Expanding the Toolbox: SNP Tools for Aquaculture and Conservation Management in the Eastern Oyster (*Crassostrea virginica*) and the Black Basses (*Micropterus* spp.)

by

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Abstract

Single nucleotide polymorphisms (SNPs) are considered as important molecular markers due to their several advantages, including their abundance, distribution in the genome, stability due to low mutation rates, ease of multiplexing, lower cost, amenability to high throughput assays, and low genotyping error rate. The rapid development of technology for SNPs has provided an efficient and cost-effective genetic marker tool for aquaculture and aquatic conservation in recent years for various purposes such as the determination of the population structure, population genomics, traceability, species identification, hybridization rates, and migratory dynamics. Here, relevant aspects of the Eastern oyster (*Crassostrea virginica*) and black basses (*Micropterus* spp.), key aquatic species in the southeastern United States, are examined.

Culture of the Eastern oyster is rapidly expanding. Combined with their continuing role as an environmental sentinel species and ecological model, this trend necessitates improved molecular tools for breeding and selection, as well as population assessment and genetic conservation. The development and validation of two panels of 58 SNPs for the species are described. Population analyses revealed three distinct populations, based upon F_{ST} values and STRUCTURE, among wild oysters sampled from Delaware Bay (1), Northwest Florida (2), Alabama (2), Louisiana (2), and the Texas Gulf Coast (3), consistent with previous microsatellite and mtDNA analyses. In addition, utilizing the developed panels for parentage assignment in cultured oysters resulted in highly accurate parent-offspring pairing (99.37%). The SNP.

markers could, furthermore, clearly discriminate between hatchery stocks and wild-sourced individuals

Black basses are apex predators in North American streams, rivers, and lakes and are important game fishes. Translocation and introductions for angling, accompanied by intrinsically weak genetic barriers, have led to widespread introgressive hybridization and genetic swamping. Species-diagnostic (fixed allele) SNP markers have been utilized successfully in salmonids to monitor hybridization and maintain genetic integrity. Here, similar resources for black basses through initial genotyping-by-sequencing (GBS), followed by extensive validation in additional samples using two panels of 64 SNPs, were developed. Results from >1300 genotyped bass indicated that the developed panels robustly and clearly delineate the majority of species and their hybrids among black basses.

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Chapter I Introduction and Literature Review

Overview

Aquatic species have long been the object of study, culture, and consumption in human civilizations. However, due to their striking diversity, their high fecundity (relative to terrestrial creatures) and the difficulty of observation posed by their aquatic niches, our understanding of most of these taxa remains surprisingly limited. Tagging vast numbers of offspring is logistically impractical, spawning practices often go unobserved in crevices and river bottoms, meristic measurements unreliably predict species membership, and anthropogenic introductions alter relationships between organisms and watersheds. Given these challenges, molecular markers are particularly critical for the management of aquatic species. While earlier marker systems allowed only basic divisions of species, often required sacrificing the tested individual, and were expensive in labor and reagents, current markers (as detailed in Chapters II and III) can be used for practical, powerful applications such as rapidly assessing levels of inbreeding, determining spawning contributions, and evaluating hybridization status. Below, aspects of molecular markers as they relate to aquaculture and conservation genetics, with a particular focus on modern SNP marker technologies, are reviewed and the focus of this dissertation. Finally, I examine relevant aspects of black basses (*Micropterus* spp.) and the Eastern oyster, *Crassostrea virginica*, the aquatic organisms targeted here.

1. Molecular Markers in Aquaculture and Conservation Genetics: Past and Present

Many types of genetic markers have been used in aquaculture and conservation, including allozymes, mitochondrial DNA, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLPs), microsatellites or simple sequence repeats (SSR), and single nucleotide polymorphisms (SNPs) [1, 2]. These techniques have provided numerous novel taxonomic and evolutionary insights, which were not previously obtained through exclusively phenotypic methods.

Allozymes are genetic variants of proteins (enzymes) produced via various alleles at a single locus. Their analysis is a method of measuring protein polymorphisms and requires gel electrophoresis [1]. Allozymes were common markers for addressing particular aspects of population genetics [e.g., 3, 4-9] and were used in aquaculture for stock identification, parentage analysis, and tracking inbreeding [e.g., 10, 11]. Many allozyme studies of marine species revealed a low level of genetic variation [e.g., 3, 4, 6, 9], indicating the need for markers with a higher resolution. Furthermore, the need to utilize tissue-specific loci often required the sacrifice of the organism in order to obtain the muscle, heart, liver, or eye.

Mitochondrial (mt) DNA evolves more rapidly than nuclear DNA, generating polymorphism that can be exploited as molecular markers [12]. Faster mutation rates in mtDNA likely stem from its lack of repair mechanisms during replication [13]. mtDNA markers have been extensively used in aquaculture and conservation in applications including species and strain identification as well as the assessments of genetic variability [e.g., 14, 15-17]. The maternal nature of the mitochondria limits its application in analyses involving putative hybrids. Additionally, mito-nuclear incongruence is well documented in fish and shellfish [18]. As a

result, studies based on 1-2 mitochondrial genes can arrive at spurious conclusions that do not reflect broader (nuclear) relationships.

RFLPs have been used to examine genetic variation based on DNA fragments lengths following digestion with specific restriction enzymes. Traditionally, RFLPs markers were visualized using Southern-blot hybridization [1, 19]. Later, RFLP analyses have replaced the tedious Southern blot method with polymerase chain reaction (PCR). RFLP markers have been broadly employed in aquaculture and conservation [e.g., 20, 21, 22]. RFLP markers have a major strength, as they are codominant markers, where both alleles in an individual are detectable in RFLP analysis. However, using RFLPs can be challenging because RFLP analysis is time consuming and labor intensive, and requires a large amount of DNA and known molecular information [1, 19].

RAPD markers rely on ten-mer, arbitrary primers amplifying genomic DNA (gDNA), where their products are separated and visualized on gel electrophoresis with staining. RAPDs have the advantages of being cost effective and of not requiring known targeted sequences, but they are a dominant marker and have low reproducibility [19, 23]. Nevertheless, RAPD makers have been beneficial for aquaculture and conservation genetics studies, including species identification [24-26], population structure determination [27], and genetic diversity [28, 29].

Later generation markers developed based on RFLP and PCR include AFLP and SSR. These markers provide better informative content and a higher resolution of genetic variation compared with RFLP and RAPD. AFLP markers are generated via restriction digest of gDNA, followed by ligation of fragment ends with adaptors for PCR amplification. The polymorphism of AFLP does not rely on the lengths of fragments; rather, it is determined based on the absence or presence of the fragments. AFLP markers are highly reproducible, and an unlimited number of

markers can be produced using the combination of specific PCR primers with restriction enzyme digestion. However, they are dominant markers, are costly, and require a high resolution of gel electrophoresis [1, 19, 23]. AFLP markers have been used for distinguishing rainbow trout, coastal cutthroat trout, and their hybrids [30], and for discriminating domestic and wild channel catfish [31].

SSRs, microsatellites, or short tandem repeats (STR) are tandem repeats of 2-6 bp sequences in a genome. SSRs are co-dominant. They are relatively abundant and highly polymorphic due their high mutation rate (10^{-2} to 10^{-6}). Microsatellites are the most extensively used marker type for parentage analysis, population genetics, and species identification in numerous aquatic animals [32-37]. While identification and evaluation of microsatellites in new species was initially cost-prohibitive, as sequencing costs declined this became a lesser concern. However, relatively low multiplexing capacity, higher costs for scoring, and their connection to low throughput, largely obsolete ABI sequencers have contributed to a transition to SNP markers. Genotyping legacy microsatellite sets through targeted next generation sequencing (NGS) protocols are currently being explored [38].

2. SNP Markers

SNPs are (generally) bi-allelic genetic markers based on single nucleotide substitutions at a given position within a genome. As sequencing-based rather than size (gel)-based markers, they are inextricably linked to NGS approaches developed over the last decade. They are also considerably less time consuming in both development and genotyping when compared with the other markers mentioned above [19]. In addition, SNPs are valued as molecular markers due to their several advantages, including their abundance, distribution in the genome [39], stability due

to low mutation rates [40], ease of multiplexing, lower cost, amenable to high throughput, and low genotyping error rate [39, 41]. SNPs have been used in aquaculture and aquatic conservation in recent years for various purposes such as the determination of the population structure, population genomics, traceability, species identification, hybridization rates, and migratory dynamics [42-49].

The rapid development of technology for SNPs has provided an efficient and cost-effective genetic-marker tool for selective breeding programs. SNP panels for the parentage assignment of aquatic species have been developed with increasing frequency and are currently available for use in the Pacific oyster, *Crassostrea gigas* [50, 51], black tiger shrimp, *Penaeus monodon* [34], blue mussel, *Mytilus galloprovincialis* [52], common carp, *Cyprinus carpio* [53], European flat oyster, *Ostrea edulis* [51], rainbow trout, *Oncorhynchus mykiss* [54], sockeye salmon, *O. nerka* [55], and steelhead, *O. mykiss* [56, 57]. Moreover, SNP markers are now commonly used to evaluate variation in quantitative trait loci (QTL), marker-assisted selection (MAS), and candidate gene approaches (CGA). QTLs are genomic regions that affect polygenic phenotypic traits with continuous variation [58]. SNP markers are ideally suited for marker-trait association analysis [59]. QTL-associated SNPs may be mapped and used to identify loci linked to or causative of important quantitative traits. However, the most effective method for identifying QTLs still requires a fine-scale linkage map. In some species, which still lack such a linkage map, CGA has been applied to identify putative associations between genotypes and phenotypes [60]. The main target of candidate genes is the known physiological function enhancing the characterization of specific functional mutations that affect variation in a specific phenotype [61-63]. Recently, CGA has been applied to identify genotype-phenotype associations in several aquaculture species [58, 61, 62, 64-66].

3. SNP Discovery

As SNPs have grown in dominance as the marker type of choice, methods for discovering them from new species have proliferated. The studies presented in Chapters II and III utilize several of these methods detailed below. Previously SNPs were identified from libraries of messenger ribonucleic acid (mRNA) transcripts, for example, expressed sequence tags (ESTs) [67-69] and Sanger-based re-sequencing [62]. The advancement of NGS has been accomplished through massively parallel sequencing that has rapidly reduced the sequencing cost and increased the throughput of genomic sequencing [70, 71]. In non-model species, several methods of SNP discovery combine reducing genome complexity and cost-effective NGS, including the RNA sequencing (RNA-seq) of pooled samples [e.g., 62, 72], genotyping by sequencing (GBS) [73], and restriction site–associated DNA sequencing (RAD-seq) [74] and their derivatives, such as 2b-RAD, that use type IIB restriction enzymes for digestion [75], double-digest RAD (ddRAD-seq) [76], and ezRAD [77]. Techniques employing restriction enzymes as a genome reduction method also include the complexity reduction of polymorphic sequences (CrOPS) [78], multiplex shotgun genotyping (MGS) [79], and RESTriction Fragment SEQuencing (RESTSeq) [80].

3.1 ESTs

ESTs are fragments of mRNA sequences that are derived from single sequencing reactions performed via the random selection of clones from complementary DNA (cDNA) libraries [81]. Typically, an EST sequence ranges from 200-800 bp in length and is likely to have errors [82]. To discover SNPs, the ESTs are subjected to cluster analysis using software, for example,

PHRAP, CAP3 [83], Vector NTI Advance™ 10 (Thermo Fisher Scientific, Waltham, MA, USA), ICAtools [84], and UIcluster v. 2.02 [85]. Variant sites are identified with software depending on the users, for example, Sequencher v4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA) [86] and autoSNP [67, 69, 87]. SNP discovery using ESTs has been carried out in various aquatic species, for example, the Eastern oyster, *C. virginica* [67, 68] et al. 2007, Pacific white shrimp, *Litopenaeus vannamei* [87], common carp, *Cyprinus carpio* [69], olive flounder, *Paralichthys olivaceus* [88], and westslope cutthroat trout, *O. clarkii* and rainbow trout, *O. mykiss* [86].

3.2 RNA-seq

RNA sequencing (RNA-seq) is transcriptome sequencing where total RNA is converted to a library of cDNA fragments with adaptors attached to one or both ends for NGS [89]. Short reads of RNA-seq can be assembled as a *de novo* transcriptome assembly or mapped to a reference genome. Various bioinformatics software exist for *de novo* transcriptome assembly, either commercial or open source, for example, CLC Genomics Workbench (version 5.5.2; CLC Bio, Aarhus, Denmark), Newbler (gsAssembler, 454 Life Sciences, Roche Diagnostics), Trinity [90], Mira [91], Trans-ABySS [92], Velvet [93], and Oases [94]. When a reference genome is available, RNA-seq analyses are subjected to the mapping of the reads onto the reference genome. Many kinds of software are available for the short read alignment to the reference genome, for example, ELAND, SSAHA [95], BLAT [96], MAQ [97], RMAP [98], SeqMap [99], SOAP [100], Bowtie [101], BWA [102], Samtools [103], Bedtools [104], and CLC Genomics Workbench. Subsequently, SNP calling is performed with various software, for

example, the Genome Analysis Toolkit (GATK 2.7) [105], Samtools [103], and the probabilistic variant detection software in CLC Genomics Workbench.

SNP discovery via RNA-seq approaches has been successfully applied in a multitude of aquatic species for both aquaculture and conservation aspects. For example, Baranski et al. (2014) [106] employed Illumina sequencing to generate transcriptomic resources for black tiger shrimp, *P. monodon*. Short reads were assembled using CLC Assembly Cell, and then contigs were assembled using Phrap software. Subsequently, 473,620 putative SNPs/indels were discovered using the "find_variations" software in CLC Assembly Cell. Of 473,620 SNPs, 6000 loci were further genotyped on 1,024 offspring samples using an Illumina iSelect SNP-array, resulting in 3,959 SNPs used for mapping to 44 linkage groups [106]. Yu et al. (2014) [107] identified 96,040 high-quality SNPs of Pacific white shrimp, *L. vannamei*, from transcriptomes generated on an Illumina HiSeq 2000. The reads were assembled using Trinity, the assembled reference was mapped using BWA, and then SNPs were called using SAMtools. These plentiful SNPs were demonstrated to be useful for high-density linkage map construction and genome-wide association studies [107]. A total of 218,777 SNPs in the eastern oyster, *C. virginica*, were identified using the SNAPE-pooled program [108]. SNP discovery from RNA-seq in the black-faced blenny, *Tripterygion delaisi*, revealed 172,430 SNP loci. The short reads of this study were obtained from Illumina Hiseq 2000, assembled with Trinity, and called for SNPs with GATK 2.0 [109]. These are examples illustrating the use of various NGS sequencers, assembly software, and SNP calling software. Although only a few examples could be mentioned here, all of these studies clearly demonstrated that RNA-seq enables the simple identification of thousands of SNPs, serving downstream analysis for particular purposes.

3.3 GBS

The GBS method of Elshire et al. (2011) [73] is relatively simple and robust for SNP discovery. GBS principles are based on generating fragments in low-copy genomic regions using restriction enzyme digestion to preferentially targeted sites and minimize reads in repetitive sequences [73, 110]. A cost-effective process is accomplished through multiplexing barcoded individuals [73, 74]. The recent improvement of the GBS library preparation also provided a sufficient number of SNPs and increased their depth of coverage, resulting in increased efficiency in cost reduction per sample [111].

According to Elshire et al. (2011) [73], the suitable restriction enzyme for each species is determined prior to initiating GBS. GSB library preparation is possible with two different types of oligonucleotide adaptors designed for both DNA strands. One adapter is used for barcoding and another is a common adapter. The adapter is designed for single-end sequencing on the Illumina platform. DNA samples, a barcode, and common adapter pairs are plated and dried. Afterward, the restriction enzymes digest the DNA samples and adapters, and then adapters are ligated to the ends of gDNA by T4 ligase. The plate is then dried to inactivate T4 ligase activity. An aliquot of each sample is pooled and purified using a commercial kit following the manufacturer's instructions (QIAquick PCR Purification Kit). Appropriate primers with binding sites on the ligated adapters are added, and then, PCR is carried out to increase fragment pools. PCR amplicons are cleaned up, and the fragment sizes of the library are examined on a DNA analyzer (BioRad Experion®) prior to performing Illumina sequencing. Raw GBS sequences can be analyzed with a non-reference pipeline using the Universal Network Enabled Analysis Kit (UNEAK) [112], which is part of the Trait Analysis by aSSociation, Evolution and Linkage

(TASSEL3) stand-alone or mapping into the reference genome using the TASSEL pipeline Version: 3.0 with default settings [113].

Several recent fish studies (including Chapter III) have used the Cornell University Biotechnology Resource Centre (BRC) to perform GBS. Li et al. (2014) [49] discovered 21,145 unfiltered SNPs from GBS in blue catfish, *Ictalurus furcatus*, and used 4,275 stringent filtered SNP loci to assess the population genetics and structure in blue catfish. Carlson et al. (2015) [114] used GBS to generate 7,956 putative SNPs in the Mexican tetra, *Astyanax mexicanus*, with which the stringent SNPs of 3,003 loci were used to construct a high-density linkage map. Moreover, Carreras et al. (2017) [115] identified a total of 51,221 putative SNPs in an east Atlantic peacock wrasse, *Symphodus tinca*. With stringent filtering, 4,155 polymorphic SNPs were obtained and directly analyzed for population genomics, observing a biodiversity complex of the east Atlantic peacock wrasse, *S. tinca*, in the Adriatic and Ionian Seas [115].

3.4 RAD-seq

Baird et al. (2008) [74] first described RAD-seq, following microarray-based RAD techniques [116]. Later years have seen numerous modifications of RAD-seq, including 2b-RAD [75], ddRAD-seq [76], and ezRAD [77]. Similar to GBS, RAD-seq is a method that uses restriction enzymes to reduce genome complexity, and then sequence DNA libraries using NGS technologies. Instead of ligating only a barcode adapter (P1), RAD-seq requires additional steps for the shearing and the ligation to a second adapter (P2). P2 is known as a divergent ‘‘Y’’ adapter that is the reverse complement of the reverse amplification primer. Thus, a P2 adapter prevents the amplification of gDNA fragments when lacking a P1 adapter. This structure ensures that all amplified fragments of RAD tags contain the P1 adapter [74].

Several modifications have been introduced for RAD-seq techniques. For example, 2b-RAD uses type IIB restriction enzymes, such as BsaXI or AlfI for gDNA digestion. Type IIB restriction enzymes are different from all other enzymes because they cut on both sides of both DNA strands at a fixed distance from their recognition sites. This produces identical sizes of short gDNA fragments at each IIB restriction enzyme site in the genome. The technique of 2b-RAD avoids random shearing step reducing the time-consuming and potentially error-prone size selection step [75]. Recently, Guo et al. (2014) [117] reported a 2b-RAD (I2b-RAD) approach that uses two adapters: barcode and common with the modification by fixed base in 3′end. This method allows for more efficient sample pooling in library preparation than the original 2b-RAD.

Bioinformatics analysis of RAD-seq is possible with general software for clustering, alignment, and SNP detection, for example, the CD-HIT software package, BWA, SAMtools, VarScan2, Bowtie, and Stacks [118]. Some have developed their own scripts, for example, custom Perl scripts [74] and Laboratory Information Management System (LIMS) using the Python programming language [76]. RAD-seq has become widely used in various aquatic species. RAD-seq has been used to construct linkage maps for numerous fish, such as the Common Pandora, *Pagellus erythrinus* [119], Midas cichlid, *Amphilophus* spp. [120], the spotted gar, *Lepisosteus oculatus* [121], gudgeon, genus *Gnathopogon* [122], blind cavefish, *A. mexicanus* [123], Nile tilapia, *Oreochromis niloticus* [124], Atlantic halibut, *Hippoglossus hippoglossus* [125], orange-spotted grouper, *Epinephelus coioides* [126], Atlantic salmon, *Salmo salar* [127], Japanese eel, *Anguilla japonica* [128], and platyfish, *Xiphophorus maculatus* [129]. RAD-seq has also been used for comparative genomics [e.g., 119, 122], sex determination [e.g., 124, 125], genome-wide association studies [e.g., 45, 130, 131], population genomics [e.g., 43,

132], fishery management [e.g., 133], reference genome assembly [e.g., 134], or providing useful SNP resources for the development of SNP array [e.g., 46, 135].

4. SNP Genotyping Platforms

Following SNP identification by means of ESTs, RNA-seq, GBS, or RAD-seq and their derivatives, SNPs are selected and validated for further uses by genotyping. SNP genotyping platforms allow utilization of screened SNPs on a larger set of individuals, populations, strains etc. It also provides a means by which to verify that SNPs are not the result of sequencing errors or mis-assembly of paralogous regions. There are several methods for SNP genotyping of known loci used in aquaculture and aquatic conservation [136]. Applications for large numbers of known loci SNPs include high-density SNP chips (e.g. 190K, 250K or 690K SNPs) which can be genotyped using Affymetrix Axiom genotyping technology (Axiom® SNP array) [137-139]. Lower number of SNPs (50-500 SNPs) may be genotyped using, for example, the TaqMan® platform (Applied Biosystems; Foster City, CA, USA), the KASPar genotyping assay (KBiosciences, Herts, England, UK), Fluidigm® SNP Type™ assays (Fluidigm, South San Francisco, CA, USA), the Illumina Golden Gate platform (Illumina, San Diego, CA, USA), and the Agena MassARRAY system (Agena Bioscience, San Diego, CA, USA) [48, 49, 51, 86, 109, 140-143]. I will review each of these briefly below.

4.1 TaqMan® platform

Each TaqMan® SNP genotyping assay includes locus-specific primers and allele specific probes. Locus-specific forward and reverse primers amplify the polymorphic sequence of interest. Two TaqMan® minor groove binder (MGB) probes are used for detecting genotypes,

with one probe being labeled with VIC® dye to detect the first allele and another probe labeled with FAM™ dye to detect the second allele. Fluorescent probes binding at the SNP site are degraded during amplification by the exonuclease activity of *Taq* DNA polymerase that leads to release of a fluorescent reporter dye [144]. The Taqman™ assay was used to genotype 188 SNP loci on 9,011 samples to assess population structure and genetic diversity among steelhead trout of the Columbia River Basin [142]. Moreover, gender identification markers of Chinook salmon, *O. tshawytscha*, have been developed using the TaqMan assay [145].

4.2 KASP™ genotyping assay

Kompetitive Allele Specific PCR (KASP) is a uniplex genotyping platform, similar to Taqman [146, 147]. KASP employs technology based on allele-specific oligo extension and fluorescence resonance energy transfer (FRET) for signal generation. The KASP technology can be performed on a variety of equipment platforms and in 96-, 384-, and 1,536-well microtiter plate formats, allowing flexibility in the number of SNPs and the number of samples [146]. The KASP assay mix contains two allele-specific forward primers and one common reverse primer. Two allele-specific forward primers contain a unique tail sequence corresponding with a universal FRET cassette, with one labelled with FAM™ dye and the other with HEX™ dye. During PCR, one of the allele-specific forward primers binds to the template with reverse primers, and then amplifies the target region. The complement of the allele-specific tail sequence is then newly synthesized during the following rounds of PCR. This allows the FRET cassette to bind to the DNA, release the fluor from the quencher, and emit a fluorescent signal. Detection of a bi-allele is accomplished through the competitive binding of the two allele-specific forward primers. If it is a homozygous genotype at a given SNP site, only one of the two possible

fluorescent signals will be emitted. If it is a heterozygous genotype, it will release a mixed fluorescent signal. Campbell et al. (2012) [86] used three strategies: ESTs, Sanger sequencing, and RAD-seq, to discover 310 SNPs for assay development. Subsequently, assay designs were performed using either Taqman™ or KASP genotyping assays. The final 200 assays were used in tests of population variation and successfully discriminated populations and subspecies in westslope cutthroat trout and rainbow trout. Palaiokostas et al. (2013) [124] used the KASP genotyping system to genotype five SNPs that showed the highest association with phenotypic sex on 96 tilapia samples. Bradbury (2010) [148] genotyped two temperature-associated SNPs on 500 Atlantic cod (adults and juveniles) using the KASP genotyping system.

4.3 Fluidigm® SNP Type™ Assays

SNP assays have also been carried out using the Fluidigm system. Samples and SNP assays are loaded into a nanofluidics chip with an Integrated Fluidic Circuit (IFC) controller that utilizes pressure to control microfluidic valves and channels on a silicon chip to combine samples and SNP assay reagents into reaction chambers [149, 150]. Then, the chips are transferred to perform PCR either using a BioMark HD system [149, 150] or the EP1 genotyping system [86, 109, 141, 143] that has thermal cycling and fluorescence detection abilities.

Several studies in aquaculture and conservation genetics have carried out genotyping using Fluidigm. Lew et al. (2015) [141] utilized RAD-seq to identify 2,317 SNP loci from 27 broodstock samples and created a linkage map from 143 additional progeny samples of the Delta Smelt, *Hypomesus transpacificus*. Then, 104 candidate SNPs were genotyped using the EP1 system on Fluidigm for assay development. A final panel of 24 independent SNPs was successfully developed. This panel provided 100% accuracy of parentage assignment by

genotyping in Fluidigm [141]. Campbell et al. (2012) [86] also used the EP1 system on Fluidigm 96.96 Dynamic Genotyping Arrays to genotype 200 SNPs in westslope cutthroat trout for observing population genetics. Schunter et al. (2014a) [109] genotyped 192 SNP loci derived from RNA-seq on 1,599 individuals of the black-faced blenny, *T. delaisi*, using the EP1 genotyping system on Fluidigm 96.96 Dynamic Genotyping Arrays. Moreover, Schunter et al. (2014b) [143] used Fluidigm to genotype 178 SNP markers to test parentage and sibship of 1,573 black-faced blenny, *T. delaisi*, from an open coastline in the Mediterranean Sea.

4.4 Illumina Golden Gate platform

The GoldenGate genotyping assay or GoldenGate bead array [151, 152] offers a flexible, accurate, and high-throughput SNP genotyping method for large-scale genetic analysis. Highly specific extension and amplification steps allow a high degree of locus multiplexing. The GoldenGate genotyping assay can genotype from a 384 to a 1,536 SNP multiplexed assays in a single reaction. Bradbury (2010) [148] used a GoldenGate assay to screen 3,072 putative SNPs in Atlantic cod. Of these, 1,641 were informative for studying adaptive evolution of that species. Recently, Hedgecock et al. (2015) [140] used the GoldenGate bead assay with 1,536 SNPs to construct high-density second generation linkage maps for Pacific oysters. A total of 1,278 cupped oyster and 1,070 flat oyster samples were genotyped using the GoldenGate assay with 384 SNP markers designed for each species [51]. These assays were tested for parentage assignment, and their application was found to contribute to future selective breeding programs and conservation [51].

4.5 The Agena MassARRAY

The Agena MassARRAY system, formerly known as the Sequenom MassARRAY, is the primary platform utilized in establishing high-throughput SNP genotyping assays developed, in part, in the studies reported in this dissertation. The MassARRAY procedure comprises an initial step of locus-specific PCR amplification, followed by a single base extension (SBE) reaction (iPLEX assay) using mass-modified dideoxynucleotide terminators of an oligonucleotide primer that anneals immediately upstream of the target polymorphic site. The primer of SBE is synthesized according to the sequence of the variant site and is a single complementary mass-modified base. The MassARRAY system can be run in 24-, 96-, and 384-well formats.

The primers for both PCR and SBE reactions are designed using Assay Design Suite v2.0. Primers are multiplexed and genotyped using the MassARRAY platform integrating the iPLEX® SBE reaction and MassARRAY® technology-based matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The distinct mass of an extended primer is determined for the alternative alleles at the polymorphic site of interest through MALDI-TOF mass spectrometry. The mass of the observed primers are automatically converted into a genotype for each reaction using SpectroTYPER (MassARRAY Typer 4.0.20) software, allowing simultaneous detection of all assays in a multiplex reaction [153].

The MassARRAY system has been described as a highly sensitive and accurate method for SNP detection and validation [154]. The use of SNP panels in the iPLEX MassARRAY technology allows rapid turn-around of results as quickly as within a day from DNA extraction to data analysis. Moreover, the multiplexed MassARRAY system has been increasingly utilized for SNP genotyping and validation of allele frequencies in several aquatic species for aquaculture selective breeding programs, and ecological and conservation genetics studies [34,

48, 49, 62, 155, 156]. For selective breeding programs in aquaculture, Jung et al. (2014) [62] applied SNP panels in the MassARRAY for detecting growth-related candidate genes in giant freshwater prawn, *Macrobrachium rosenbergii*. SNPs were identified from transcriptomic resources (i.e. EST and 454 pyrosequencing). Sellars et al. (2014) [34] developed 122 SNP markers (63 SNPs in Panel A and 59 SNPs in Panel B) for parentage assignment in black tiger shrimp, *P. monodon*.

In the realm of conservation genetics, Krück et al. (2013) [155] employed the MassARRAY to validate 161 SNPs obtained from RAD-seq, ultimately selecting 92 SNPs for population genetic analysis of eastern Australian sea mullet, *Mugil cephalus.* Li et al. (2015) [48] discovered thousands of SNPs from RNA-seq of largemouth bass and multiplexed 25 SNPs for the MassARRAY in order to assess the hybridization of northern largemouth bass and Florida largemouth bass in 227 samples. Malde et al. (2017) [156] developed five panels of diagnostic SNPs for use in the MassARRAY to investigate hybridization of minke whale (n=1101). These SNPs were derived from whole genome sequencing and were found to be highly effective in terms of their ability to differentiate species and subspecies.

5. All in One Approaches to SNP Genotyping

Several studies in aquaculture selective breeding programs and conservation genetics have been carried out using an "all-in-one" approach. This approach takes advantage of GBS or RAD-seq pipelines that are capable of genotyping each individual sample, and, subsequently, individual genotypes are analyzed for their objectives without any additional SNP genotyping or validation. For example, Campbell et al. (2014) [157] utilized RAD-seq on 429 individuals of rainbow trout and steelhead, *O. mykiss*, to find candidate SNPs for marker-assisted selection in

aquaculture broodstock selective programs. They discovered 4,661 polymorphic SNP loci after stringent filtering and used TASSEL to test for association between disease resistance and genotype from individual survivors and mortalities.

Carreras et al. (2017) [115] carried out GBS to assess population diversity of 176 individuals of the east Atlantic peacock wrasse, *S. tinca*, from six locations in the Adriatic and Ionian seas. With strict filtering, a total of 4,155 polymorphic SNPs were obtained. These genotypes were analyzed using STRUCTURE to observe population structure. They detected two substantial barriers to gene flow that were useful in understanding species biodiversity related to environmental complexity.

The "all-in-one" approach is increasingly common, as biological samples can be outsourced to sequencing labs and resulting data can be processed through standard bioinformatics pipelines for population genetics and genomics measurements. This approach minimizes the burden of costly equipment which has traditionally fallen on genetics laboratories. However, several potential drawbacks to this approach exist. In non-model species lacking reference genomes, follow-up studies are often impossible as different tag sets are genotyped in each experiment. In cases where a standardized set of markers are needed for testing multiple populations over a prolonged time period (monitoring, assessments), or rapid turnaround of results are needed, the all-in-one approach often falls short. The all-in-one approach may also be more vulnerable to errors and ascertainment bias, as the SNP sets are never tested by another method or with additional external samples. A recent study, furthermore, reveals that different variants on the RAD-seq approach generate different SNP sets and different population genetics conclusions [158].

6. Genotyping-in-Thousands by Sequencing (GT-seq)

A final noteworthy approach here is the new method of genotyping-in-thousands by sequencing (GT-seq). This method takes a well-characterized, validated set of SNPs and targets it efficiently and robustly through amplicon sequencing on the Illumina platform. This method was first utilized for genotyping 2,068 individual steelhead trout by a set of 192 SNP markers [159]. GT-seq was used to sequence multiplexed PCR amplicons targeting SNPs in a single Illumina HiSeq lane. The technique is appropriate to use with well characterized SNPs in small panels of 50–500 SNPs, making it applicable to SNPs developed in this dissertation. Labs with ready access to Illumina sequencing could easily convert the MassARRAY SNPs described in Chapters II and III for genotyping via GT-seq.

7. Eastern Oyster Population Genetics

The eastern oyster (*Crassostrea virginica* Gmelin) is an economically and ecologically important species that naturally resides in near-shore estuaries along the Atlantic and Gulf coasts of the United States. In terms of an ecological role, oysters and oyster reefs provide nursery and foraging habitats for finfish, crabs, and other aquatic species [160-164], provide shoreline stabilization and erosion control [162], play a role as primary consumers of suspended phytoplankton [165], filter water, and improve water quality [160, 162, 166]. In addition to their ecosystem services, oysters are also important for their economic value in fisheries and aquaculture. In 2014, the value of eastern oysters in the United States was over 90 million US dollars [167]. Oyster aquaculture has expanded very rapidly to meet the market demand and is being carried out in many areas [168]. Overfishing, pollution, and habitat degradation are considered threats to eastern oyster populations [169]. Moreover, diseases outbreaks of

protozoan parasites such as *Perkinsus marinus* (dermo) and *Haplosporidium nelsoni* (MSX) are recognized as major threats for both cultured and wild populations of the oyster species [170]. Due to their importance for ecological functions and market demand for consumption, reliable molecular markers for selective breeding improvement and genetic diversity assessment for eastern oysters are required.

