.14140	268	213
Altamaha River	LMBALT069	LMB	31.90144	-82.14140	203	78
Altamaha River	LMBALT070	LMB	31.90144	-82.14140	220	119
Altamaha River	MYST 130	LMB	31.95148	-82.50669	356	531
Altamaha River	MYST 131	LMB	31.95148	-82.50669	369	600
Altamaha River	MYST 132	LMB	31.95148	-82.50669	375	622
Altamaha River	MYST 133	LMB	31.95148	-82.50669	294	304
Altamaha River	MYST 134	LMB	31.95148	-82.50669	312	348
Altamaha River	MYST 135	LMB	31.95148	-82.50669	293	287
Altamaha River	MYST 136	LMB	31.95148	-82.50669	286	235
Altamaha River	MYST 137	LMB	31.95148	-82.50669	315	334
Altamaha River	MYST 138	LMB	31.95148	-82.50669	276	237
Altamaha River	MYST 139	LMB	31.95148	-82.50669	247	123
Altamaha River	MYST 140	LMB	31.95148	-82.50669	260	178
Altamaha River	MYST 141	LMB	31.95148	-82.50669	258	190
Altamaha River	MYST 142	LMB	31.95148	-82.50669	251	161
Altamaha River	MYST 143	LMB	31.95148	-82.50669	242	144
Altamaha River	MYST 144	LMB	31.95148	-82.50669	189	71
Altamaha River	MYST 145	LMB	31.95148	-82.50669	203	78
Oconee River	MYST 121	LMB	31.98172	-82.55125	399	831
Oconee River	MYST 122	LMB	31.98172	-82.55125	375	732
Oconee River	MYST 123	LMB	31.98172	-82.55125	326	422

Oconee River	MYST 125	LMB	31.98172	-82.55125	305	330
Oconee River	MYST 126	LMB	31.98172	-82.55125	370	594
Oconee River	MYST 127	LMB	31.98172	-82.55125	260	193
Oconee River	MYST 128	LMB	31.98172	-82.55125	270	218
Oconee River	MYST 129	LMB	31.98172	-82.55125	186	73
Oconee River	LMBOCR001	LMB	32.45709	-82.84090	258	217
Oconee River	LMBOCR002	LMB	32.45709	-82.84090	407	917
Oconee River	LMBOCR003	LMB	32.45709	-82.84090	383	753
Oconee River	LMBOCR004	LMB	32.45709	-82.84090	465	1607
Oconee River	LMBOCR005	LMB	32.45709	-82.84090	417	1189
Oconee River	LMBOCR006	LMB	32.45709	-82.84090	409	950
Oconee River	LMBOCR007	LMB	32.45709	-82.84090	422	1159
Oconee River	LMBOCR008	LMB	32.45709	-82.84090	407	1050
Oconee River	LMBOCR009	LMB	32.45709	-82.84090	432	1299
Oconee River	LMBOCR010	LMB	32.45709	-82.84090	405	879
Oconee River	LMBOCR011	LMB	32.45709	-82.84090	415	987
Oconee River	LMBOCR012	LMB	32.45709	-82.84090	370	700
Oconee River	LMBOCR013	LMB	32.45709	-82.84090	357	580
Oconee River	LMBOCR014	LMB	32.45709	-82.84090	284	295
Oconee River	LMBOCR015	LMB	32.45709	-82.84090	297	325
Oconee River	LMBOCR016	LMB	32.45709	-82.84090	309	357
Oconee River	LMBOCR017	LMB	32.45709	-82.84090	302	351
Oconee River	LMBOCR018	LMB	32.45709	-82.84090	392	830
Oconee River	LMBOCR019	LMB	32.45709	-82.84090	425	1135
Oconee River	LMBOCR020	LMB	32.45709	-82.84090	317	369
Oconee River	LMBOCR021	LMB	32.40934	-82.81452	254	192
Oconee River	LMBOCR022	LMB	32.40934	-82.81452	132	23
Oconee River	LMBOCR023	LMB	32.40934	-82.81452	345	512
Oconee River	LMBOCR024	LMB	32.40934	-82.81452	345	561
Oconee River	LMBOCR025	LMB	32.40934	-82.81452	455	1350
Oconee River	LMBOCR026	LMB	32.40934	-82.81452	372	812

Oconee River	LMBOCR027	LMB	32.40934	-82.81452	122	23
Oconee River	LMBOCR028	LMB	32.40934	-82.81452	325	486
Oconee River	LMBOCR029	LMB	32.40934	-82.81452	460	1403
Oconee River	LMBOCR030	LMB	32.40934	-82.81452	280	285
Oconee River	LMBOCR031	LMB	32.34665	-82.73204	371	740
Oconee River	LMBOCR032	LMB	32.34665	-82.73204	134	22
Oconee River	LMBOCR033	LMB	32.34665	-82.73204	292	31
Oconee River	LMBOCR034	LMB	32.34665	-82.73204	328	452
Oconee River	LMBOCR035	LMB	32.34665	-82.73204	314	410
Oconee River	LMBOCR036	LMB	32.34665	-82.73204	151	36
Oconee River	LMBOCR037	LMB	32.34665	-82.73204	318	434
Oconee River	LMBOCR038	LMB	32.34665	-82.73204	400	888
Oconee River	LMBOCR039	LMB	32.34665	-82.73204	431	1193
Oconee River	LMBOCR040	LMB	32.34665	-82.73204	40	
Oconee River	LMBOCR041	LMB	32.30327	-82.70732	357	635
Oconee River	LMBOCR042	LMB	32.30327	-82.70732	385	780
Oconee River	LMBOCR043	LMB	32.30327	-82.70732	473	1640
Oconee River	LMBOCR044	LMB	32.30327	-82.70732	405	990
Oconee River	LMBOCR045	LMB	32.30327	-82.70732	395	950
Oconee River	LMBOCR046	LMB	32.27518	-82.64409	246	178
Oconee River	LMBOCR047	LMB	32.27518	-82.64409	183	75
Oconee River	LMBOCR048	LMB	32.27518	-82.64409	301	336
Oconee River	LMBOCR049	LMB	32.27518	-82.64409	254	164
Oconee River	LMBOCR050	LMB	32.27518	-82.64409	157	39
Oconee River	LMBOCR051	LMB	32.06864	-82.61354	345	505
Oconee River	LMBOCR052	LMB	32.06864	-82.61354	370	743
Oconee River	LMBOCR053	LMB	32.06864	-82.61354	333	467
Oconee River	LMBOCR054	LMB	32.06864	-82.61354	300	347
Oconee River	LMBOCR055	LMB	32.06864	-82.61354	325	405
Oconee River	LMBOCR056	LMB	32.06864	-82.61354	189	73
Oconee River	LMBOCR057	LMB	32.06864	-82.61354	241	160

Oconee River	LMBOCR058	LMB	32.06864	-82.61354	213	103
Oconee River	LMBOCR059	LMB	32.06864	-82.61354	244	177
Oconee River	LMBOCR060	LMB	32.06864	-82.61354	345	520
Oconee River	LMBOCR061	LMB	32.06864	-82.61354	205	96
Oconee River	LMBOCR062	LMB	32.07535	-82.61098	333	455
Oconee River	LMBOCR063	LMB	32.07535	-82.61098	361	571
Oconee River	LMBOCR064	LMB	32.07535	-82.61098	309	324
Oconee River	LMBOCR065	LMB	32.07535	-82.61098	406	956
Oconee River	LMBOCR066	LMB	32.07535	-82.61098	310	396
Oconee River	LMBOCR067	LMB	32.07535	-82.61098	185	70
Oconee River	LMBOCR068	LMB	32.07535	-82.61098	197	90
Oconee River	LMBOCR069	LMB	32.07535	-82.61098	250	184
Oconee River	LMBOCR070	LMB	32.07535	-82.61098	349	600
Oconee River	LMBOCR071	LMB	32.07535	-82.61098	164	53
Oconee River	LMBOCR072	LMB	32.07535	-82.61098	316	370
Oconee River	LMBOCR073	LMB	32.07535	-82.61098	455	1550
Oconee River	LMBOCR074	LMB	32.07535	-82.61098	326	465
Oconee River	LMBOCR075	LMB			635	4672
Lake Sinclair	GA16SCL001	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL002	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL003	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL004	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL005	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL006	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL007	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL008	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL009	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL010	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL011	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL012	LMB	33.15180	-83.21696		
Lake Sinclair	GA16SCL013	LMB	33.15180	-83.21696		

Lake Sinclair	GA16SCL014	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL015	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL016	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL017	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL018	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL019	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL020	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL021	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL022	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL023	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL024	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL025	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL026	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL027	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL028	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL029	LMB	33.15180	-83.21696
Lake Sinclair	GA16SCL030	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL039	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL040	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL041	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL042	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL043	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL044	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL045	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL046	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL047	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL048	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL049	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL050	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL051	LMB	33.16718	-83.23910
Lake Sinclair	GA16SCL052	LMB	33.16718	-83.23910

Lake Sinclair	GA16SCL053	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL054	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL055	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL056	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL057	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL058	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL059	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL060	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL061	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL062	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL063	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL064	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL065	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL066	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL067	LMB	33.16718	-83.23910		
Lake Sinclair	GA16SCL068	LMB	33.16718	-83.23910		
Ocmulgee River	MYST 101	LMB	31.78351	-82.91664	438	1166
Ocmulgee River	MYST 102	LMB	31.78351	-82.91664	279	237
Ocmulgee River	MYST 103	LMB	31.78351	-82.91664	300	371
Ocmulgee River	MYST 104	LMB	31.78351	-82.91664	288	285
Ocmulgee River	MYST 105	LMB	31.78351	-82.91664	197	70
Ocmulgee River	MYST 106	LMB	31.78351	-82.91664	243	152
Ocmulgee River	MYST 107	LMB	31.78351	-82.91664	142	26
Ocmulgee River	MYST 108	LMB	31.78351	-82.91664	187	62
Ocmulgee River	MYST 109	LMB	31.78351	-82.91664	281	264
Ocmulgee River	MYST 110	LMB	31.93525	-82.58941	289	268
Ocmulgee River	MYST 111	LMB	31.93525	-82.58941	442	889
Ocmulgee River	MYST 112	LMB	31.93525	-82.58941	341	492
Ocmulgee River	MYST 113	LMB	31.93525	-82.58941	310	311
Ocmulgee River	MYST 114	LMB	31.93525	-82.58941	365	534
Ocmulgee River	MYST 115	LMB	31.93525	-82.58941	340	390