Previous assessments of population genetics of eastern oysters have been conducted in an area ranging from the St. Lawrence River estuary in eastern Canada to the northern Yucatan Peninsula of the Gulf of Mexico using several molecular markers including allozymes, single-copy nuclear DNA (scnDNA), mtDNA, RFLP, microsatellites, and a few nuclear SNPs [3, 21, 22, 36, 171-179]. Buroker (1983) [3] first reported a genetically homogeneous population from Cape Cod, Massachusetts to Corpus Christi, Texas using 32 allozymes with the estimation as 99% of genetic similarities among populations. As mentioned earlier, allozymes provide lower genetic variation than other types of DNA markers because allozymes represent variations in the sequence of amino acids comprising a protein. Variation at the amino acid sequence level may miss underlying changes at the DNA sequence level.

Reeb and Avise (1990) [22] utilized RFLP of mtDNA to explore genetic variations of oysters from the Gulf of St. Lawrence, Canada to Brownsville, Texas. The results of mtDNA haplotype frequencies revealed two distinct populations between the Atlantic and Gulf with a transition zone at West Palm Beach, Florida. Karl and Avise (1992) [21] also found a similar genetic pattern of genetic differentiation between an Atlantic population and a Gulf of Mexico population using nuclear RFLP. The genetic transition in this case was identified at Stuart, Florida, which is approximately 40 miles north of West Palm Beach. Discrepancies between mtDNA RFLP data [22] and allozyme data [3] likely is due to selection on the allozyme loci

assayed that balances allozyme frequencies in restrictions on gene flow [22]. Subsequently, Cunningham and Collins (1994) [171] re-analyzed Buroker's (1983) [3] allozyme data and found geographic structure within the allozyme data, but the structure demonstrated the clustering of the peninsular Florida population with the Atlantic population that still disagreed with the findings of RFLP on nuclear and mtDNA.

From 1996–1998, a number studies on population genetics of eastern oyster were performed using numerous molecular techniques [172-175, 180]. Some found genetic structure between Atlantic and Gulf populations [172, 180] that supported the studies of Reeb and Avise (1990) [22] and Karl and Avise (1992) [21], while others showed no genetic structure [173, 174] that was consistent with the finding of Buroker (1983) [3]. Hare and Avise (1998) [175] used the same samples and the same nuclear DNA loci that were previously analyzed by Karl and Avise (1992) [21] to examine nuclear gene genealogies. Nuclear loci were amplified from gDNA using PCR and then haplotypes of heterozygotes were isolated using single-strand conformational polymorphism (SSCP), re-amplified, and directly sequenced. Phylogenetic analysis revealed no population structure at any nuclear loci. This illustrated that different techniques could lead to varying conclusions. Subsequently, Milbury et al. (2004) [176] employed SNPs in mtDNA (16s ribosomal gene) to distinguish Gulf oysters planted in the Chesapeake Bay from local bay oysters. Similarly, Hoover and Gaffney (2005) [177] found two distinct population structures between Atlantic and Gulf oysters using RFLP of four non-anonymous nuclear loci from samples from Prince Edward Island, Canada to Tabasco, Mexico.

In addition of two genetic structures between Atlantic and Gulf oysters, a distinct population was observed in the Laguna Madre, Texas using allozymes by several groups. Groue and Lester (1982) [181] surveyed genetic variation of eastern oysters in the Gulf of Mexico

including Biloxi Bay, Mississippi; West Bay, Texas; Drum Bay, Texas; Aransas Bay, Texas; lower Laguna Madre, Texas and discovered different allozyme allele frequencies between the lower Laguna Madre and all other sites. Buroker (1983) [3] found that the genetic similarities of populations between Corpus Christi and Brownsville, Texas were estimated as 93%, suggesting a transition of population structures between the Laguna Madre of southern Texas and estuaries of central and eastern Texas. Hedgecock and Okazaki (1984) [182] conducted allozyme analysis and found allozyme frequency differences between oysters from Campeche, Mexico and Turkey Bayou, Florida. King et al. (1994) [5] evaluated nine oyster populations on the Texas coast from East Matagorda Bay to South Bay and found a significant difference in allele frequencies in the Laguna Madre area. Genetic differences of eastern oyster population in the Laguna Madre may be a result of adaptation to hypersaline conditions (salinities from 35 to 60 ppt) due to low precipitation, high evaporation, and lack of freshwater inflow as well as natural selection and random genetic drift that may occur due to isolation from oyster populations in north [5].

Microsatellite loci in the eastern oyster have been characterized by several research groups [67, 183-186]. Microsatellite markers were widely utilized to evaluate genetic diversity, genetic differentiation, population structure, and isolation by distance [36, 179, 187, 188], effective population size (*Ne*) in Chesapeake Bay and Delaware Bay [187, 188], and parentage assignment [189]. Rose et al. (2006) [187] used eight microsatellite loci to observe geographic differentiation using genotypic exact tests and a subtle pattern of isolation by distance in Chesapeake Bay. Oyster *Ne* in the James River was estimated at 535, whereas an estimate of the pseudo-likelihood was 1,516 [187]. He et al. (2009) [188] studied eastern oysters in Delaware Bay using seven microsatellite markers and found no genetic differentiation and *Ne* estimates for spat and adults were 140–440 and 589–2,779, respectively. Galindo-Sánchez et al. (2008) [36]

evaluated eastern oysters from six sampling sites in the coast of Veracruz, eastern Gulf of Mexico using five microsatellites and observed neither a pattern of isolation by distance nor genetic differentiation among these populations. The major factors of genetic homogeneity may be due to gene flow of eastern oysters in these areas. Anderson et al. (2014) [179] applied 11 microsatellite markers to evaluate genetic structure and migration of oyster population in the Gulf coast of Texas and found a shift of the secondary contact zone towards the north. Moreover, microsatellite markers were used for parentage analysis of eastern oysters [189]. Wang et al. (2010) [189] used 16 microsatellite loci on 160 progeny from a putative pool of 81 full-sib families in a multiplex PCR protocol and gained 100% accurate parentage assignment in the eastern oysters. However, the utility of microsatellites was limited in terms of its potential for null alleles, the expensive, labor-intensive, and time-consuming nature of the method, and difficulties in adapting it to NGS [19, 23, 41, 190].

Recently, SNP markers of eastern oysters have been identified from expressed sequence tag (EST) resources, Sanger resequencing [67, 68] and transcriptomic resources using high-throughput sequencing technology (RNA-sequencing) [108, 191]. Quilang et al. (2007) [67] identified a total of 6,533 putative SNPs from EST resources of the eastern oysters. Zhang and Guo (2010) [68] characterized 46 SNPs in the eastern oyster using ESTs and resequencing. Eierman and Hare (2014) [108] conducted RNA-seq from pooled eastern oysters using 454 sequencing technology and obtained 98,729 contigs with a total of 218,777 SNPs. Two years later, Eierman and Hare (2016) [191] used another NGS platform (Hi-Seq Illumina 2000) to sequence the multiplexed libraries on five lanes of 100 bp and gained 42,072 annotated contigs. The Genome Analysis Toolkit (GATK version 2.8) was used to discover a total of 1,345,639 SNPs, and after stringent filtering, there were 79,660 SNPs remaining. However, these SNPs

from pooled samples lacked the validation and genotyping in individual samples necessary for downstream use. Varney et al. (2009) [178] examined genetic variation of eastern oysters from 13 sites in the Gulf of Mexico using the combined analysis of mtDNA sequencing, mtDNA RFLP, and nuclear SNPs. Genetic differentiation and structure among samples were observed using 12 SNPs, indicating significant population subdivision in the Gulf of Mexico [178].

8. Black Basses

Black basses are the common name given to a group of fish in the genus *Micropterus*, family Centrarchidae, order Perciformes, class Actinopterygii, phylum Chordata. The important ecological role of this genus is as a top-level predator of North American freshwater fishes [37, 192, 193]. In addition to their ecological values, black basses are one of the most popular game fish groups in the United States, and are known for their social and economic importance [37, 192-194]. Due to their importance related to their ecological benefits and recreational fishing, the black basses have become the target of intense conservation efforts.

8.1 Black Bass Diversity

Black basses are highly diverse and possess an uncertain taxonomic classification. Up to the present, black basses were recognized as comprising 14 species including northern largemouth bass, *M. salmoides*; Florida largemouth bass, *M. floridanus*; Alabama bass, *M. henshalli*; spotted bass, *M. punctulatus*; redeye bass, *M. coosae*; shoal bass, *M. cataractae*; smallmouth bass, *M. dolomieu*; Suwannee bass, *M. notius*; Guadalupe bass, *M. treculii*; Cahaba bass, *M. cahabae*; Tallapoosa bass, *M. tallapoosae*; Warrior bass, *M. warriorensis*; Chattahoochee bass, *M. chattahoochae*; Choctaw bass, *M. haiaka* [16, 195-198]; two subspecies

of Neosho smallmouth bass, *M. dolomieu velox* and Ouachita-lineage smallmouth bass, *M*. sp. cf. *dolomieu velox* [199]; and three undescribed species: Bartram's bass, *M.* sp. cf. *cataractae* from the Savannah River drainage [17, 200], Altamaha bass, *M*. sp. cf. *cataractae* from the Altamaha-Ogeechee River drainage [17], and Lobina Negra de Cuatro Ciénegas bass, *M.* sp. cf. *salmoides* in southwest Texas and Mexico [201].

However, various factors (i.e., invasive species, pollution and contamination, habitat loss and destruction, and hybridization) are common threats to black bass biodiversity [192]. Furthermore, several species (i.e. shoal bass, Choctaw bass, Cahaba bass, Coosa bass, Guadalupe bass, Suwannee bass, Tallapoosa bass, Warrior bass, Altamaha bass, Bartram's bass, and Chattahoochee bass) are regional endemic taxa occupying restricted areas of the United States, requiring appropriate conservation management [17, 196, 198, 202-204]. In contrast, northern largemouth bass, spotted bass, and smallmouth bass are well adapted and can be invasive when stocked outside of their native ranges. They have been stocked throughout the United States and have been introduced into Africa, Europe and South America, as well as Canada [205-208].

8.2 Hybridization

Hybridization and introgression occurs widely within this genus [209]. For example, smallmouth bass was documented to interbreed with at least six other basses including largemouth bass, redeye bass, shoal bass, spotted bass, Guadalupe bass, and Bartram's bass [209-217]. Guadalupe bass has been documented as seriously threatened by introgressive swamping due to the introduction of smallmouth bass into its native range [214, 216, 218]. The hybrid of Guadalupe bass and smallmouth bass is fertile and capable of backcrossing to the parent species [216].

The introduction of non-native spotted bass in a north Georgia reservoir led to hybridization and dramatic genetic and demographic changes [219]. Barwick et al. (2006) [220] reported the hybridization of non-native Alabama bass and native Bartram's bass in the Savannah river drainage, resulting in difficult differentiation in offspring. Later, Leitner et al. (2015) [213] also found hybrids of non-native smallmouth bass and Bartram's bass in the Savannah River drainage. Shoal bass are native to the Apalachicola drainage, including the Chattahoochee River in Alabama and Georgia, the Flint River in Georgia, and the Chipola River in Florida. The occurrence of hybridization in shoal bass was documented with non-native spotted bass, largemouth bass, smallmouth bass, Alabama bass, and Chattahoochee bass in the upper Chattahoochee River basin [212], with the invasive spotted bass in the Lower Flint River, Georgia [221], and with non-native spotted bass, largemouth bass intergrades, and Choctaw bass in the Chipola River, Florida [222].

Incomplete reproductive isolation and slow divergence times in Centrarchid fishes may explain the high rate of interspecific hybridization and introgression in this genus. The phenomenon is typically observed after the introduction of a black bass species outside its native range [223, 224]. Hybridization in black basses impacts genetic diversity and genetic structure. It also causes loss of genetic integrity, genetic variation and fitness [221]. A number of black basses have been documented to exhibit lowered fitness for their environments following introductions and hybridization [e.g., 218, 219, 225]. These effects in part may come through outbreeding depression, due to the incompatibilities of physiological and biochemical characteristics between genes in different populations or the swamping [226]. Not only interspecific hybridization, but also intraspecific breeding has been shown to lead to outbreeding depression. Experimental studies have shown that breeding between genetically distinct stocks of

largemouth bass can produce offspring with slower growth rates, lower fitness, and compromised immune systems [227-231]. The prevalence and associated consequences of black bass hybridization illustrate the necessity of developing effective tools to monitor and protect genetic integrity.

8.3. The Use of Molecular Markers in Black Basses

In the early days of black bass analysis, Bailey and Hubbs (1949) [232] distinguished two subspecies of largemouth bass: northern largemouth bass and Florida largemouth bass based on meristic and morphological differences. The first critical biochemical genetic analysis of largemouth bass was carried out by Philipp et al. (1983) [8] using 28 enzyme loci (2 fixed loci) in a nationwide study on 90 different populations. The results of this study showed distinct population level variations among largemouth bass rather than differences at the subspecies level. In 2002, Kassler et al. [233] investigated the phylogenetic relationships among different taxa under *Micropterus* using RFLP of mtDNA and mtDNA sequences and proposed that the Florida largemouth bass should be elevated to a new species, as well as suggesting a termination of any management programs that involved stocking Florida bass outside their native range. The estimates for rates of speciation and diversification in the genus *Micropterus* were calculated using a phylogenetic analysis of two mtDNA genes (cytochrome b and ND2) [197]. Bagley et al. (2011) [16] used mtDNA sequencing and found monophyletic and genetic distinctions in coastal *M. punctulatus* populations (3.4–12.9% sequence divergence), suggesting cryptic species. Baker et al. (2013) [196] also relied on sequences of ND2 as a genetic tool to establish four new species within the redeye bass, *M. coosae* species group. Freeman et al. (2015) [17] employed internal

transcribed spacer 2 (ITS2) and ND2 to identity two undescribed taxa native to the Altamaha, Ogeechee, and Savannah River systems.

These previous genetic studies of black basses relied heavily on electrophoretic techniques such as allozymes [7, 8, 215, 234], RFLP analysis of mtDNA [211, 233, 235, 236] and mtDNA sequencing [16, 17, 197, 220]. The techniques were limited by factors such as insufficient number of informative/fixed loci, the need to sacrifice the fish and difficulty in reproducing the procedure. More recently, microsatellites have been widely used as a molecular tool for genetic analyses to monitor and manage the introduction of non-native micropterids [37, 198, 211, 212, 221, 222, 236-238]. Although microsatellites are useful for genetic differentiation within the genus *Micropterus*, they suffer from the drawbacks discussed above. Additionally, fixed, species-specific alleles (of high utility in determining hybridization status) are limited among existing microsatellite sets.

Recently, SNPs have been employed as the newest type of molecular markers in black basses [48]. The gene-linked diagnostic SNP markers were successfully developed from transcriptomic resources (RNA-seq) and employed fixed-alleles as a tool for distinguishing between northern largemouth bass, *M. salmoides* and Florida largemouth bass, *M. floridanus*, in intergrade zones [48]. Although this panel is able to evaluate hybridization with highly accurate and reliable assessment and offers a significantly informative source of fixed markers for assessing the hybridization patterns, its uses remain limited solely to largemouth bass.

9. Dissertation Overview

Due to the advantages of SNP markers for applied applications in aquaculture and conservation (reviewed above), they were the focus of two marker resource development studies in key aquatic species in the southeastern United States. C**hapter II** focuses on the development

of SNP marker panels for use in the genetic analysis of the eastern oyster. The studies include the identification of SNPs from transcriptomes, SNP validation, the development of multiplexed SNPs for use in the MassARRAY system, a comparison of effectiveness between microsatellite and SNP markers, and an examination of the utility of the developed SNP panels for assessments of genetic diversity, structure and parentage analysis. In **Chapter III**, GBS techniques were employed to identify diagnostic SNP markers for use in all black bass species. Subsequent validation and extension of these SNPs in >1300 black bass individuals with differing pure and hybridized backgrounds, again utilizing the MassARRAY system, are described. Taken together, the results of the dissertation studies expand the molecular tools available for aquaculture and conservation genetics. These tools, properly applied, should aid biologists and fish and shellfish culturists in making informed decisions relevant to stocking, hatchery operations and population management.

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Chapter II Development of SNP panels as a new tool to assess the genetic diversity, population structure, and parentage analysis of the eastern oyster (*Crassostrea virginica*)

Abstract

Culture of the eastern oyster, *Crassostrea virginica*, is rapidly expanding. Combined with their continuing role as an environmental sentinel species and ecological model, this trend necessitates improved molecular tools for breeding and selection, as well as population assessment and genetic conservation. Here, the development and validation of two panels of 58 single nucleotide polymorphism markers (SNPs) for the species are described. Population analyses revealed three distinct populations, based on F_{ST} values and STRUCTURE, among wild oysters sampled from Delaware Bay (1), northwest Florida (2), Alabama (2), Louisiana (2), and the Texas Gulf Coast (3), consistent with previous microsatellite and mtDNA analyses. In addition, utilizing the developed panels for parentage assignment in cultured oysters (Rutgers, New Jersey) resulted in a highly accurate parent-offspring pairing (99.37%). Furthermore, the SNP markers could clearly discriminate between hatchery stocks and wild-sourced individuals. The developed SNP panels may serve as an important tool for more rapid and affordable genetic analyses in eastern oyster.

1. Introduction

The eastern oyster, *Crassostrea virginica*, naturally inhabits estuarine environments along the Atlantic and Gulf coasts of the United States providing a vital ecological service in these

areas. The ecological influences of oysters and oyster reefs include providing nursery and foraging habitats for other aquatic species [1-5], stabilizing shorelines [3], consuming suspended phytoplankton [6], filtering water, and improving water quality [1, 3, 7]. In addition to their ecological importance, oysters are also a valued commodity in commercial fisheries and aquaculture. In fact, the value of eastern oysters in the United States surpassed 90 million US dollars in 2014 [8]. Moreover, cultivation of the eastern oyster is rapidly expanding to satisfy increased market demand [9]. Combined with their role as a biological indicator species, ecological benefits, and the growing demand for their consumption, eastern oysters are in need of improved molecular tools for breeding and selection, as well as population assessment and genetic conservation.

The population genetics of eastern oysters have been investigated using several marker types including allozymes, single-copy nuclear DNA (scnDNA), mitochondrial DNA (mtDNA), restriction fragment length polymorphisms (RFLP), microsatellites, and a few nuclear single nucleotide polymorphisms (SNPs) [10-22]. Microsatellites have been widely exploited in eastern oysters for studies of genetic diversity [20, 22] and parentage assignment [23]. However, microsatellites have several limitations in this regard including their potential for null alleles and, more importantly, higher expense in reagents and labor [24-27]. On the other hand, while less informative on a per marker basis, bi-allelic SNPs are abundant, stable, evenly distributed throughout the genome and lend themselves to high levels of multiplexing [24, 28, 29].

SNP markers of eastern oysters have been previously mined and developed from expressed sequence tags (ESTs) resources and Sanger resequencing [30, 31]. Recently, a larger number of SNPs (218,777 and 1,345,639) from pooled eastern oysters have been identified from transcriptomic resources using high-throughput sequencing technology (RNA-sequencing) [32,

33]. However, SNP marker panels for use in the genetic analysis of the eastern oyster have not yet been implemented. In addition, SNPs from pooled samples still require validation and genotyping in individuals for downstream use.

Although individual genotyping using techniques which reduce genome complexity (e.g., restriction site associated DNA sequencing (RAD-seq) [34, 35] and derivatives such as double-digest restriction-site associated DNA sequencing (ddRAD-seq) [36], and genotyping by sequencing (GBS) [37])—is more advantageous and suitable for population genetics, data processing and analysis are onerous and, without further validation and extension, results are limited in application to other samples and experiments. At the same time, the medium- to high-throughput SNP-genotyping techniques, e.g., the MassARRAY system [38-41], and GoldenGate Genotyping technology (the Illumina BeadXpress genotyping system) [42], have been increasingly employed for SNP genotyping in large numbers of individuals in non-model species.

In this study, new multiplex marker panels for genetic analysis of the eastern oyster using the MassARRAY System (Agena Bioscience® Inc., San Diego, CA) are reported. Utilizing this tool, genetic diversity and population structure in eastern oyster population along the U.S. Atlantic and Gulf of Mexico coastlines were analyzed. Furthermore, the effectiveness of microsatellites and SNP panels for these analyses were compared. Finally, the SNP multiplexes were utilized for parentage analysis demonstrating their potential applications in conservation hatcheries and selective breeding programs.

2. Materials and Methods

2.1 Sample collection and DNA extraction

A total of 618 oysters were used in the present study. Oyster collection sites and sample sizes (N) are summarized in Table 1. SNP primers used in the MassARRAY System were pre-screened using the following individuals: 45 (FL) individuals from Apalachicola Bay, FL; 50 (ALPP) individuals from Perdido Pass, AL; 50 (ALCP) individuals from Cedar Point, AL; and 45 (LA) individuals from Caillou Lake, LA. After screening, 144 DNA samples from Texas oysters were provided by the Perry R. Bass Marine Fisheries Research Station (Texas Parks and Wildlife Department) and 284 oyster tissue samples were supplied from the Haskin Shellfish Research Laboratory (Rutgers University) in Port Norris (NJ) (Table 1).

The Texas oysters were the subset samples of those used in Anderson et al. (2014) [22] with 36 individuals from each of the four sites: 1. Sabine Lake (TXSL), 2. Redfish Bay in Corpus Christi (TXRB), 3. Packery Channel in Upper Laguna Madre (TXPC), and 4. South Bay in Lower Laguna Madre (TXSB). Samples from Haskin Shellfish Research Laboratory consisted of 96 wild-caught samples collected from Delaware Bay and 188 individuals from hatcheries. The wild-caught Delaware Bay oysters were the subset samples of those used in He et al. (2012) [43], for which 32 individuals were collected from each of the three sites: 1. Hope Creek (DBHC), 2. Shell Rock (DBSR), and 3. Cape Shore (DBCS). The hatchery samples containing six, single-pair mating families (six males and six females) and 160 progeny were initially used for parentage analysis (designated as HRL). The progeny were equivalently pooled in four replicated vats at the early larval stage and cultured to approximately14 months of age. Forty individuals were subsampled from bags on racks (Delaware Bay) and were labelled A, B, C, and

D (A = 1-40, B = 41-80, C = 81-120, D = 121-160). An additional 16 samples from another culture line (15G) were included to ensure parentage assignment accuracy.

Table 1 Sample collection sites and sample size (N) of *Crassostrea virginica*. Wild populations were collected from the U.S. Atlantic coast (Delaware Bay) and the Gulf of Mexico (FL, AL, LA, TX), while cultured oysters were obtained from Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ.

	Locality	**Abbreviation**	**N**
1	Hope Creek, Delaware Bay	DBHC	32
2	Shell Rock, Delaware Bay	DBSR	32
3	Cape Shore, Delaware Bay	DBCS	32
4	Apalachicola Bay, FL	FL	45
5	Perdido Pass, AL	ALPP	50
6	Cedar Point, AL	ALCP	50
7	Caillou Lake, LA	LA	45
8	Sabine Lake, TX	TXSL	36
9	Redfish Bay, Corpus Christi, TX	TXRB	36
10	Packery Channel, Upper Laguna Madre, TX	TXPC	36
11	South Bay, Lower Laguna Madre, TX	TXSB	36
12	Haskin Shellfish Research Laboratory, Port Norris, NJ	HRL	172
13	15G Haskin Shellfish Research Laboratory, Port Norris, NJ	15G	16
	Total		**618**

The collected tissue samples were preserved in 95% ethanol at room temperature prior to DNA extraction. DNA was extracted from 30 mg of tissues incubated overnight in cell lysis solution using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocols. DNA concentration and purity were measured using a NanoDrop ND-2000 UV-VIS Spectrophotometer. The samples were diluted to 15 ng/µl for use in the MassARRAY System.

2.2 SNP identification and panel development

Publicly-available raw short read sequencing data was mined from NCBI SRA **SRP042090**. Raw reads were *de novo* assembled into contigs using CLC Genomics Workbench (version 5.5.2; CLC Bio, Aarhus, Denmark). Prior to assembly, raw reads were trimmed by removing adapter sequences and ambiguous nucleotides. Also, reads with quality scores less than 20 and lengths below 30 bp were removed to retain high quality sequences. The assembled contigs were examined for SNPs using the probabilistic variant-detection function in CLC Genomics Workbench and annotated using BLASTX. To obtain working SNPs for assay design in the MassARRAY System, SNPs from contigs were initially selected based on the following criteria: a flanking region at least 100 bp with no other variants, a minimum read coverage at 100, and a minor allele frequency (MAF) greater than 0.10.

SNP marker panels were developed using the MassARRAY System. Briefly, SNP assays were designed using ASSAY DESIGN software with a maximum of 40 multiplexed SNPs per well. The first amplification step used 30 ng of DNA per sample and followed with extension reactions using the iPLEX Gold Reagent Kit (Agena Bioscience® Inc., San Diego, CA) according to the manufacturer's instructions. SNP genotypes were called using the MassARRAY TYPER Analyzer 4.0 software. Initially, 200 annotated contigs without neighboring polymorphisms were used for assay design. Of 200 contigs, 148 contigs obtained from assay design for 4 panels were subjected to pre-screening process. After screening, two panels of 62 SNPs were developed (Table 2) and utilized for genotyping on all 618 eastern oyster samples with a flexible number of SNPs used for each data set, depending on the analyses conducted.

Table 2 The 58 SNP primers for the eastern oyster, *Crassostrea virginica*.

SNP_ID	UEP_MASS		Primer Sequences
Contig414_697CT_PCMTD2_LL	4544.0	PCR1:	ACGTTGGATGTGATGCCCCCTGTTGATTTG
		PCR2:	ACGTTGGATGGATCTCTGATTCTTGCCAGC
		EXT:	CTTGCCAGCAGGCTT
Contig271_550CT_IQCG_VV	4593.0	PCR1:	ACGTTGGATGACAGGGTGCACTTTTTCTGG
		PCR2:	ACGTTGGATGACATGGAGGGAAAGTACCTG
		EXT:	TGCAGAGGTCACCGT
Contig296_664CT_Vigilin_PP	4698.1	PCR1:	ACGTTGGATGTGTCACCTTCACTTGATCAC
		PCR2:	ACGTTGGATGTGCGAGCAATGATGAAGCAG
		EXT:	GCTCAATCCCTCCCCC
Contig7_283GA_Rabgef1_PP	4971.2	PCR1:	ACGTTGGATGTGCCGGCTTTGATCTTCATC
		PCR2:	ACGTTGGATGGCAAACCTTGTGACGTACTG
		EXT:	TGGACTGCAGCAGAGG
Contig462_498CT_HPD_TT	5139.4	PCR1:	ACGTTGGATGATTTGTGACAACGATGGAGC
		PCR2:	ACGTTGGATGTCATCGTTTCTGGTCAGTGG
		EXT:	GACTCGCAGATCCATAC
Contig367_1372AT_MIB2_TS	5170.4	PCR1:	ACGTTGGATGGTTTGGAAACGAGACTCGTG
		PCR2:	ACGTTGGATGATTGGACTAGGACCATTGGC
		EXT:	ACCATTGGCTTCCAAGG
Contig460_509TC_CathB_VV	5412.5	PCR1:	ACGTTGGATGCTTCGATTCCCGCACACAGT
		PCR2:	ACGTTGGATGAAAAGCCCAGCAGGATCCG
		EXT:	CCCGGCTCCCTGGTCACG
Contig553_1147CT_NCOA4_LL	5554.6	PCR1:	ACGTTGGATGTATCCAATGGATTGGTCCCC
		PCR2:	ACGTTGGATGTATTGGGCCTGTGGATACTG
		EXT:	GATACTGGTGCATGGCTA
Contig316_156GA_TGFBI_QQ	5580.7	PCR1:	ACGTTGGATGGACACTCTAACATTGGGCAC
		PCR2:	ACGTTGGATGGTTTTTGAAGGCGTTGTCCG
		EXT:	TGGAGCAAACAGAGTATA
Contig131_734CT_CDC42_PS	5748.7	PCR1:	ACGTTGGATGAGCCGTGGCGAACTTAAATC
		PCR2:	ACGTTGGATGAGAGCACCGAGGTGATTAAC
		EXT:	CCAAAAGCGTCCGGTTTCC
Contig617_111CT_HSP90_YY	5760.7	PCR1:	ACGTTGGATGGACCTCACTGAAGGAATATG
		PCR2:	ACGTTGGATGAAAGCAGAGTTCTGCACCAC
		EXT:	TGCTCTCTCCTGTGATGTA
Contig360_453AG_Collagen6_NS	5940.8	PCR1:	ACGTTGGATGGTGACGCTCTACAGTTCATC
		PCR2:	ACGTTGGATGACGACGAAGAGGGAGGAGTT
		EXT:	GGGTTTGGCCGGCAGGATG
Contig444_1394TC_Myosin_AA	6142.0	PCR1:	ACGTTGGATGCGAGCTTCTTCCTGATTCTC
		PCR2:	ACGTTGGATGACCAGAGAGCCCTTGATTCC
		EXT:	GACAGTGATTCCATGCAGGC

SNP_ID	UEP_MASS		Primer Sequences
Contig306_684AC_SAMD7_AA	6220.0	PCR1:	ACGTTGGATGGTTTCCCGCTTTTGCAGAAC
		PCR2:	ACGTTGGATGTTGCCGGATTTGACCGTTTG
		EXT:	GGTGTGACCGTTTGAGGTCG
Contig48_519CT_KPNA3_SS	6307.1	PCR1:	ACGTTGGATGCTAAGAAAGAAGCAGCATGG
		PCR2:	ACGTTGGATGTTTTGACGACTTCAGCCACC
		EXT:	AGCCACCTGTTCTTTATTACC
Contig50_2398CT_ALOX5_TT	6429.2	PCR1:	ACGTTGGATGCGATCTGGTGAATCTCATCC
		PCR2:	ACGTTGGATGTCGTGACGATTTTGAGCGTG
		EXT:	TGGTCAGATTGACAAATTCAC
Contig250_896CT_frizzled_LL	6431.2	PCR1:	ACGTTGGATGCACCATCAATGAGGTCATCC
		PCR2:	ACGTTGGATGAATAGCTTGGTGTCCGGATG
		EXT:	GGCAGGATGACACTGTACTCC
Contig172_579CT_MYH9_VV	6621.3	PCR1:	ACGTTGGATGAGTGACAGGAGTAGTTCAGG
		PCR2:	ACGTTGGATGCTTTGGTCTGAGCTTTGGTG
		EXT:	ACCTTGTGACATAGTCCCTTCC
Contig416_359AC_SNX6_RR	6646.3	PCR1:	ACGTTGGATGGGATTCACAAGCTGCCAAAG
		PCR2:	ACGTTGGATGCAGACGGGCTTTTTCTAAGG
		EXT:	CCTGCATAATCTGCTAGTGCCC
Contig763_190GA_Basigin_VI	6797.5	PCR1:	ACGTTGGATGTAGTTGCAGAGGTCATCGTC
		PCR2:	ACGTTGGATGAGCCTCATCCTCCATTTCTC
		EXT:	TTCTCATAGATGAAGATGATGA
Contig6_677AT_B3galt5_RR	6822.4	PCR1:	ACGTTGGATGCCCATCACTGTTTTAAGGGT
		PCR2:	ACGTTGGATGACACGGATGTTCTAGTGGAG
		EXT:	GTGGTCTAGTGGAGCATGCTCG
Contig351_534GA_GALE_EE	6998.6	PCR1:	ACGTTGGATGAATGCCATTCATAGCCCAGG
		PCR2:	ACGTTGGATGTCTCGTACTCCTGTTCCATC
		EXT:	TGGTTACTGCCATATACACTCAG
Contig32_81TC_LMBR1_CC	7001.6	PCR1:	ACGTTGGATGCCTCCATTTTGACTATCGCC
		PCR2:	ACGTTGGATGTTCGGATCTGGAACACACTG
		EXT:	CCCCAACTGTAGTATGCACAGAA
Contig284_591AC_KY_ED	7179.7	PCR1:	ACGTTGGATGTCAAAACGGAAAGTGTCGGC
		PCR2:	ACGTTGGATGCTTCCCCGGTATGACAGATA
		EXT:	GGGATGCAGAGAACAAACGATGA
Contig100_1030CT_solutecarrier35_NN	7214.7	PCR1:	ACGTTGGATGAGCTGCTAGTGTCAATGGTG
		PCR2:	ACGTTGGATGGAGATCCCTAACAGACACAC
		EXT:	CCCTCGTCCTACTCCGTTAATGTA
Contig368_348AG_hnRNPQ_SS	7351.8	PCR1:	ACGTTGGATGAGTTGACAGCTTGCGTTTGG
		PCR2:	ACGTTGGATGCCAGAAAAACCGTGGGTTTG
		EXT:	TCGGGAATATGATTCTCACAAGTC

SNP_ID	UEP_MASS		Primer Sequences
Contig380_1135AG_Nkx26_TT	7394.8	PCR1:	ACGTTGGATGTCATGTCTGGAGAAGACTGC
		PCR2:	ACGTTGGATGTGCAACCAGGTGAAACTTCC
		EXT:	GAAAGGTGAAACTTCCGTCAAGAC
Contig105_310CT_CaM_TT	7567.9	PCR1:	ACGTTGGATGTACTCCTGCTGAATAGCCTG
		PCR2:	ACGTTGGATGCCACAGTGATGAGGTCTATC
		EXT:	CTAGTTCTATCGGATTCAGTCCCAC
Contig236_324TC_RNF19A_PP	7582.9	PCR1:	ACGTTGGATGCAGGTAGATGTAGACTCCAC
		PCR2:	ACGTTGGATGGGTGAACTTTGACCTGACTG
		EXT:	CTTACCTTTGACCTGACTGATTACG
Contig333_245CT_Tetraspanin33_AA	7731.1	PCR1:	ACGTTGGATGTTCTTTCTGGATCCAGCCAC
		PCR2:	ACGTTGGATGATGCTCCCATGCATCCAAAG
		EXT:	GACAGAAAGTGATAAACACTATGAT
Contig273_447GA_Amyloid_KK	7772.1	PCR1:	ACGTTGGATGTGGTCGAGTTCTATTCTGGG
		PCR2:	ACGTTGGATGGGATCTCTCTGATCATCTTG
		EXT:	AGGAGCATCTTGAAGAAGTCAAGAA
Contig129_1732TC_HSP70_TT	7966.2	PCR1:	ACGTTGGATGTATTGCTGGCATTGAGCAGG
		PCR2:	ACGTTGGATGGAAAACTCAGTCATCGGAGC
		EXT:	ACGGCGCTGCTGGAGTCCCCAAAGAC
Contig463_137CT_OAZ1_SS	4488.9	PCR1:	ACGTTGGATGTCTCCCAGCTGACCTTGTG
		PCR2:	ACGTTGGATGATAACAGCAGGCACTCGTTG
		EXT:	CACCGTGCACCTGTC
Contig1883_386GA_MGAM_GG	4590.0	PCR1:	ACGTTGGATGTCTTTCGCAGAGTTCTGGTG
		PCR2:	ACGTTGGATGTCAATCTCTTTGGCATTCCG
		EXT:	ATGCGGTTTCTTCGG
Contig188_1597AC_actinin_TT	4618.0	PCR1:	ACGTTGGATGAGTTCCGCGTCTCTTTCAAC
		PCR2:	ACGTTGGATGTGTTGTATCCCAAGCTGACC
		EXT:	AGGTTCCAGGCGACG
Contig1025_907CT_LAMC1_PL	4787.1	PCR1:	ACGTTGGATGCATCGCAAATCTGACACCTG
		PCR2:	ACGTTGGATGACCAACACCTGTGGACTTTC
		EXT:	CCCACCGAGTACTGCC
Contig1755_481CT_IK_NN	5059.3	PCR1:	ACGTTGGATGTCTGGGTCAACTTGTTGATG
		PCR2:	ACGTTGGATGCACCACAATCCGCAGTAAGG
		EXT:	TTCACCCTGACCACCAA
Contig1791_792CT_EIF3A_II	5178.4	PCR1:	ACGTTGGATGTTGTACTGGTACAAGCCCTC
		PCR2:	ACGTTGGATGACCCATCATGGAGAAGTACC
		EXT:	TTACGCAAGAGTCACAT
Contig1556_192AG_DRP_LL	5179.4	PCR1:	ACGTTGGATGCAGCGAACGTCATAACTAGC
		PCR2:	ACGTTGGATGGACATCGGACTTATGCGTTC
		EXT:	ACGGCCATAGCAGACTT

SNP_ID	UEP_MASS		Primer Sequences
Contig1801_301AC_CD63_NT	5278.4	PCR1:	ACGTTGGATGAACAAAACCGAGAGCGAGAC
		PCR2:	ACGTTGGATGAACCCTGATTCATGTAGGAG
		EXT:	TTTTGTTCGTGGTGGGA
Contig1856_219TC_Calpain5_II	5700.7	PCR1:	ACGTTGGATGGTTCCTTTGCTTAGGCACTC
		PCR2:	ACGTTGGATGCTGATCTAGGTGCTGAGTAG
		EXT:	CACAAAAGTACTCCTCCAT
Contig1640_690CT_CDC25_SS	5741.8	PCR1:	ACGTTGGATGGACTTGAGACGGAAATGTCG
		PCR2:	ACGTTGGATGGCTTTCTGTGAACCTCAGAC
		EXT:	CGTTGCACAAAGACCACTC
Contig301_147CT_PTPRF_HH	6094.0	PCR1:	ACGTTGGATGTCAGATTCCCGTACACTTCC
		PCR2:	ACGTTGGATGTGCTGTTTTGCTGGATGAGG
		EXT:	TCTGAGCGGCCCCAGGTTCA
Contig1748_360TC_FCGBP_YY	6205.1	PCR1:	ACGTTGGATGGTAATAGGACGGCGCATAAG
		PCR2:	ACGTTGGATGCCGATGATGCTTACTTAGCC
		EXT:	GTCTTAGGAAAGGAGTACTA
Contig18_345AG_headcase_GG	6232.1	PCR1:	ACGTTGGATGACGTCCTGCCAGTTTTTGAC
		PCR2:	ACGTTGGATGACGCTTTCATCGTACGCTTG
		EXT:	TGCGGAGACAAAAAGAAAGT
Contig1660_554TC_COX15_HH	6386.2	PCR1:	ACGTTGGATGACAGATGTTCCCAGGGTCAG
		PCR2:	ACGTTGGATGGCCACAGAAACATGGAGTAG
		EXT:	CCCCCTGAAGGCAGAGCCCAG
Contig455_425GA_HES1_TT	6586.3	PCR1:	ACGTTGGATGCAAGATGGTACGGGTGTATG
		PCR2:	ACGTTGGATGCTCTCAACGTGCCTTCAAAC
		EXT:	GCCCTCAAACATCCAACAACAC
Contig2189_797GA_QPCT_RR	6730.4	PCR1:	ACGTTGGATGGGCAAAGAAAGAACAACACC
		PCR2:	ACGTTGGATGATACCTCCCCCATAAGACTG
		EXT:	TTCGATTATTGTTACTCAGTAG
Contig1793_251GA_Septin4_TT	6764.4	PCR1:	ACGTTGGATGTGATAAGCACCCACATGCAG
		PCR2:	ACGTTGGATGTGTTCCGCTCGATAGTTCTC
		EXT:	AGTTCTCATAATGGATGTCTGA
Contig122_737GC_PCSK1_AA	6946.5	PCR1:	ACGTTGGATGAGACGAGAGCAAATATGCCG
		PCR2:	ACGTTGGATGCGATGCACCACACAGCATAC
		EXT:	CCTCCTACGGGAACCTCCGCGGC
Contig1742_382CT_ALDH7A1_II	7135.7	PCR1:	ACGTTGGATGATTTCTGCACCACTAGTGGG
		PCR2:	ACGTTGGATGGCTGTATTCAGATGGCTAGG
		EXT:	CAAAAAGGATCTGATTGTGGAAT
Contig37_761GA_USP46_AA	7138.6	PCR1:	ACGTTGGATGAGGGCTAAAATCTTTGGAAG
		PCR2:	ACGTTGGATGTATTACTGTGAGGTGTGCGG
		EXT:	GGGTTCGGCACTAAACAGGAGGC

SNP_ID	UEP_MASS		Primer Sequences
Contig1610_183GA_SPTB_EE	7289.8	PCR1:	ACGTTGGATGGCATTAGAAAAGTTAACAACC
		PCR2:	ACGTTGGATGTTGCCTGCGTTTCTCTTCTC
		EXT:	TATTTCTTTTGCAATGTCCATTTG
Contig293_183CT_MnSOD_HH	7334.8	PCR1:	ACGTTGGATGGCCAGTTTCTCTTCTGCAAC
		PCR2:	ACGTTGGATGACTAGAGCCCTACATCTCAG
		EXT:	ATCTGATATCATGAAATTGCATCA
Contig1185_393TC_PSMB1_FF	7688.0	PCR1:	ACGTTGGATGCCATCTTCATCTATTCCTGC
		PCR2:	ACGTTGGATGATAGCAGCCATGCTGTCAAC
		EXT:	TGAAAATGCTTTATCACAGAAGATT
Contig1781_860TA_DPF6_GG	7713.0	PCR1:	ACGTTGGATGCTGGAAGACCTCAGATGATG
		PCR2:	ACGTTGGATGGCCTGGTAGTCCTTATTCTG
		EXT:	CCGTATTCTGAGGGCTGTACAGGTA
Contig1589_794GT_CHE_LF	7865.1	PCR1:	ACGTTGGATGGCAATTACAGTAGATCCCCC
		PCR2:	ACGTTGGATGTCTTCGGTTGTAACACCTTC
		EXT:	CCCTCTGTAACACCTTCAGTGAAATT
Contig1074_219CT_CalpA_FF	8067.3	PCR1:	ACGTTGGATGGACTGACTTTCGATGACGAC
		PCR2:	ACGTTGGATGCCAGGTTACAGATCTCTAGC
		EXT:	GGAGAAAAGTTCTTGGAGAAATCATC

2.3 Genetic data analysis

Prior to performing data analysis, MAF and call rate were calculated for each data set (genotype data). SNPs, with MAF greater than 0.05 and call rates greater than 0.95, were used for genetic data analysis. Therefore, each data set could have SNP markers used in different numbers – contingent only on satisfying the criteria listed above. The GenAlEx 6.5 program [44, 45] was used to determine the observed heterozygosity (Ho), expected heterozygosity (He), pairwise Fst, and Hardy-Weinberg equilibrium (HWE) during the assessment of the effectiveness between the new SNP panels and existing microsatellite markers. Ternary Hardy-Weinberg equilibrium plots were performed using package of HardyWeinberg version 1.5.6 in Rstudio [46]. Evolutionary analyses based on pairwise Fst values were conducted using the unweighted pair group method with arithmetic mean (UPGMA) method in Molecular Evolutionary Genetics

Analysis version 6.0 (MEGA6) [47]. The degree of linkage disequilibrium (LD) was calculated between all pairs of loci using the exact genotypic disequilibrium test in GENEPOP 4.5.1 [48]. A Bonferroni correction was applied for significance threshold at $\alpha = 0.05$ in both HWE and LD tests to reduce false positive from multiple tests. The population structures of the 618 eastern oysters were obtained from group-membership coefficients derived from the program STRUCTURE 2.3.4 [49, 50]. The program was used with the admixture model and a burn-in of 10,000 iterations followed by 100,000 iterations of Markov Chain Monte Carlo (MCMC) repetitions.

A total of 252 samples (160 HRL progenies, 12 HRL parents, 16 cultured oysters of 15G line, and 64 wild Delaware Bay samples) were used to run parentage assignment. Parentage assignments were performed using the programs CERVUS 3.0.7 [51] and SNPPIT 1.0 [52]. Parentage analyses based on CERVUS 3.0.7 consist of three continuous modules: 1. allele frequency analysis; 2. simulation of parentage analysis; and 3. parentage analysis. The threshold log-likelihood (LOD) scores for the true parental pair were determined during step 2 through simulating the parents and offspring. The simulations of 10,000 offspring were run for each tested population with the default genotyping error rate (0.01) and default confidence levels (strict confidence at 0.95 and relaxed confidence at 0.80). The parentage analysis module assigns the most-likely candidate parent pair to each offspring tested with a pre-determined population-wide assignment confidence (strict confidence at 0.95). In contrast, SNPPIT 1.0 uses a likelihood-based categorical assignment method and Monte Carlo simulation to assess the confidence of parentage assignment. SNPPIT also uses false-discovery rates (FDR) instead of the population-wide assignment confidence used in CERVUS to determine the parentage assignments. FDR levels of <0.01 were accepted for parentage assignment in SNPPIT.

2.4 Statistical analyses

Data were analyzed for means and standard error of measurement (SEM), repeated measures one-way analysis of variance (ANOVA) on ranks followed by Tukey for multiple comparison procedure and *t*-tests by means of GraphPad Prism (GraphPad Software, Inc, La Jolla, CA, USA).

3. Results and Discussion

3.1 Transcriptome assembly, contig annotation, and SNP identification

A total of 67,501 contigs were assembled from NCBI SRA **SRP042090**. Afterwards, 36,185 contigs were annotated against the non-redundant (NR) database in which 531,654 variant sites were identified from a total of 40 million bp (1/75 bp). This number is consistent with previous reports that oysters have high genetic variability [53]. Several studies have shown higher SNP density in the eastern oyster with estimates of 1 per 20 bp [31] and 1 per 54 bp [32]. In the Pacific oyster, *Crassostrea gigas*, SNPs were present at rates of approximately 1 per 60 bp in coding regions and 1 per 40 bp in non-coding regions [54]. Furthermore, in the European flat oyster, *Ostrea edulis*, SNP frequency was calculated at 1 per 76 bp and 1 per 47 bp, for coding regions and non-coding regions, respectively [55]. Two hundred SNPs were selected (irrespective of gene function) for primer-assay design and screening.

3.2 SNP validation using Agena MassARRAY

Following a previously established strategy of screening and redesigning mass-specific extension primers [38], two final panels of 32- and 26-multiplexes were developed for running

on the MassARRAY system. The primer sequences of 58 SNPs are shown in Table 2. The featured summary of 58 SNP markers is listed in Table 3, including contig ID, SNP position, SNP alleles, SNP types (synonymous; S vs. nonsynonymous; NS SNPs), and gene annotation. Of the 58 SNPs, 8 sites are non-synonymous SNPs and 50 are synonymous SNPs.

The consistency of genotype calling among technical replicates was examined using a total of 46 individuals, with a 98.25% of genotypes matching across multiple plates (data not shown). Similar technical replication scores were also observed for SNP markers from largemouth bass [38]. The number of SNPs containing in an array for this oyster study was similar to previous studies of 52-SNP array for European flat oysters, and 77-SNP array for the Pacific oyster [42].

Table 3 The features of the SNP multiplex including contig, position, SNP alleles (genotypes), SNP types (synonymous; S and non-synonymous; NS SNPs), and gene annotation.

Contig	Position	Alleles	SNP types	Gene Annotation
Contig100	1030	T/C	S (NN)	Solute carrier family 35 member C2
Contig105	310	C/T	S (TT)	Calmodulin
Contig129	1732	C/T	S (TT)	Heat shock 70 kDa protein 12A
Contig131	734	C/T	NS (PS)	Cell division control protein 42
Contig172	579	T/C	S (VV)	Myosin heavy chain, non-muscle, partial
Contig236	324	T/C	S (PP)	E3 ubiquitin-protein ligase RNF19A
Contig250	896	T/C	S (LL)	Secreted frizzled-related protein 5
Contig271	550	C/T	S (VV)	IQ domain-containing protein G
Contig273	447	A/G	S (KK)	Amyloid beta A4 protein
Contig284	591	C/A	NS (ED)	Kyphoscoliosis peptidase
Contig296	664	C/T	S (PP)	Vigilin
Contig306	684	C/A	S (AA)	Sterile alpha motif domain-containing protein 7
Contig316	156	G/A	S (QQ)	Transforming growth factor-beta-induced protein ig-h3
Contig32	81	T/C	S (CC)	LMBR1 domain-containing protein 2
Contig333	245	T/C	S (AA)	Tetraspanin-33
Contig351	534	G/A	S (EE)	UDP-glucose 4-epimerase
Contig360	453	G/A	NS (NS)	Collagen alpha-6(VI) chain
Contig367	1372	T/A	NS (TS)	E3 ubiquitin-protein ligase MIB2
Contig368	348	A/G	S (SS)	Heterogeneous nuclear ribonucleoprotein Q
Contig380	1135	A/G	S (TT)	Homeobox protein Nkx-2.6

Contig	Position	Alleles	SNP types	Gene Annotation
Contig414	697	C/T	S (LL)	L-isoaspartate O-methyltransferase domain-containing protein 2
Contig416	359	C/A	S (RR)	Sorting nexin-6
Contig444	1394	C/T	S (AA)	Myosin heavy chain, striated muscle
Contig460	509	T/C	S (VV)	Cathepsin B
Contig462	498	C/T	S (TT)	4-hydroxyphenylpyruvate dioxygenase
Contig48	519	T/C	S (SS)	Importin subunit alpha-3
Contig50	2398	C/T	S (TT)	Allene oxide synthase-lipoxygenase protein
Contig553	1147	C/T	S (LL)	Nuclear receptor coactivator 4
Contig6	677	A/T	S (RR)	Beta-1,3-galactosyltransferase brn
Contig617	111	T/C	S (YY)	Heat shock protein HSP 90-alpha 1
Contig7	283	G/A	S (PP)	Rab5 GDP/GTP exchange factor
Contig763	190	G/A	NS (VI)	Basigin
Contig1025	907	C/T	NS (PL)	Laminin subunit gamma-1
Contig1074	219	T/C	S (FF)	Calpain-A
Contig1185	393	C/T	S (FF)	Proteasome subunit beta type-1
Contig122	737	C/G	S (AA)	Neuroendocrine convertase 1
Contig1556	192	A/G	S (LL)	dopamine receptor protein
Contig1589	794	G/T	NS (LF)	Cholinesterase
Contig1610	183	G/A	S (EE)	Spectrin beta chain
Contig1640	690	C/T	S (SS)	M-phase inducer phosphatase
Contig1660	554	T/C	S (HH)	Cytochrome c oxidase assembly protein COX15-like protein
Contig1742	382	C/T	S (II)	Alpha-aminoadipic semialdehyde dehydrogenase
Contig1748	360	C/T	S (YY)	IgGFc-binding protein
Contig1755	481	C/T	S (NN)	IK cytokine
Contig1781	860	T/A	S (GG)	Dipeptidyl peptidase family member 6
Contig1791	792	C/T	S (II)	Eukaryotic translation initiation factor 3 subunit A
Contig1793	251	G/A	S (TT)	Septin-4
Contig18	345	G/A	S (GG)	headcase protein homolog isoform X1
Contig1801	301	C/A	NS (NT)	CD63 antigen
Contig1856	219	C/T	S (II)	Calpain-5
Contig188	1597	C/A	S (TT)	Alpha-actinin, sarcomeric
Contig1883	386	A/G	S (GG)	Maltase-glucoamylase, intestinal
Contig2189	797	G/A	S (RR)	Glutaminyl-peptide cyclotransferase
Contig293	183	C/T	S (HH)	Manganese superoxide dismutase
Contig301	147	C/T	S (HH)	Tyrosine-protein phosphatase Lar
Contig37	761	A/G	S (AA)	Ubiquitin carboxyl-terminal hydrolase 46
Contig455	425	A/G	S (TT)	Transcription factor HES-1
Contig463	137	C/T	S (SS)	Ornithine decarboxylase antizyme 1

3.3 A comparison of microsatellite markers and SNP panels

To examine the effectiveness of the new SNP panels, samples that had previously been analyzed using microsatellites (Delaware Bay) [43], the northeast Gulf of Mexico (unpublished data), and western (TX) Gulf of Mexico [22] were re-genotyped using SNP panels. The Delaware Bay samples (32 each from DBHC, DBSR, and DBCS; Table 1) had mean observed heterozygosity (Ho) values from SNP markers ranging from 0.329 to 0.343 (Table 4). While these heterozygosity values were lower than those obtained from microsatellites, inter-population comparisons with both marker types indicated no significant differences in genetic diversity. Lack of raw data from this study prevented comparison with standard errors. Similarly, pairwise Fst values ranged from 0.008 to 0.009 and from -0.002 to 0.001 [43] for the SNPs and 7 microsatellites, respectively (Table 5), indicating similar genetic population structure of Delaware Bay samples, i.e., a lack of genetic differentiation throughout the bay.

Eastern oysters from the northeast Gulf of Mexico (50 samples each from ALPP and ALCP) were also genotyped using SNP panels. The mean Ho values for ALPP and ALCP samples for SNPs were 0.339±0.029 and 0.338±0.024, respectively; whereas, the mean Ho values of these samples for 11 microsatellites were 0.371±0.093 and 0.369±0.071, respectively (Fig. 1A). The pairwise Fst between ALPP and ALCP samples for SNPs and 11 microsatellites was 0.032 and 0.043, respectively (Table 5). Based on Ho, He, and Fst values ($P \geq 0.05$), it can be concluded that both SNP markers and microsatellites show similar genetic diversities and population structure among the oysters listed above (Fig. 1A; Table 5).

Table 4 The genetic diversity derived from SNP panels. N is sample size, Ho is average observed heterozygosity, and He is average expected heterozygosity.

Sample Sites		N	Ho	He
Delaware Bay				
Hope Creek	Mean	32	0.329	0.357
	SE		0.020	0.017
Shell Rock	Mean	32	0.343	0.361
	SE		0.019	0.017
Cape Shore	Mean	32	0.334	0.351
	SE		0.020	0.017
Alabama Gulf Coast				
Perdido Pass, AL	Mean	50	0.339	0.324
	SE		0.029	0.024
Cedar Point, AL	Mean	50	0.338	0.340
	SE		0.024	0.021
Texas Gulf Coast				
Sabine Reef, Sabine Lake	Mean	36	0.342	0.360
	SE		0.021	0.019
Redfish Bay, Aransas	Mean	36	0.228	0.285
	SE		0.020	0.020
Packery Channel, Upper Laguna Madre	Mean	36	0.199	0.216
	SE		0.029	0.024
South Bay, Lower Laguna Madre	Mean	36	0.189	0.215
	SE		0.022	0.023

Table 5 The comparison of SNPs and microsatellite markers in the estimation of population structure by pairwise Fst values. Abbreviations are as listed in Table 1

	SNPs				**Microsatellites**			
	DBHC	DBSR	DBCS		DBHC	DBSR	DBCS	
DBHC	0.000				0.000			
DBSR	0.009	0.000			-0.002	0.000		
DBCS	0.009	0.008	0.000		0.001	0.000	0.000	
	ALPP	ALCP			ALPP	ALCP		
ALPP	0.000				0.000			
ALCP	0.032	0.000			0.043	0.000		
	TXSL	TXRB	TXPC	TXSB	TXSL	TXRB	TXPC	TXSB
TXSL	0.000				0.000			
TXRB	0.085	0.000			0.066	0.000		
TXPC	0.145	0.023	0.000		0.096	0.012	0.000	
TXSB	0.150	0.021	0.013	0.000	0.104	0.013	0.008	0.000

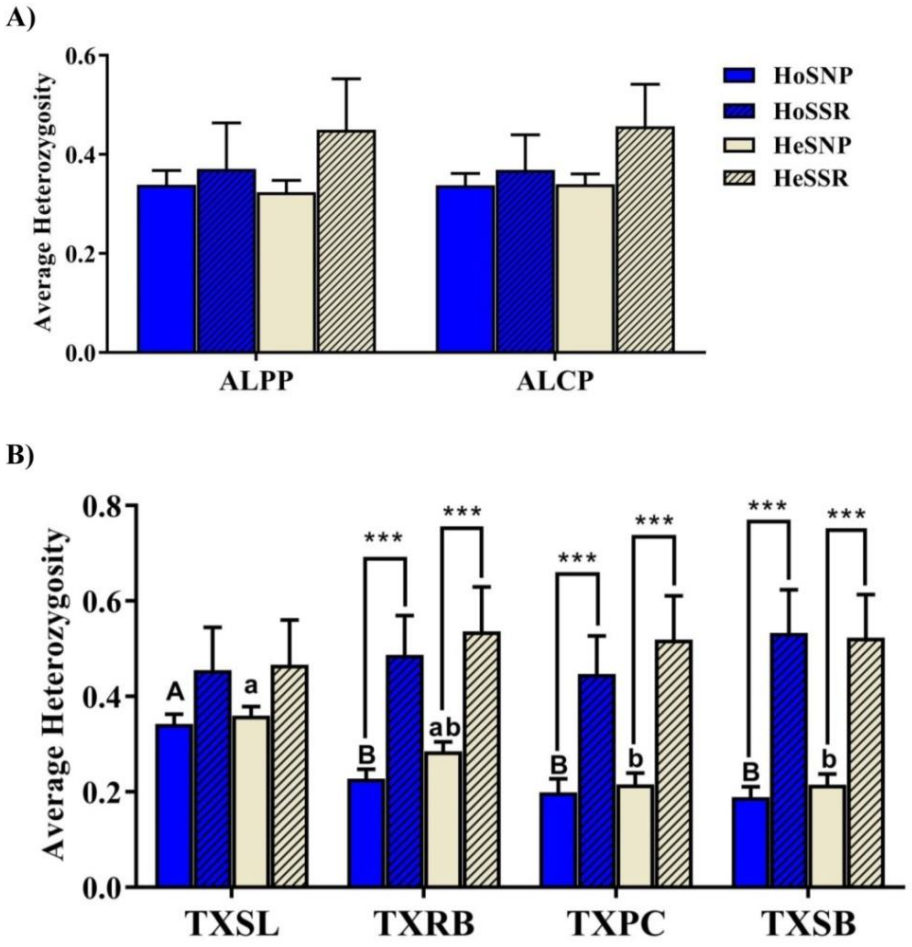

Fig. 1 Mean observed and expected heterozygosity (Ho and He) obtained from SNP and short-sequence DNA repeat (SSR) or microsatellite markers in oyster samples from Alabama (A) and Texas gulf coast (B). Statistical significance at $P < 0.05$ is indicated with upper case letters for the differences of Ho from SNPs and with lower case letters for the differences of He from SNPs, while asterisks show the significant differences between SNP and microsatellite markers at $P < 0.001$.

SNP results for the Texas Gulf Coast (36 oysters each from TXSL, TXRB, TXPC, and TXSB) were also compared. Mean Ho values of these samples ranged from 0.189 to 0.342; whereas, the mean Ho values for 11 microsatellites ranged from 0.447 to 0.553 (Fig. 1B) [22]. Despite lower SNP heterozygosity levels, highly similar genetic population structures were observed based on pairwise Fst values with SNPs and microsatellite markers (correlation

coefficient of 0.99; Table 5). Interestingly, m SNP panels captured significantly lower heterozygosity and differentiated population structure between Laguna Madre (TXRB, TXPC and TXSB) and the Galveston region (TXSL). This lower genetic diversity may imply that oyster populations in Laguna Madre may be more susceptible in the face of environmental change [56] and, as suggested previously, may warrant additional protection [57].

3.4 The assessment of population genetics of the eastern oyster

Beyond the samples genotyped above, samples from the Gulf of Mexico (northwest FL and LA) and hatchery-reared oysters for parentage analysis (mentioned in the following section) from the Haskin Shellfish Research Laboratory (HRL) were additionally genotyped (Table 1). These samples provided the assessment of the genetic diversity and population structure of eastern oysters along the U.S. Atlantic coastline and the Gulf of Mexico using SNP markers. Before conducting population genetic analyses I examined loci for deviations from HWE were examined.

Upon pooling the results of 430 wild samples, 36 out of 58 SNP loci deviated from HWE assumptions –due to heterozygote deficiencies (Fig. 2A). However, when examining results on a population levels, deviations from HWE in each sampling site were observed in both the direction of heterozygote deficiency and heterozygote excess and loci outside of HWE differed among populations (Fig. 2B). The highest numbers of SNPs that deviated from HWE assumptions were observed in hatchery samples (HRL). These results are most likely due to selective breeding (Fig. 2B; Table 6). Of the 58 loci, 8 loci were monomorphic SNPs, 21 loci were in HWE, and 29 loci deviated from HWE assumption. In addition to the high number of HWE deviations, HRL had higher rates of linkage disequilibrium (data not shown).

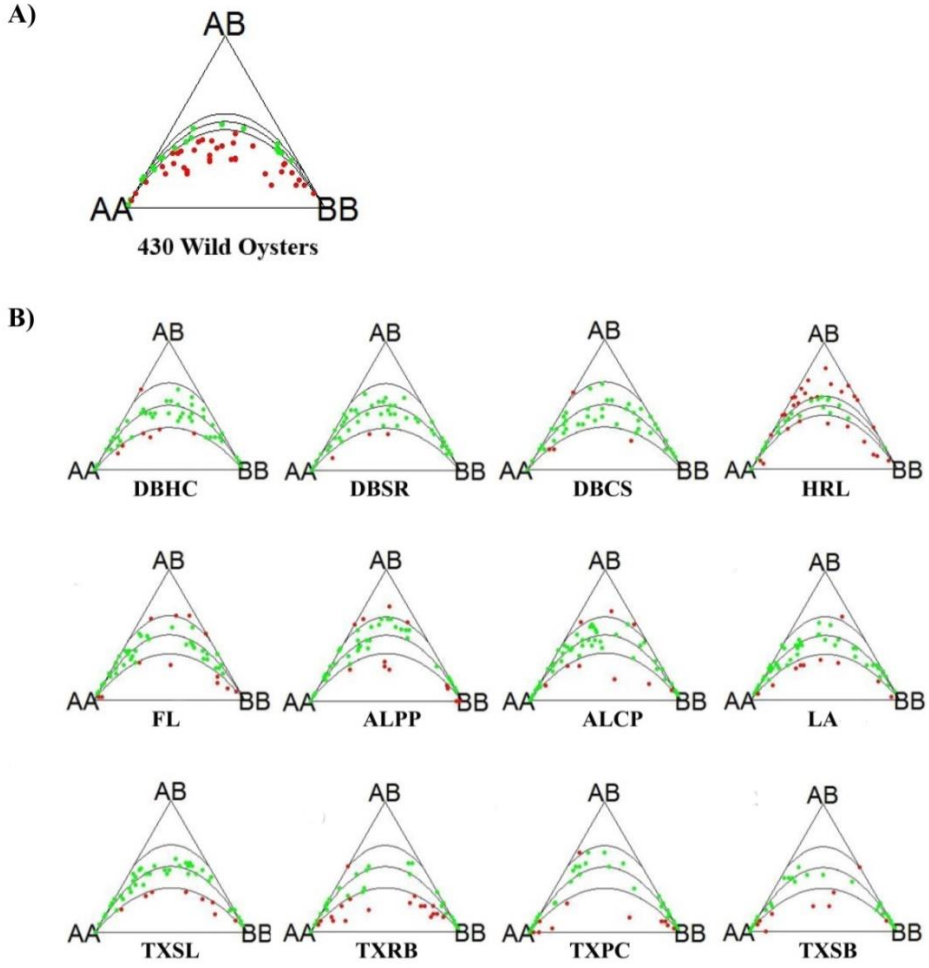

Fig. 2 Ternary Hardy-Weinberg Equilibrium (HWE) plots showing heterozygote deficiencies or excesses in 12 populations. Green dots are loci following HWE and red dots are loci departing from HWE. The upper and lower curved lines are the exact test cutoff boundaries. The ideal HWE proportion is the middle curve. A is from pooling wild oysters and B is for each geographical sample.

In contrast to hatchery samples, wild oysters from Laguna Madre had HWE deviations caused by heterozygote deficiencies (Fig. 2B; Table 6). These deficiencies were also detected previously along the central Texas coast where differences in spawning time may lead to non-random mating and population subdivision [22]. Buroker (1983) [10] also pointed out the establishment of reproductive isolation in the Laguna Madre; whereas, King et al. (1994) [57]

postulated that hydrographic isolation, genetic drift, and/or natural selection contribute to population subdivision in Laguna Madre samples. Although these situations would lead to unfavorable SNP markers with respect to HWE, discarding loci deviating from HWE expectations in a particular population could result in a loss of the most informative markers in others [58].

SNP markers showed the mean Ho ranging from a minimum of 0.165 ±0.021 in TXSB to a maximum of 0.375±0.030 in hatchery stock from HRL (Fig. 3; Table 6). Comparing wild populations, sample observations from Delaware Bay and the northeastern Gulf of Mexico showed similar genetic diversity (Ho = 0.297-0.333); whereas, samples from the Laguna Madre region revealed significantly lower genetic diversity (Ho = 0.165-0.175) than other populations (as previously discussed). When cultured populations were compared to wild populations, the cultured populations had greater genetic diversity (Ho = 0.375-0.346), suggesting their composite nature.