Ocmulgee River	MYST 116	LMB	31.93525	-82.58941	266	198
Ocmulgee River	MYST 117	LMB	31.93525	-82.58941	245	158
Ocmulgee River	MYST 118	LMB	31.93525	-82.58941	172	44
Ocmulgee River	MYST 119	LMB	31.93525	-82.58941	161	39
Ocmulgee River	MYST 120	LMB	31.93525	-82.58941	102	9
Ocmulgee River	LMBOMR101	LMB	32.56479	-83.54838	355	734
Ocmulgee River	LMBOMR102	LMB	32.56479	-83.54838	389	956
Ocmulgee River	LMBOMR103	LMB	32.56479	-83.54838	380	864
Ocmulgee River	LMBOMR104	LMB	32.56479	-83.54838	445	1504
Ocmulgee River	LMBOMR105	LMB	32.56479	-83.54838	450	1484
Ocmulgee River	LMBOMR106	LMB	32.56479	-83.54838	346	748
Ocmulgee River	LMBOMR107	LMB	32.56479	-83.54838	175	85
Ocmulgee River	LMBOMR108	LMB	32.56479	-83.54838	199	113
Ocmulgee River	LMBOMR109	LMB	32.56479	-83.54838	162	58
Ocmulgee River	LMBOMR110	LMB	32.56479	-83.54838	135	34
Ocmulgee River	LMBOMR111	LMB	32.56479	-83.54838	143	37
Ocmulgee River	LMBOMR112	LMB	32.56479	-83.54838	238	559
Ocmulgee River	LMBOMR113	LMB	32.56479	-83.54838	356	577
Ocmulgee River	LMBOMR114	LMB	32.56479	-83.54838	293	390
Ocmulgee River	LMBOMR115	LMB	32.56479	-83.54838	211	128
Ocmulgee River	LMBOMR116	LMB	32.56479	-83.54838	355	666
Ocmulgee River	LMBOMR117	LMB	32.56479	-83.54838	393	772
Ocmulgee River	LMBOMR118	LMB	32.56479	-83.54838	381	832
Ocmulgee River	LMBOMR119	LMB	32.56479	-83.54838	312	478
Ocmulgee River	LMBOMR120	LMB	32.56479	-83.54838	210	149
Ocmulgee River	LMBOMR121	LMB	32.56479	-83.54838	216	162
Ocmulgee River	LMBOMR122	LMB	32.56479	-83.54838	356	657
Ocmulgee River	LMBOMR123	LMB	32.56479	-83.54838	200	104
Ocmulgee River	LMBOMR124	LMB	32.56479	-83.54838	431	1309
Ocmulgee River	LMBOMR125	LMB	32.56479	-83.54838	157	53
Ocmulgee River	LMBOMR126	LMB	32.56479	-83.54838	219	162

Ocmulgee River	LMBOMR127	LMB	32.56479	-83.54838	227	167
Ocmulgee River	LMBOMR128	LMB	31.78447	-82.92231	238	154
Ocmulgee River	LMBOMR129	LMB	31.78447	-82.92231	208	97
Ocmulgee River	LMBOMR130	LMB	31.78447	-82.92231	109	16
Ocmulgee River	LMBOMR131	LMB	31.78447	-82.92231	214	100
Ocmulgee River	LMBOMR132	LMB	31.78447	-82.92231	204	92
Ocmulgee River	LMBOMR133	LMB	31.78447	-82.92231	209	101
Ocmulgee River	LMBOMR134	LMB	31.78447	-82.92231	210	113
Ocmulgee River	LMBOMR135	LMB	31.78447	-82.92231	208	102
Ocmulgee River	LMBOMR136	LMB	31.78447	-82.92231	173	61
Ocmulgee River	LMBOMR137	LMB	31.78447	-82.92231	259	206
Ocmulgee River	LMBOMR138	LMB	31.78447	-82.92231	115	15
Ocmulgee River	LMBOMR139	LMB	31.78447	-82.92231	106	11
Ocmulgee River	LMBOMR140	LMB	33.13460	-83.81590	451	1192
Ocmulgee River	LMBOMR141	LMB	33.13460	-83.81590	568	2434
Ocmulgee River	LMBOMR142	LMB	33.13460	-83.81590	307	341
Ocmulgee River	LMBOMR143	LMB	33.13460	-83.81590	198	85
Ocmulgee River	LMBOMR144	LMB	33.13460	-83.81590	111	14
Ocmulgee River	LMBOMR145	LMB	33.13460	-83.81590	414	825
Ocmulgee River	LMBOMR146	LMB	32.99120	-83.72350	310	369
Ocmulgee River	LMBOMR147	LMB	32.56110	-83.54660	561	2725
Ocmulgee River	LMBOMR148	LMB	32.56110	-83.54660	549	2946
Ocmulgee River	LMBOMR149	LMB	32.56110	-83.54660	505	1881
Ocmulgee River	LMBOMR150	LMB	32.56110	-83.54660	337	510
Ocmulgee River	LMBOMR151	LMB	32.56110	-83.54660	516	1937
Ocmulgee River	LMBOMR152	LMB	32.56110	-83.54660	471	1332
Ocmulgee River	LMBOMR153	LMB	32.56110	-83.54660	538	2395
Ocmulgee River	LMBOMR154	LMB	32.56110	-83.54660	380	703
Ocmulgee River	LMBOMR155	LMB	32.72960	-83.59930	497	1788
Ocmulgee River	LMBOMR156	LMB	32.72960	-83.59930	464	1550
Ocmulgee River	LMBOMR157	LMB	32.72960	-83.59930	367	643

Ocmulgee River	LMBOMR158	LMB	32.72960	-83.59930	456	1134
Ocmulgee River	LMBOMR159	LMB	32.72960	-83.59930	313	360
Ocmulgee River	LMBOMR160	LMB	32.72960	-83.59930	217	116
Ocmulgee River	LMBOMR161	LMB	32.63930	-83.54870	365	708
Ocmulgee River	LMBOMR162	LMB	32.63930	-83.54870	197	81
Ocmulgee River	LMBOMR163	LMB	32.63930	-83.54870	636	4781
Ocmulgee River	LMBOMR164	LMB	32.63930	-83.54870	505	2075
Ocmulgee River	LMBOMR165	LMB	32.63930	-83.54870	332	496
Ocmulgee River	LMBOMR166	LMB	32.63930	-83.54870	372	755
Ocmulgee River	LMBOMR167	LMB	33.31360	-83.83810	418	1016
Ocmulgee River	LMBOMR168	LMB	33.31360	-83.83810	374	558
Ocmulgee River	LMBOMR169	LMB	33.31360	-83.83810	528	1919
Ocmulgee River	LMBOMR170	LMB	33.31360	-83.83810	304	302
Ocmulgee River	LMBOMR171	LMB	33.31360	-83.83810	296	314
Ocmulgee River	LMBOMR172	LMB	33.31360	-83.83810	475	1634
Ocmulgee River	LMBOMR173	LMB	33.18620	-83.81740	495	2048
Ocmulgee River	LMBOMR174	LMB	33.18620	-83.81740	347	556
Ocmulgee River	LMBOMR175	LMB	33.18620	-83.81740	283	285
Ocmulgee River	LMBOMR176	LMB	33.26550	-83.82790	135	23
Ocmulgee River	LMBOMR177	LMB	33.26550	-83.82790	166	38
Ocmulgee River	LMBOMR178	LMB	33.26550	-83.82790	401	796
Ocmulgee River	LMBOMR179	LMB	33.26550	-83.82790	435	978
Ocmulgee River	LMBOMR180	LMB	33.26550	-83.82790	477	1453
Ocmulgee River	LMBOMR181	LMB	33.26550	-83.82790	390	710
Ocmulgee River	LMBOMR182	LMB	33.10750	-83.80460	407	1072
Ocmulgee River	LMBOMR183	LMB	33.10750	-83.80460	525	2180
Ocmulgee River	LMBOMR184	LMB	33.10750	-83.80460	309	343
Ocmulgee River	LMBOMR185	LMB	33.10750	-83.80460	348	539
Ocmulgee River	LMBOMR186	LMB	33.10750	-83.80460	304	399
Ocmulgee River	LMBOMR187	LMB	33.10750	-83.80460	303	354
Ocmulgee River	GA16OMR001	LMB				

Ocmulgee River	GA16OMR002	LMB
Ocmulgee River	GA16OMR003	LMB
Ocmulgee River	GA16OMR004	LMB
Ocmulgee River	GA16OMR005	LMB
Ocmulgee River	GA16OMR007	LMB
Ocmulgee River	GA16OMR008	LMB
Ocmulgee River	GA16OMR009	LMB
Ocmulgee River	GA16OMR016	LMB
Ocmulgee River	GA16OMR022	LMB
Ocmulgee River	GA16OMR023	LMB
Ocmulgee River	GA16OMR026	LMB
Ocmulgee River	GA16OMR027	LMB
Ocmulgee River	GA16OMR028	LMB
Ocmulgee River	GA16OMR029	LMB
Ocmulgee River	GA16OMR030	LMB
Ocmulgee River	GA16OMR032	LMB
Ocmulgee River	GA16OMR033	LMB
Ocmulgee River	GA16OMR036	LMB
Ocmulgee River	GA16OMR037	LMB
Ocmulgee River	GA16OMR038	LMB
Ocmulgee River	GA16OMR039	LMB
Ocmulgee River	GA16OMR040	LMB
Ocmulgee River	GA16OMR043	LMB
Ocmulgee River	GA16OMR047	LMB
Ocmulgee River	GA16OMR048	LMB
Ocmulgee River	GA16OMR053	LMB
Ocmulgee River	GA16OMR057	LMB
Ocmulgee River	GA16OMR059	LMB
Ocmulgee River	GA16OMR062	LMB
Ocmulgee River	GA16OMR063	LMB
Ocmulgee River	GA16OMR064	LMB

Ocmulgee River	GA16OMR065	LMB		
Ocmulgee River	GA16OMR066	LMB		
Ocmulgee River	GA16OMR067	LMB		
Ocmulgee River	GA16OMR068	LMB		
Ocmulgee River	GA16OMR069	LMB		
Ocmulgee River	GA16OMR070	LMB		
Ocmulgee River	GA16OMR071	LMB		
Ocmulgee River	GA16OMR072	LMB		
Ocmulgee River	GA16OMR077	LMB		
Ocmulgee River	GA16OMR079	LMB		
Ocmulgee River	GA16OMR081	LMB		
Ocmulgee River	GA16OMR084	LMB		
Ocmulgee River	GA16OMR087	LMB		
Ocmulgee River	GA16OMR089	LMB		
Ocmulgee River	GA16OMR090	LMB		
Ocmulgee River	GA16OMR091	LMB		
Ocmulgee River	GA16OMR092	LMB		
Ocmulgee River	GA16OMR093	LMB		
Ocmulgee River	GA16OMR094	LMB		
Ocmulgee River	GA16OMR095	LMB		
Ocmulgee River	GA16OMR096	LMB		
Ocmulgee River	GA16OMR100	LMB		
Ocmulgee River	GA16OMR104	LMB		
Ocmulgee River	GA16OMR105	LMB		
Ocmulgee River	GA16OMR107	LMB		
Ocmulgee River	GA16OMR110	LMB		
Ocmulgee River	GA16OMR115	LMB		
Ocmulgee River	GA16OMR119	LMB		
Lake Juliette	GA16JUL001	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL002	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL010	LMB	33.04740	-83.78626

Lake Juliette	GA16JUL014	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL016	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL017	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL019	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL020	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL021	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL023	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL026	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL027	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL028	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL029	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL030	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL031	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL032	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL033	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL034	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL035	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL036	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL037	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL038	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL039	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL040	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL041	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL042	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL043	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL044	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL045	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL046	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL048	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL049	LMB	33.04740	-83.78626
Lake Juliette	GA16JUL050	LMB	33.04740	-83.78626

Lake Juliette	GA16JUL051	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL052	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL053	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL054	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL055	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL056	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL057	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL058	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL059	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL060	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL061	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL062	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL063	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL064	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL065	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL066	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL067	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL068	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL069	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL070	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL071	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL072	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL073	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL074	LMB	33.04740	-83.78626		
Lake Juliette	GA16JUL075	LMB	33.04740	-83.78626		
Ocmulgee River	SPBOMR001	SPB	32.13160	-83.35980	323	363
Ocmulgee River	SPBOMR002	SPB	32.13160	-83.35980	330	397
Ocmulgee River	SPBOMR003	SPB	32.13160	-83.35980	262	190
Ocmulgee River	SPBOMR004	SPB	32.13160	-83.35980	270	211
Ocmulgee River	SPBOMR005	SPB	32.13160	-83.35980	267	200
Ocmulgee River	SPBOMR006	SPB	32.13160	-83.35980	333	430

Ocmulgee River	SPBOMR007	SPB	32.13160	-83.35980	129	22
Ocmulgee River	SPBOMR008	SPB	32.13160	-83.35980	371	698
Ocmulgee River	SPBOMR009	SPB	32.13160	-83.35980	248	172
Ocmulgee River	SPBOMR010	SPB	32.13160	-83.35980	370	712
Ocmulgee River	SPBOMR011	SPB	32.13160	-83.35980	262	192
Ocmulgee River	SPBOMR012	SPB	32.13160	-83.35980	301	286
Ocmulgee River	SPBOMR013	SPB	32.21670	-83.42060	229	122
Ocmulgee River	SPBOMR014	SPB	32.29940	-83.46260	378	669
Ocmulgee River	SPBOMR015	SPB	32.29940	-83.46260	374	571
Ocmulgee River	SPBOMR016	SPB	31.98010	-83.28430	361	508
Ocmulgee River	SPBOMR017	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR018	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR019	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR020	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR021	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR022	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR023	SPB	33.13460	-83.81590		
Ocmulgee River	SPBOMR024	SPB	32.99120	-83.72350	274	228
Ocmulgee River	SPBOMR025	SPB	32.99120	-83.72350	226	84
Ocmulgee River	SPBOMR026	SPB	32.99120	-83.72350	226	89
Ocmulgee River	SPBOMR027	SPB	32.99120	-83.72350	401	752
Ocmulgee River	SPBOMR028	SPB	32.99120	-83.72350	402	595
Ocmulgee River	SPBOMR029	SPB	32.99120	-83.72350	312	334
Ocmulgee River	SPBOMR030	SPB	32.56110	-83.54660	325	429
Ocmulgee River	SPBOMR031	SPB	32.56110	-83.54660	186	61
Ocmulgee River	SPBOMR032	SPB	32.72960	-83.59930	403	795
Ocmulgee River	SPBOMR033	SPB	32.72960	-83.59930	298	277
Ocmulgee River	SPBOMR034	SPB	32.72960	-83.59930	101	16
Ocmulgee River	SPBOMR035	SPB	32.72960	-83.59930	367	539
Ocmulgee River	SPBOMR036	SPB	32.72960	-83.59930	413	839
Ocmulgee River	SPBOMR037	SPB	32.72960	-83.59930	248	151

Ocmulgee River	SPBOMR038	SPB	32.63930	-83.54870	407	794
Ocmulgee River	SPBOMR039	SPB	32.63930	-83.54870	215	100
Ocmulgee River	SPBOMR040	SPB	32.63930	-83.54870	273	222
Ocmulgee River	SPBOMR041	SPB	32.63930	-83.54870	271	218
Ocmulgee River	SPBOMR042	SPB	32.63930	-83.54870	337	405
Ocmulgee River	SPBOMR043	SPB	32.63930	-83.54870	207	100
Ocmulgee River	SPBOMR044	SPB	33.31360	-83.83810	478	1350
Ocmulgee River	SPBOMR045	SPB	33.31360	-83.83810	256	122
Ocmulgee River	SPBOMR046	SPB	33.31360	-83.83810	511	1375
Ocmulgee River	SPBOMR047	SPB	33.31360	-83.83810	394	690
Ocmulgee River	SPBOMR048	SPB	33.31360	-83.83810	521	1777
Ocmulgee River	SPBOMR049	SPB	33.31360	-83.83810	282	228
Ocmulgee River	SPBOMR050	SPB	33.18620	-83.81740	503	1638
Ocmulgee River	SPBOMR051	SPB	33.18620	-83.81740	229	126
Ocmulgee River	SPBOMR052	SPB	33.18620	-83.81740	212	91
Ocmulgee River	SPBOMR053	SPB	33.18620	-83.81740	130	26
Ocmulgee River	SPBOMR054	SPB	33.18620	-83.81740	186	71
Ocmulgee River	SPBOMR055	SPB	33.18620	-83.81740	180	51
Ocmulgee River	SPBOMR056	SPB	33.18620	-83.81740	160	45
Ocmulgee River	SPBOMR057	SPB	33.26550	-83.82790	461	1165
Ocmulgee River	SPBOMR058	SPB	33.26550	-83.82790	318	330
Ocmulgee River	SPBOMR059	SPB	33.26550	-83.82790	283	230
Ocmulgee River	SPBOMR060	SPB	33.26550	-83.82790	206	75
Ocmulgee River	SPBOMR061	SPB	33.26550	-83.82790	361	462
Ocmulgee River	SPBOMR062	SPB	33.26550	-83.82790	389	619
Ocmulgee River	SPBOMR063	SPB	33.10750	-83.80460	212	86
Ocmulgee River	SPBOMR064	SPB	33.10750	-83.80460	110	14
Ocmulgee River	SPBOMR065	SPB	33.10750	-83.80460	224	110
Ocmulgee River	SPBOMR066	SPB	33.10750	-83.80460	220	121
Ocmulgee River	SHBOMR001	SHB	32.13154	-83.35980	315	465
Ocmulgee River	SHBOMR003	SHB	32.13154	-83.35980	341	490

Ocmulgee River	SHBOMR004	SHB	32.13154	-83.35980	325	455
Ocmulgee River	SHBOMR005	SHB	32.21670	-83.42060	364	655
Ocmulgee River	SHBOMR006	SHB	32.21670	-83.42060	398	838
Ocmulgee River	SHBOMR008	SHB	32.21670	-83.42060	376	784
Ocmulgee River	SHBOMR009	SHB	32.21670	-83.42060	298	351
Ocmulgee River	SHBOMR010	SHB	32.21670	-83.42060	283	312
Ocmulgee River	SHBOMR011	SHB	32.21670	-83.42060	262	219
Ocmulgee River	SHBOMR012	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR013	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR014	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR015	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR016	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR017	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR018	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR019	SHB	33.13460	-83.81590		
Ocmulgee River	SHBOMR020	SHB	32.99120	-83.72350	282	263
Ocmulgee River	SHBOMR021	SHB	32.99120	-83.72350	298	301
Ocmulgee River	SHBOMR022	SHB	32.99120	-83.72350	447	1116
Ocmulgee River	SHBOMR023	SHB	32.99120	-83.72350	270	230
Ocmulgee River	SHBOMR024	SHB	32.99120	-83.72350	222	126
Ocmulgee River	SHBOMR025	SHB	32.99120	-83.72350	130	22
Ocmulgee River	SHBOMR026	SHB	32.99120	-83.72350	238	150
Ocmulgee River	SHBOMR027	SHB	32.56110	-83.54660	420	920
Ocmulgee River	SHBOMR028	SHB	32.56110	-83.54660	368	603
Ocmulgee River	SHBOMR029	SHB	32.72960	-83.59930	123	23
Ocmulgee River	SHBOMR030	SHB	32.72960	-83.59930	351	573
Ocmulgee River	SHBOMR031	SHB	32.72960	-83.59930	480	1716
Ocmulgee River	SHBOMR032	SHB	32.72960	-83.59930	435	1103
Ocmulgee River	SHBOMR033	SHB	32.72960	-83.59930	360	592
Ocmulgee River	SHBOMR034	SHB	32.72960	-83.59930	450	1272
Ocmulgee River	SHBOMR035	SHB	32.63930	-83.54870	392	745