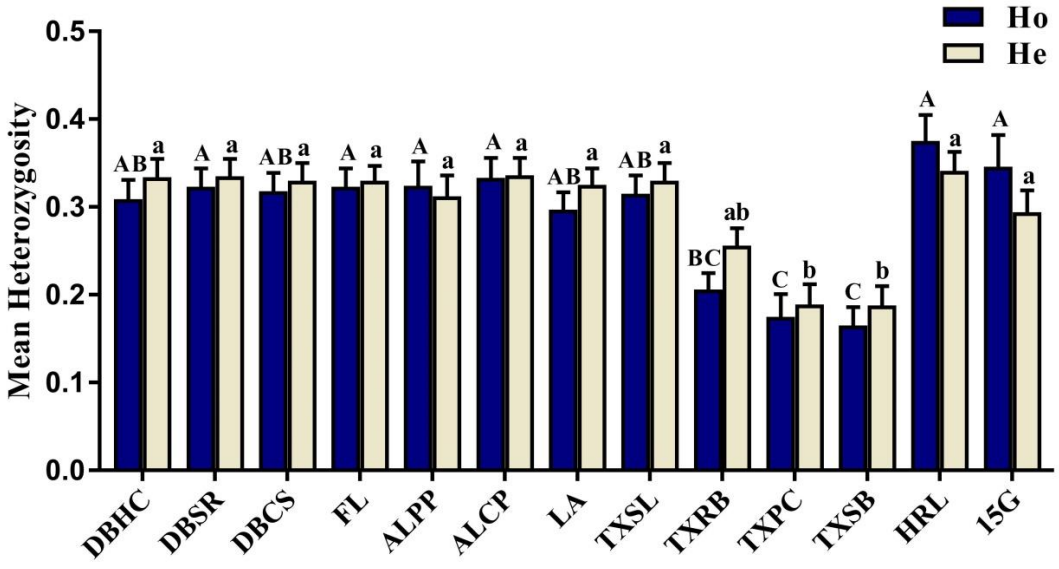

Fig. 3 Mean observed and expected heterozygosity (Ho and He) for 13 populations of eastern oysters using SNP markers. Statistical significance at $P < 0.05$ is indicated with upper case letters for the differences of Ho and with lower case letters for the differences of He.

Table 6 The evaluation of SNP panels in 618 eastern oysters. N is sample size. MAF is minor allele frequency. Ho and He are observed and expected heterozygosity, respectively. F_{IS} is fixation index and HWE indicates significance of Chi-square tests for Hardy-Weinberg equilibrium where ns is not significant, * indicates $P<0.05$, ** indicates $P<0.01$, and *** indicates $P<0.001$.

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig100_1030CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.344	0.375	0.266	0.478	0.750	0.590	0.433	0.472	0.611	0.625	0.611	0.334	0.813
	Ho	0.375	0.563	0.469	0.556	0.340	0.580	0.422	0.556	0.500	0.528	0.444	0.657	0.250
	He	0.451	0.469	0.390	0.499	0.375	0.484	0.491	0.498	0.475	0.469	0.475	0.445	0.305
	F_{IS}	0.169	-0.200	-0.202	-0.113	0.093	-0.199	0.140	-0.115	-0.052	-0.126	0.065	-0.476	0.179
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Contig105_310CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.375	0.203	0.250	0.656	0.530	0.340	0.322	0.264	0.097	0.056	0.042	0.302	0.000
	Ho	0.438	0.344	0.313	0.422	0.620	0.600	0.467	0.417	0.194	0.111	0.083	0.570	0.000
	He	0.469	0.324	0.375	0.452	0.498	0.449	0.437	0.389	0.176	0.105	0.080	0.422	0.000
	F_{IS}	0.067	-0.062	0.167	0.065	-0.244	-0.337	-0.068	-0.072	-0.108	-0.059	-0.043	-0.351	#N/A
	HWE	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	***	
Contig129_1732TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.031	0.078	0.078	0.278	0.170	0.240	0.300	0.403	0.333	0.375	0.306	0.166	0.000
	Ho	0.063	0.156	0.156	0.378	0.260	0.440	0.333	0.528	0.444	0.528	0.389	0.331	0.000
	He	0.061	0.144	0.144	0.401	0.282	0.365	0.420	0.481	0.444	0.469	0.424	0.276	0.000
	F_{IS}	-0.032	-0.085	-0.085	0.058	0.079	-0.206	0.206	-0.097	0.000	-0.126	0.084	-0.199	#N/A
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	
Contig131_734CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.000	0.016	0.000	0.144	0.190	0.120	0.189	0.264	0.694	0.903	0.931	0.000	0.000
	Ho	0.000	0.031	0.000	0.244	0.380	0.240	0.244	0.361	0.278	0.083	0.139	0.000	0.000
	He	0.000	0.031	0.000	0.247	0.308	0.211	0.306	0.389	0.424	0.176	0.129	0.000	0.000
	F_{IS}	#N/A	-0.016	#N/A	0.011	-0.235	-0.136	0.202	0.071	0.345	0.525	-0.075	#N/A	#N/A
	HWE		ns		ns	ns	ns	ns	ns	*	**	ns		
Contig172_579CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.156	0.266	0.234	0.222	0.100	0.270	0.222	0.292	0.069	0.014	0.042	0.282	0.125
	Ho	0.250	0.469	0.344	0.356	0.200	0.380	0.400	0.472	0.139	0.028	0.083	0.564	0.250
	He	0.264	0.390	0.359	0.346	0.180	0.394	0.346	0.413	0.129	0.027	0.080	0.405	0.219
	F_{IS}	0.052	-0.202	0.042	-0.029	-0.111	0.036	-0.157	-0.143	-0.075	-0.014	-0.043	-0.393	-0.143
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Contig236_324TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.344	0.484	0.344	0.767	0.690	0.460	0.411	0.333	0.611	0.833	0.931	0.061	0.000
	Ho	0.438	0.281	0.500	0.378	0.460	0.680	0.467	0.444	0.167	0.222	0.083	0.064	0.000
	He	0.451	0.500	0.451	0.358	0.428	0.497	0.484	0.444	0.475	0.278	0.129	0.115	0.000
	F_{IS}	0.030	0.437	-0.108	-0.056	-0.075	-0.369	0.036	0.000	0.649	0.200	0.355	0.442	#N/A
	HWE	ns	*	ns	ns	ns	**	ns	ns	***	ns	*	***	

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig250_896CT	**N**	32	32	32	45	42	40	42	33	34	36	36	164	16
	MAF	0.156	0.188	0.156	0.944	0.905	0.688	0.857	0.924	0.985	0.986	0.986	0.662	1.000
	Ho	0.125	0.250	0.313	0.067	0.095	0.325	0.143	0.091	0.029	0.028	0.028	0.335	0.000
	He	0.264	0.305	0.264	0.105	0.172	0.430	0.245	0.140	0.029	0.027	0.027	0.448	0.000
	FIS	0.526	0.179	-0.185	0.365	0.447	0.244	0.417	0.351	-0.015	-0.014	-0.014	0.251	#N/A
	HWE	**	ns	ns	*	**	ns	**	*	ns	ns	ns	**	
Contig271_550CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.016	0.000	0.031	0.033	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.212	0.063
	Ho	0.031	0.000	0.063	0.067	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.424	0.125
	He	0.031	0.000	0.061	0.064	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.334	0.117
	FIS	-0.016	#N/A	-0.032	-0.034	#N/A	#N/A	-0.023	#N/A	#N/A	#N/A	#N/A	-0.269	-0.067
	HWE	ns		ns	ns			ns					***	ns
Contig273_447GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.344	0.297	0.250	0.356	0.310	0.430	0.311	0.361	0.347	0.389	0.264	0.523	0.313
	Ho	0.438	0.469	0.438	0.489	0.540	0.460	0.400	0.444	0.306	0.611	0.417	0.360	0.625
	He	0.451	0.417	0.375	0.458	0.428	0.490	0.429	0.461	0.453	0.475	0.389	0.499	0.430
	FIS	0.030	-0.123	-0.167	-0.067	-0.262	0.062	0.067	0.037	0.326	-0.286	-0.072	0.278	-0.455
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Contig284_591AC	**N**	32	32	32	45	50	50	45	35	36	36	36	170	16
	MAF	0.328	0.313	0.234	0.278	0.330	0.350	0.311	0.300	0.806	0.986	0.889	0.185	0.313
	Ho	0.469	0.438	0.406	0.378	0.420	0.460	0.356	0.486	0.111	0.028	0.222	0.288	0.625
	He	0.441	0.430	0.359	0.401	0.442	0.455	0.429	0.420	0.313	0.027	0.198	0.302	0.430
	FIS	-0.063	-0.018	-0.132	0.058	0.050	-0.011	0.171	-0.156	0.645	-0.014	-0.125	0.045	-0.455
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns
Contig296_664CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.125	0.156	0.078	0.067	0.090	0.040	0.133	0.111	0.028	0.028	0.028	0.183	0.188
	Ho	0.250	0.313	0.156	0.133	0.180	0.080	0.267	0.222	0.056	0.056	0.056	0.343	0.375
	He	0.219	0.264	0.144	0.124	0.164	0.077	0.231	0.198	0.054	0.054	0.054	0.299	0.305
	FIS	-0.143	-0.185	-0.085	-0.071	-0.099	-0.042	-0.154	-0.125	-0.029	-0.029	-0.029	-0.146	-0.231
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Contig306_684AC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.391	0.297	0.359	0.633	0.320	0.150	0.333	0.333	0.819	0.875	0.931	0.323	0.250
	Ho	0.406	0.469	0.344	0.644	0.400	0.260	0.444	0.444	0.139	0.194	0.139	0.517	0.500
	He	0.476	0.417	0.460	0.464	0.435	0.255	0.444	0.444	0.296	0.219	0.129	0.437	0.375
	FIS	0.147	-0.123	0.253	-0.388	0.081	-0.020	0.000	0.000	0.531	0.111	-0.075	-0.184	-0.333
	HWE	ns	ns	ns	**	ns	ns	ns	ns	**	ns	ns	*	ns
Contig316_156GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.219	0.469	0.297	0.389	0.480	0.670	0.544	0.389	0.153	0.069	0.042	0.866	0.938
	Ho	0.250	0.438	0.281	0.422	0.720	0.340	0.511	0.444	0.139	0.139	0.083	0.267	0.125
	He	0.342	0.498	0.417	0.475	0.499	0.442	0.496	0.475	0.259	0.129	0.080	0.232	0.117
	FIS	0.269	0.122	0.326	0.112	-0.442	0.231	-0.030	0.065	0.463	-0.075	-0.043	-0.154	-0.067
	HWE	ns	ns	ns	ns	**	ns	ns	ns	**	ns	ns	*	ns

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig32_81TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.438	0.438	0.516	0.244	0.000	0.030	0.078	0.056	0.028	0.083	0.139	0.919	0.000
	Ho	0.500	0.438	0.656	0.311	0.000	0.060	0.111	0.111	0.056	0.056	0.167	0.151	0.000
	He	0.492	0.492	0.500	0.369	0.000	0.058	0.143	0.105	0.054	0.153	0.239	0.150	0.000
	FIS	-0.016	0.111	-0.314	0.158	#N/A	-0.031	0.225	-0.059	-0.029	0.636	0.303	-0.011	#N/A
	HWE	ns	ns	ns	ns		ns	ns	ns	ns	***	ns	ns	
Contig333_245CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.047	0.063	0.109	0.344	0.360	0.430	0.311	0.292	0.125	0.042	0.028	0.509	0.688
	Ho	0.094	0.125	0.156	0.556	0.600	0.500	0.400	0.472	0.194	0.028	0.056	0.483	0.500
	He	0.089	0.117	0.195	0.452	0.461	0.490	0.429	0.413	0.219	0.080	0.054	0.500	0.430
	FIS	-0.049	-0.067	0.198	-0.230	-0.302	-0.020	0.067	-0.143	0.111	0.652	-0.029	0.035	-0.164
	HWE	ns	ns	ns	ns	*	ns	ns	ns	ns	***	ns	ns	ns
Contig351_534GA	**N**	32	32	32	45	50	40	45	35	36	35	36	170	16
	MAF	0.047	0.125	0.063	0.178	0.090	0.213	0.111	0.086	0.750	0.829	0.889	0.550	0.250
	Ho	0.094	0.188	0.125	0.356	0.180	0.125	0.222	0.171	0.278	0.229	0.167	0.571	0.375
	He	0.089	0.219	0.117	0.292	0.164	0.335	0.198	0.157	0.375	0.284	0.198	0.495	0.375
	FIS	-0.049	0.143	-0.067	-0.216	-0.099	0.627	-0.125	-0.094	0.259	0.195	0.156	-0.153	0.000
	HWE	ns	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	*	ns
Contig360_453AG	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.141	0.188	0.203	0.333	0.630	0.930	0.911	0.875	0.306	0.111	0.097	0.517	0.500
	Ho	0.281	0.375	0.406	0.400	0.460	0.140	0.178	0.250	0.278	0.222	0.139	0.570	0.750
	He	0.242	0.305	0.324	0.444	0.466	0.130	0.162	0.219	0.424	0.198	0.176	0.499	0.500
	FIS	-0.164	-0.231	-0.255	0.100	0.013	-0.075	-0.098	-0.143	0.345	-0.125	0.209	-0.141	-0.500
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*
Contig367_1372AT	**N**	32	32	32	45	46	22	45	35	36	32	36	169	16
	MAF	0.172	0.203	0.172	0.200	0.163	0.250	0.122	0.171	0.236	0.063	0.431	0.062	0.000
	Ho	0.219	0.219	0.219	0.267	0.283	0.409	0.156	0.171	0.083	0.000	0.194	0.065	0.000
	He	0.285	0.324	0.285	0.320	0.273	0.375	0.215	0.284	0.361	0.117	0.490	0.117	0.000
	FIS	0.232	0.324	0.232	0.167	-0.035	-0.091	0.275	0.397	0.769	1.000	0.603	0.441	#N/A
	HWE	ns	ns	ns	ns	ns	ns	ns	*	***	***	***	***	
Contig368_348AG	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.000	0.016	0.000	0.067	0.000	0.010	0.133	0.097	0.056	0.069	0.069	0.000	0.000
	Ho	0.000	0.031	0.000	0.133	0.000	0.020	0.222	0.194	0.056	0.139	0.083	0.000	0.000
	He	0.000	0.031	0.000	0.124	0.000	0.020	0.231	0.176	0.105	0.129	0.129	0.000	0.000
	FIS	#N/A	-0.016	#N/A	-0.071	#N/A	-0.010	0.038	-0.108	0.471	-0.075	0.355	#N/A	#N/A
	HWE		ns		ns		ns	ns	ns	**	ns	*		
Contig380_1135AG	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.203	0.125	0.281	0.356	0.370	0.170	0.289	0.361	0.111	0.042	0.014	0.654	0.688
	Ho	0.344	0.250	0.375	0.444	0.540	0.300	0.400	0.500	0.222	0.028	0.028	0.634	0.625
	He	0.324	0.219	0.404	0.458	0.466	0.282	0.411	0.461	0.198	0.080	0.027	0.453	0.430
	FIS	-0.062	-0.143	0.072	0.030	-0.158	-0.063	0.026	-0.084	-0.125	0.652	-0.014	-0.400	-0.455
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig414_697CT	**N**	32	32	32	45	50	49	45	36	36	36	36	172	16
	MAF	0.359	0.484	0.313	0.178	0.050	0.061	0.033	0.028	0.042	0.014	0.000	0.477	0.438
	Ho	0.406	0.469	0.438	0.178	0.100	0.122	0.067	0.056	0.083	0.028	0.000	0.547	0.750
	He	0.460	0.500	0.430	0.292	0.095	0.115	0.064	0.054	0.080	0.027	0.000	0.499	0.492
	FIS	0.118	0.062	-0.018	0.392	-0.053	-0.065	-0.034	-0.029	-0.043	-0.014	#N/A	-0.095	-0.524
	HWE	ns	ns	ns	**	ns	ns	ns	ns	ns	ns		ns	*
Contig416_359AC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.078	0.063	0.109	0.067	0.290	0.230	0.122	0.319	0.069	0.028	0.028	0.093	0.000
	Ho	0.156	0.125	0.156	0.133	0.580	0.420	0.244	0.361	0.139	0.056	0.056	0.174	0.000
	He	0.144	0.117	0.195	0.124	0.412	0.354	0.215	0.435	0.129	0.054	0.054	0.169	0.000
	FIS	-0.085	-0.067	0.198	-0.071	-0.408	-0.186	-0.139	0.169	-0.075	-0.029	-0.029	-0.034	#N/A
	HWE	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	
Contig444_1394TC	**N**	32	32	32	45	50	50	45	36	36	36	36	171	16
	MAF	0.328	0.359	0.234	0.100	0.200	0.070	0.144	0.097	0.069	0.000	0.014	0.228	0.219
	Ho	0.281	0.594	0.344	0.200	0.400	0.100	0.244	0.194	0.083	0.000	0.028	0.433	0.438
	He	0.441	0.460	0.359	0.180	0.320	0.130	0.247	0.176	0.129	0.000	0.027	0.352	0.342
	FIS	0.362	-0.290	0.042	-0.111	-0.250	0.232	0.011	-0.108	0.355	#N/A	-0.014	-0.229	-0.280
	HWE	*	ns	ns	ns	ns	ns	ns	ns	*		ns	**	ns
Contig460_509TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.438	0.406	0.328	0.033	0.000	0.150	0.044	0.097	0.375	0.528	0.500	0.276	0.719
	Ho	0.313	0.500	0.344	0.067	0.000	0.220	0.044	0.194	0.472	0.500	0.444	0.436	0.563
	He	0.492	0.482	0.441	0.064	0.000	0.255	0.085	0.176	0.469	0.498	0.500	0.400	0.404
	FIS	0.365	-0.036	0.220	-0.034	#N/A	0.137	0.477	-0.108	-0.007	-0.003	0.111	-0.091	-0.391
	HWE	*	ns	ns	ns		ns	**	ns	ns	ns	ns	ns	ns
Contig462_498CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.406	0.469	0.453	0.756	0.510	0.580	0.756	0.667	0.806	0.722	0.847	0.747	0.781
	Ho	0.438	0.500	0.531	0.311	0.300	0.560	0.267	0.444	0.333	0.389	0.194	0.401	0.063
	He	0.482	0.498	0.496	0.369	0.500	0.487	0.369	0.444	0.313	0.401	0.259	0.378	0.342
	FIS	0.093	-0.004	-0.072	0.158	0.400	-0.149	0.278	0.000	-0.064	0.031	0.249	-0.062	0.817
	HWE	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	**
Contig48_519CT	**N**	32	32	32	45	50	47	45	35	36	36	36	172	16
	MAF	0.313	0.219	0.297	0.067	0.000	0.000	0.033	0.057	0.111	0.000	0.042	0.000	0.000
	Ho	0.625	0.438	0.594	0.133	0.000	0.000	0.067	0.114	0.167	0.000	0.083	0.000	0.000
	He	0.430	0.342	0.417	0.124	0.000	0.000	0.064	0.108	0.198	0.000	0.080	0.000	0.000
	FIS	-0.455	-0.280	-0.422	-0.071	#N/A	#N/A	-0.034	-0.061	0.156	#N/A	-0.043	#N/A	#N/A
	HWE	*	ns	*	ns			ns	ns	ns		ns		
Contig50_2398CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.313	0.313	0.328	0.844	0.950	0.880	0.911	0.958	0.944	0.958	0.903	0.509	0.406
	Ho	0.438	0.375	0.344	0.267	0.100	0.240	0.178	0.083	0.111	0.083	0.139	0.797	0.813
	He	0.430	0.430	0.441	0.263	0.095	0.211	0.162	0.080	0.105	0.080	0.176	0.500	0.482
	FIS	-0.018	0.127	0.220	-0.015	-0.053	-0.136	-0.098	-0.043	-0.059	-0.043	0.209	-0.594	-0.684
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	**

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig553_1147CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.156	0.125	0.141	0.111	0.350	0.160	0.178	0.111	0.042	0.014	0.014	0.000	0.063
	Ho	0.250	0.188	0.156	0.178	0.620	0.280	0.267	0.222	0.083	0.028	0.028	0.000	0.125
	He	0.264	0.219	0.242	0.198	0.455	0.269	0.292	0.198	0.080	0.027	0.027	0.000	0.117
	FIS	0.052	0.143	0.354	0.100	-0.363	-0.042	0.088	-0.125	-0.043	-0.014	-0.014	#N/A	-0.067
	HWE	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns		ns
Contig6_677AT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.219	0.234	0.188	0.367	0.650	0.480	0.500	0.486	0.153	0.153	0.111	0.776	0.531
	Ho	0.375	0.406	0.313	0.467	0.540	0.400	0.467	0.472	0.083	0.028	0.000	0.215	0.563
	He	0.342	0.359	0.305	0.464	0.455	0.499	0.500	0.500	0.259	0.259	0.198	0.347	0.498
	FIS	-0.097	-0.132	-0.026	-0.005	-0.187	0.199	0.067	0.055	0.678	0.893	1.000	0.381	-0.129
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	***	***	***	***	ns
Contig617_111CT	**N**	32	32	32	44	50	50	45	36	36	36	36	172	16
	MAF	0.000	0.000	0.000	0.023	0.000	0.020	0.078	0.097	0.028	0.000	0.000	0.000	0.000
	Ho	0.000	0.000	0.000	0.045	0.000	0.040	0.156	0.194	0.056	0.000	0.000	0.000	0.000
	He	0.000	0.000	0.000	0.044	0.000	0.039	0.143	0.176	0.054	0.000	0.000	0.000	0.000
	FIS	#N/A	#N/A	#N/A	-0.023	#N/A	-0.020	-0.084	-0.108	-0.029	#N/A	#N/A	#N/A	#N/A
	HWE				ns		ns	ns	ns	ns				
Contig7_283GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.156	0.094	0.047	0.178	0.080	0.160	0.133	0.208	0.014	0.000	0.028	0.334	0.500
	Ho	0.250	0.125	0.094	0.133	0.160	0.240	0.178	0.139	0.028	0.000	0.056	0.401	0.750
	He	0.264	0.170	0.089	0.292	0.147	0.269	0.231	0.330	0.027	0.000	0.054	0.445	0.500
	FIS	0.052	0.264	-0.049	0.544	-0.087	0.107	0.231	0.579	-0.014	#N/A	-0.029	0.099	-0.500
	HWE	ns	ns	ns	***	ns	ns	ns	***	ns		ns	ns	*
Contig763_190GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.375	0.391	0.391	0.278	0.130	0.280	0.178	0.250	0.736	0.931	0.944	0.413	0.438
	Ho	0.250	0.281	0.406	0.378	0.260	0.400	0.267	0.389	0.194	0.139	0.111	0.558	0.500
	He	0.469	0.476	0.476	0.401	0.226	0.403	0.292	0.375	0.389	0.129	0.105	0.485	0.492
	FIS	0.467	0.409	0.147	0.058	-0.149	0.008	0.088	-0.037	0.500	-0.075	-0.059	-0.151	-0.016
	HWE	**	*	ns	ns	ns	ns	ns	ns	**	ns	ns	*	ns
Contig1025_907CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.109	0.109	0.172	0.256	0.510	0.400	0.300	0.389	0.111	0.000	0.014	0.041	0.250
	Ho	0.219	0.156	0.156	0.333	0.620	0.560	0.422	0.500	0.222	0.000	0.028	0.081	0.500
	He	0.195	0.195	0.285	0.380	0.500	0.480	0.420	0.475	0.198	0.000	0.027	0.078	0.375
	FIS	-0.123	0.198	0.451	0.124	-0.240	-0.167	-0.005	-0.052	-0.125	#N/A	-0.014	-0.042	-0.333
	HWE	ns	ns	*	ns	ns	ns	ns	ns	ns		ns	ns	ns
Contig1074_219CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.188	0.156	0.094	0.056	0.020	0.190	0.044	0.069	0.139	0.028	0.153	0.369	0.531
	Ho	0.188	0.250	0.188	0.111	0.040	0.380	0.089	0.139	0.111	0.000	0.139	0.599	0.688
	He	0.305	0.264	0.170	0.105	0.039	0.308	0.085	0.129	0.239	0.054	0.259	0.466	0.498
	FIS	0.385	0.052	-0.103	-0.059	-0.020	-0.235	-0.047	-0.075	0.535	1.000	0.463	-0.286	-0.380
	HWE	*	ns	ns	ns	ns	ns	ns	ns	**	***	**	***	ns

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig1185_393TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.016	0.000	0.000	0.167	0.040	0.020	0.144	0.111	0.056	0.014	0.014	0.000	0.000
	Ho	0.031	0.000	0.000	0.244	0.000	0.040	0.244	0.167	0.111	0.028	0.028	0.000	0.000
	He	0.031	0.000	0.000	0.278	0.077	0.039	0.247	0.198	0.105	0.027	0.027	0.000	0.000
	FIS	-0.016	#N/A	#N/A	0.120	1.000	-0.020	0.011	0.156	-0.059	-0.014	-0.014	#N/A	#N/A
	HWE	ns			ns	***	ns	ns	ns	ns	ns	ns		
Contig122_737GC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.141	0.109	0.109	0.856	0.690	0.650	0.667	0.764	0.236	0.139	0.125	0.395	0.750
	Ho	0.219	0.219	0.219	0.289	0.380	0.420	0.267	0.306	0.194	0.278	0.194	0.500	0.125
	He	0.242	0.195	0.195	0.247	0.428	0.455	0.444	0.361	0.361	0.239	0.219	0.478	0.375
	FIS	0.095	-0.123	-0.123	-0.169	0.112	0.077	0.400	0.153	0.461	-0.161	0.111	-0.046	0.667
	HWE	ns	ns	ns	ns	ns	ns	**	ns	**	ns	ns	ns	**
Contig1556_192AG	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.500	0.438	0.328	0.111	0.020	0.060	0.056	0.028	0.042	0.125	0.069	0.137	0.219
	Ho	0.438	0.500	0.219	0.089	0.000	0.080	0.022	0.056	0.083	0.083	0.083	0.099	0.313
	He	0.500	0.492	0.441	0.198	0.039	0.113	0.105	0.054	0.080	0.219	0.129	0.236	0.342
	FIS	0.125	-0.016	0.504	0.550	1.000	0.291	0.788	-0.029	-0.043	0.619	0.355	0.581	0.086
	HWE	ns	ns	**	***	***	*	***	ns	ns	***	*	***	ns
Contig1589_794GT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.438	0.531	0.516	0.456	0.070	0.040	0.156	0.083	0.042	0.042	0.000	0.765	0.844
	Ho	0.625	0.438	0.406	0.644	0.140	0.080	0.267	0.167	0.083	0.083	0.000	0.378	0.313
	He	0.492	0.498	0.500	0.496	0.130	0.077	0.263	0.153	0.080	0.080	0.000	0.360	0.264
	FIS	-0.270	0.122	0.187	-0.299	-0.075	-0.042	-0.015	-0.091	-0.043	-0.043	#N/A	-0.050	-0.185
	HWE	ns	ns	ns	*	ns	ns	ns	ns	ns	ns		ns	ns
Contig1610_183GA	**N**	32	32	32	45	50	49	45	36	36	36	36	172	16
	MAF	0.328	0.516	0.422	0.489	0.600	0.531	0.411	0.403	0.792	0.958	0.944	0.084	0.219
	Ho	0.281	0.344	0.344	0.267	0.560	0.449	0.289	0.306	0.194	0.083	0.111	0.169	0.188
	He	0.441	0.500	0.488	0.500	0.480	0.498	0.484	0.481	0.330	0.080	0.105	0.154	0.342
	FIS	0.362	0.312	0.295	0.466	-0.167	0.099	0.403	0.365	0.411	-0.043	-0.059	-0.092	0.451
	HWE	*	ns	ns	**	ns	ns	**	*	*	ns	ns	ns	ns
Contig1640_690CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.016	0.000	0.094	0.378	0.450	0.210	0.356	0.375	0.819	0.958	0.958	0.291	0.250
	Ho	0.031	0.000	0.188	0.622	0.580	0.300	0.267	0.306	0.194	0.083	0.083	0.500	0.500
	He	0.031	0.000	0.170	0.470	0.495	0.332	0.458	0.469	0.296	0.080	0.080	0.412	0.375
	FIS	-0.016	#N/A	-0.103	-0.324	-0.172	0.096	0.418	0.348	0.343	-0.043	-0.043	-0.212	-0.333
	HWE	ns		ns	*	ns	ns	**	*	*	ns	ns	**	ns
Contig1660_554TC	**N**	32	32	32	45	50	44	45	36	36	36	36	171	16
	MAF	0.188	0.094	0.078	0.856	0.810	0.898	0.789	0.764	0.931	0.944	0.944	0.462	0.188
	Ho	0.250	0.188	0.156	0.200	0.380	0.114	0.422	0.306	0.083	0.111	0.056	0.444	0.375
	He	0.305	0.170	0.144	0.247	0.308	0.184	0.333	0.361	0.129	0.105	0.105	0.497	0.305
	FIS	0.179	-0.103	-0.085	0.191	-0.235	0.381	-0.268	0.153	0.355	-0.059	0.471	0.106	-0.231
	HWE	ns	ns	ns	ns	ns	*	ns	ns	*	ns	**	ns	ns