Ocmulgee River	SHBOMR036	SHB	32.63930	-83.54870	225	131
Ocmulgee River	SHBOMR037	SHB	32.63930	-83.54870	113	22
Ocmulgee River	SHBOMR038	SHB	32.63930	-83.54870	277	243
Ocmulgee River	SHBOMR039	SHB	32.63930	-83.54870	274	227
Ocmulgee River	SHBOMR040	SHB	32.63930	-83.54870	220	114
Ocmulgee River	SHBOMR041	SHB	33.31360	-83.83810	524	2240
Ocmulgee River	SHBOMR042	SHB	33.31360	-83.83810	321	377
Ocmulgee River	SHBOMR043	SHB	33.31360	-83.83810	460	1222
Ocmulgee River	SHBOMR044	SHB	33.31360	-83.83810	194	72
Ocmulgee River	SHBOMR045	SHB	33.31360	-83.83810	211	98
Ocmulgee River	SHBOMR046	SHB	33.31360	-83.83810	374	780
Ocmulgee River	SHBOMR047	SHB	33.18620	-83.81740	554	2436
Ocmulgee River	SHBOMR048	SHB	33.18620	-83.81740	193	73
Ocmulgee River	SHBOMR049	SHB	33.18620	-83.81740	271	215
Ocmulgee River	SHBOMR050	SHB	33.18620	-83.81740	201	90
Ocmulgee River	SHBOMR051	SHB	33.18620	-83.81740	140	30
Ocmulgee River	SHBOMR052	SHB	33.18620	-83.81740	199	77
Ocmulgee River	SHBOMR053	SHB	33.26550	-83.82790	296	292
Ocmulgee River	SHBOMR054	SHB	33.26550	-83.82790	440	1043
Ocmulgee River	SHBOMR055	SHB	33.26550	-83.82790	214	101
Ocmulgee River	SHBOMR056	SHB	33.26550	-83.82790	186	70
Ocmulgee River	SHBOMR057	SHB	33.26550	-83.82790	171	48
Ocmulgee River	SHBOMR058	SHB	33.26550	-83.82790	499	1693
Ocmulgee River	SHBOMR059	SHB	33.10750	-83.80460	352	503
Ocmulgee River	SHBOMR060	SHB	33.10750	-83.80460	291	248
Ocmulgee River	SHBOMR061	SHB	33.10750	-83.80460	193	75

Appendix II The 38-plex FLNB results for Largemouth Bass samples from the Altamaha, Oconee, and Ocmulgee Rivers. Samples highlighted in blue are pure (>95%) FLMB. Largemouth Bass samples with unknown genetic signatures were run on the 64-plex black bass panels to determine their identity. Individuals highlighted in yellow are the novel bass form, red are pure Shoal Bass, orange are pure Alabama Bass, and the purple sample is a hybrid specimen of Alabama Bass, Altamaha Bass, and Shoal Bass.

Location	ID	FLMB %	NLMB %	Heterozygous %	Homozygous %	Species
Altamaha River	LMBALT001	91.89	8.11	5.41	94.59	
Altamaha River	LMBALT002	98.65	1.35	2.70	97.30	FLMB
Altamaha River	LMBALT003	94.74	5.26	5.26	94.74	
Altamaha River	LMBALT004	97.14	2.86	5.71	94.29	FLMB
Altamaha River	LMBALT005	90.63	9.38	12.50	87.50	
Altamaha River	LMBALT006	95.95	4.05	2.70	97.30	FLMB
Altamaha River	LMBALT007	84.21	15.79	21.05	78.95	
Altamaha River	LMBALT008	93.55	6.45	12.90	87.10	
Altamaha River	LMBALT009	59.09	40.91	3.03	96.97	Novel Form
Altamaha River	LMBALT010	61.84	38.16	2.63	97.37	Novel Form
Altamaha River	LMBALT011	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT012	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT013	93.24	6.76	13.51	86.49	
Altamaha River	LMBALT014	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT015	93.42	6.58	7.89	92.11	
Altamaha River	LMBALT016	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT017	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT018	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT019	93.42	6.58	7.89	92.11	
Altamaha River	LMBALT020	94.74	5.26	5.26	94.74	
Altamaha River	LMBALT021	93.42	6.58	2.63	97.37	
Altamaha River	LMBALT022	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT023	93.42	6.58	7.89	92.11	
Altamaha River	LMBALT024	92.11	7.89	15.79	84.21	
Altamaha River	LMBALT025	94.74	5.26	10.53	89.47	

Altamaha River	LMBALT026	92.11	7.89	5.26	94.74	
Altamaha River	LMBALT027	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT028	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT029	96.05	3.95	2.63	97.37	FLMB
Altamaha River	LMBALT030	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT031	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT032	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT033	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT034	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT035	92.11	7.89	10.53	89.47	
Altamaha River	LMBALT036	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT037	89.47	10.53	5.26	94.74	
Altamaha River	LMBALT038	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT039	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT040	93.42	6.58	13.16	86.84	
Altamaha River	LMBALT041	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT042	93.42	6.58	13.16	86.84	
Altamaha River	LMBALT043	94.74	5.26	5.26	94.74	
Altamaha River	LMBALT044	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT045	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT046	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT047	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT048	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT049	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT050	96.05	3.95	2.63	97.37	FLMB
Altamaha River	LMBALT051	63.16	36.84	0.00	100.00	Novel Form
Altamaha River	LMBALT052	94.74	5.26	5.26	94.74	
Altamaha River	LMBALT053	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT054	100.00	0.00	0.00	100.00	FLMB
Altamaha River	LMBALT055	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT056	97.37	2.63	5.26	94.74	FLMB

Altamaha River	LMBALT057	94.74	5.26	5.26	94.74	
Altamaha River	LMBALT058	94.74	5.26	10.53	89.47	
Altamaha River	LMBALT059	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT060	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT061	97.37	2.63	5.26	94.74	FLMB
Altamaha River	LMBALT062	63.16	36.84	0.00	100.00	Novel Form
Altamaha River	LMBALT063	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT064	98.68	1.32	2.63	97.37	FLMB
Altamaha River	LMBALT065	63.16	36.84	0.00	100.00	Novel Form
Altamaha River	LMBALT066	63.16	36.84	0.00	100.00	Novel Form
Altamaha River	LMBALT067	96.05	3.95	2.63	97.37	FLMB
Altamaha River	LMBALT068	96.05	3.95	7.89	92.11	FLMB
Altamaha River	LMBALT069	63.16	36.84	0.00	100.00	Novel Form
Altamaha River	LMBALT070	97.37	2.63	5.26	94.74	FLMB
Altamaha River	MYST 130	96.05	3.95	2.63	97.37	FLMB
Altamaha River	MYST 131	60.53	39.47	5.26	94.74	Novel Form
Altamaha River	MYST 132	96.05	3.95	7.89	92.11	FLMB
Altamaha River	MYST 133	92.11	7.89	10.53	89.47	
Altamaha River	MYST 134	92.11	7.89	10.53	89.47	
Altamaha River	MYST 135	73.68	26.32	42.11	57.89	
Altamaha River	MYST 136	97.37	2.63	5.26	94.74	FLMB
Altamaha River	MYST 137	94.74	5.26	5.26	94.74	
Altamaha River	MYST 138	94.74	5.26	10.53	89.47	
Altamaha River	MYST 139	93.42	6.58	7.89	92.11	
Altamaha River	MYST 140	93.42	6.58	13.16	86.84	
Altamaha River	MYST 141	98.68	1.32	2.63	97.37	FLMB
Altamaha River	MYST 142	97.37	2.63	5.26	94.74	FLMB
Altamaha River	MYST 143	96.05	3.95	2.63	97.37	FLMB
Altamaha River	MYST 144	61.84	38.16	2.63	97.37	Novel Form
Altamaha River	MYST 145	60.53	39.47	5.26	94.74	Novel Form
Oconee River	MYST 121	93.42	6.58	13.16	86.84	

Oconee River	MYST 122	94.74	5.26	10.53	89.47	
Oconee River	MYST 123	97.37	2.63	5.26	94.74	FLMB
Oconee River	MYST 125	94.74	5.26	10.53	89.47	
Oconee River	MYST 126	94.74	5.26	5.26	94.74	
Oconee River	MYST 127	90.79	9.21	13.16	86.84	
Oconee River	MYST 128	97.37	2.63	5.26	94.74	FLMB
Oconee River	MYST 129	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR001	84.21	15.79	21.05	78.95	
Oconee River	LMBOCR002	94.74	5.26	5.26	94.74	
Oconee River	LMBOCR003	90.79	9.21	18.42	81.58	
Oconee River	LMBOCR004	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR005	89.47	10.53	21.05	78.95	
Oconee River	LMBOCR006	92.11	7.89	10.53	89.47	
Oconee River	LMBOCR007	78.95	21.05	36.84	63.16	
Oconee River	LMBOCR008	100.00	0.00	0.00	100.00	FLMB
Oconee River	LMBOCR009	80.26	19.74	39.47	60.53	
Oconee River	LMBOCR010	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR011	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR012	85.53	14.47	18.42	81.58	
Oconee River	LMBOCR013	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR014	68.42	31.58	31.58	68.42	
Oconee River	LMBOCR015	84.21	15.79	21.05	78.95	
Oconee River	LMBOCR016	90.79	9.21	13.16	86.84	
Oconee River	LMBOCR017	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR018	76.32	23.68	42.11	57.89	
Oconee River	LMBOCR019	90.79	9.21	13.16	86.84	
Oconee River	LMBOCR020	90.79	9.21	18.42	81.58	
Oconee River	LMBOCR021	92.11	7.89	10.53	89.47	
Oconee River	LMBOCR022	75.00	25.00	34.21	65.79	
Oconee River	LMBOCR023	92.11	7.89	10.53	89.47	
Oconee River	LMBOCR024	78.95	21.05	36.84	63.16	