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig1742_382CT	**N**	32	32	32	45	50	50	45	36	36	36	36	171	16
	MAF	0.422	0.359	0.484	0.689	0.740	0.610	0.678	0.542	0.639	0.750	0.694	0.667	0.688
	Ho	0.406	0.406	0.531	0.489	0.240	0.460	0.422	0.472	0.333	0.222	0.389	0.421	0.625
	He	0.488	0.460	0.500	0.429	0.385	0.476	0.437	0.497	0.461	0.375	0.424	0.444	0.430
	FIS	0.167	0.118	-0.064	-0.141	0.376	0.033	0.033	0.049	0.278	0.407	0.084	0.053	-0.455
	HWE	ns	ns	ns	ns	**	ns	ns	ns	ns	*	ns	ns	ns
Contig1748_360TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.109	0.219	0.250	0.056	0.120	0.220	0.144	0.139	0.250	0.306	0.236	0.410	0.406
	Ho	0.156	0.375	0.375	0.022	0.200	0.360	0.244	0.167	0.500	0.500	0.417	0.541	0.813
	He	0.195	0.342	0.375	0.105	0.211	0.343	0.247	0.239	0.375	0.424	0.361	0.484	0.482
	FIS	0.198	-0.097	0.000	0.788	0.053	-0.049	0.011	0.303	-0.333	-0.178	-0.155	-0.118	-0.684
	HWE	ns	ns	ns	***	ns	ns	ns	ns	*	ns	ns	ns	**
Contig1755_481CT	**N**	29	29	31	45	50	48	43	35	36	36	36	154	16
	MAF	0.017	0.034	0.000	0.033	0.030	0.052	0.047	0.029	0.028	0.000	0.000	0.104	0.031
	Ho	0.034	0.069	0.000	0.022	0.060	0.104	0.047	0.057	0.056	0.000	0.000	0.208	0.063
	He	0.034	0.067	0.000	0.064	0.058	0.099	0.089	0.056	0.054	0.000	0.000	0.186	0.061
	FIS	-0.018	-0.036	#N/A	0.655	-0.031	-0.055	0.476	-0.029	-0.029	#N/A	#N/A	-0.116	-0.032
	HWE	ns	ns		***	ns	ns	**	ns	ns			ns	ns
Contig1781_860TA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.156	0.250	0.234	0.311	0.390	0.650	0.533	0.625	0.889	0.931	0.944	0.529	0.563
	Ho	0.313	0.313	0.344	0.489	0.460	0.580	0.578	0.528	0.111	0.139	0.111	0.558	0.500
	He	0.264	0.375	0.359	0.429	0.476	0.455	0.498	0.469	0.198	0.129	0.105	0.498	0.492
	FIS	-0.185	0.167	0.042	-0.141	0.033	-0.275	-0.161	-0.126	0.438	-0.075	-0.059	-0.120	-0.016
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
Contig1791_792CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.406	0.500	0.391	0.300	0.490	0.270	0.167	0.167	0.306	0.333	0.333	0.180	0.281
	Ho	0.438	0.563	0.469	0.511	0.260	0.380	0.289	0.278	0.444	0.444	0.500	0.291	0.313
	He	0.482	0.500	0.476	0.420	0.500	0.394	0.278	0.278	0.424	0.444	0.444	0.295	0.404
	FIS	0.093	-0.125	0.015	-0.217	0.480	0.036	-0.040	0.000	-0.047	0.000	-0.125	0.016	0.227
	HWE	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	ns
Contig1793_251GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.344	0.172	0.203	0.389	0.360	0.590	0.544	0.444	0.347	0.500	0.431	0.456	0.938
	Ho	0.563	0.281	0.281	0.422	0.480	0.420	0.600	0.500	0.528	0.611	0.306	0.669	0.125
	He	0.451	0.285	0.324	0.475	0.461	0.484	0.496	0.494	0.453	0.500	0.490	0.496	0.117
	FIS	-0.247	0.012	0.131	0.112	-0.042	0.132	-0.210	-0.013	-0.164	-0.222	0.377	-0.347	-0.067
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	***	ns
Contig18_345AG	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.438	0.422	0.516	0.833	0.970	0.780	0.733	0.819	0.875	0.667	0.861	0.666	0.250
	Ho	0.375	0.344	0.469	0.333	0.060	0.360	0.311	0.250	0.250	0.611	0.222	0.378	0.375
	He	0.492	0.488	0.500	0.278	0.058	0.343	0.391	0.296	0.219	0.444	0.239	0.445	0.375
	FIS	0.238	0.295	0.062	-0.200	-0.031	-0.049	0.205	0.155	-0.143	-0.375	0.071	0.151	0.000
	HW	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig1801_301AC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.156	0.141	0.234	0.300	0.380	0.310	0.300	0.222	0.264	0.333	0.292	0.084	0.000
	Ho	0.188	0.094	0.281	0.289	0.400	0.500	0.244	0.333	0.250	0.500	0.417	0.041	0.000
	He	0.264	0.242	0.359	0.420	0.471	0.428	0.420	0.346	0.389	0.444	0.413	0.154	0.000
	FIS	0.289	0.612	0.216	0.312	0.151	-0.169	0.418	0.036	0.357	-0.125	-0.008	0.736	#N/A
	HWE	ns	***	ns	*	ns	ns	**	ns	*	ns	ns	***	
Contig1856_219TC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.266	0.281	0.359	0.722	0.740	0.750	0.856	0.667	0.958	1.000	1.000	0.294	0.563
	Ho	0.469	0.375	0.469	0.289	0.280	0.260	0.111	0.278	0.083	0.000	0.000	0.576	0.500
	He	0.390	0.404	0.460	0.401	0.385	0.375	0.247	0.444	0.080	0.000	0.000	0.415	0.492
	FIS	-0.202	0.072	-0.018	0.280	0.272	0.307	0.550	0.375	-0.043	#N/A	#N/A	-0.388	-0.016
	HWE	ns	ns	ns	ns	ns	*	***	*	ns			***	ns
Contig188_1597AC	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.484	0.516	0.484	0.789	0.900	0.840	0.944	0.903	0.972	1.000	1.000	0.166	0.281
	Ho	0.531	0.531	0.531	0.378	0.160	0.280	0.111	0.194	0.056	0.000	0.000	0.110	0.313
	He	0.500	0.500	0.500	0.333	0.180	0.269	0.105	0.176	0.054	0.000	0.000	0.276	0.404
	FIS	-0.064	-0.064	-0.064	-0.134	0.111	-0.042	-0.059	-0.108	-0.029	#N/A	#N/A	0.600	0.227
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns			***	ns
Contig1883_386GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.344	0.297	0.281	0.256	0.040	0.100	0.178	0.236	0.111	0.083	0.056	0.544	0.406
	Ho	0.500	0.406	0.313	0.511	0.080	0.160	0.267	0.417	0.222	0.111	0.111	0.552	0.563
	He	0.451	0.417	0.404	0.380	0.077	0.180	0.292	0.361	0.198	0.153	0.105	0.496	0.482
	FIS	-0.108	0.027	0.227	-0.343	-0.042	0.111	0.088	-0.155	-0.125	0.273	-0.059	-0.113	-0.166
	HWE	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Contig2189_797GA	**N**	32	32	32	45	50	50	45	35	36	36	36	172	16
	MAF	0.000	0.000	0.000	0.144	0.440	0.440	0.389	0.229	0.153	0.083	0.111	0.000	0.063
	Ho	0.000	0.000	0.000	0.200	0.560	0.160	0.644	0.229	0.306	0.167	0.222	0.000	0.125
	He	0.000	0.000	0.000	0.247	0.493	0.493	0.475	0.353	0.259	0.153	0.198	0.000	0.117
	FIS	#N/A	#N/A	#N/A	0.191	-0.136	0.675	-0.356	0.352	-0.180	-0.091	-0.125	#N/A	-0.067
	HWE				ns	ns	***	*	*	ns	ns	ns		ns
Contig293_183CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.141	0.172	0.063	0.244	0.530	0.270	0.289	0.389	0.097	0.028	0.111	0.273	0.156
	Ho	0.219	0.281	0.125	0.356	0.620	0.420	0.400	0.500	0.139	0.056	0.222	0.547	0.313
	He	0.242	0.285	0.117	0.369	0.498	0.394	0.411	0.475	0.176	0.054	0.198	0.397	0.264
	FIS	0.095	0.012	-0.067	0.037	-0.244	-0.065	0.026	-0.052	0.209	-0.029	-0.125	-0.376	-0.185
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Contig301_147CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.250	0.375	0.406	0.211	0.190	0.440	0.344	0.250	0.069	0.069	0.028	0.000	0.000
	Ho	0.250	0.438	0.625	0.378	0.380	0.520	0.422	0.444	0.139	0.083	0.056	0.000	0.000
	He	0.375	0.469	0.482	0.333	0.308	0.493	0.452	0.375	0.129	0.129	0.054	0.000	0.000
	FIS	0.333	0.067	-0.296	-0.134	-0.235	-0.055	0.065	-0.185	-0.075	0.355	-0.029	#N/A	#N/A
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns		

MARKERS		SAMPLE SITES												
		DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
Contig37_761GA	**N**	32	32	32	44	46	41	44	36	36	36	36	170	16
	MAF	0.250	0.172	0.156	0.273	0.402	0.561	0.545	0.528	0.625	0.708	0.667	0.609	0.750
	Ho	0.250	0.281	0.313	0.318	0.543	0.537	0.500	0.444	0.472	0.472	0.500	0.700	0.500
	He	0.375	0.285	0.264	0.397	0.481	0.493	0.496	0.498	0.469	0.413	0.444	0.476	0.375
	FIS	0.333	0.012	-0.185	0.198	-0.130	-0.089	-0.008	0.108	-0.007	-0.143	-0.125	-0.470	-0.333
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Contig455_425GA	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.266	0.188	0.172	0.244	0.100	0.310	0.222	0.250	0.319	0.306	0.250	0.381	0.500
	Ho	0.406	0.313	0.344	0.356	0.120	0.580	0.267	0.389	0.528	0.333	0.500	0.610	0.750
	He	0.390	0.305	0.285	0.369	0.180	0.428	0.346	0.375	0.435	0.424	0.375	0.472	0.500
	FIS	-0.041	-0.026	-0.208	0.037	0.333	-0.356	0.229	-0.037	-0.214	0.215	-0.333	-0.294	-0.500
	HWE	ns	ns	ns	ns	*	*	ns	ns	ns	ns	*	***	*
Contig463_137CT	**N**	32	32	32	45	50	50	45	36	36	36	36	172	16
	MAF	0.484	0.453	0.438	0.811	0.520	0.330	0.467	0.708	0.667	0.667	0.542	0.250	0.219
	Ho	0.531	0.531	0.375	0.244	0.240	0.260	0.311	0.250	0.222	0.111	0.194	0.314	0.188
	He	0.500	0.496	0.492	0.306	0.499	0.442	0.498	0.413	0.444	0.444	0.497	0.375	0.342
	FIS	-0.064	-0.072	0.238	0.202	0.519	0.412	0.375	0.395	0.500	0.750	0.608	0.163	0.451
	HWE	ns	ns	ns	ns	***	**	*	*	**	***	***	*	ns

Table 7 Pairwise population Fst values (below diagonal) using SNP markers in 618 *Crassostrea virginica* from 13 sites (11 wild populations along the Atlantic and Gulf coasts of the United States and 2 cultured lines).

	DBHC	**DBSR**	**DBCS**	**FL**	**ALPP**	**ALCP**	**LA**	**TXSL**	**TXRB**	**TXPC**	**TXSB**	**HRL**	**15G**
DBHC	0.000												
DBSR	0.009	0.000											
DBCS	0.009	0.009	0.000										
FL	0.088	0.092	0.084	0.000									
ALPP	0.126	0.129	0.119	0.037	0.000								
ALCP	0.111	0.110	0.104	0.048	0.032	0.000							
LA	0.107	0.108	0.099	0.031	0.025	0.014	0.000						
TXSL	0.109	0.114	0.103	0.032	0.028	0.020	0.009	0.000					
TXRB	0.151	0.152	0.145	0.079	0.093	0.088	0.079	0.077	0.000				
TXPC	0.191	0.194	0.188	0.126	0.149	0.144	0.130	0.133	0.021	0.000			
TXSB	0.195	0.196	0.192	0.128	0.149	0.144	0.136	0.138	0.020	0.012	0.000		
HRL	0.093	0.093	0.091	0.102	0.142	0.110	0.104	0.108	0.170	0.222	0.222	0.000	
15G	0.123	0.123	0.128	0.127	0.156	0.120	0.118	0.123	0.180	0.227	0.234	0.067	0.000

Pairwise population Fst values ranged from 0.009-0.234 (Table 7). The minimum pairwise Fst of 0.009 was found among population samples from Delaware Bay (DBHC, DBSR, and DBCS). The maximum pairwise Fst of 0.196 was observed between DBSR and TXSB. The genetic differentiation of samples within the northeast Gulf of Mexico in this study (from Apalachicola, FL to Caillou Lake, LA with a pairwise Fst of 0.014-0.048) was consistent with the findings of Varney et al. (2009) [21]. In Varney et al. (2009) [21], they utilized 12 SNPs on samples between Apalachicola, FL and Grand Isle, LA with a pairwise Fst of 0.022.

STRUCTURE analysis revealed three distinct populations among 11 wild populations; 1. Delaware Bay, 2. northeast Gulf of Mexico and north of Port Aransas, and 3. south of Port Aransas (Fig. 4). These results were consistent with the previous studies from Reeb and Avise (1990) [11], Karl and Avise (1992) [12], King et al. (1994) [57], Hare and Avise (1998) [17], Hoover and Gaffney (2005) [19], Varney et al. (2009) [21], and Anderson et al. (2014) [22]. Using mitochondrial restriction site polymorphisms (RSP) and RFLP, the genetic discontinuity of Gulf and Atlantic eastern oysters were discovered on the east coast of Florida [11, 12, 14, 19]. The genetic differentiations of samples in Laguna Madre between north and south of Port Aransas (TX) were consistent with allozyme results from King et al. (1994) [57], and studies with mtDNA, mtDNA RFLP, and a limited number of nuclear SNPs [21], and microsatellites [22]. Furthermore, when hatchery samples were included in STRUCTURE analysis with wild samples, four distinct populations resulted (Fig. 4). These findings indicate that these marker panels may be of utility in differentiating between cultured and wild populations, such as in monitoring restoration programs [59].

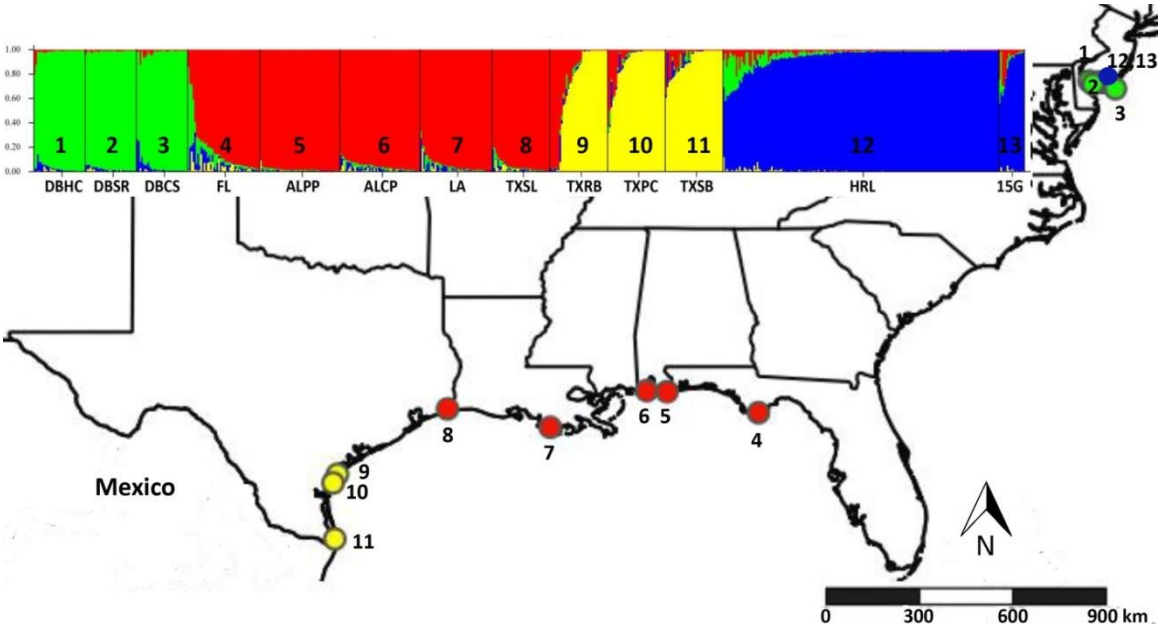

Fig. 4 Population structure bar plot result of a total of 618 *Crassostrea virginica* from 12 sites (13 populations) that include 11 sites of wild populations and 1 site of cultured sample population, but 2 different culture lines. STRUCTURE analysis showed four populations (*K*) in green, red, yellow and blue bars that are sorted by *Q* within each site.

Eastern oysters inhabiting the Laguna Madre represent a unique population (Fig. 4; Fig. 5) that should be further investigated for patterns of genetic exchange. This population should be considered as a distinct management unit – as previously suggested by King et al. (1994) [57]. Low level of genetic differences within the populations of Delaware Bay or within the northeast Gulf of Mexico populations are likely due to a homogenizing effect caused by high gene flow. Due to the limitation on the number of sampling locations, there was not sufficient data to determine the presence of eastern oyster subpopulations along the U.S. Atlantic coast or a genetic discontinuity of eastern oyster populations along the east coast of Florida. However – similar to the findings by Varney et al. (2009) [21] –results obtained in the present study using this new genetic tool confirmed the presence of a subpopulation of eastern oysters in the Gulf of Mexico.

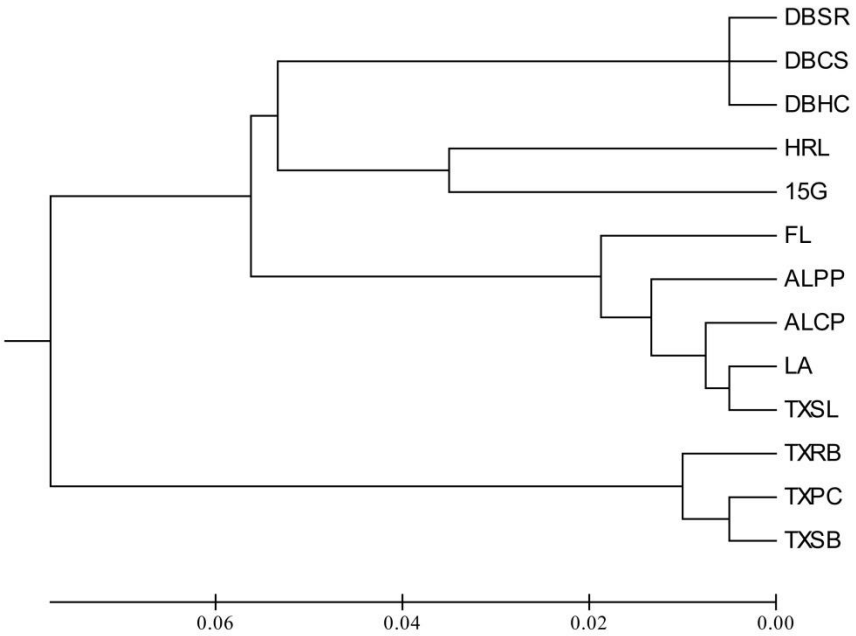

Fig. 5 Evolutionary relationship of oyster populations based on pairwise population Fst values using UPGMA method in MEGA6.

3.5 Examination of SNPs for parentage analysis

Following comparison with published microsatellite studies, the utility of the developed panels for parentage analyses were also examined. As described in the Methods, a subset of progeny reared in common garden fashion in replicated bags on racks in Delaware Bay, as well as all potentially contributing parents were genotyped. Based on SNP results, 99.375% (159/160) of the HRL samples were assigned to paired parents. The results obtained from CERVUS 3.0.7 (see Appendix 1) and SNPPIT 1.0 were consistent (see Appendix 2). In addition, a comparative assessment between SNP panels and 16 microsatellite markers [23] for assigning parentage showed 98.74% concurrence of assignment matches (data not shown). Similar contributions from each family were observed in each replicate, likely indicating differential survival of spat and differing genetic contributions to cohort (Table 8, [23]).

A minimum of 8 microsatellite loci have been reported to be required for correct parentage assignments in eastern oyster [23]. An estimation of approximately 6 SNPs is needed to generate the same assignment power as one microsatellite [27, 60]. Thus, the expected number of SNP markers needed for parentage assignment of eastern oyster would be approximately 48 loci. Similarly, a recent report from Pacific oyster demonstrated that as few as 40 SNPs were sufficient for 100% parentage-assignment accuracy [61]. These factors, together with initial analyses in Atlantic populations, suggest that SNP markers should perform robustly in tracking parentage in hatcheries rearing the eastern oyster.

Table 8 Parentage contributions of 160 eastern oyster progenies from 6 families (4 replicates: A, B, C, and D), using SNP markers with CERVUS and SNPPIT.

	A (1-40)	**B (41-80)**	**C (81-120)**	**D (121-160)**	**Total**
Family 1	17	12	16	13	58
Family 2	0	0	1	1	2
Family 3	8	7	7	8	30
Family 4	6	6	5	9	26
Family 5	7	10	11	9	37
Family 6	2	4	0	0	6
Total	40	39	40	40	159

4. Conclusions

The current study developed a rapid and effective tool for analyzing the genetic variation of eastern oysters, *C. virginica*. Results indicate the utility of the developed 58 SNP loci markers for genetic differentiation, population structure, parentage assignment, and discrimination between hatchery stocks and wild-sourced individuals. Nevertheless, other technologies are still required to obtain higher density SNP arrays for conducting quantitative trait locus (QTL) mapping. This will lead to genome-wide association studies to link genetic variations to phenotypic traits and be used for marker assisted selection (MAS). MAS will be useful for

eastern oyster breeding and farming to increase oyster growth rate and minimize impacts of disease. Moreover, future work with eastern oysters should focus on considering the evolutionary aspects of eastern oysters (e.g. the potential factors in speciation) to improve management of oyster hatcheries and wild fisheries.

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Chapter III Species-diagnostic SNP markers for the black basses (*Micropterus* spp.): A new tool for black bass conservation

Abstract

Black basses (genus *Micropterus*) are apex predators in North American streams, rivers, and lakes and important game fishes. Translocation and introductions for angling, accompanied by intrinsically weak genetic barriers, have led to widespread introgressive hybridization and genetic swamping. Species-diagnostic (fixed allele) SNP markers have been utilized successfully in salmonids to monitor hybridization and maintain genetic integrity. Here, similar resources were developed for black basses through initial genotyping-by-sequencing, followed by extensive validation in additional samples using two panels of 64 SNPs. Results from >1300 genotyped bass indicated that the developed panels robustly and clearly delineate the majority of species and their hybrids among black basses. The panels represent a flexible, rapid turnaround (~1 d) and cost-effective tool that should augment ongoing efforts toward black bass conservation and management.

1. Introduction

Black basses (*Micropterus* spp.), members of the family Centrarchidae, are apex predators in diverse aquatic systems throughout the Southeastern United States, and, as such, hold an important place as indicators of overall aquatic ecosystem health [1-3]. In addition to their ecological value, black basses comprise one of the most highly sought after groups of game

fishes in the United States and around the world [2, 3]. This has led to high rates of translocation and, in some cases, adverse effects on native fish communities around the globe [4-6]. One adverse outcome associated with these introductions in the United States has been hybridization and introgression among black basses, particularly in several geographically limited species that have only recently begun to be characterized [e.g., 7, 8, 9]. The weak genetic barriers of black basses [10] make them particularly prone to genetic swamping, a phenomenon documented with alarming regularity in southeastern US populations [11-13]. In some cases, conservation and fisheries agencies have responded to threatened endemic populations through genetic restoration (repatriation) efforts focused on propagation and release of pure individuals and/or removal of invasive species [12, 14].

Monitoring for introductions, assessing population hybridization status, determining broodstock purity, and evaluating the success of restoration efforts all require molecular genetic tools as phenotypic determination of species identification and purity in black basses is notoriously unreliable [8]. If these genetic assessments can be made rapidly (days rather than weeks or months), affordably, and reliably, fisheries biologists and conservation managers are more likely to proactively monitor populations on a regular basis and will possess the necessary evidence to make real-time decisions in the field or hatchery [15]. Single nucleotide polymorphism (SNP) markers with higher multiplexing potential, automated scoring, and associated lower costs are enabling tighter integration of genetic tools into aquatic conservation [16]. For example, biologists in the northwestern United States monitoring hybridization and introgession of invasive rainbow trout into threatened westslope cutthroat trout populations have transitioned away from microsatellites (where few diagnostic markers are typically identified) to multiplexed SNP assays, which can provide individual level estimates of admixture proportion at

a fraction of the cost and time [17, 18]. While diagnostic SNP panels [19, 20] for the northern largemouth bass (*Micropterus salmoides*) and Florida largemouth bass (*Micropterus floridanus*) have recently been developed, other black basses, including those most threatened by introductions, lack these resources.

Reduced-representation sequencing of individual genomes, e.g., restriction-site associated DNA sequencing (RAD-seq) [21] and genotyping-by-sequencing (GBS) [22], allow affordable generation of thousands of SNPs and rapid identification of species-diagnostic markers [18]. In the present study, therefore, GBS was utilized to sequence thousands of SNPs from key black bass species. Further screening and validation (1376 individual samples) led to the development of two SNP panels of diagnostic markers highly informative for assessments of genetic purity and monitoring hybridization among 15 species (described and under description) within the genus.

2. Materials and Methods

2.1 Sample collection for genotyping-by-sequencing (GBS)

As part of an ongoing project involving coastal largemouth bass in the Mobile-Tensaw Delta, a total of 190 black bass were collected for GBS library construction and downstream analysis. Samples for GBS included coastal largemouth bass (n=34), northern largemouth bass (*M. salmoides*; n=43), Florida largemouth bass (*M. floridanus*; n=29), intergrade largemouth bass (n=55), Coosa bass (*M. coosae*; n=12), and smaller numbers of samples from other key black bass species including Alabama bass (*M. henshalli*; n=2), spotted bass (*M. punctulatus*; n=2), shoal bass (*M. cataractae*; n=6), smallmouth bass (*M. dolomieu*; n=3), and Guadalupe bass (*M. treculii*; n=4). Genomic DNA from all samples were extracted from blood or fin clips using

the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Quantity of genomic DNA was determined using a Qubit dsDNA BR assay kit (Invitrogen by Life Technologies, Carlsbad, CA, USA) with a Qubit fluorometer (Life Technologies) following the manufacturer's protocol. Quality of DNA was examined by running 100 ng DNA sample on 1% agarose gels. In addition, 300 ng of each DNA sample was digested with HindIII at 37°C for 3 h and run on 1% agarose gels for an image of trial digestion by non-methylated sensitive restriction enzyme (RE) prior to submitting DNA samples to the Institute for Genomic Diversity, Cornell University, for GBS.

2.2 Library preparation and Illumina sequencing

A 96-plex GBS library was prepared according to Elshire et al. (2011). *Pst*I (6-base cutters, CTGCA*G) was selected as the most suitable restriction enzyme to reduce genome complexity [20]. Unique barcode adapters (4-8 bp barcode at the 3ˈend) were designed for every sample along with a common adapter. The lower strand of the barcode adapter and the upper strand of the common adapter were designed to have a 5ˈoverhang compatible to the sticky end produced by the specific restriction enzyme. Aliquots from the DNA samples were then added to a 96-well plate that already contained the adapters. DNA samples were digested in the plates using the restriction enzyme. Adapters were also ligated to the ends of DNA fragments. Adapter-ligated DNA fragments were then pooled and purified using QIAquick PCR purification kit (Qiagen Valencia, CA, USA), followed by amplification using primers with sequences complimentary to the oligonucleotides coating the Illumina flow cell. PCR profiles consisted of initial denaturation at 72°C for 5 minutes, 98° C for 30 seconds, followed by 18 cycles of denaturation at 98°C for 30 minutes, annealing at 65°C for 30 seconds, extension at 72°C for 30 seconds and a final

extension at 72°C for 5 minutes. Single-end sequencing of DNA fragments adjacent to the cut sites was performed on a Genome Analyzer II (Illumina Inc., San Diego, CA).

2.3 DNA sequence processing and SNP calling

Raw read sequence data and SNP calls were processed using the non-reference GBS Universal Network Enabled Analysis Kit (UNEAK) pipeline [23], which is part of the Trait Analysis by aSSociation, Evolution and Linkage (TASSEL3) stand-alone program [24, 25]. The pipeline trimmed off ambiguous nucleotides to avoid sequencing errors and removed barcodes, chimeric sequences, cut sites and adapter sequences. After removing low-quality reads and trimming sequences to 64 bases, remaining reads were clustered by their sequence similarity within individuals as tags (the tagCount files). All tagCount files were then merged within taxa, and tags with coverage ≥100X (-c = 100) retained. These criteria removed rare or singleton tags that possibly resulted from sequencing errors. The merged tagCount output file was then used as a master tag list for the network filter in order to identify tag pairs with 1 bp mismatch in associated reads as candidate SNPs, and in order to generate a TagsByTaxa file for the tags within the tagPair file.

The HapMap output file (MapInfo) was generated based on the tagPair file. SNP calling on the Hapmap file was performed with the following criteria: minimum minor allele frequency (mnMAF:0.05), maximum minor allele frequency (mxMAF:0.5), and minimum call rate (mnC:0.70).

2.4 Fixed SNP filtering and SNP panel development and validation

SNPs called from the UNEAK pipeline were further filtered for species-specificity using TASSEL5 [24, 25]. A subset of 95 samples including northern largemouth bass, Florida largemouth bass, Alabama bass, spotted bass, Coosa bass, shoal bass, and smallmouth bass was utilized for stringent filtering. SNPs with MAF of 0.05- 0.15 and proportion of heterozygous genotypes less than 0.05 were filtered for fixed alleles between largemouth bass (n=72) and others (n=6-14). SNPs matching these criteria were connected to their Fasta tag pair sequences and mapped to a preliminary genomic assembly of largemouth bass contigs (DDBJ/ENA/ GenBank under the accession NRCI00000000) [20]. Only SNP loci or their complementary sites that mapped to a single location of the genomic reference (with no adjacent SNPs) were selected, and the tag sequences were required to perfectly match the reference. Assays were designed from 100 bp flanking region on either side of SNPs (based on largemouth bass contigs) using the MassARRAY Assay Design Software (Agena Bioscience® Inc., San Diego, CA).

The MassARRAY software utilized default settings for multiplexing with a maximum of 40 SNPs per well. A subset of filtered SNPs passing assay design was validated on the MassARRAY system using the iPLEX Gold Reagent Kit according to the manufacturer's protocols. In order to validate the SNPs, some of which were derived from limited GBS sequencing data, an initial screening step was carried out. Given existing Florida largemouth bass and northern largemouth bass panels [19], both of these were combined into "largemouth bass" here for further analysis (n=32) and compared with Alabama bass (n=32), spotted bass (n=32), Coosa bass (n=32), smallmouth bass (n=40), and shoal bass (n=32). Samples utilized at this step were separately validated for purity based on existing microsatellite genotypes [1] and morphology. All samples run on the MassARRAY were derived from fin clips with DNA

extracted using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). Amplification and extension reactions (iPLEX™ Gold Assay) were performed using 2 µl of 15 ng concentration DNA. SNP genotypes were automatically called and manually confirmed using the MassARRAY Typer 4.0 Analysis software. SNPs not consistently amplified in the multiplex, found to have resulted from sequencing errors, absent polymorphism (potentially duplicated regions), or lacking species specificity were not retained (Fig. 1). The final assay was performed using pure, hybridized, and unknown samples from the original six species as well as Guadalupe bass, Choctaw bass (*M. haiaka*), Suwannee bass (*M. notius*), Cahaba bass (*M. cahabae*), Tallapoosa bass (*M. tallapoosae*), Warrior bass (*M. warriorensis*), Chattahoochee bass (*M. chattahoochae*), and two undescribed putative species, Bartram's bass (*M. sp. cf. coosae*) from the Savannah River drainage and Altamaha bass (*M. sp. cf. coosae*) from the Altamaha-Ogeechee River drainage.

2.5 Genetic Analysis

Genotype data was analyzed using STRUCTURE version 2.3.4 [26, 27] to identify the species status of unknown samples or evaluate genetic purity. The data analysis used different numbers of assumed population genetic clusters (K) depending on species inclusion for each run. 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. According to previous methodologies, individuals with membership coefficients of ≥0.95 were assigned to a single species ("pure"); otherwise, they were assigned as hybrids depending on species proportion [28].

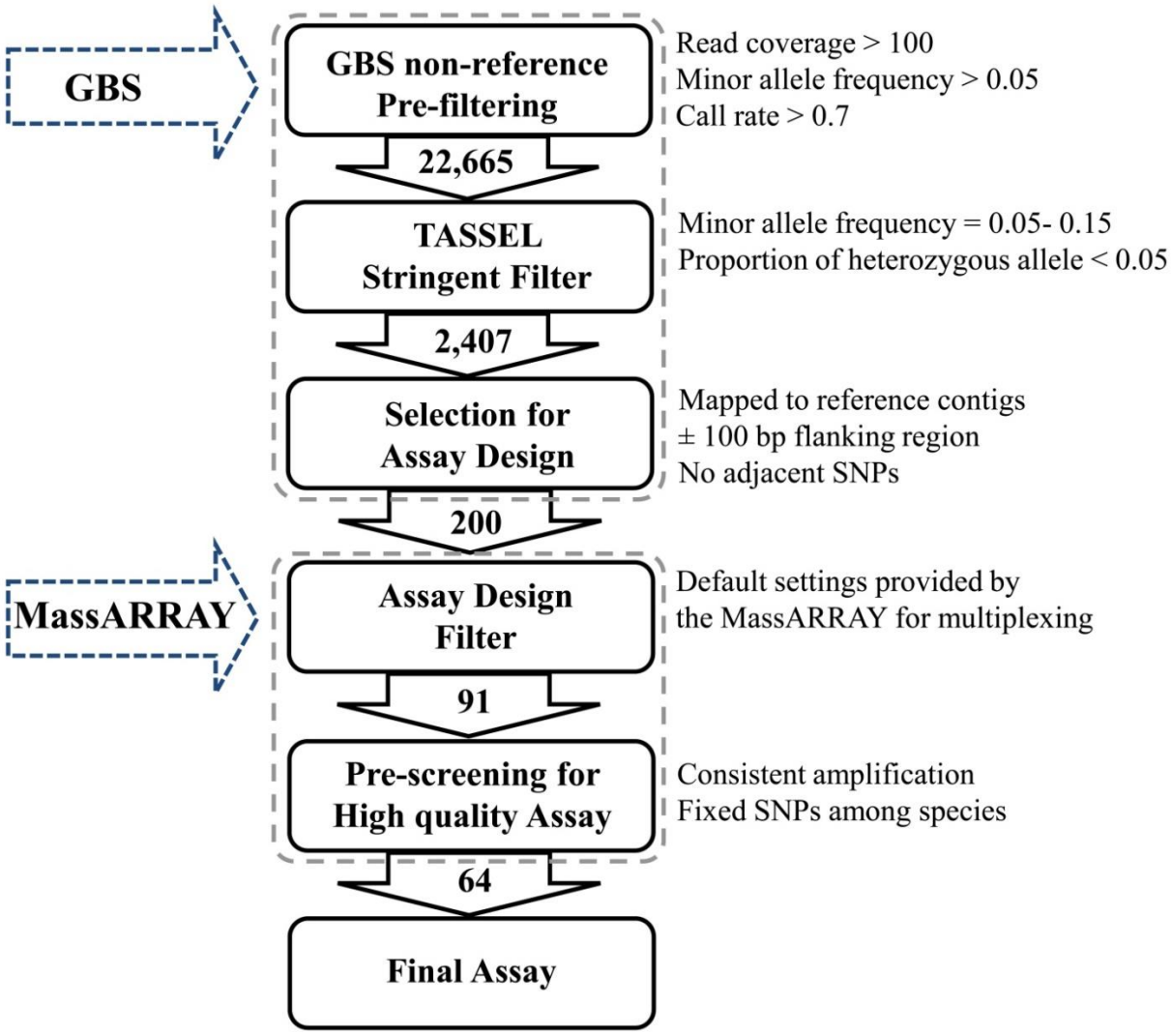

Fig. 1 Workflow demonstrating the steps used in marker identification and selection of SNP panels for species-diagnostic markers.

3. Results

3.1 SNP discovery by GBS

Reduced-representation sequencing of individual samples (genotyping-by-sequencing) were employed to generate a total of 428,984,624 high-quality short reads, including an average of 2,257,813 reads for each sequenced sample (n=190 including six failed samples). Raw reads were deposited in NCBI SRA database with accession number **PRJNA417468**. Based on SNP

calling parameters (c≥100, MAF 0.05, and MCR 0.7) a total of 22,665 SNPs were identified in the UNEAK pipeline. Further filtering aimed at enriching species-specific fixed SNP (MAF between 0.05- 0.15 and proportion of heterozygous genotypes less than 0.05) retained 2,407 SNPs (data not shown). Of these, a total of 200 putative fixed SNPs were selected for MassARRAY assay design.

3.2 MassARRAY pre-screening and validation

Assay design and multiplexing generated 91 putative fixed/diagnostic SNPs. These were pre-screened in the MassARRAY system and validated for specificity using 200 well-characterized samples from six black bass species (see Methods; Appendix 3). Of 91 putative fixed SNPs, 67 SNPs were consistently amplified and species diagnosed in these samples. Further panel refinement and redesign of extension primers resulted in a final assay of 64 highly informative SNPs, used in subsequent genotyping of additional samples and STRUCTURE analyses. Primer and contig sequences for SNP loci are listed in Table 1. An overview of the workflow for SNP screening and development is presented in Fig. 1.

Table 1 The 67 SNP primers of 2 assay panels for black bass (*Micropterus* spp.).

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
SMShTP5873G	G/A	4488.9	PCR1:	ACGTTGGATGAGCTGCCTTTCTGTAAGTCC
			PCR2:	ACGTTGGATGACAGTCACAGGAGAAGGTC
			EXT:	GGTCTCCCAGCTCAC
SMShTP1235G	G/A	4540	PCR1:	ACGTTGGATGTAACTGTGCAGACACAGAGG
			PCR2:	ACGTTGGATGTGCCGGTATGCATACATCAG
			EXT:	CAGCGCACACAAGAC
SPMhRETP21822A	G/A	4680	PCR1:	ACGTTGGATGAAGCTGTCAGTCACCTGGAG
			PCR2:	ACGTTGGATGAGGTGAAGCTGGATCAGTGG
			EXT:	TCAGTGGGCGTGGTG

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
SPMhRETP1192T	A/T	5069.3	PCR1:	ACGTTGGATGACTTGTCGTGCTCTACACAG
			PCR2:	ACGTTGGATGCTGAACAGACTGCAGAACTC
			EXT:	TCTTTTGTTCCTCGCTC
MpRETP30468C	C/G	5201.4	PCR1:	ACGTTGGATGACTTGACAATTTTCAGGCCC
			PCR2:	ACGTTGGATGTTGTCTGCAGTTTCAGAGCG
			EXT:	GCAAGGGTACTCTTCTG
RETP7594T	T/C	5290.5	PCR1:	ACGTTGGATGAACATCTGTCTCTGCCGCTG
			PCR2:	ACGTTGGATGGCTTATCAGAGGGACACTTG
			EXT:	GGACACTTGTGGTAGAG
RETP4454T	C/T	5516.6	PCR1:	ACGTTGGATGGTTTGTGCTCTTGTTCTGGG
			PCR2:	ACGTTGGATGTGAAGCGTGGAGACAGAGTG
			EXT:	AAACCTGACTGCAGAGAT
SPMhRETP18863T	C/T	5653.7	PCR1:	ACGTTGGATGCCATCAACAGCAGCACTATC
			PCR2:	ACGTTGGATGCTTAATCCCTGCAGGCAAAC
			EXT:	GGGAACGGCTGAGGAATG
RETP4714A	T/A	5818.8	PCR1:	ACGTTGGATGTCATCTGCTGCTTTGGCGTC
			PCR2:	ACGTTGGATGAAGAAGCAGATGCTGGAGAG
			EXT:	AAATCTGGACGCTGCTGTT
RETP5317T	C/T	5873.8	PCR1:	ACGTTGGATGACGTCCCTTTTTCCCACTTG
			PCR2:	ACGTTGGATGAGAGGCAACAACTGCTGAGG
			EXT:	AACTGCTGAGGATTGTTTG
MpRETP8149T	C/T	6039.9	PCR1:	ACGTTGGATGAGGCTGAAGTCATTCTGGTC
			PCR2:	ACGTTGGATGAGCCTGAGATTGCCAAGATG
			EXT:	CCTCTCGCCGGGGCCGCCAA
SPMhRETP12009T	A/T	6066	PCR1:	ACGTTGGATGGGACTGCAGTACAACATGTG
			PCR2:	ACGTTGGATGGAGTGAGCGTTGGAAACGAC
			EXT:	CCCCCAACGACGCAGAGACG
MpSHTP18868C	C/T	6172	PCR1:	ACGTTGGATGGAGACCTGCTCCACTTTTTC
			PCR2:	ACGTTGGATGAGTTCAACCTGCAGGCAAAG
			EXT:	GCAAAGGTTGAGGATCTTCT
ShTP20328A	A/G	6176	PCR1:	ACGTTGGATGTTTCTACATCCTCTGGCTCG
			PCR2:	ACGTTGGATGTGGCAAGCACAAACACCAAC
			EXT:	GGACTAGCTGCAGGGACAAC
MpRETP16597T	G/T	6534.3	PCR1:	ACGTTGGATGCTTACCTGCAGCTTTCAGAG
			PCR2:	ACGTTGGATGAACATACTGCAGCTGCAGGG
			EXT:	GATCAGTGTCAGGAGTGTAAA
RETP5475A	A/G	6568.3	PCR1:	ACGTTGGATGGTGTTGCGATGAAGCTTCAG
			PCR2:	ACGTTGGATGCGCAGTGGATGGTAATGTTC
			EXT:	GCGGGCAGTTAGAGAAATAGA
SPMhRETP7161A	G/A	6712.4	PCR1:	ACGTTGGATGGCAGATGTGCTGCATTTTCC
			PCR2:	ACGTTGGATGGAGAGACAACTGCAGCAGAT
			EXT:	CTTATTGTGGTTTTGATTCCTA

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
SPMhRETP18151G	G/A	6748.4	PCR1:	ACGTTGGATGGGACTGAGGGTGTCAACAAG
			PCR2:	ACGTTGGATGGTGTCAGGCAGACTGACTTT
			EXT:	GGCAGACTGACTTTAATAATTT
RETP3652A	T/A	6926.5	PCR1:	ACGTTGGATGCGTACTCAACTCTACAGCTC
			PCR2:	ACGTTGGATGGTTCATTCTGCAGGAAGCTC
			EXT:	CCCCCGCTTACCTGTGGATCTTA
SPMhRETP6389C	C/T	7110.6	PCR1:	ACGTTGGATGCATCAAAACGGCTTGCCGAC
			PCR2:	ACGTTGGATGTGTAGATGTTGAGTCTGCCG
			EXT:	GGGGTAGATATCTTGCTGGCATC
MpRETP5605T	C/T	7137.7	PCR1:	ACGTTGGATGTCATCCTGCCATGTTTCCTC
			PCR2:	ACGTTGGATGGCAGGTTTAAGCAAGTGCAG
			EXT:	GTGACGAAAGCTGCAGAGGTTAC
SPMhRETP7458C	C/T	7165.7	PCR1:	ACGTTGGATGTGAATGAGGCTGCAGCTGAG
			PCR2:	ACGTTGGATGTATCGCTCCGGTTTGATTGC
			EXT:	GTGGGTTGATTGCTGCAGATTGA
RETP5033T	T/A	7319.8	PCR1:	ACGTTGGATGTTACAAAATGTCTGCAGAGC
			PCR2:	ACGTTGGATGAAGGCCTAGTAACAGTCCAG
			EXT:	AAGCATTTCGAAGACCATTTTAAC
SMNBTP5121T§	T/A	7560.9	PCR1:	ACGTTGGATGTTCCTAAGGAACTCCACCTC
			PCR2:	ACGTTGGATGGACGTGTGTGGTATTAGGTG
			EXT:	CCCTGTTACCAAAGTTGCAATCCAT
SPMhRETP8383G	G/C	7678	PCR1:	ACGTTGGATGACAATCTGCAGCACACAAGG
			PCR2:	ACGTTGGATGCAACAGGATCTGTCACAATG
			EXT:	GGTTTTTTTCAGGGACACTTCAGTA
RETP5103T	T/C	7735.1	PCR1:	ACGTTGGATGCAACTGTGTTCCTGCAGAGC
			PCR2:	ACGTTGGATGACACCGGGCATAGAGTAAAC
			EXT:	TGTAGAACATGTAGTATTTGTGAAC
RETP7076G	G/T	8053.3	PCR1:	ACGTTGGATGACTGCAGATGGTGAGAAGGC
			PCR2:	ACGTTGGATGGTGCAGAGAGAGCTGACATT
			EXT:	GATGTAAAACCAGACAGGTGGCGTAC
SPMhRETP2910G	G/C	8155.3	PCR1:	ACGTTGGATGGTCGCGTTTGGTGCAATTTG
			PCR2:	ACGTTGGATGTGCAGAGATCCACTCAGAGC
			EXT:	CTGTTTTCTATCACATTTATTAATTCT
MpRETP29431T	C/T	8221.4	PCR1:	ACGTTGGATGAGTTGGAGAAGACAGATGGG
			PCR2:	ACGTTGGATGCTCGCGTGAAACACACAAAC
			EXT:	GCCGGCAGTTATCCACTAATAAAACAC
ShTP9651T	C/T	4465.9	PCR1:	ACGTTGGATGTGACTCATGTGAAATGGTGG
			PCR2:	ACGTTGGATGCCACAGAGGATGAGTGAGTG
			EXT:	ACAGCTCCATCAACC
RETP5360T	C/T	4571	PCR1:	ACGTTGGATGTAGGCACAGGTTAATGAAGC
			PCR2:	ACGTTGGATGTGCCTGTCAGCATGTTACTC
			EXT:	TGCAGAGGCCCCAAA

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
ShTP9722A§	G/A	4601	PCR1:	ACGTTGGATGCAAGTCGGACACATTCTACC
			PCR2:	ACGTTGGATGTTTTCCCCGACTGCACCAAG
			EXT:	TGCACCAAGTTGGAA
SPMhRETP18435T	C/T	4626	PCR1:	ACGTTGGATGAAAAGTTGCTGCCTGCTCTG
			PCR2:	ACGTTGGATGAACATATCTGCAGGAGTCGG
			EXT:	GCCAATGAGAGCAGT
SPMhRETP11109C	C/T	4738.1	PCR1:	ACGTTGGATGAGTGTGTACTAGTACTCGGG
			PCR2:	ACGTTGGATGAGCAGCATCCTGAAGTCGTC
			EXT:	ACCCCGTCCATCGCTC
SMShTP5127T	T/A	4768.1	PCR1:	ACGTTGGATGGCAGAGCTTGTTGGAAAACC
			PCR2:	ACGTTGGATGGAGTTGTAACCCAACTCTGC
			EXT:	CAGCTCTCATCTGTCC
SPMhRETP1486CG	C/G	5152.4	PCR1:	ACGTTGGATGATGTGGTTCCAGACAGAGTG
			PCR2:	ACGTTGGATGTTACCCTCTCAATCTCGTGG
			EXT:	TCGTGGCACTTCTTCAG
RETP5172C	C/A	5192.4	PCR1:	ACGTTGGATGCTGTGTGTATCTGTAATCTG
			PCR2:	ACGTTGGATGCTGGTTTTATAGCCGTGGTC
			EXT:	CGTGGTCTCATTGATGC
RETP5306A	A/T	5283.5	PCR1:	ACGTTGGATGGCTTGAAGTTTGAGGGTCAC
			PCR2:	ACGTTGGATGGAAATTCGGCACTTGAGGAG
			EXT:	AGCTGCAGAGGATTAAG
SMShTP21440A	A/G	5372.5	PCR1:	ACGTTGGATGCGGATTTTGTCTGCCTCTGC
			PCR2:	ACGTTGGATGTCAATCATCACCACCCTGAC
			EXT:	ACCTGACCACTACATCAC
SMShTP13277T	T/C	5581.7	PCR1:	ACGTTGGATGAGACGTAGATGAGCCCGTTG
			PCR2:	ACGTTGGATGACACAAGAACAGAGTCGTGG
			EXT:	AACGTGGCAGGTGAAAAC
SPMhRETP28164T	G/T	5705.7	PCR1:	ACGTTGGATGCAGCAGATTAATCCCTGAGC
			PCR2:	ACGTTGGATGGTAACTGTGACCACCAGAAG
			EXT:	CCCGGCACTTTGCCATTTT
MpRETP9363A	A/T	5731.8	PCR1:	ACGTTGGATGGCGTCTTATTTGTGGATGGG
			PCR2:	ACGTTGGATGGCGTGACAATCAGGTCTTTT
			EXT:	AGATTAATGATCTCCACCC
SPMhFLTP25746A§	G/A	5784.8	PCR1:	ACGTTGGATGTCCCAGGTACCTCACAATAG
			PCR2:	ACGTTGGATGCAGCTCCACTAGTTTACCTG
			EXT:	TGGTCACAGCTTTTCTTAG
RETP5089T	G/T	5901.9	PCR1:	ACGTTGGATGTCTATCTGAGCCAGCGAGTC
			PCR2:	ACGTTGGATGTTGGAGAGCCAGCTGGTAG
			EXT:	CCTAGGAGGCTGAGAAGAT
RETP4504A	C/A	5922.9	PCR1:	ACGTTGGATGAATCACAGCTGGTCATGCAC
			PCR2:	ACGTTGGATGACCACCAGTCATTGTTAGAG
			EXT:	AGTTAGAGATGGGATTCGT

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
SMShTP7448C	C/A	6086	PCR1:	ACGTTGGATGGATGGCTGCAGATTGAACAC
			PCR2:	ACGTTGGATGAGATGTGTGTGAACGTGACC
			EXT:	GAGTGACCCGATCTACCAAT
MpSMBTP16142A	C/A	6283.1	PCR1:	ACGTTGGATGTCAGCAGTGGGACCAAATTC
			PCR2:	ACGTTGGATGTTTTCTTACTCTCCTTCTGC
			EXT:	CTCATCTCCTTCTGCAGCTTA
RETP10556A	G/A	6334.1	PCR1:	ACGTTGGATGGGCTGCTCACATAAACACAC
			PCR2:	ACGTTGGATGGCAGCACATGAAATCAACAC
			EXT:	CGTGAAACACTTCTAACACTC
ShTP6906C	T/C	6347.1	PCR1:	ACGTTGGATGAGCCTTTCATACTTTGGCCC
			PCR2:	ACGTTGGATGGCCTTCACTGCATTGATTTC
			EXT:	CCATTGATTTCACGTTAGTCC
SPMhRETP26317T	C/T	6460.2	PCR1:	ACGTTGGATGCGCCTTCCAGTTTTCTTGTC
			PCR2:	ACGTTGGATGGGGTATGTCTGTGGAAATGC
			EXT:	TGTCTGTGGAAATGCTTCAAA
SPMhRETP24673A	G/A	6504.2	PCR1:	ACGTTGGATGCAGCGTTATCTCCTACTTTG
			PCR2:	ACGTTGGATGATTACCTTGTCTGTTGTGTG
			EXT:	GTGTCTGTTGTGTGTAATGTT
SPMhRETP8660C	T/C	6921.5	PCR1:	ACGTTGGATGATGTGTGTTACTGCAGCACC
			PCR2:	ACGTTGGATGCCCATCACAACTTTCACACC
			EXT:	CACCCCTTCAATAAGAAGCCTCA
SMRETP25045A	A/G	6962.5	PCR1:	ACGTTGGATGCTCTGTTCCACGGTCTTTTC
			PCR2:	ACGTTGGATGATGACCACTGCATAACCCAC
			EXT:	AAAACCACGCAGGTTCCTCCCAG
RETP4763G	A/G	7006.5	PCR1:	ACGTTGGATGCAAAGTAGTGGTGCAGGTTG
			PCR2:	ACGTTGGATGACCCAGCCAATATGATCCAG
			EXT:	GCCGGCTCTTTCAGACTTGCAGT
SMRETP6977C	C/A	7074.6	PCR1:	ACGTTGGATGCTGTGCACTGTTCTGAAATG
			PCR2:	ACGTTGGATGCAGATGGAGACAGTTGCTTG
			EXT:	TTGAGACAGTTGCTTGATTTTTA
SPMhRETP3437TC	T/C	7118.6	PCR1:	ACGTTGGATGTTGAACTGCAGACTCACAGC
			PCR2:	ACGTTGGATGTACAGGCTTCATGATCAGGG
			EXT:	AGCAGGGATGGTTTTTAGATCAC
MpRETP17639A	A/G	7161.7	PCR1:	ACGTTGGATGGATGCTTCTGTGCTTATTCG
			PCR2:	ACGTTGGATGAGTACTGTATGCGCAACCTG
			EXT:	GGGCAGAGGCTCGTAAAGATATA
RETP4967G	G/C	7520.9	PCR1:	ACGTTGGATGCCTGTTGCACACATAAACCC
			PCR2:	ACGTTGGATGAATGGACAACTGGGACTTGG
			EXT:	GGCCCAGCTCACTCCTTGTCGCCTT
RETP4592A	C/A	7621	PCR1:	ACGTTGGATGTTTGATGTCCTGCTCCGCTC
			PCR2:	ACGTTGGATGACACGCTCAGCCACGCACA
			EXT:	CGCTAAGCCACGCACACCAGTGGAT

SNP_ID	SNP Alleles	UEP_MASS		Primer Sequences
RETP13743C	C/G	7690	PCR1:	ACGTTGGATGAGAGCCTGCTCGTAACCTTG
			PCR2:	ACGTTGGATGCAGCTAACTGGCTTTCTCTC
			EXT:	CTGTGGCGATAAAGCAGCTCTATAA
SPMhRETP15885T	G/T	7737.1	PCR1:	ACGTTGGATGAGCACATTTTCAGCACATTG
			PCR2:	ACGTTGGATGCCTGCAGCTGTAAACAACAC
			EXT:	GAGTTAACTAAAACAGTAAATTGTG
SPMhRETP4105A	C/A	7843.1	PCR1:	ACGTTGGATGCAGAGACAGCCAGTCAGAAC
			PCR2:	ACGTTGGATGTGTGCGAACAAACTGTTCGG
			EXT:	GGTTGGATAGAAAGATCAGGGTAAA
RETP3097A	G/A	8100.3	PCR1:	ACGTTGGATGCTGGTTTCCCTGACAACATC
			PCR2:	ACGTTGGATGAGTCAAACGCAGTCCGAGAG
			EXT:	GGAGGTCCGAGAGGTGAAACTTCAGT
ShTP9781T	T/C	8104.3	PCR1:	ACGTTGGATGTTAAGAGCCGCAGAGTTTCC
			PCR2:	ACGTTGGATGCAAACAGCAGCAGGAGGTC
			EXT:	GGAGACAGCAGCAGGAGGTCAACGGC
SMRETP23527A	G/A	8230.4	PCR1:	ACGTTGGATGAAATAGCAGGAGGAAGGTTG
			PCR2:	ACGTTGGATGTACAGGTAACAGTGTGCCTC
			EXT:	TATGATTTTTTCAACAATCGGCCTTTG
SPMhRETP8469A	G/A	8257.4	PCR1:	ACGTTGGATGAAGAATGGCATGCACTCAGG
			PCR2:	ACGTTGGATGCAGTGCCGGTGTGTTTTATG
			EXT:	TCTTGTTATGGCTGCAGTCCAACGGTC
SPMhRETP6034T	C/T	8285.4	PCR1:	ACGTTGGATGAGCGTTTCAGAATTTCCTCC
			PCR2:	ACGTTGGATGCCTGCAGAGTTGTGAAGAAC
			EXT:	AATGACAAACACTCAGCAGATGTTCAG

§ Excluded from STRUCTURE analysis

3.3 Genetic analysis using STRUCTURE

3.3.1 Panel validation and testing on largemouth bass, Alabama bass, spotted bass, Coosa bass, shoal bass, and smallmouth bass

The genotypes of pure representatives of the initial six species (largemouth bass-Florida and northern combined), Alabama bass, spotted bass, Coosa bass, shoal bass, and smallmouth bass) were analyzed using STRUCTURE assuming K=6. All initial 200 samples of each species had membership coefficients (Q-value) >0.95 in STRUCTURE analysis (see Appendix 3) and

were subsequently utilized as putative reference genotypes for resolving the taxonomic status of additional samples of each species. A total of 1005 samples of these six species were genotyped and analyzed in STRUCTURE assuming K=6. Of 1005 samples, 883 were resolved as pure representatives of their given species and 122 were resolved as hybrids (Table 2; Table 3; Appendix 4). Reference genotypes were further expanded based on maximum Q-values (≥0.99) to include larger sample sizes (n=70 additional samples each of Alabama bass, largemouth bass, shoal bass, smallmouth bass, spotted bass, and Coosa bass). These results are given in Appendix 5 and Fig. 2A.

Table 2 Species, numbers of individuals (N), and their hybridization status. Samples were genotyped using the presented diagnostic SNP panels. Note that *M. floridanus* and *M. salmoides* were not differentiated here, given existing diagnostic markers for those two species (Li et al., 2015) [19].

	Scientific Name	Common Name	Abbreviation	N	Pure	Hybrid
1	*M. henshalli*	Alabama bass	ALB	189	126	63
2	*M. floridanus/ M. salmoides*	Florida bass/ Northern largemouth bass	LMB	195	193	2
3	*M. cataractae*	Shoal bass	SHB	290	267	23
4	*M. dolomieu*	Smallmouth bass	SMB	126	108	18
5	*M. punctulatus*	Spotted bass	SPB	123	117	6
6	*M. treculii*	Guadalupe bass	GLB	55	31	24
7	*M. haiaka*	Choctaw bass	CTB	20	17	3
8	*M. notius*	Suwannee bass	SWB	19	19	0
9	*M. cahabae*	Cahaba bass	CHB	54	29	25
10	*M. coosae*	Coosa bass	CSB	82	61	21
11	*M. tallapoosae*	Tallapoosa bass	TLPB	89	66	23
12	*M. warriorensis*	Warrior bass	WRB	67	37	30
13	*M. sp. cf. coosae*†	Altamaha bass	ALTB	15	12	3
14	*M. sp. cf. coosae* †	Bartram's bass	BRTB	31	25	6
15	*M. chattahoochae*	Chattahoochee bass	CHTB	21	6	15
		Total		**1376**	**1114**	**262**

† Referred to as *Micropterus* sp. cf. *cataractae* in Freeman et al., 2015 [8].

Table 3 List of *Micropterus* samples based on localities genotyped with 64 diagnostic SNP markers

	SOURCE	CODE	COMMON NAME	N
1	Chattahoochee River GA Hwy 255	GMNHTC	Alabama bass	1
2	Tallapoosa River, AL, 2015	MhAL15_T	Alabama bass	28
3	Tallapoosa River, AL, 2016	MhTLR	Alabama bass	15
4	Neely Henry Reservoir, AL	MhNHER	Alabama bass	3
5	Lake Norman, NC, 2015	MpMhNC15	Alabama bass	21
6	Ocmulgee River	SPBOMR	Alabama bass	65
7	Juliette River	SPBJUL	Alabama bass	3
8	Flint River	SPBFR	Alabama bass	37
9	Cahaba River	CahabaS	Alabama bass	2
10	Clarks Hill, GA 2016	GA16CLA	Alabama bass	2
11	Juliette, GA 2016	GA16JUL	Alabama bass	1
12	Ocmulgee River	LMBOMR	Alabama bass	4
13	Upper Ocmulgee River	LMBOMR	Alabama bass	1
14	Tallapoosa River, AL, 2016	ReTLR	Alabama bass	1
15	Chattahoochee River, 2017	SHB	Alabama bass	1
16	Ocmulgee River	SHBOMR	Alabama bass	4
17	Big Bayou Canot, AL	LDBCANOT	Coastal largemouth bass	5
18	D'Olive Bay, AL	LDB2	Coastal largemouth bass	3
19	Tensaw Lake, AL	LDBTN	Coastal largemouth bass	1
20	Sipsey River, AL	LDBSIPS	Coastal largemouth bass	1
21	Florida ASF, FL	LFLAL	Florida bass	4
22	Florida Bass Conservation Center, FL	LFL	Florida bass	6
23	Northern ASF, IL	LNB	Northern largemouth bass	4
24	Lake Mattoon, Little Wabash, IL	LNBMATT	Northern largemouth bass	4
25	Sugar Lake, MN	LNBSL	Northern largemouth bass	4
26	Lake Martin, AL	LMA	Intergrade largemouth bass	1
27	Flint River	LMBFR	Intergrade largemouth bass	83
28	Ocmulgee River	LMBOMR	Intergrade largemouth bass	70
29	Choctawhatchee River, FL	CCTCB	Intergrade largemouth bass	2
30	Chattahoochee River, 2013	SHB	Intergrade largemouth bass	1
31	Chattahoochee River, 2013	SPB	Intergrade largemouth bass	4
32	Virginia Department of Game and Inland Fisheries	VADGIFSPB001	Intergrade largemouth bass	1
33	Georgia Museum of Natural History	GMNHTC	Intergrade largemouth bass	1
34	Big Canoe Creek, Coosa River, AL 2011	REBigCan	Coosa bass	8
35	Big Willis Creek, Coosa River, AL, 2011	REBigW	Coosa bass	8
36	Cheaha Creek, Coosa River, AL, 2011	REChea	Coosa bass	9
37	Choccolocco Creek, Coosa River, AL, 2011	REChoc	Coosa bass	8
38	Little Willis Creek, Coosa River, AL, 2011	REHat	Coosa bass	8
39	Little Canoe Creek, Coosa River, AL, 2011	REL_Can	Coosa bass	8
40	Hatchet Creek, Coosa River, AL, 2011	RELitW	Coosa bass	8
41	Little River, Coosa River, AL, 2011	RELR	Coosa bass	8
42	Terrapin Creek, Coosa River, AL, 2011	RETerra	Coosa bass	8
43	Walnut Creek, Coosa River, AL, 2011	REWal	Coosa bass	8

	SOURCE	CODE	COMMON NAME	N
44	Chattahoochee River	SHB	Coosa bass	1
45	Flint River, 2013	GASHB13	shoal bass	14
46	Flint River, 2014	USFWS178	shoal bass	24
47	Flint River, 2015	USFWS19	shoal bass	28
48	Georgia Museum of Natural History	GMNHTC	shoal bass	2
49	Flint River, 2016	GASHB16	shoal bass	20
50	Chattahoochee River, 2017	GASHB17	shoal bass	34
51	Flint River, 2017	USFWS179	shoal bass	25
52	Flint River, 2017	USFWS248	shoal bass	29
53	Chattahoochee River, ADCNR (Steven Rider)	SHB	shoal bass	29
54	Little Uchee, 2008 (Steven Sammon)	SHBLUC	shoal bass	23
55	Ocmulgee River	SHBOMR	shoal bass	56
56	Hybrid Project, 2012	GASHHY12	shoal bass	4
57	Ocmulgee River, GA 2016	GA16OMR	shoal bass	1
58	Ocmulgee River	LMBOMR	shoal bass	1
59	Dale Hollow Reservoir, TN	TWRA	smallmouth bass	51
60	GO Fish Education Center, GA--GA	GFEC	smallmouth bass	46
61	ARKSMB Fourche La Fave River, 1997	SMBARFLFR	smallmouth bass	9
62	ARKSMB South Fourche La Fave River, 1997	SMBARSFLFR	smallmouth bass	10
63	SMB Kentucky, Left Fork Beaver Creek, 2003	SMBKYLFBC	smallmouth bass	10
64	Kentucky Lake, TN (Tenn Tech samples), 2015	MpTN15	spotted bass	20
65	Centerhill Lake, Caney Fork, TN, 2015	MpTNSPB	spotted bass	29
66	Dale Hollow Reservoir, TN	TWRA	spotted bass	13
67	Watauga Reservoir TN TWRA2017	TWRA	spotted bass	50
68	Center Hill Lake, TN 2010	SPBCH	spotted bass	7
69	Lower Flint River	LMBFR	spotted bass	1
70	Chattahoochee River	SHB	spotted bass	2
71	Chattahoochee River	SPB001	spotted bass	1
72	Choctawhatchee River, FL, 2015	CCTCB	Choctaw bass	17
73	Conecuh, 2012	CTB	Choctaw bass	3
74	Withlacoochee River, GA	SWBWTL	Suwannee bass	19
75	James River, TX	GBJAMR	Guadalupe bass	1
76	Cibolo creek, TX	GBJAMR	Guadalupe bass	1
77	Sabine river, TX	GBLLAR	Guadalupe bass	1
78	Denton creek, TX	GBLLAR	Guadalupe bass	1
79	Guadalupe Bass from Dijar J. Lutz-Carrillo	GLB	Guadalupe bass	51
80	Caffee Creek	CHBCFC	Cahaba bass	13
81	Little Cahaba River	CHBLCR	Cahaba bass	20
82	Cahaba River 2011	Cahaba	Cahaba bass	4
83	Cahaba ADCNR 2010	CHB	Cahaba bass	8
84	Little Cahaba River 2011	LtCahaba	Cahaba bass	9
85	Crooked Creek, Tallapoosa River, AL, 2011	RECrok	Tallapoosa bass	8
86	Enitachopco Creek, Tallapoosa River, AL, 2011	REEnita	Tallapoosa bass	8
87	Horseshoe Bend Creek, Tallapoosa River, AL, 2011	REHB	Tallapoosa bass	8
88	Mad Indian Creek, Tallapoosa River, AL, 2011	REMadi	Tallapoosa bass	10
89	Price Island Creek, Tallapoosa River, AL, 2011	REPI	Tallapoosa bass	6

	SOURCE	CODE	COMMON NAME	N
90	Shoal Creek, Tallapoosa River, AL, 2011	REShol	Tallapoosa bass	12
91	Tallapoosa River, AL (2016)	ReTLR	Tallapoosa bass	33
92	Wadley Creek, Tallapoosa River, AL, 2011	REWAD	Tallapoosa bass	4
93	Blackburn Fork	WRBBBF	Warrior bass	25
94	Blue Creek	WRBBLC	Warrior bass	13
95	Border Creek	WRBBOC	Warrior bass	9
96	Sipsey Fork	WRBSSF	Warrior bass	11
97	Turkey Creek	WRBTKC	Warrior bass	9
98	Georgia Museum of Natural History (pure)	GMNHTC	Altamaha bass	6
99	Georgia Museum of Natural History (hybrid)	GMNHTC	Altamaha bass	3
100	Oconee River	ALTB/GAUNK	Altamaha bass	5
101	Ocmulgee River	SPBOMR	Altamaha bass	1
102	Georgia Museum of Natural History (pure)	GMNHTC	Bartram's bass	5
103	Clemson University	CLEM	Bartram's bass	26
104	Georgia Museum of Natural History (pure)	GMNHTC	Chattahoochee bass	6
105	Georgia Museum of Natural History (hybrid)	GMNHTC	Chattahoochee bass	7
106	Austin Peay State University Museum of Zoology	APSU	Chattahoochee bass	8
	Total			**1376**

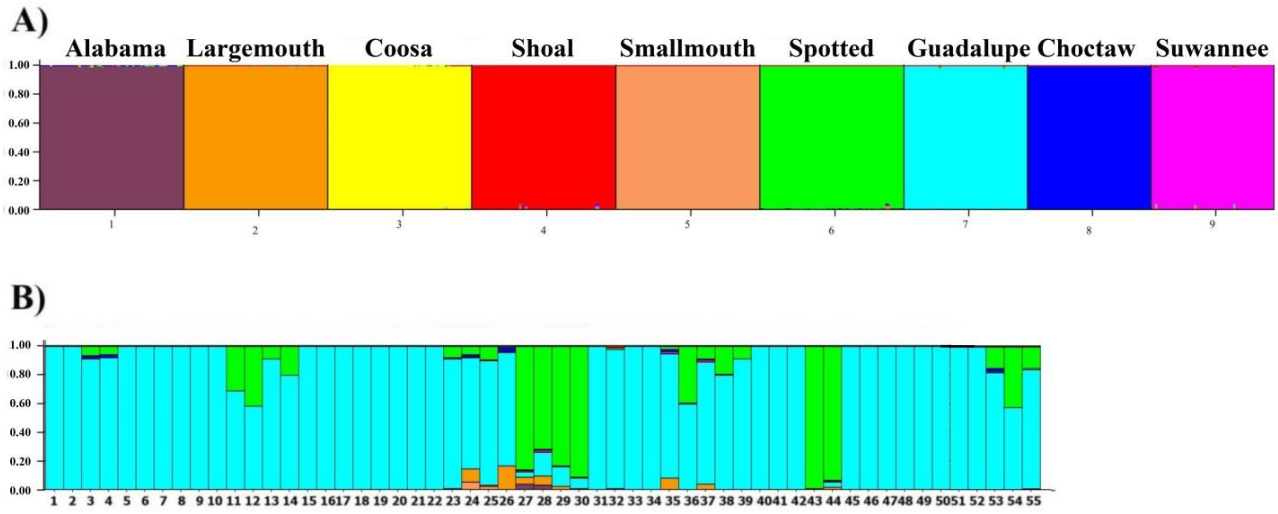

Fig. 2 Bar plot results for individual genetic assignments of *Micropterus* specimens from Bayesian clustering analysis in STRUCTURE based on 64 SNP markers using the admixture model. (A) Reference genotypes of nine species (B) An example data subset from Guadalupe bass (n=55).