Oconee River	LMBOCR025	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR026	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR027	93.42	6.58	13.16	86.84	
Oconee River	LMBOCR028	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR029	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR030	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR031	90.79	9.21	13.16	86.84	
Oconee River	LMBOCR032	88.89	11.11	22.22	77.78	
Oconee River	LMBOCR033	92.11	7.89	15.79	84.21	
Oconee River	LMBOCR034	84.21	15.79	21.05	78.95	
Oconee River	LMBOCR035	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR036	67.11	32.89	23.68	76.32	
Oconee River	LMBOCR037	96.05	3.95	2.63	97.37	FLMB
Oconee River	LMBOCR038	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR039	92.11	7.89	10.53	89.47	
Oconee River	LMBOCR040	82.89	17.11	18.42	81.58	
Oconee River	LMBOCR041	93.42	6.58	7.89	92.11	
Oconee River	LMBOCR042	85.53	14.47	28.95	71.05	
Oconee River	LMBOCR043	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR044	88.16	11.84	23.68	76.32	
Oconee River	LMBOCR045	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR046	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR047	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR048	92.11	7.89	10.53	89.47	
Oconee River	LMBOCR049	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR050	63.16	36.84	0.00	100.00	Novel Form
Oconee River	LMBOCR051	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR052	100.00	0.00	0.00	100.00	FLMB
Oconee River	LMBOCR053	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR054	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR055	96.05	3.95	7.89	92.11	FLMB

Oconee River	LMBOCR056	97.37	2.63	0.00	100.00	FLMB
Oconee River	LMBOCR057	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR058	96.05	3.95	2.63	97.37	FLMB
Oconee River	LMBOCR059	96.05	3.95	2.63	97.37	FLMB
Oconee River	LMBOCR060	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR061	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR062	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR063	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR064	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR065	94.74	5.26	5.26	94.74	
Oconee River	LMBOCR066	92.11	7.89	5.26	94.74	
Oconee River	LMBOCR067	96.05	3.95	7.89	92.11	FLMB
Oconee River	LMBOCR068	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR069	94.74	5.26	10.53	89.47	
Oconee River	LMBOCR070	98.68	1.32	2.63	97.37	FLMB
Oconee River	LMBOCR071	94.74	5.26	5.26	94.74	
Oconee River	LMBOCR072	97.37	2.63	5.26	94.74	FLMB
Oconee River	LMBOCR073	96.05	3.95	2.63	97.37	FLMB
Oconee River	LMBOCR074	89.47	10.53	10.53	89.47	
Oconee River	LMBOCR075	96.05	3.95	7.89	92.11	FLMB
Lake Sinclair	GA16SCL001	78.69	21.31	29.51	70.49	
Lake Sinclair	GA16SCL002	63.93	36.07	36.07	63.93	
Lake Sinclair	GA16SCL003	76.23	23.77	31.15	68.85	
Lake Sinclair	GA16SCL004	82.50	17.50	21.67	78.33	
Lake Sinclair	GA16SCL005	70.83	29.17	35.00	65.00	
Lake Sinclair	GA16SCL006	62.30	37.70	39.34	60.66	
Lake Sinclair	GA16SCL007	74.17	25.83	35.00	65.00	
Lake Sinclair	GA16SCL008	77.50	22.50	25.00	75.00	
Lake Sinclair	GA16SCL009	77.05	22.95	29.51	70.49	
Lake Sinclair	GA16SCL010	71.31	28.69	37.70	62.30	
Lake Sinclair	GA16SCL011	80.83	19.17	28.33	71.67	

Lake Sinclair	GA16SCL012	74.59	25.41	34.43	65.57
Lake Sinclair	GA16SCL013	80.00	20.00	33.33	66.67
Lake Sinclair	GA16SCL014	71.67	28.33	33.33	66.67
Lake Sinclair	GA16SCL015	70.83	29.17	31.67	68.33
Lake Sinclair	GA16SCL016	74.59	25.41	27.87	72.13
Lake Sinclair	GA16SCL017	78.33	21.67	30.00	70.00
Lake Sinclair	GA16SCL018	75.83	24.17	35.00	65.00
Lake Sinclair	GA16SCL019	67.50	32.50	28.33	71.67
Lake Sinclair	GA16SCL020	64.17	35.83	45.00	55.00
Lake Sinclair	GA16SCL021	75.00	25.00	36.67	63.33
Lake Sinclair	GA16SCL022	70.49	29.51	32.79	67.21
Lake Sinclair	GA16SCL023	69.17	30.83	35.00	65.00
Lake Sinclair	GA16SCL024	72.50	27.50	38.33	61.67
Lake Sinclair	GA16SCL025	69.17	30.83	45.00	55.00
Lake Sinclair	GA16SCL026	59.84	40.16	40.98	59.02
Lake Sinclair	GA16SCL027	76.67	23.33	30.00	70.00
Lake Sinclair	GA16SCL028	72.13	27.87	42.62	57.38
Lake Sinclair	GA16SCL029	74.17	25.83	35.00	65.00
Lake Sinclair	GA16SCL030	65.00	35.00	46.67	53.33
Lake Sinclair	GA16SCL039	76.23	23.77	37.70	62.30
Lake Sinclair	GA16SCL040	79.51	20.49	27.87	72.13
Lake Sinclair	GA16SCL041	77.87	22.13	24.59	75.41
Lake Sinclair	GA16SCL042	65.57	34.43	36.07	63.93
Lake Sinclair	GA16SCL043	74.59	25.41	40.98	59.02
Lake Sinclair	GA16SCL044	74.59	25.41	37.70	62.30
Lake Sinclair	GA16SCL045	78.69	21.31	22.95	77.05
Lake Sinclair	GA16SCL046	72.13	27.87	39.34	60.66
Lake Sinclair	GA16SCL047	77.05	22.95	36.07	63.93
Lake Sinclair	GA16SCL048	72.95	27.05	44.26	55.74
Lake Sinclair	GA16SCL049	68.85	31.15	42.62	57.38
Lake Sinclair	GA16SCL050	76.23	23.77	37.70	62.30

Lake Sinclair	GA16SCL051	83.61	16.39	22.95	77.05	
Lake Sinclair	GA16SCL052	73.77	26.23	39.34	60.66	
Lake Sinclair	GA16SCL053	73.77	26.23	45.90	54.10	
Lake Sinclair	GA16SCL054	81.15	18.85	14.75	85.25	
Lake Sinclair	GA16SCL055	67.21	32.79	26.23	73.77	
Lake Sinclair	GA16SCL056	78.69	21.31	26.23	73.77	
Lake Sinclair	GA16SCL057	72.13	27.87	36.07	63.93	
Lake Sinclair	GA16SCL058	68.03	31.97	34.43	65.57	
Lake Sinclair	GA16SCL059	74.59	25.41	34.43	65.57	
Lake Sinclair	GA16SCL060	77.87	22.13	34.43	65.57	
Lake Sinclair	GA16SCL061	72.13	27.87	39.34	60.66	
Lake Sinclair	GA16SCL062	72.13	27.87	39.34	60.66	
Lake Sinclair	GA16SCL063	81.15	18.85	34.43	65.57	
Lake Sinclair	GA16SCL064	79.51	20.49	24.59	75.41	
Lake Sinclair	GA16SCL065	69.67	30.33	44.26	55.74	
Lake Sinclair	GA16SCL066	72.13	27.87	32.79	67.21	
Lake Sinclair	GA16SCL067	71.31	28.69	34.43	65.57	
Lake Sinclair	GA16SCL068	60.66	39.34	49.18	50.82	
Ocmulgee River	MYST 101	94.74	5.26	10.53	89.47	
Ocmulgee River	MYST 102	94.74	5.26	10.53	89.47	
Ocmulgee River	MYST 103	82.89	17.11	28.95	71.05	
Ocmulgee River	MYST 104	80.26	19.74	28.95	71.05	
Ocmulgee River	MYST 105	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	MYST 106	89.47	10.53	15.79	84.21	
Ocmulgee River	MYST 107	60.53	39.47	0.00	100.00	Novel Form
Ocmulgee River	MYST 108	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	MYST 109	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	MYST 110	88.16	11.84	13.16	86.84	
Ocmulgee River	MYST 111	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	MYST 112	98.68	1.32	2.63	97.37	FLMB
Ocmulgee River	MYST 113	97.37	2.63	5.26	94.74	FLMB

Ocmulgee River	MYST 114	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	MYST 115	59.46	40.54	0.00	100.00	Novel Form
Ocmulgee River	MYST 116	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	MYST 117	93.06	6.94	13.89	86.11	
Ocmulgee River	MYST 118	61.84	38.16	2.63	97.37	Novel Form
Ocmulgee River	MYST 119	61.84	38.16	2.63	97.37	Novel Form
Ocmulgee River	MYST 120	100.00	0.00	0.00	100.00	FLMB
Ocmulgee River	LMBOMR101	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR102	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR103	39.19	60.81	2.70	97.30	SHB
Ocmulgee River	LMBOMR104	91.80	8.20	16.39	83.61	
Ocmulgee River	LMBOMR105	90.83	9.17	11.67	88.33	
Ocmulgee River	LMBOMR106	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR107	96.67	3.33	6.67	93.33	FLMB
Ocmulgee River	LMBOMR108	95.95	4.05	8.11	91.89	FLMB
Ocmulgee River	LMBOMR109	97.30	2.70	5.41	94.59	FLMB
Ocmulgee River	LMBOMR110	92.50	7.50	8.33	91.67	
Ocmulgee River	LMBOMR111	98.65	1.35	2.70	97.30	FLMB
Ocmulgee River	LMBOMR112	38.16	61.84	2.63	97.37	ALB
Ocmulgee River	LMBOMR113	39.19	60.81	2.70	97.30	ALB
Ocmulgee River	LMBOMR114	89.47	10.53	15.79	84.21	
Ocmulgee River	LMBOMR115	94.26	5.74	8.20	91.80	
Ocmulgee River	LMBOMR116	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR117	36.76	63.24	2.94	97.06	ALB
Ocmulgee River	LMBOMR118	77.03	22.97	29.73	70.27	
Ocmulgee River	LMBOMR119	94.17	5.83	11.67	88.33	
Ocmulgee River	LMBOMR120	95.83	4.17	2.78	97.22	FLMB
Ocmulgee River	LMBOMR121	93.42	6.58	13.16	86.84	
Ocmulgee River	LMBOMR122	93.42	6.58	7.89	92.11	
Ocmulgee River	LMBOMR123	88.16	11.84	23.68	76.32	
Ocmulgee River	LMBOMR124	92.11	7.89	15.79	84.21	