3.3.2 SNP panels testing with Choctaw bass, Suwannee bass, Guadalupe bass, and Redeye bass Complex

The next question asked was whether the diagnostic SNPs identified from the initial six black bass species were informative for differentiating additional black bass species (putative and described). Therefore individual samples of Choctaw bass (n=20), Suwannee bass (n=19), Guadalupe bass (n=54), Cahaba bass (n=54), Tallapoosa bass (n=89), Warrior bass (n=54), Altamaha bass (n=12), Bartram's bass (n=30), and Chattahoochee bass (n=21) were genotyped and the results analyzed alongside reference genotypes of Alabama bass, largemouth bass, shoal bass, smallmouth bass, spotted bass, and Coosa bass using STRUCTURE (Table 2; Table 3).

Analysis of Choctaw bass, Suwannee bass, and Guadalupe bass along with the reference genotypes of the previous six species (Alabama bass, largemouth bass, shoal bass, smallmouth bass, spotted bass, and Coosa bass) using STRUCTURE at K=9, resolved individuals to their respective species groups, identified hybrids, and revealed misidentified samples (Table 2; Table 4). STRUCTURE analysis performs best with evenness among samples in each population [29]. Therefore, based on genotypes of pure individuals, reference genotypes were simulated for these three species for future testing of unknown individuals using STRUCTURE analysis (Fig. 2A). An example data subset from Guadalupe bass (n=55) is presented in Fig. 2B, illustrating both pure individuals and those hybridized to varying degrees with spotted bass and largemouth bass in various riverine localities (Table 4).

Table 4 Samples of Choctaw bass (CTB), Suwannee bass (SWB), and Guadalupe bass (GLB) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. STRUCTURE analysis were run along with pure samples of Alabama bass (ALB), largemouth bass (LMB), Coosa bass (CSB), shoal bass (SHB), smallmouth bass (SMB), and spotted bass (SPB).

SPECIES	SAMPLE_NAME	ALB	LMB	CSB	SHB	SMB	SPB	CTB	SWB	GLB
CTB	CCTCB_01	0.001	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_02	0.001	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_03	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_04	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_05	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_07	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_08	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_09	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_10	0.008	0.002	0.005	0.001	0.001	0.001	0.981	0.001	0.001
CTB	CCTCB_11	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_12	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_13	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_14	0	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_15	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_18	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_19	0	0.002	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB	CCTCB_20	0.001	0.001	0	0.001	0.001	0.001	0.993	0.001	0.001
CTB/ALB/LMB	CTB001	0.098	0.064	0.002	0.006	0.003	0.014	0.781	0.004	0.029
CTB/ALB	CTB002	0.11	0.022	0.001	0.003	0.003	0.012	0.808	0.002	0.04
CTB/ALB	CTB003	0.125	0.014	0.001	0.001	0.001	0.008	0.823	0.003	0.024
SWB	SWBWTL002	0.001	0.002	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL003	0	0.002	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL004	0	0.023	0	0.017	0.006	0.005	0.001	0.939	0.009
SWB	SWBWTL015	0	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL016	0	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL017	0.001	0.001	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL018	0.001	0.002	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL019	0.001	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL020	0	0.001	0	0.001	0.001	0.001	0.001	0.993	0.001
SWB	SWBWTL022	0	0.001	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL038	0.001	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL039	0.001	0.001	0	0.001	0.001	0.001	0.001	0.993	0.001
SWB	SWBWTL040	0	0.001	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL041	0	0.002	0	0.001	0.001	0.001	0.001	0.993	0.001
SWB	SWBWTL042	0	0.002	0	0.002	0.001	0.001	0.002	0.992	0.001
SWB	SWBWTL043	0	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL044	0	0.001	0	0.001	0.001	0.001	0.001	0.993	0.001
SWB	SWBWTL045	0	0.002	0	0.001	0.001	0.001	0.001	0.992	0.001
SWB	SWBWTL046	0	0.002	0	0.002	0.001	0.001	0.001	0.992	0.001

SPECIES	SAMPLE_NAME	ALB	LMB	CSB	SHB	SMB	SPB	CTB	SWB	GLB
GLB	GBJAMR_40-0004(1)	0.001	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GBJAMR_40-0008(2)	0	0.002	0	0.001	0.001	0.003	0.001	0.001	0.991
GLB/SPB	GBLLAR_43-0003(3)	0	0.002	0.001	0.001	0.001	0.058	0.012	0.001	0.924
GLB/SPB	GBLLAR_43-0004(4)	0	0.002	0.001	0.001	0.001	0.056	0.014	0.001	0.925
GLB	GLBBEC0432992(5)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBBEC0432993(6)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBBTC0432997(7)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBBTC0432998(8)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBBTC0432999(9)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBBTC0433000(10)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB/SPB	GLBCLR0433022(11)	0	0.002	0	0.001	0.001	0.29	0.001	0.001	0.704
GLB/SPB	GLBCLR0433024(12)	0	0.002	0	0.001	0.001	0.409	0.001	0.001	0.584
GLB/SPB	GLBCLR0433025(13)	0	0.002	0.001	0.001	0.001	0.051	0.001	0.001	0.943
GLB/SPB	GLBCLR0433026(14)	0.001	0.002	0.001	0.001	0.001	0.187	0.001	0.001	0.807
GLB	GLBDOC0433042(15)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBDOC0433082(16)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBDOC0433083(17)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBDOC0433084(18)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBJAR0433106(19)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBJAR0433107(20)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBJAR0433108(21)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBJAR0433109(22)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB/SPB	GLBMED0420001(23)	0.001	0.002	0	0.001	0.007	0.065	0.001	0.001	0.923
GLB/LMB/SMB/SPB	GLBMED0420002(24)	0.001	0.101	0	0.004	0.049	0.044	0.016	0.01	0.775
GLB/SPB	GLBMED0420003(25)	0.001	0.012	0	0.002	0.02	0.086	0.004	0.004	0.87
GLB/LMB	GLBMED0420004(26)	0.001	0.186	0	0.004	0.003	0.007	0.017	0.012	0.769
SPB/LMB/ALB/GLB	GLBMED0420023(27)	0.032	0.059	0.002	0.004	0.006	0.857	0.004	0.011	0.026
SPB/GLB/LMB/ALB	GLBMED0420024(28)	0.032	0.052	0.002	0.004	0.004	0.706	0.006	0.009	0.185
SPB/GLB	GLBMED0420025(29)	0.001	0.023	0	0.003	0.004	0.825	0.002	0.007	0.136
SPB/GLB	GLBMED0420026(30)	0.001	0.011	0	0.003	0.003	0.907	0.006	0.004	0.065
GLB	GLBNLl0432866(31)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBNLl0432867(32)	0.001	0.002	0	0.015	0.012	0.016	0.001	0.001	0.952
GLB	GLBNLl0432868(33)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBNLl0432869(34)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB/LMB	GLBONC433037(35)	0.001	0.088	0	0.006	0.005	0.016	0.014	0.013	0.858
GLB/SPB	GLBONC433038(36)	0	0.003	0	0.001	0.001	0.393	0.002	0.001	0.599
GLB/SPB	GLBONC433039(37)	0.002	0.028	0.001	0.005	0.004	0.05	0.009	0.008	0.893
GLB/SPB	GLBONC433040(38)	0	0.002	0.001	0.001	0.001	0.193	0.002	0.001	0.8
GLB/SPB	GLBPER0433170(39)	0	0.002	0.001	0.001	0.001	0.05	0.001	0.001	0.943
GLB	GLBPER0433171(40)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBPER0433172(41)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBPER0433173(42)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
SPB	GLBSAR0420055(43)	0.001	0.003	0	0.001	0.001	0.987	0.002	0.001	0.004
SPB/GLB	GLBSAR0420056(44)	0.001	0.012	0	0.003	0.003	0.929	0.007	0.004	0.041
GLB	GLBSLl0432880(45)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBSLl0432881(46)	0	0.002	0	0.001	0.001	0.003	0.001	0.001	0.991

SPECIES	SAMPLE_NAME	ALB	LMB	CSB	SHB	SMB	SPB	CTB	SWB	GLB
GLB	GLBSLl0432882(47)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBSLl0432883(48)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBSSB0433011(49)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBSSB0433012(50)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.991
GLB	GLBSSB0433013(51)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB	GLBSSB0433014(52)	0	0.002	0	0.001	0.001	0.002	0.001	0.001	0.992
GLB/SPB	GLBWAL0433056(53)	0	0.006	0	0.001	0.001	0.137	0.025	0.002	0.828
GLB/SPB	GLBWAL0433057(54)	0	0.002	0	0.001	0.001	0.415	0.002	0.001	0.577
GLB/SPB	GLBWAL0433058(55)	0.002	0.012	0.001	0.002	0.002	0.143	0.005	0.004	0.83

A similar approach was taken in analyzing the recently described species belonging to the redeye bass complex within the Mobile River drainage (Coosa bass, Cahaba bass, Tallapoosa bass, and Warrior bass) [7]. Sample numbers were >50 for each of these species (Table 2; Table 3). Using the SNP panel, STRUCTURE analyses assuming K=10 or K=12 were unable to differentiate Coosa bass, Cahaba bass, Tallapoosa bass, or Warrior bass when analyzed with the Coosa reference group and other non-redeye bass species (Fig. 3). Therefore, in order to capture hybridization signatures with species outside the redeye bass group, Coosa, Cahaba, Warrior, and Tallapoosa individuals were analyzed separately assuming K=9 (Fig. 4). These results demonstrated that Coosa bass, Cahaba bass, Tallapoosa bass, and Warrior bass predominantly hybridize (outside their group) with Alabama bass (Fig. 4). Next, a STRUCTURE analysis of the Mobile-Redeye group (Coosa bass, Cahaba bass, Tallapoosa bass, and Warrior bass) with Alabama bass was conducted assuming K=5. This allowed documentation of hybridization among redeye complex members and identification of pure individuals (Fig. 5; Appendix 6). These individuals were sorted based on Q-values (≥0.95) and further utilized for reference genotypes of these species (Fig. 6).

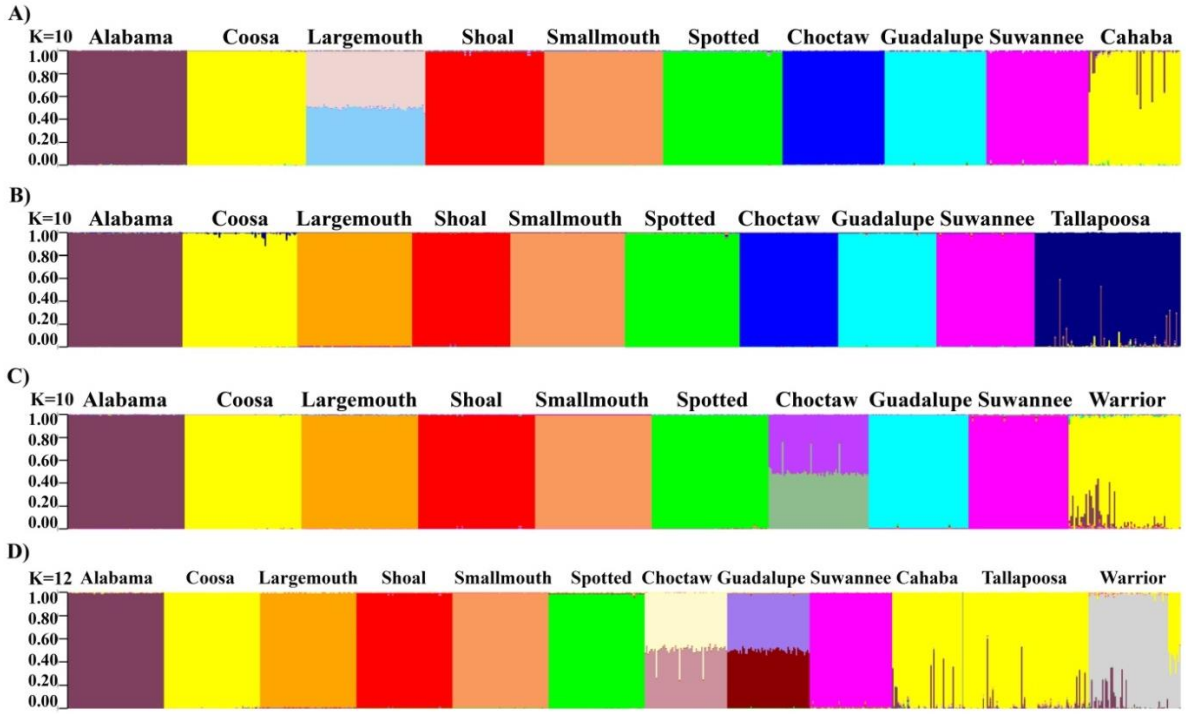

Fig. 3 STRUCTURE analyses with K=10 were unable to differentiate Coosa bass, Cahaba bass (A. yellow bars), or Warrior bass (C. yellow bars) while Tallapoosa bass (B) could be distinguished due to the effect of large sample size. STRUCTURE analyses with K=12 were unable to differentiate Coosa bass, Cahaba bass, or Tallapoosa bass (D. yellow bars).

Fig. 4 STRUCTURE analyses with K=9 using the SNP markers demonstrated genetic assignments of each redeye bass species; (A) Coosa bass, (B) Cahaba bass, (C) Tallapoosa bass, and (D) Warrior bass when analyzed with other non-redeye bass species.

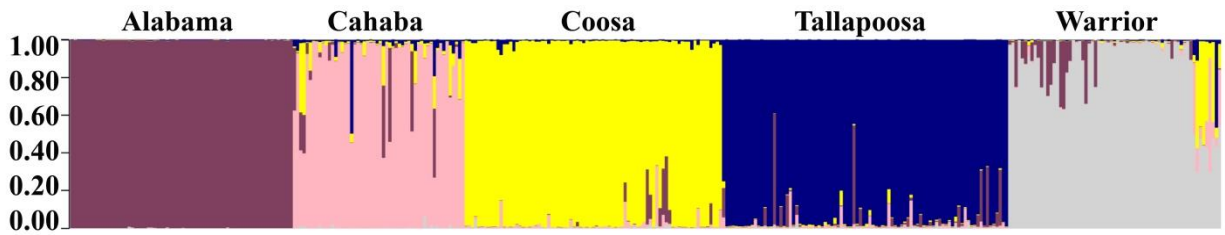

Fig. 5 STRUCTURE analyses with K=5 using the SNP markers revealed hybridization within the Mobile River drainage redeye bass group and outgroup (Alabama bass).

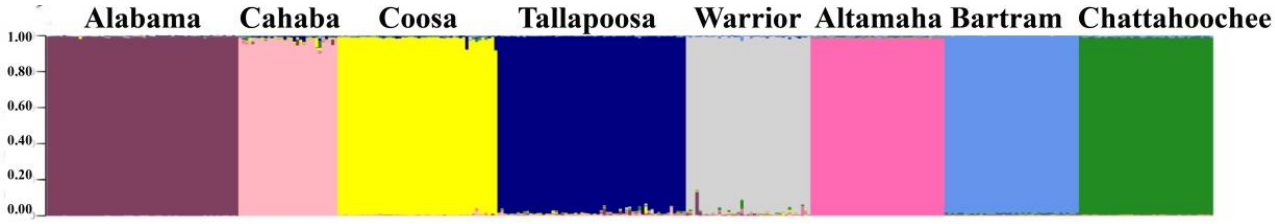

Fig. 6 Reference genotypes of the redeye bass group alongside the outgroup species (Alabama bass).

Despite having access to only a small number of verified pure specimens of Georgia redeye bass (Altamaha bass (n=6), Bartram's bass (n=4), and Chattahoochee bass (n=6) received from the Georgia Museum of Natural History (GMNHTC)), conserved, distinguishing genotypes were observed for each putative species. As in the case of Choctaw, Guadalupe and Suwannee bass, these genotypes were utilized to simulate reference genotypes for further STRUCTURE analyses (Fig. 6). The substitution of Coosa bass reference genotypes with Altamaha bass, Bartram's bass or Chattahoochee bass simulated reference genotypes (from pure individuals) successfully detected pure individuals and hybrids from additional samples from each species/group (Table 2; Table 5; 6; 7).

Table 5 Samples of Altamaha bass (ALTB) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. STRUCTURE analysis were run along with pure samples of Alabama bass (ALB), largemouth bass (LMB), shoal bass (SHB), smallmouth bass (SMB), spotted bass (SPB) and simulated genotypes based on pure ALTB, Guadalupe bass (GLB), Choctaw bass (CTB), and Suwannee bass (SWB).

SPECIES	SAMPLE_NAME	ALB	LMB	ALTB	SHB	SMB	SPB	CTB	SWB	GLB
ALTB	ALTB001	0	0	0.997	0	0	0	0	0	0
ALTB	ALTB002	0	0	0.998	0	0	0	0	0	0
ALTB	ALTB003	0	0	0.998	0	0	0	0	0	0
ALTB	ALTB004	0	0	0.998	0	0	0	0	0	0
ALTB	GAUNK001/ALTB	0	0.002	0.984	0.001	0.002	0.003	0.002	0.002	0.003
ALTB	SPBOMR056/ALTB	0.001	0.005	0.97	0.003	0.002	0.008	0.001	0.004	0.007
ALTB	GMNHTC12150/ALTB	0	0	0.997	0	0	0	0	0	0
ALTB	GMNHTC12152/ALTB	0	0	0.997	0	0	0	0	0	0
ALTB	GMNHTC12216/ALTB	0	0	0.997	0	0	0	0	0	0
ALTB	GMNHTC12219/ALTB	0	0	0.998	0	0	0	0	0	0
ALTB	GMNHTC12222/ALTB	0	0	0.997	0	0	0	0	0	0
ALTB	GMNHTC12437/ALTB	0	0	0.997	0	0	0	0	0	0
ALTB/ALB	GMNHTC9214/ALTBHY	0.508	0.001	0.487	0	0	0.001	0.001	0.001	0.001
ALTB/ALB	GMNHTC9215/ALTBHY	0.84	0.005	0.134	0.002	0.002	0.004	0.005	0.003	0.004
ALTB/ALB	GMNHTC12239/ALTBHY	0.739	0.001	0.256	0	0	0.001	0.001	0.001	0.001

Table 6 Samples of Bartram's bass (BRTB) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. STRUCTURE analysis were run along with pure samples of Alabama bass (ALB), largemouth bass (LMB), shoal bass (SHB), smallmouth bass (SMB), spotted bass (SPB) and simulated genotypes based on pure BRTB, Guadalupe bass (GLB), Choctaw bass (CTB), and Suwannee bass (SWB).

SPECIES	SAMPLE_NAME	ALB	LMB	BTRB	SHB	SMB	SPB	CTB	SWB	GLB
BRTB/SMB	Clem001	0.001	0.001	0.92	0.001	0.075	0.001	0.001	0.001	0.001
BRTB/SMB	Clem002	0.001	0.004	0.886	0.001	0.092	0.003	0.002	0.002	0.009
BRTB	Clem003	0	0.001	0.95	0.007	0.014	0.001	0.026	0	0.001
BRTB	Clem004	0.001	0	0.997	0	0	0	0	0	0
BRTB/ALB	Clem005	0.224	0.001	0.772	0	0	0.001	0.001	0.001	0.001
BRTB	Clem006	0	0	0.997	0	0	0	0	0	0
BRTB/ALB	Clem007	0.162	0.001	0.832	0.001	0	0.001	0.001	0.001	0.001
ALB	Clem008	0.955	0.002	0.003	0.001	0.006	0.001	0.03	0.001	0.001
BRTB	Clem009	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem010	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem011	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem012	0	0	0.997	0	0	0	0	0	0
BRTB	Clem013	0.019	0	0.979	0	0	0	0	0	0
BRTB	Clem014	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem015	0	0	0.997	0	0	0	0	0	0
BRTB	Clem016	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem017	0.006	0	0.983	0	0	0	0.005	0.004	0
BRTB	Clem018	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem019	0	0	0.997	0	0	0	0	0	0
BRTB	Clem020	0	0	0.997	0	0	0	0	0	0
BRTB	Clem021	0	0	0.997	0	0	0	0	0	0
BRTB	Clem022	0.001	0	0.997	0	0	0	0	0	0
BRTB	Clem023	0.005	0.002	0.981	0.001	0.002	0.003	0.002	0.002	0.002
BRTB	Clem024	0	0	0.997	0	0	0	0	0	0
BRTB	Clem025	0	0	0.997	0	0	0	0	0	0
BRTB	Clem026	0	0	0.997	0	0	0	0	0	0
BRTB	GMNHTC12141/BRTB	0	0	0.997	0	0	0	0	0	0
BRTB	GMNHTC12143/BRTB	0	0	0.997	0	0	0	0	0	0
BRTB	GMNHTC12146/BRTB	0	0	0.997	0	0	0	0	0	0
BRTB	GMNHTC12148/BRTB	0.001	0	0.997	0	0	0	0	0	0
BRTB/ALB	GMNHTC12215/BRTB	0.074	0.001	0.921	0	0	0.001	0.001	0.001	0.001

Table 7 Samples of Chattahoochee bass (CHTB) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. STRUCTURE analyses were run along with pure samples of Alabama bass (ALB), largemouth bass (LMB), shoal bass (SHB), smallmouth bass (SMB), spotted bass (SPB).

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CHTB	SHB	SMB	SPB
CHTB	GMNHTC10390	Chattahoochee River	0	0	0.998	0	0	0
CHTB	GMNHTC12124	Marico River	0.001	0.001	0.978	0.018	0	0.001
CHTB	GMNHTC12440	Chattahoochee River	0.001	0	0.998	0	0	0
CHTB	GMNHTC3540	Chattahoochee River	0.003	0.003	0.986	0.002	0.002	0.004
CHTB	GMNHTC9217	Chattahoochee River	0	0	0.998	0	0	0
CHTB	GMNHTC9219	Chattahoochee River	0	0	0.998	0	0	0
CHTB/ALB	GMNHTC4882	Hillabahatchee Creek	0.055	0.004	0.933	0.002	0.003	0.003
CHTB/ALB	GMNHTC4887	Hillabahatchee Creek	0.086	0.001	0.912	0	0	0.001
CHTB/ALB/SPB	GMNHTC12427	Snake Creek	0.209	0.004	0.66	0.006	0.001	0.12
CHTB/LMB	GMNHTC12260	Maricao River	0.001	0.523	0.467	0.001	0.001	0.007
CHTB/SHB/ALB	GMNHTC12255	Snake Creek	0.071	0.009	0.524	0.376	0.001	0.018
CHTB/SHB/ALB	GMNHTC12428	Snake Creek	0.018	0.002	0.897	0.079	0.001	0.002
CHTB/SPB	GMNHTC12259	Maricao River	0.001	0.017	0.93	0.003	0.008	0.041
CHTB/ALB/SPB	APSU1082.01	Wehadkee Creek	0.242	0.001	0.445	0.001	0.001	0.309
CHTB/ALB/SMB/SPB	APSU1122.01(L)	Hillabahatchee Creek	0.259	0.005	0.51	0.008	0.125	0.093
CHTB/ALB/SPB	APSU1123.01	Centralhatchee Creek	0.111	0.001	0.732	0.001	0.012	0.143
CHTB/ALB/SPB/SMB	APSU1123.02	Centralhatchee Creek	0.201	0.016	0.546	0.021	0.042	0.173
CHTB/ALB/SHB	APSU1123.03(J)	Centralhatchee Creek	0.118	0.019	0.545	0.308	0.003	0.008
CHTB/ALB/SPB/SHB	APSU1124.01(K)	Centralhatchee Creek	0.096	0.003	0.499	0.096	0.001	0.305
CHTB/ALB/SPB/SMB	APSU1137.01(L)	Hillabahatchee Creek	0.253	0.004	0.52	0.007	0.132	0.083
CHTB/SPB/SHB	APSU1138.01	Centralhatchee Creek	0.004	0.002	0.797	0.032	0.022	0.143

Table 8 Proposed reference genotypes of *Micropterus* species. A single letter, "G" for example, represent a homozygous GG genotype. The slash (/) indicates polymorphic markers found in a particular species.

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

Based on analyses of 1376 samples from 15 black bass species (putative and described), reference genotypes were proposed for each group in Table 8. Across 64 loci, pairwise differentiation of species was enabled, allowing identification of unknown black bass individuals. Fixed allelic differences between species ranged from 58 between shoal bass and Coosa bass to only one fixed difference among Coosa bass, Cahaba bass, and Tallapoosa bass, reflecting the minimal genetic distances within this redeye complex (Table 9) [7]. Similarly, Altamaha, Bartram's, and Chattahoochee basses had only 2-3 fixed differences among them. While fixed marker numbers were low within the redeye basses, additional differences in allele frequencies are utilized by STRUCTURE for clustering and identification of unknowns.

Table 9 Pairwise fixed allele differences among black bass species, out of 64 markers.

	ALB	LMB	SHB	SMB	SPB	GLB	CTB	SWB	CHB	CSB	TLPB	WRB	ALTB	BRTB
LMB	19													
SHB	29	10												
SMB	28	9	9											
SPB	23	6	13	12										
GLB	23	6	14	15	4									
CTB	24	8	14	14	8	10								
SWB	22	7	7	13	11	11	9							
CHB	24	46	55	47	40	42	43	48						
CSB	27	48	58	50	43	45	44	50	1					
TLPB	24	43	52	44	39	43	40	44	1	1				
WRB	21	40	45	38	35	36	37	39	8	8	8			
ALTB	23	38	48	42	32	35	38	42	10	11	10	8		
BRTB	23	39	47	41	31	33	37	41	10	12	10	10	3	
CHTB	22	39	49	43	33	35	37	41	8	10	8	8	3	2

4. Discussion

Conservation genetics is currently in a time of transition. Sets of microsatellites, long the mainstay of population genetic analyses, increasingly either a) lack the power needed to resolve currently recognized biodiversity and/or b) are cost-prohibitive in the face of declining instrument support and rising labor costs. Reduced representation sequencing approaches have come to dominate newer studies, often in an all-in-one-approach where markers are generated and genotyped in a single all-encompassing experiment [e.g., 30]. However, in species lacking a reference genome, direct analysis of variants requires caution in interpretation as putative SNPs can be formed by homologous or paralogous loci [31]. Additionally, comparisons among datasets generated by different RAD-seq approaches has revealed that varying biases in the methods lead to varying population genetics results (heterozygosity, allele frequencies, etc.) from the same population [32]. SNPs, easily identified by sequencing, should be validated in additional individuals, separate from those contained in the discovery sequencing. This validation/extension of discovered markers can be via parallelized amplicon sequencing [33, 34] or through use of a multiplex genotyping platform [17, 35]. These smaller-scale assays offer the features required by conservation agencies (rapid turn-around of results, reproducibility, and lower costs).

Towards that end, SNP assays enriched for diagnostic markers necessary for assessment of purity and hybridization in black basses (*Micropterus* spp.) were developed. Starting from a relatively small number of individual samples from representative species sequenced by GBS, putative fixed SNP markers were bioinformatically screened. These markers were then validated and tested on progressively larger individual sample sizes and species numbers utilizing MassARRAY multiplex panels, ultimately genotyping 1376 samples (>88,000 individual

genotypes), one of the largest genetic surveys in *Micropterus* to-date (Fig. 1; Table 8). Samples were sourced, whenever possible, from diverse geographical localities to identify and minimize ascertainment biases in the SNP panels (Table 3).

Alabama bass, spotted bass and smallmouth bass are often the introduced aggressors in cases of introgressive hybridization, affecting more restricted populations of shoal bass, Guadalupe bass, redeye bass, etc. [8, 11, 12, 36-38]. The present SNP panels are well-suited for these cases, clearly differentiating pure and hybridized individuals (see Table 4 and Fig. 2B for an example of hybridized spotted bass/Guadalupe bass and Table S10 for examples of hybrids of Alabama bass and Bartram's bass). Alabama bass, for example, increasingly problematic as an introduced species throughout the Southeastern US, can be differentiated from other bass species by 19-29 diagnostic markers, allowing accurate assessment of its hybridization with other species. Due to permissive DNA quality requirements and simplified SNP scoring, results can commonly be returned within 24 hours of receipt of samples, critical for cases where fish are being held for spawning or biologists need guidance during field surveys.

While the 64 SNP markers proved to be highly diagnostic for most *Micropterus* species, including those not originally targeted by GBS or included in low numbers (e.g. Suwannee bass, Guadalupe bass, Choctaw bass), they were far less informative in differentiating among the redeye bass complex members based on fixed alleles alone. The recently described Mobile River basin redeye species (Tallapoosa, Coosa, Cahaba, Warrior basses) [7] are still controversial. Consistent fixed marker signatures in this group were rare (Table 8 and Table 9) and STRUCTURE analyses were unable to consistently differentiate among these when analyzed alongside other black basses (Fig. 3). The exception within this subgroup was the Warrior bass with 8 fixed markers distinguishing it from the Coosa, Cahaba, and Tallapoosa basses. As noted

previously [7], hybridization with Alabama bass was common (Fig. 4). Signatures of hybridization among these four species (Fig. 5) were also detected, potentially attributable to headwater capture events and/or historical introductions from one watershed into another. While the status of this group clearly needs revisiting with additional approaches, unknown redeye individuals can be resolved to their correct species group utilizing the current panels. After analysis with core reference genotypes (Fig. 2A) identifies that an individual belongs to the redeye bass complex, STRUCTURE analysis within the redeye group alone (Fig. 6) can be conducted to assign watershed/species. Further analyses, as in Fig. 4, can be carried out to examine hybridization patterns. Identification of hybridized individuals is a critical precursor to assessing lineage separation, and hybrid inclusion in past analyses likely obscured phylogenetic relationships [7].

Also problematic were the redeye bass of Georgia and South Carolina (Bartram's bass, Altamaha bass, and Chattahoochee bass). These basses have been recently proposed to be members of the shoal bass clade [8]. Although the present study was not focused on delineating species boundaries, I note that the currently described shoal bass (*M. cataractae*; n=290) is differentiated from these species by 47-49 fixed markers, while only 8-12 markers separated them from the Mobile River drainage redeye bass (Table 9). This may indicate a potentially closer relationship with the redeye group with which they have been traditionally associated. Available sample numbers in these three species were low, limiting the depth of analyses that could be conducted. Initial analysis indicated that 2-3 diagnostic markers differentiated Bartram's, Altamaha, and Chattahoochee bass samples from each other. Chattahoochee bass samples showed a high degree of hybridization (15/21 individuals; Table 7), and further pure samples would be needed to confirm the putative diagnostic "barcode" for this species (Table 8).

SNP markers supported the identification of pure and hybridized samples from Freeman (based on morphological and meristic characters and mitochondrial/nuclear sequences; [8]; and pers. comm.). Samples reported to have conflicting meristic characters and ND2 genotypes (GMNHTC9214 and GMNHTC9215) [8] were clearly revealed to be hybridized with Alabama bass based on SNP markers (Table 5). In another example, while sample GMNHTC3540 was identified using microsatellites as spotted bass [36], results demonstrated that this individual was a Chattahoochee bass (Table 7), similar to ND2 results [8]. The same specimens of Chattahoochee bass (K and L) from Baker et al. (2013) [7] and Freeman et al. (2015) [8] were also re-evaluated using SNP panels. I confirmed that those specimens were not pure individuals, but were rather complex hybrids of Chattahoochee bass, Alabama bass, spotted bass, smallmouth bass, and shoal bass (Table 7). Taken together, these examples demonstrate the utility of the described SNP panels in helping to delineate hybridization status and, thereby, clarifying the extent of existing black bass diversity. As the species relationships and boundaries of the redeye complex are examined in greater depth in coming years, expanded SNP panels may need to be crafted specifically for this group.

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Appendices

Appendix 1 Parentage assignments, determined by 51 SNPs with CERVUS 3.0.7, of 160 oyster progenies from Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ (CvHRL).