Ocmulgee River	LMBOMR125	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR126	83.78	16.22	21.62	78.38	
Ocmulgee River	LMBOMR127	54.17	45.83	41.67	58.33	
Ocmulgee River	LMBOMR128	93.42	6.58	7.89	92.11	
Ocmulgee River	LMBOMR129	61.84	38.16	2.63	97.37	Novel Form
Ocmulgee River	LMBOMR130	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR131	63.16	36.84	0.00	100.00	Novel Form
Ocmulgee River	LMBOMR132	63.16	36.84	0.00	100.00	Novel Form
Ocmulgee River	LMBOMR133	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR134	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR135	61.84	38.16	2.63	97.37	Novel Form
Ocmulgee River	LMBOMR136	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	LMBOMR137	94.74	5.26	5.26	94.74	
Ocmulgee River	LMBOMR138	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR139	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR140	78.95	21.05	26.32	73.68	
Ocmulgee River	LMBOMR141	75.00	25.00	28.95	71.05	
Ocmulgee River	LMBOMR142	78.95	21.05	26.32	73.68	
Ocmulgee River	LMBOMR143	65.79	34.21	42.11	57.89	
Ocmulgee River	LMBOMR144	75.00	25.00	44.74	55.26	
Ocmulgee River	LMBOMR145	77.63	22.37	39.47	60.53	
Ocmulgee River	LMBOMR146	89.47	10.53	15.79	84.21	
Ocmulgee River	LMBOMR147	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	LMBOMR148	86.84	13.16	26.32	73.68	
Ocmulgee River	LMBOMR149	96.05	3.95	7.89	92.11	FLMB
Ocmulgee River	LMBOMR150	89.47	10.53	21.05	78.95	
Ocmulgee River	LMBOMR151	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR152	89.47	10.53	15.79	84.21	
Ocmulgee River	LMBOMR153	94.74	5.26	5.26	94.74	
Ocmulgee River	LMBOMR154	36.84	63.16	0.00	100.00	ALB
Ocmulgee River	LMBOMR155	88.16	11.84	23.68	76.32	

Ocmulgee River	LMBOMR156	94.74	5.26	5.26	94.74	
Ocmulgee River	LMBOMR157	94.74	5.26	5.26	94.74	
Ocmulgee River	LMBOMR158	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR159	93.42	6.58	13.16	86.84	
Ocmulgee River	LMBOMR160	55.26	44.74	31.58	68.42	
Ocmulgee River	LMBOMR161	97.37	2.63	0.00	100.00	FLMB
Ocmulgee River	LMBOMR162	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR163	96.05	3.95	2.63	97.37	FLMB
Ocmulgee River	LMBOMR164	94.74	5.26	5.26	94.74	
Ocmulgee River	LMBOMR165	93.42	6.58	2.63	97.37	
Ocmulgee River	LMBOMR166	93.42	6.58	13.16	86.84	
Ocmulgee River	LMBOMR167	100.00	0.00	0.00	100.00	FLMB
Ocmulgee River	LMBOMR168	76.32	23.68	31.58	68.42	
Ocmulgee River	LMBOMR169	73.68	26.32	36.84	63.16	
Ocmulgee River	LMBOMR170	72.37	27.63	28.95	71.05	
Ocmulgee River	LMBOMR171	65.79	34.21	42.11	57.89	
Ocmulgee River	LMBOMR172	75.00	25.00	34.21	65.79	
Ocmulgee River	LMBOMR173	68.42	31.58	31.58	68.42	
Ocmulgee River	LMBOMR174	75.00	25.00	18.42	81.58	
Ocmulgee River	LMBOMR175	61.84	38.16	55.26	44.74	
Ocmulgee River	LMBOMR176	36.84	63.16	5.26	94.74	ALB/ALTB/SHB
Ocmulgee River	LMBOMR177	78.95	21.05	26.32	73.68	
Ocmulgee River	LMBOMR178	71.05	28.95	36.84	63.16	
Ocmulgee River	LMBOMR179	73.68	26.32	31.58	68.42	
Ocmulgee River	LMBOMR180	60.53	39.47	63.16	36.84	
Ocmulgee River	LMBOMR181	65.79	34.21	36.84	63.16	
Ocmulgee River	LMBOMR182	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	LMBOMR183	97.37	2.63	5.26	94.74	FLMB
Ocmulgee River	LMBOMR184	80.26	19.74	34.21	65.79	
Ocmulgee River	LMBOMR185	94.74	5.26	10.53	89.47	
Ocmulgee River	LMBOMR186	90.79	9.21	7.89	92.11	

Ocmulgee River	LMBOMR187	76.32	23.68	42.11	57.89	
Ocmulgee River	GA16OMR001	95.90	4.10	4.92	95.08	FLMB
Ocmulgee River	GA16OMR002	93.44	6.56	9.84	90.16	
Ocmulgee River	GA16OMR003	95.83	4.17	5.00	95.00	FLMB
Ocmulgee River	GA16OMR004	96.72	3.28	3.28	96.72	FLMB
Ocmulgee River	GA16OMR005	95.90	4.10	4.92	95.08	FLMB
Ocmulgee River	GA16OMR007	96.67	3.33	6.67	93.33	FLMB
Ocmulgee River	GA16OMR008	92.62	7.38	8.20	91.80	
Ocmulgee River	GA16OMR009	97.46	2.54	5.08	94.92	FLMB
Ocmulgee River	GA16OMR016	95.83	4.17	5.00	95.00	FLMB
Ocmulgee River	GA16OMR022	96.67	3.33	3.33	96.67	FLMB
Ocmulgee River	GA16OMR023	96.67	3.33	6.67	93.33	FLMB
Ocmulgee River	GA16OMR026	90.00	10.00	20.00	80.00	
Ocmulgee River	GA16OMR027	95.08	4.92	6.56	93.44	FLMB
Ocmulgee River	GA16OMR028	95.83	4.17	8.33	91.67	FLMB
Ocmulgee River	GA16OMR029	92.62	7.38	11.48	88.52	
Ocmulgee River	GA16OMR030	96.67	3.33	6.67	93.33	FLMB
Ocmulgee River	GA16OMR032	95.00	5.00	6.67	93.33	FLMB
Ocmulgee River	GA16OMR033	98.33	1.67	3.33	96.67	FLMB
Ocmulgee River	GA16OMR036	94.26	5.74	8.20	91.80	
Ocmulgee River	GA16OMR037	95.90	4.10	4.92	95.08	FLMB
Ocmulgee River	GA16OMR038	95.08	4.92	3.28	96.72	FLMB
Ocmulgee River	GA16OMR039	94.26	5.74	4.92	95.08	
Ocmulgee River	GA16OMR040	94.26	5.74	8.20	91.80	
Ocmulgee River	GA16OMR043	95.90	4.10	4.92	95.08	FLMB
Ocmulgee River	GA16OMR047	94.26	5.74	8.20	91.80	
Ocmulgee River	GA16OMR048	94.26	5.74	4.92	95.08	
Ocmulgee River	GA16OMR053	93.44	6.56	6.56	93.44	
Ocmulgee River	GA16OMR057	96.72	3.28	3.28	96.72	FLMB
Ocmulgee River	GA16OMR059	80.83	19.17	31.67	68.33	
Ocmulgee River	GA16OMR062	94.26	5.74	1.64	98.36	

Ocmulgee River	GA16OMR063	95.08	4.92	6.56	93.44	FLMB
Ocmulgee River	GA16OMR064	93.44	6.56	9.84	90.16	
Ocmulgee River	GA16OMR065	91.80	8.20	9.84	90.16	
Ocmulgee River	GA16OMR066	86.89	13.11	22.95	77.05	
Ocmulgee River	GA16OMR067	89.34	10.66	21.31	78.69	
Ocmulgee River	GA16OMR068	78.69	21.31	42.62	57.38	
Ocmulgee River	GA16OMR069	93.33	6.67	6.67	93.33	
Ocmulgee River	GA16OMR070	91.67	8.33	13.33	86.67	
Ocmulgee River	GA16OMR071	92.62	7.38	11.48	88.52	
Ocmulgee River	GA16OMR072	97.50	2.50	1.67	98.33	FLMB
Ocmulgee River	GA16OMR077	94.92	5.08	6.78	93.22	FLMB
Ocmulgee River	GA16OMR079	95.00	5.00	10.00	90.00	FLMB
Ocmulgee River	GA16OMR081	96.61	3.39	0.00	100.00	FLMB
Ocmulgee River	GA16OMR084	91.67	8.33	10.00	90.00	
Ocmulgee River	GA16OMR087	94.17	5.83	8.33	91.67	
Ocmulgee River	GA16OMR089	87.50	12.50	15.00	85.00	
Ocmulgee River	GA16OMR090	88.33	11.67	16.67	83.33	
Ocmulgee River	GA16OMR091	92.50	7.50	15.00	85.00	
Ocmulgee River	GA16OMR092	85.00	15.00	20.00	80.00	
Ocmulgee River	GA16OMR093	93.44	6.56	3.28	96.72	
Ocmulgee River	GA16OMR094	91.80	8.20	13.11	86.89	
Ocmulgee River	GA16OMR095	95.08	4.92	6.56	93.44	FLMB
Ocmulgee River	GA16OMR096	88.52	11.48	16.39	83.61	
Ocmulgee River	GA16OMR100	95.00	5.00	6.67	93.33	FLMB
Ocmulgee River	GA16OMR104	91.80	8.20	16.39	83.61	
Ocmulgee River	GA16OMR105	90.83	9.17	11.67	88.33	
Ocmulgee River	GA16OMR107	96.67	3.33	6.67	93.33	FLMB
Ocmulgee River	GA16OMR110	92.50	7.50	8.33	91.67	
Ocmulgee River	GA16OMR115	94.26	5.74	8.20	91.80	
Ocmulgee River	GA16OMR119	94.17	5.83	11.67	88.33	
Lake Juliette	GA16JUL001	69.17	30.83	31.67	68.33	

Lake Juliette	GA16JUL002	65.00	35.00	46.67	53.33
Lake Juliette	GA16JUL010	62.30	37.70	45.90	54.10
Lake Juliette	GA16JUL014	78.69	21.31	26.23	73.77
Lake Juliette	GA16JUL016	63.11	36.89	50.82	49.18
Lake Juliette	GA16JUL017	77.05	22.95	32.79	67.21
Lake Juliette	GA16JUL019	61.67	38.33	36.67	63.33
Lake Juliette	GA16JUL020	68.85	31.15	32.79	67.21
Lake Juliette	GA16JUL021	71.31	28.69	34.43	65.57
Lake Juliette	GA16JUL023	66.39	33.61	34.43	65.57
Lake Juliette	GA16JUL026	74.59	25.41	47.54	52.46
Lake Juliette	GA16JUL027	77.87	22.13	31.15	68.85
Lake Juliette	GA16JUL028	73.77	26.23	39.34	60.66
Lake Juliette	GA16JUL029	75.41	24.59	42.62	57.38
Lake Juliette	GA16JUL030	71.31	28.69	34.43	65.57
Lake Juliette	GA16JUL031	75.41	24.59	32.79	67.21
Lake Juliette	GA16JUL032	81.15	18.85	34.43	65.57
Lake Juliette	GA16JUL033	73.77	26.23	32.79	67.21
Lake Juliette	GA16JUL034	73.77	26.23	26.23	73.77
Lake Juliette	GA16JUL035	68.85	31.15	36.07	63.93
Lake Juliette	GA16JUL036	72.13	27.87	32.79	67.21
Lake Juliette	GA16JUL037	77.87	22.13	31.15	68.85
Lake Juliette	GA16JUL038	79.51	20.49	18.03	81.97
Lake Juliette	GA16JUL039	77.87	22.13	31.15	68.85
Lake Juliette	GA16JUL040	71.31	28.69	37.70	62.30
Lake Juliette	GA16JUL041	78.69	21.31	29.51	70.49
Lake Juliette	GA16JUL042	77.87	22.13	27.87	72.13
Lake Juliette	GA16JUL043	73.77	26.23	32.79	67.21
Lake Juliette	GA16JUL044	75.41	24.59	22.95	77.05
Lake Juliette	GA16JUL045	74.59	25.41	27.87	72.13
Lake Juliette	GA16JUL046	74.59	25.41	27.87	72.13
Lake Juliette	GA16JUL048	67.21	32.79	32.79	67.21

Lake Juliette	GA16JUL049	76.23	23.77	37.70	62.30
Lake Juliette	GA16JUL050	72.95	27.05	34.43	65.57
Lake Juliette	GA16JUL051	71.31	28.69	27.87	72.13
Lake Juliette	GA16JUL052	76.23	23.77	37.70	62.30
Lake Juliette	GA16JUL053	68.85	31.15	45.90	54.10
Lake Juliette	GA16JUL054	68.85	31.15	45.90	54.10
Lake Juliette	GA16JUL055	71.31	28.69	47.54	52.46
Lake Juliette	GA16JUL056	79.51	20.49	24.59	75.41
Lake Juliette	GA16JUL057	74.59	25.41	27.87	72.13
Lake Juliette	GA16JUL058	75.41	24.59	26.23	73.77
Lake Juliette	GA16JUL059	72.13	27.87	32.79	67.21
Lake Juliette	GA16JUL060	72.13	27.87	42.62	57.38
Lake Juliette	GA16JUL061	82.79	17.21	34.43	65.57
Lake Juliette	GA16JUL062	80.33	19.67	36.07	63.93
Lake Juliette	GA16JUL063	77.87	22.13	34.43	65.57
Lake Juliette	GA16JUL064	70.49	29.51	45.90	54.10
Lake Juliette	GA16JUL065	79.51	20.49	34.43	65.57
Lake Juliette	GA16JUL066	69.67	30.33	40.98	59.02
Lake Juliette	GA16JUL067	75.41	24.59	39.34	60.66
Lake Juliette	GA16JUL068	71.31	28.69	37.70	62.30
Lake Juliette	GA16JUL069	76.23	23.77	37.70	62.30
Lake Juliette	GA16JUL070	86.89	13.11	19.67	80.33
Lake Juliette	GA16JUL071	77.87	22.13	31.15	68.85
Lake Juliette	GA16JUL072	72.95	27.05	34.43	65.57
Lake Juliette	GA16JUL073	72.95	27.05	24.59	75.41
Lake Juliette	GA16JUL074	81.15	18.85	14.75	85.25
Lake Juliette	GA16JUL075	78.69	21.31	36.07	63.93

Appendix III The STRUCTURE results (Q-values) for the 64-plex genotype data on the Largemouth Bass, Spotted Bass, and Shoal Bass samples collected from the Ocmulgee River. The highlighted Q-values are genomic proportions ≥ 0.05.

Sample ID	SHB	SPB	LMB	ALB	REB	SMB	Species
LMBOMR101	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR102	0.02	0.00	0.94	0.02	0.00	0.02	LMB
LMBOMR103	0.97	0.01	0.03	0.00	0.00	0.00	SHB
LMBOMR106	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR108	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR109	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR111	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR112	0.00	0.00	0.01	0.98	0.00	0.00	ALB
LMBOMR113	0.01	0.01	0.02	0.94	0.02	0.01	ALB
LMBOMR114	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR116	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR117	0.00	0.03	0.00	0.96	0.01	0.00	ALB
LMBOMR118	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR120	0.02	0.00	0.94	0.04	0.00	0.00	LMB
LMBOMR121	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR122	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR123	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR124	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR125	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR126	0.00	0.00	0.99	0.00	0.00	0.00	LMB
LMBOMR127	0.00	0.00	0.99	0.00	0.00	0.00	LMB
LMBOMR128	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR129	0.00	0.02	0.97	0.00	0.00	0.01	MYST
LMBOMR130	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR131	0.00	0.02	0.97	0.00	0.00	0.01	MYST
LMBOMR132	0.00	0.02	0.97	0.00	0.00	0.01	MYST
LMBOMR133	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR134	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR135	0.00	0.02	0.96	0.00	0.00	0.01	MYST
LMBOMR136	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR137	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR138	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR139	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR140	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR141	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR142	0.00	0.00	0.99	0.00	0.00	0.00	LMB
LMBOMR143	0.00	0.00	0.99	0.00	0.00	0.00	LMB
LMBOMR144	0.00	0.00	1.00	0.00	0.00	0.00	LMB

LMBOMR145	0.00	0.00	0.99	0.00	0.00	0.00	LMB
LMBOMR146	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR147	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR148	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR149	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR150	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR151	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR152	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR153	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR154	0.00	0.03	0.01	0.95	0.01	0.00	ALB
LMBOMR155	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR156	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR157	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR158	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR159	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR160	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR161	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR162	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR163	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR164	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR165	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR166	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR167	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR168	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR169	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR170	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR171	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR172	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR173	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR174	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR175	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR176	0.19	0.13	0.01	0.42	0.25	0.01	ALB/REB/SHB/SPB
LMBOMR177	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR178	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR179	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR180	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR181	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR182	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR183	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR184	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR185	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR186	0.00	0.00	1.00	0.00	0.00	0.00	LMB
LMBOMR187	0.00	0.00	0.99	0.00	0.00	0.00	LMB

SHBOMR001	0.56	0.16	0.00	0.00	0.27	0.01	SHB/REB/SPB
SHBOMR003	0.94	0.00	0.00	0.00	0.05	0.01	SHB
SHBOMR004	0.91	0.03	0.00	0.00	0.06	0.00	SHB
SHBOMR005	0.95	0.00	0.04	0.00	0.01	0.00	SHB
SHBOMR006	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR008	0.68	0.01	0.01	0.26	0.05	0.00	SHB/ALB/REB
SHBOMR009	0.97	0.00	0.00	0.02	0.00	0.00	SHB
SHBOMR010	0.95	0.00	0.00	0.00	0.04	0.00	SHB
SHBOMR011	0.94	0.01	0.01	0.01	0.02	0.00	SHB
SHBOMR012	0.49	0.02	0.01	0.48	0.00	0.00	SHB/ALB
SHBOMR013	0.97	0.01	0.02	0.00	0.00	0.00	SHB
SHBOMR014	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR015	0.24	0.00	0.01	0.67	0.08	0.00	ALB/SHB/REB
SHBOMR016	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR017	0.12	0.01	0.01	0.69	0.18	0.00	ALB/REB/SHB
SHBOMR018	0.01	0.01	0.01	0.97	0.00	0.01	ALB
SHBOMR019	0.19	0.01	0.00	0.55	0.24	0.01	ALB/REB/SHB
SHBOMR020	0.97	0.00	0.00	0.02	0.00	0.00	SHB
SHBOMR021	0.97	0.01	0.02	0.00	0.00	0.00	SHB
SHBOMR022	0.97	0.00	0.00	0.02	0.00	0.00	SHB
SHBOMR023	0.73	0.14	0.00	0.00	0.12	0.00	SHB/SPB/REB
SHBOMR024	0.98	0.02	0.00	0.00	0.00	0.00	SHB
SHBOMR025	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR026	0.88	0.03	0.00	0.00	0.01	0.08	SHB/SMB
SHBOMR027	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR028	0.97	0.00	0.00	0.00	0.02	0.00	SHB
SHBOMR029	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR030	0.72	0.00	0.01	0.26	0.00	0.00	SHB/ALB
SHBOMR031	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR032	0.98	0.01	0.00	0.00	0.00	0.02	SHB
SHBOMR033	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR034	0.97	0.00	0.00	0.00	0.02	0.01	SHB
SHBOMR035	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR036	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR037	0.97	0.00	0.00	0.01	0.02	0.00	SHB
SHBOMR038	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR039	0.96	0.00	0.00	0.00	0.03	0.00	SHB
SHBOMR040	0.97	0.01	0.00	0.00	0.01	0.02	SHB
SHBOMR041	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR042	0.99	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR043	0.98	0.00	0.00	0.00	0.02	0.00	SHB
SHBOMR044	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR045	1.00	0.00	0.00	0.00	0.00	0.00	SHB

SHBOMR046	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR047	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR048	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR049	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR050	0.96	0.00	0.00	0.00	0.00	0.04	SHB
SHBOMR051	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR052	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR053	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR054	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR055	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR056	0.98	0.01	0.00	0.00	0.00	0.02	SHB
SHBOMR057	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR058	0.89	0.03	0.00	0.00	0.01	0.07	SHB/SMB
SHBOMR059	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR060	1.00	0.00	0.00	0.00	0.00	0.00	SHB
SHBOMR061	0.84	0.00	0.01	0.01	0.14	0.00	SHB/REB
SPBOMR001	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR002	0.00	0.01	0.00	0.99	0.00	0.00	ALB
SPBOMR003	0.26	0.00	0.00	0.73	0.00	0.00	ALB/SHB
SPBOMR004	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR005	0.00	0.03	0.01	0.95	0.00	0.00	ALB
SPBOMR006	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR007	0.01	0.00	0.01	0.97	0.00	0.01	ALB
SPBOMR008	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR009	0.05	0.03	0.00	0.87	0.01	0.04	ALB/SHB
SPBOMR010	0.00	0.00	0.00	0.99	0.00	0.00	ALB
SPBOMR011	0.00	0.01	0.01	0.98	0.00	0.00	ALB
SPBOMR012	0.01	0.01	0.00	0.98	0.00	0.00	ALB
SPBOMR013	0.00	0.05	0.00	0.89	0.01	0.05	ALB/SPB/SMB
SPBOMR014	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR015	0.00	0.05	0.01	0.94	0.00	0.00	ALB
SPBOMR016	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR017	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR018	0.01	0.01	0.02	0.96	0.00	0.01	ALB
SPBOMR019	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR020	0.00	0.13	0.00	0.57	0.29	0.00	ALB/REB/SPB
SPBOMR021	0.00	0.00	0.00	0.97	0.02	0.00	ALB
SPBOMR022	0.00	0.00	0.01	0.99	0.00	0.00	ALB
SPBOMR023	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR024	0.07	0.00	0.00	0.92	0.00	0.00	ALB
SPBOMR025	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR026	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR027	0.00	0.00	0.00	1.00	0.00	0.00	ALB

SPBOMR028	0.00	0.01	0.00	0.98	0.00	0.00	ALB
SPBOMR029	0.00	0.00	0.00	0.99	0.00	0.01	ALB
SPBOMR030	0.49	0.00	0.00	0.50	0.00	0.00	ALB/SHB
SPBOMR031	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR032	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR033	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR034	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR035	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR036	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR037	0.01	0.01	0.03	0.95	0.00	0.01	ALB
SPBOMR038	0.01	0.01	0.04	0.93	0.00	0.01	ALB
SPBOMR039	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR040	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR041	0.00	0.00	0.00	0.98	0.02	0.00	ALB
SPBOMR042	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR043	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR044	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR045	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR046	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR047	0.01	0.01	0.02	0.96	0.00	0.01	ALB
SPBOMR048	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR049	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR050	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR051	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR052	0.18	0.00	0.00	0.81	0.00	0.01	ALB/SHB
SPBOMR053	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR054	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR055	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR056	0.00	0.30	0.00	0.00	0.69	0.00	REB/SPB
SPBOMR057	0.00	0.00	0.00	0.99	0.00	0.00	ALB
SPBOMR058	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR059	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR060	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR061	0.00	0.01	0.01	0.97	0.01	0.01	ALB
SPBOMR062	0.00	0.01	0.01	0.98	0.00	0.00	ALB
SPBOMR063	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR064	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR065	0.00	0.00	0.00	1.00	0.00	0.00	ALB
SPBOMR066	0.00	0.00	0.00	1.00	0.00	0.00	ALB

Appendix IV The reanalyzed STRUCTURE results (Q-values) for the 64-plex genotype data using Altamaha Bass reference genotypes in analysis. The highlighted Q-values are genomic proportions ≥ 0.05.

Sample ID	ALB	ALTB	LMB	SHB	SMB	SPB	Species
LMBOMR176	0.43	0.33	0.01	0.22	0.01	0.01	ALB/ALTB/SHB
SHBOMR001	0.00	0.38	0.00	0.59	0.01	0.01	SHB/ALTB
SHBOMR003	0.00	0.06	0.00	0.93	0.01	0.00	SHB/ALTB
SHBOMR004	0.00	0.08	0.00	0.91	0.00	0.00	SHB/ALTB
SHBOMR008	0.33	0.01	0.01	0.65	0.00	0.00	SHB/ALB
SHBOMR010	0.00	0.04	0.00	0.95	0.01	0.00	SHB
SHBOMR011	0.01	0.03	0.01	0.94	0.00	0.01	SHB
SHBOMR015	0.71	0.06	0.00	0.22	0.00	0.00	ALB/SHB/ALTB
SHBOMR017	0.67	0.24	0.00	0.08	0.00	0.00	ALB/ALTB/SHB
SHBOMR019	0.54	0.30	0.00	0.15	0.00	0.00	ALB/ALTB/SHB
SHBOMR020	0.02	0.01	0.00	0.97	0.00	0.00	SHB
SHBOMR022	0.02	0.01	0.00	0.97	0.00	0.00	SHB
SHBOMR023	0.00	0.20	0.01	0.79	0.00	0.01	SHB/ALTB
SHBOMR028	0.00	0.03	0.00	0.96	0.01	0.00	SHB
SHBOMR030	0.23	0.01	0.02	0.68	0.06	0.00	SHB/ALB/SMB
SHBOMR034	0.00	0.01	0.00	0.93	0.06	0.00	SHB/SMB
SHBOMR037	0.00	0.03	0.00	0.96	0.00	0.00	SHB
SHBOMR039	0.00	0.04	0.00	0.95	0.00	0.00	SHB
SHBOMR040	0.00	0.01	0.00	0.95	0.03	0.01	SHB
SHBOMR043	0.00	0.02	0.00	0.97	0.00	0.00	SHB
SHBOMR061	0.00	0.17	0.01	0.81	0.00	0.00	SHB/ALTB
SPBOMR005	0.94	0.04	0.01	0.00	0.00	0.01	ALB/ALTB
SPBOMR007	0.96	0.01	0.01	0.00	0.02	0.00	ALB
SPBOMR008	0.99	0.01	0.00	0.00	0.00	0.00	ALB
SPBOMR009	0.88	0.01	0.00	0.04	0.05	0.02	ALB/SMB
SPBOMR020	0.56	0.44	0.00	0.00	0.00	0.00	ALB/ALTB
SPBOMR021	0.98	0.02	0.00	0.00	0.00	0.00	ALB
SPBOMR024	0.91	0.01	0.00	0.07	0.00	0.01	ALB/SHB
SPBOMR028	0.98	0.01	0.00	0.00	0.01	0.00	ALB
SPBOMR056	0.00	0.97	0.01	0.00	0.00	0.01	ALTB
SPBOMR061	0.96	0.03	0.00	0.00	0.00	0.00	ALB

Appendix V Details of the sampling locations, length (mm), weight (g), and 38-plex FLNB results for the cryptic black bass individuals from the Altamaha, Oconee, and Ocmulgee Rivers.

River	ID	Latitude	Longitude	Length (mm)	Weight (g)	FLMB %	NLMB %	Hetero %	Homo%
Altamaha	LMBALT009	31.963876	-82.454308	337	559	59.09	40.91	3.03	96.97
	LMBALT010	31.963876	-82.454308	334	519	61.84	38.16	2.63	97.37
	MYST 131	31.951484	-82.506691	369	600	60.53	39.47	5.26	94.74
	MYST 144	31.951484	-82.506691	189	71	61.84	38.16	2.63	97.37
	MYST 145	31.951484	-82.506691	203	78	60.53	39.47	5.26	94.74
	LMBALT051	31.905851	-82.195989	395	866	63.16	36.84	0.00	100.00
	LMBALT062	31.901442	-82.141395	421	1165	63.16	36.84	0.00	100.00
	LMBALT065	31.901442	-82.141395	242	156	63.16	36.84	0.00	100.00
	LMBALT066	31.901442	-82.141395	339	458	63.16	36.84	0.00	100.00
	LMBALT069	31.901442	-82.141395	203	78	63.16	36.84	0.00	100.00
Oconee	LMBOCR050	32.068641	-82.613544	157	39	63.16	36.84	0.00	100.00
Ocmulgee	MYST 107	31.783509	-82.916638	142	26	60.53	39.47	0.00	100.00
	LMBOMR129	31.784470	-82.922310	208	97	61.84	38.16	2.63	97.37
	LMBOMR131	31.784470	-82.922310	214	100	63.16	36.84	0.00	100.00
	LMBOMR132	31.784470	-82.922310	204	92	63.16	36.84	0.00	100.00
	LMBOMR135	31.784470	-82.922310	208	102	61.84	38.16	2.63	97.37
	MYST 115	31.935254	-82.589414	340	390	59.46	40.54	0.00	100.00
	MYST 118	31.935254	-82.589414	172	44	61.84	38.16	2.63	97.37
	MYST 119	31.935254	-82.589414	161	39	61.84	38.16	2.63	97.37