Offspring ID	Candidate mother ID	Pair LOD score	Pair confidence	Candidate father ID
CvHRL001	P1FHRL010	3.64E+00	*	P1MHRL004
CvHRL002	P5FHRL029	6.40E+00	*	P5MHRL001
CvHRL003	P5FHRL029	5.55E+00	*	P5MHRL001
CvHRL004	P1FHRL010	3.97E+00	*	P1MHRL004
CvHRL005	P5FHRL029	6.67E+00	*	P5MHRL001
CvHRL006	P4FHRL023	4.44E+00	*	P4MHRL036
CvHRL007	P1FHRL010	6.94E+00	*	P1MHRL004
CvHRL008	P3FHRL019	6.82E+00	*	P3MHRL012
CvHRL009	P1FHRL010	5.29E+00	*	P1MHRL004
CvHRL010	P6FHRL038	6.03E+00	*	P6MHRL045
CvHRL011	P1FHRL010	5.26E+00	*	P1MHRL004
CvHRL012	P1FHRL010	5.02E+00	*	P1MHRL004
CvHRL013	P4FHRL023	3.92E+00	*	P4MHRL036
CvHRL014	P1FHRL010	4.27E+00	*	P1MHRL004
CvHRL015	P5FHRL029	8.41E+00	*	P5MHRL001
CvHRL016	P1FHRL010	3.97E+00	*	P1MHRL004
CvHRL017	P1FHRL010	3.93E+00	*	P1MHRL004
CvHRL018	P1FHRL010	5.35E+00	*	P1MHRL004
CvHRL019	P3FHRL019	6.89E+00	*	P3MHRL012
CvHRL020	P1FHRL010	3.85E+00	*	P1MHRL004
CvHRL021	P3FHRL019	4.48E+00	*	P3MHRL012
CvHRL022	P1FHRL010	2.35E+00	*	P1MHRL004
CvHRL023	P1FHRL010	4.45E+00	*	P1MHRL004
CvHRL024	P5FHRL029	5.92E+00	*	P5MHRL001
CvHRL025	P1FHRL010	1.51E+00	*	P1MHRL004
CvHRL026	P1FHRL010	3.22E+00	*	P1MHRL004
CvHRL027	P5FHRL029	4.60E+00	*	P5MHRL001
CvHRL028	P4FHRL023	6.72E+00	*	P4MHRL036
CvHRL029	P5FHRL029	8.15E+00	*	P5MHRL001
CvHRL030	P3FHRL019	4.44E+00	*	P3MHRL012
CvHRL031	P4FHRL023	6.96E+00	*	P4MHRL036
CvHRL032	P3FHRL019	2.84E+00	*	P3MHRL012
CvHRL033	P4FHRL023	6.15E+00	*	P4MHRL036
CvHRL034	P3FHRL019	5.98E+00	*	P3MHRL012
CvHRL035	P1FHRL010	2.03E+00	*	P1MHRL004

Offspring ID	Candidate mother ID	Pair LOD score	Pair confidence	Candidate father ID
CvHRL036	P6FHRL038	3.65E+00	*	P6MHRL045
CvHRL037	P3FHRL019	7.08E+00	*	P3MHRL012
CvHRL038	P4FHRL023	3.92E+00	*	P4MHRL036
CvHRL039	P1FHRL010	4.97E+00	*	P1MHRL004
CvHRL040	P3FHRL019	5.76E+00	*	P3MHRL012
CvHRL041	P5FHRL029	5.50E+00	*	P5MHRL001
CvHRL042	P5FHRL029	5.83E+00	*	P5MHRL001
CvHRL043	P1FHRL010	4.87E+00	*	P1MHRL004
CvHRL044	P1FHRL010	7.01E+00	*	P1MHRL004
CvHRL045	P1FHRL010	1.21E-01	*	P1MHRL004
CvHRL046	P5FHRL029	6.80E+00	*	P5MHRL001
CvHRL047	P4FHRL023	3.12E+00	*	P4MHRL036
CvHRL048	P5FHRL029	9.94E+00	*	P5MHRL001
CvHRL049	P3FHRL019	5.54E+00	*	P3MHRL012
CvHRL050	P1FHRL010	5.53E+00	*	P1MHRL004
CvHRL051	P1FHRL010	7.78E+00	*	P1MHRL004
CvHRL052	P5FHRL029	7.28E+00	*	P5MHRL001
CvHRL053	P1FHRL010	5.57E+00	*	P1MHRL004
CvHRL054	P1FHRL010	3.54E+00	*	P1MHRL004
CvHRL055	P1FHRL010	3.33E+00	*	P1MHRL004
CvHRL056	P3FHRL019	7.90E+00	*	P3MHRL012
CvHRL057	P5FHRL029	4.37E+00	*	P5MHRL001
CvHRL058	P3FHRL019	6.68E+00	*	P3MHRL012
CvHRL059	P5FHRL029	6.79E+00	*	P5MHRL001
CvHRL060	P1FHRL010	2.17E+00	*	P1MHRL004
CvHRL061	P6FHRL038	3.59E+00	*	P6MHRL045
CvHRL062	P3FHRL019	2.97E+00	*	P3MHRL012
CvHRL063	P5FHRL029	3.34E+00	*	P5MHRL001
CvHRL064	P1FHRL010	4.38E+00	*	P5MHRL001
CvHRL065	P6FHRL038	-4.20E-01		P6MHRL045
CvHRL066	P3FHRL019	4.03E+00		P3MHRL012
CvHRL067	P4FHRL023	3.51E+00	*	P4MHRL036
CvHRL068	P4FHRL023	4.90E+00	*	P4MHRL036
CvHRL069	P5FHRL029	9.09E+00	*	P5MHRL001
CvHRL070	P1FHRL010	5.59E+00	*	P1MHRL004
CvHRL071	P3FHRL019	4.81E+00	*	P3MHRL012
CvHRL072	P4FHRL023	3.21E+00	*	P4MHRL036
CvHRL073	P4FHRL023	6.32E+00	*	P4MHRL036
CvHRL074	P1FHRL010	3.24E+00	*	P1MHRL004
CvHRL075	P4FHRL023	5.12E+00	*	P4MHRL036
CvHRL076	P5FHRL029	6.31E+00	*	P5MHRL001
CvHRL077	P1FHRL010	4.21E+00	*	P1MHRL004
CvHRL078	P3FHRL019	4.17E+00	*	P3MHRL012
CvHRL079	P6FHRL038	3.99E+00	*	P6MHRL045
CvHRL080	P6FHRL038	5.30E+00	*	P6MHRL045
CvHRL081	P5FHRL029	7.88E+00	*	P5MHRL001
CvHRL082	P5FHRL029	9.48E+00	*	P5MHRL001
CvHRL083	P5FHRL029	7.54E+00	*	P5MHRL001
CvHRL084	P3FHRL019	7.06E+00	*	P3MHRL012

Offspring ID	Candidate mother ID	Pair LOD score	Pair confidence	Candidate father ID
CvHRL085	P1FHRL010	3.20E+00	*	P1MHRL004
CvHRL086	P4FHRL023	5.08E+00	*	P4MHRL036
CvHRL087	P1FHRL010	3.29E+00	*	P1MHRL004
CvHRL088	P5FHRL029	5.13E+00	*	P5MHRL001
CvHRL089	P1FHRL010	5.76E+00	*	P1MHRL004
CvHRL090	P1FHRL010	5.26E+00	*	P1MHRL004
CvHRL091	P1FHRL010	5.45E+00	*	P1MHRL004
CvHRL092	P4FHRL023	8.01E+00	*	P4MHRL036
CvHRL093	P5FHRL029	5.51E+00	*	P5MHRL001
CvHRL094	P1FHRL010	5.15E+00	*	P1MHRL004
CvHRL095	P5FHRL029	9.05E+00	*	P5MHRL001
CvHRL096	P1FHRL010	4.21E+00	*	P1MHRL004
CvHRL097	P3FHRL019	4.20E+00	*	P3MHRL012
CvHRL098	P1FHRL010	3.92E+00	*	P1MHRL004
CvHRL099	P4FHRL023	5.74E+00	*	P4MHRL036
CvHRL100	P1FHRL010	4.16E+00	*	P1MHRL004
CvHRL101	P5FHRL029	7.31E+00	*	P5MHRL001
CvHRL102	P4FHRL023	2.76E+00	*	P4MHRL036
CvHRL103	P3FHRL019	9.68E+00	*	P3MHRL012
CvHRL104	P1FHRL010	6.09E+00	*	P1MHRL004
CvHRL105	P3FHRL019	2.25E+00	*	P3MHRL012
CvHRL106	P2FHRL014	9.50E+00	*	P2MHRL018
CvHRL107	P1FHRL010	3.19E+00	*	P1MHRL004
CvHRL108	P4FHRL023	6.35E+00	*	P4MHRL036
CvHRL109	P3FHRL019	4.44E+00	*	P3MHRL012
CvHRL110	P5FHRL029	7.84E+00	*	P5MHRL001
CvHRL111	P3FHRL019	7.26E+00	*	P3MHRL012
CvHRL112	P1FHRL010	3.21E+00	*	P1MHRL004
CvHRL113	P3FHRL019	3.77E+00	*	P3MHRL012
CvHRL114	P1FHRL010	5.67E+00	*	P1MHRL004
CvHRL115	P1FHRL010	4.44E+00	*	P1MHRL004
CvHRL116	P5FHRL029	8.11E+00	*	P5MHRL001
CvHRL117	P5FHRL029	9.08E+00	*	P5MHRL001
CvHRL118	P1FHRL010	5.20E+00	*	P1MHRL004
CvHRL119	P5FHRL029	8.25E+00	*	P5MHRL001
CvHRL120	P1FHRL010	6.34E+00	*	P1MHRL004
CvHRL121	P5FHRL029	7.72E+00	*	P5MHRL001
CvHRL122	P1FHRL010	3.44E+00	*	P1MHRL004
CvHRL123	P5FHRL029	6.45E+00	*	P5MHRL001
CvHRL124	P4FHRL023	1.96E+00	*	P4MHRL036
CvHRL125	P5FHRL029	4.74E+00	*	P5MHRL001
CvHRL126	P5FHRL029	5.61E+00	*	P5MHRL001
CvHRL127	P5FHRL029	7.24E+00	*	P5MHRL001
CvHRL128	P5FHRL029	4.00E+00	*	P5MHRL001
CvHRL129	P3FHRL019	6.29E+00	*	P3MHRL012
CvHRL130	P5FHRL029	5.78E+00	*	P5MHRL001
CvHRL131	P4FHRL023	6.70E+00	*	P4MHRL036
CvHRL132	P3FHRL019	2.62E+00		P3MHRL012

Offspring ID	Candidate mother ID	Pair LOD score	Pair confidence	Candidate father ID
CvHRL133	P1FHRL010	5.76E+00	*	P1MHRL004
CvHRL134	P1FHRL010	4.58E+00	*	P1MHRL004
CvHRL135	P4FHRL023	8.52E+00	*	P4MHRL036
CvHRL136	P4FHRL023	2.98E+00	*	P4MHRL036
CvHRL137	P1FHRL010	2.53E+00	*	P1MHRL004
CvHRL138	P4FHRL023	5.70E+00	*	P4MHRL036
CvHRL139	P1FHRL010	2.82E+00	*	P1MHRL004
CvHRL140	P4FHRL023	2.57E+00	*	P4MHRL036
CvHRL141	P5FHRL029	7.88E+00	*	P5MHRL001
CvHRL142	P3FHRL019	7.36E+00	*	P3MHRL012
CvHRL143	P1FHRL010	4.52E+00	*	P1MHRL004
CvHRL144	P1FHRL010	3.68E+00	*	P1MHRL004
CvHRL145	P4FHRL023	6.04E+00	*	P4MHRL036
CvHRL146	P3FHRL019	6.65E+00	*	P3MHRL012
CvHRL147	P3FHRL019	9.08E+00	*	P3MHRL012
CvHRL148	P3FHRL019	6.19E+00	*	P3MHRL012
CvHRL149	P4FHRL023	6.52E+00	*	P4MHRL036
CvHRL150	P2FHRL014	2.46E+00	*	P2MHRL018
CvHRL151	P4FHRL023	8.25E-01	*	P4MHRL036
CvHRL152	P1FHRL010	3.47E+00	*	P1MHRL004
CvHRL153	P1FHRL010	5.12E+00	*	P1MHRL004
CvHRL154	P1FHRL010	2.87E+00	*	P1MHRL004
CvHRL155	P3FHRL019	3.43E+00	*	P3MHRL012
CvHRL156	P3FHRL019	4.95E+00	*	P3MHRL012
CvHRL157	P1FHRL010	3.73E+00	*	P1MHRL004
CvHRL158	P1FHRL010	5.72E+00	*	P1MHRL004
CvHRL159	P5FHRL029	9.34E+00	*	P5MHRL001
CvHRL160	P1FHRL010	4.72E+00	*	P1MHRL004

Appendix 2 Parentage assignments, determined by 51 SNPs with SNPPIT 1.0, of 160 oyster progenies from Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ (CvHRL).

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring1	CvHRL001	P1MHRL004	P1FHRL010	ParentPool1	0.000197	0.004	10.10935
Offspring1	CvHRL002	P5MHRL001	P5FHRL029	ParentPool1	0.000087	0.001	15.1174
Offspring1	CvHRL003	P5MHRL001	P5FHRL029	ParentPool1	0.000086	0.001	12.2532
Offspring1	CvHRL004	P1MHRL004	P1FHRL010	ParentPool1	0.000126	0.002	10.44815
Offspring1	CvHRL005	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.52967
Offspring1	CvHRL006	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.99953
Offspring1	CvHRL007	P1MHRL004	P1FHRL010	ParentPool1	0.000241	0.005	9.878462
Offspring1	CvHRL008	P3MHRL012	P3FHRL019	ParentPool1	0.000085	0.001	14.18477
Offspring1	CvHRL009	P1MHRL004	P1FHRL010	ParentPool1	0.000111	0.002	9.776012
Offspring1	CvHRL010	P6MHRL045	P6FHRL038	ParentPool1	0	0	14.49956
Offspring1	CvHRL011	P1MHRL004	P1FHRL010	ParentPool1	0.000326	0.007	9.700503
Offspring1	CvHRL012	P1MHRL004	P1FHRL010	ParentPool1	0.000204	0.004	10.38826
Offspring1	CvHRL013	P4MHRL036	P4FHRL023	ParentPool1	0	0	11.89566
Offspring1	CvHRL014	P1MHRL004	P1FHRL010	ParentPool1	0.000202	0.004	9.529762
Offspring1	CvHRL015	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.71602
Offspring1	CvHRL016	P1MHRL004	P1FHRL010	ParentPool1	0.000201	0.004	9.748843
Offspring1	CvHRL017	P1MHRL004	P1FHRL010	ParentPool1	0.000079	0.001	10.956
Offspring1	CvHRL018	P1MHRL004	P1FHRL010	ParentPool1	0.000112	0.002	10.88021
Offspring1	CvHRL019	P3MHRL012	P3FHRL019	ParentPool1	0	0	16.45924
Offspring1	CvHRL020	P1MHRL004	P1FHRL010	ParentPool1	0	0	12.1589
Offspring1	CvHRL021	P3MHRL012	P3FHRL019	ParentPool1	0	0	11.26248
Offspring1	CvHRL022	P1MHRL004	P1FHRL010	ParentPool1	0.000368	0.008	8.215683
Offspring1	CvHRL023	P1MHRL004	P1FHRL010	ParentPool1	0.000199	0.004	8.898591
Offspring1	CvHRL024	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.74268
Offspring1	CvHRL025	P1MHRL004	P1FHRL010	ParentPool1	0.000283	0.006	8.365432
Offspring1	CvHRL026	P1MHRL004	P1FHRL010	ParentPool1	0.000071	0.001	11.39931
Offspring1	CvHRL027	P5MHRL001	P5FHRL029	ParentPool1	0.000119	0.002	12.77714
Offspring1	CvHRL028	P4MHRL036	P4FHRL023	ParentPool1	0	0	16.62162
Offspring1	CvHRL029	P5MHRL001	P5FHRL029	ParentPool1	0	0	13.42537
Offspring1	CvHRL030	P3MHRL012	P3FHRL019	ParentPool1	0.000121	0.002	9.695469
Offspring1	CvHRL031	P4MHRL036	P4FHRL023	ParentPool1	0	0	15.31673
Offspring1	CvHRL032	P3MHRL012	P3FHRL019	ParentPool1	0.000123	0.002	10.0817
Offspring1	CvHRL033	P4MHRL036	P4FHRL023	ParentPool1	0.000068	0.001	13.38231
Offspring1	CvHRL034	P3MHRL012	P3FHRL019	ParentPool1	0	0	12.18058
Offspring1	CvHRL035	P1MHRL004	P1FHRL010	ParentPool1	0.000125	0.002	11.27547
Offspring1	CvHRL036	P6MHRL045	P6FHRL038	ParentPool1	0.001783	0.04	6.320094
Offspring1	CvHRL037	P3MHRL012	P3FHRL019	ParentPool1	0	0	14.56802
Offspring1	CvHRL038	P4MHRL036	P4FHRL023	ParentPool1	0	0	15.07009

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring1	CvHRL039	P1MHRL004	P1FHRL010	ParentPool1	0.000129	0.002	11.35158
Offspring1	CvHRL040	P3MHRL012	P3FHRL019	ParentPool1	0	0	15.32672
Offspring1	CvHRL041	P5MHRL001	P5FHRL029	ParentPool1	0.000067	0.001	12.55565
Offspring1	CvHRL042	P5MHRL001	P5FHRL029	ParentPool1	0.000065	0.001	11.73829
Offspring1	CvHRL043	P1MHRL004	P1FHRL010	ParentPool1	0.000198	0.004	9.89178
Offspring1	CvHRL044	P1MHRL004	P1FHRL010	ParentPool1	0.000066	0.001	13.56299
Offspring1	CvHRL045	P1MHRL004	P1FHRL010	ParentPool1	0.000243	0.005	8.171191
Offspring1	CvHRL046	P5MHRL001	P5FHRL029	ParentPool1	0	0	13.80391
Offspring1	CvHRL047	P4MHRL036	P4FHRL023	ParentPool1	0	0	13.43949
Offspring1	CvHRL048	P5MHRL001	P5FHRL029	ParentPool1	0	0	15.76345
Offspring1	CvHRL049	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.65441
Offspring1	CvHRL050	P1MHRL004	P1FHRL010	ParentPool1	0.000124	0.002	11.77856
Offspring1	CvHRL051	P1MHRL004	P1FHRL010	ParentPool1	0.00007	0.001	11.66141
Offspring1	CvHRL052	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.06006
Offspring1	CvHRL053	P1MHRL004	P1FHRL010	ParentPool1	0.00016	0.003	12.8255
Offspring1	CvHRL054	P1MHRL004	P1FHRL010	ParentPool1	0.000366	0.008	9.340086
Offspring1	CvHRL055	P1MHRL004	P1FHRL010	ParentPool1	0.000072	0.001	9.401147
Offspring1	CvHRL056	P3MHRL012	P3FHRL019	ParentPool1	0	0	15.12986
Offspring1	CvHRL057	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.18411
Offspring1	CvHRL058	P3MHRL012	P3FHRL019	ParentPool1	0	0	16.30589
Offspring1	CvHRL059	P5MHRL001	P5FHRL029	ParentPool1	0	0	11.54823
Offspring1	CvHRL060	P1MHRL004	P1FHRL010	ParentPool1	0.000111	0.002	9.976107
Offspring1	CvHRL061	P6MHRL045	P6FHRL038	ParentPool1	0.000077	0.001	10.86517
Offspring1	CvHRL062	P3MHRL012	P3FHRL019	ParentPool1	0.000078	0.001	13.35099
Offspring1	CvHRL063	P5MHRL001	P5FHRL029	ParentPool1	0	0	11.16626
Offspring1	CvHRL064	P5MHRL001	P1FHRL010	ParentPool1	0	0	10.97267
Offspring1	CvHRL065	P6MHRL045	P6FHRL038	ParentPool1	0.000628	0.014	6.015656
Offspring1	CvHRL066	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.41061
Offspring1	CvHRL067	P4MHRL036	P4FHRL023	ParentPool1	0.00012	0.002	13.31539
Offspring1	CvHRL068	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.94044
Offspring1	CvHRL069	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.488
Offspring1	CvHRL070	P1MHRL004	P1FHRL010	ParentPool1	0.000122	0.002	12.58544
Offspring1	CvHRL071	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.14518
Offspring1	CvHRL072	P4MHRL036	P4FHRL023	ParentPool1	0	0	13.84666
Offspring1	CvHRL073	P4MHRL036	P4FHRL023	ParentPool1	0	0	15.03768
Offspring1	CvHRL074	P1MHRL004	P1FHRL010	ParentPool1	0.000165	0.003	9.208339
Offspring1	CvHRL075	P4MHRL036	P4FHRL023	ParentPool1	0.000067	0.001	13.6844
Offspring1	CvHRL076	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.9724
Offspring1	CvHRL077	P1MHRL004	P1FHRL010	ParentPool1	0	0	11.24701
Offspring1	CvHRL078	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.8441
Offspring1	CvHRL079	P6MHRL045	P6FHRL038	ParentPool1	0	0	11.7999
Offspring1	CvHRL080	P6MHRL045	P6FHRL038	ParentPool1	0	0	15.59497
Offspring1	CvHRL081	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.53136

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring1	CvHRL082	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.43632
Offspring1	CvHRL083	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.38696
Offspring1	CvHRL084	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.34773
Offspring1	CvHRL085	P1MHRL004	P1FHRL010	ParentPool1	0.000497	0.011	8.08364
Offspring1	CvHRL086	P4MHRL036	P4FHRL023	ParentPool1	0.000083	0.001	15.01644
Offspring1	CvHRL087	P1MHRL004	P1FHRL010	ParentPool1	0	0	9.869027
Offspring1	CvHRL088	P5MHRL001	P5FHRL029	ParentPool1	0.000081	0.001	11.20291
Offspring1	CvHRL089	P1MHRL004	P1FHRL010	ParentPool1	0.000162	0.003	9.234109
Offspring1	CvHRL090	P1MHRL004	P1FHRL010	ParentPool1	0.000116	0.002	10.31941
Offspring1	CvHRL091	P1MHRL004	P1FHRL010	ParentPool1	0.000078	0.001	12.74791
Offspring1	CvHRL092	P4MHRL036	P4FHRL023	ParentPool1	0	0	17.9954
Offspring1	CvHRL093	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.39644
Offspring1	CvHRL094	P1MHRL004	P1FHRL010	ParentPool1	0.000076	0.001	12.74068
Offspring1	CvHRL095	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.38425
Offspring1	CvHRL096	P1MHRL004	P1FHRL010	ParentPool1	0.000409	0.009	9.487687
Offspring1	CvHRL097	P3MHRL012	P3FHRL019	ParentPool1	0.000115	0.002	10.83978
Offspring1	CvHRL098	P1MHRL004	P1FHRL010	ParentPool1	0.000073	0.001	10.71237
Offspring1	CvHRL099	P4MHRL036	P4FHRL023	ParentPool1	0	0	15.09188
Offspring1	CvHRL100	P1MHRL004	P1FHRL010	ParentPool1	0.000161	0.003	8.090096
Offspring1	CvHRL101	P5MHRL001	P5FHRL029	ParentPool1	0	0	13.13352
Offspring1	CvHRL102	P4MHRL036	P4FHRL023	ParentPool1	0	0	13.38044
Offspring1	CvHRL103	P3MHRL012	P3FHRL019	ParentPool1	0.000069	0.001	13.47485
Offspring1	CvHRL104	P1MHRL004	P1FHRL010	ParentPool1	0.000157	0.003	9.796356
Offspring1	CvHRL105	P3MHRL012	P3FHRL019	ParentPool1	0	0	10.67918
Offspring1	CvHRL106	P2MHRL018	P2FHRL014	ParentPool1	0.000156	0.003	14.36564
Offspring1	CvHRL107	P1MHRL004	P1FHRL010	ParentPool1	0	0	9.678482
Offspring1	CvHRL108	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.50041
Offspring1	CvHRL109	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.62707
Offspring1	CvHRL110	P5MHRL001	P5FHRL029	ParentPool1	0.000065	0.001	11.77257
Offspring1	CvHRL111	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.95685
Offspring1	CvHRL112	P1MHRL004	P1FHRL010	ParentPool1	0.000324	0.007	9.532411
Offspring1	CvHRL113	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.0803
Offspring1	CvHRL114	P1MHRL004	P1FHRL010	ParentPool1	0	0	11.51948
Offspring1	CvHRL115	P1MHRL004	P1FHRL010	ParentPool1	0	0	10.71945
Offspring1	CvHRL116	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.08251
Offspring1	CvHRL117	P5MHRL001	P5FHRL029	ParentPool1	0.000069	0.001	10.40615
Offspring1	CvHRL118	P1MHRL004	P1FHRL010	ParentPool1	0	0	10.05603
Offspring1	CvHRL119	P5MHRL001	P5FHRL029	ParentPool1	0.000071	0.001	13.52481
Offspring1	CvHRL120	P1MHRL004	P1FHRL010	ParentPool1	0	0	13.15048
Offspring1	CvHRL121	P5MHRL001	P5FHRL029	ParentPool1	0	0	14.50353
Offspring1	CvHRL122	P1MHRL004	P1FHRL010	ParentPool1	0.000163	0.003	11.55263
Offspring1	CvHRL123	P5MHRL001	P5FHRL029	ParentPool1	0	0	13.34413
Offspring1	CvHRL124	P4MHRL036	P4FHRL023	ParentPool1	0.000074	0.001	10.37573

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring1	CvHRL125	P5MHRL001	P5FHRL029	ParentPool1	0.000074	0.001	10.09807
Offspring1	CvHRL126	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.37062
Offspring1	CvHRL127	P5MHRL001	P5FHRL029	ParentPool1	0	0	12.26615
Offspring1	CvHRL128	P5MHRL001	P5FHRL029	ParentPool1	0.000155	0.003	10.76378
Offspring1	CvHRL129	P3MHRL012	P3FHRL019	ParentPool1	0.000075	0.001	12.4179
Offspring1	CvHRL130	P5MHRL001	P5FHRL029	ParentPool1	0	0	11.84597
Offspring1	CvHRL131	P4MHRL036	P4FHRL023	ParentPool1	0.000117	0.002	16.45062
Offspring1	CvHRL132	P3MHRL012	P3FHRL019	ParentPool1	0.00008	0.001	9.671497
Offspring1	CvHRL133	P1MHRL004	P1FHRL010	ParentPool1	0.000118	0.002	10.96059
Offspring1	CvHRL134	P1MHRL004	P1FHRL010	ParentPool1	0.000082	0.001	9.954643
Offspring1	CvHRL135	P4MHRL036	P4FHRL023	ParentPool1	0	0	17.31754
Offspring1	CvHRL136	P4MHRL036	P4FHRL023	ParentPool1	0	0	12.39016
Offspring1	CvHRL137	P1MHRL004	P1FHRL010	ParentPool1	0.000282	0.006	9.305155
Offspring1	CvHRL138	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.03739
Offspring1	CvHRL139	P1MHRL004	P1FHRL010	ParentPool1	0	0	12.07881
Offspring1	CvHRL140	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.37426
Offspring1	CvHRL141	P5MHRL001	P5FHRL029	ParentPool1	0	0	13.32572
Offspring1	CvHRL142	P3MHRL012	P3FHRL019	ParentPool1	0	0	14.77779
Offspring1	CvHRL143	P1MHRL004	P1FHRL010	ParentPool1	0.000158	0.003	11.8346
Offspring1	CvHRL144	P1MHRL004	P1FHRL010	ParentPool1	0.000238	0.005	9.729578
Offspring1	CvHRL145	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.72149
Offspring1	CvHRL146	P3MHRL012	P3FHRL019	ParentPool1	0.000195	0.004	13.1303
Offspring1	CvHRL147	P3MHRL012	P3FHRL019	ParentPool1	0	0	15.08289
Offspring1	CvHRL148	P3MHRL012	P3FHRL019	ParentPool1	0	0	14.32732
Offspring1	CvHRL149	P4MHRL036	P4FHRL023	ParentPool1	0	0	14.49066
Offspring1	CvHRL150	P2MHRL018	P2FHRL014	ParentPool1	0.000239	0.005	8.440407
Offspring1	CvHRL151	P4MHRL036	P4FHRL023	ParentPool1	0.000114	0.002	10.62982
Offspring1	CvHRL152	P1MHRL004	P1FHRL010	ParentPool1	0.000084	0.001	10.52007
Offspring1	CvHRL153	P1MHRL004	P1FHRL010	ParentPool1	0	0	12.02579
Offspring1	CvHRL154	P1MHRL004	P1FHRL010	ParentPool1	0	0	10.85327
Offspring1	CvHRL155	P3MHRL012	P3FHRL019	ParentPool1	0.000127	0.002	11.464
Offspring1	CvHRL156	P3MHRL012	P3FHRL019	ParentPool1	0	0	13.56043
Offspring1	CvHRL157	P1MHRL004	P1FHRL010	ParentPool1	0	0	13.91611
Offspring1	CvHRL158	P1MHRL004	P1FHRL010	ParentPool1	0.000113	0.002	12.70184
Offspring1	CvHRL159	P5MHRL001	P5FHRL029	ParentPool1	0	0	15.79936
Offspring1	CvHRL160	P1MHRL004	P1FHRL010	ParentPool1	0.000205	0.004	10.08586
Offspring2	CV15G002	P1MHRL004	P4FHRL023	ParentPool1	0.037035	0.862	-3.21629
Offspring2	CV15G003	---	---	---	---	---	---
Offspring2	CV15G004	---	---	---	---	---	---
Offspring2	CV15G005	P5MHRL001	P3FHRL019	ParentPool1	0.031169	0.708	-2.11075
Offspring2	CV15G006	P5MHRL001	P3FHRL019	ParentPool1	0.03566	0.825	-3.84858
Offspring2	CV15G007	---	---	---	---	---	---
Offspring2	CV15G008	---	---	---	---	---	---

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring2	CV15G009	P5MHRL001	P3FHRL019	ParentPool1	0.03251	0.743	0.809622
Offspring2	CV15G010	---	---	---	---	---	---
Offspring2	CV15G011	P5MHRL001	P3FHRL019	ParentPool1	0.012182	0.275	1.286833
Offspring2	CV15G012	---	---	---	---	---	---
Offspring2	CV15G013	---	---	---	---	---	---
Offspring2	CV15G014	---	---	---	---	---	---
Offspring2	CV15G015	---	---	---	---	---	---
Offspring2	CV15G016	P1MHRL004	P4FHRL023	ParentPool1	0.035834	0.824	-4.50762
Offspring2	CV15G017	---	---	---	---	---	---
Offspring3	HC001	---	---	---	---	---	---
Offspring3	HC002	---	---	---	---	---	---
Offspring3	HC003	---	---	---	---	---	---
Offspring3	HC004	---	---	---	---	---	---
Offspring3	HC005	---	---	---	---	---	---
Offspring3	HC006	---	---	---	---	---	---
Offspring3	HC007	---	---	---	---	---	---
Offspring3	HC008	---	---	---	---	---	---
Offspring3	HC009	---	---	---	---	---	---
Offspring3	HC010	---	---	---	---	---	---
Offspring3	HC011	---	---	---	---	---	---
Offspring3	HC012	---	---	---	---	---	---
Offspring3	HC013	---	---	---	---	---	---
Offspring3	HC014	---	---	---	---	---	---
Offspring3	HC015	---	---	---	---	---	---
Offspring3	HC016	---	---	---	---	---	---
Offspring3	HC017	---	---	---	---	---	---
Offspring3	HC018	---	---	---	---	---	---
Offspring3	HC019	---	---	---	---	---	---
Offspring3	HC020	---	---	---	---	---	---
Offspring3	HC021	---	---	---	---	---	---
Offspring3	HC022	---	---	---	---	---	---
Offspring3	HC023	---	---	---	---	---	---
Offspring3	HC024	---	---	---	---	---	---
Offspring3	HC025	---	---	---	---	---	---
Offspring3	HC026	---	---	---	---	---	---
Offspring3	HC027	---	---	---	---	---	---
Offspring3	HC028	---	---	---	---	---	---
Offspring3	HC029	---	---	---	---	---	---
Offspring3	HC030	---	---	---	---	---	---
Offspring3	HC031	---	---	---	---	---	---
Offspring3	HC032	---	---	---	---	---	---
Offspring3	SR001	---	---	---	---	---	---
Offspring3	SR002	---	---	---	---	---	---

OffspCollection	Kid	Pa	Ma	PopName	FDR	Pvalue	LOD
Offspring3	SR003	---	---	---	---	---	---
Offspring3	SR004	---	---	---	---	---	---
Offspring3	SR005	---	---	---	---	---	---
Offspring3	SR006	---	---	---	---	---	---
Offspring3	SR007	---	---	---	---	---	---
Offspring3	SR008	---	---	---	---	---	---
Offspring3	SR009	---	---	---	---	---	---
Offspring3	SR010	---	---	---	---	---	---
Offspring3	SR011	---	---	---	---	---	---
Offspring3	SR012	---	---	---	---	---	---
Offspring3	SR013	---	---	---	---	---	---
Offspring3	SR014	---	---	---	---	---	---
Offspring3	SR015	---	---	---	---	---	---
Offspring3	SR016	---	---	---	---	---	---
Offspring3	SR017	---	---	---	---	---	---
Offspring3	SR018	---	---	---	---	---	---
Offspring3	SR019	---	---	---	---	---	---
Offspring3	SR020	---	---	---	---	---	---
Offspring3	SR021	---	---	---	---	---	---
Offspring3	SR022	---	---	---	---	---	---
Offspring3	SR023	---	---	---	---	---	---
Offspring3	SR024	---	---	---	---	---	---
Offspring3	SR025	---	---	---	---	---	---
Offspring3	SR026	---	---	---	---	---	---
Offspring3	SR027	---	---	---	---	---	---
Offspring3	SR028	---	---	---	---	---	---
Offspring3	SR029	---	---	---	---	---	---
Offspring3	SR030	---	---	---	---	---	---
Offspring3	SR031	---	---	---	---	---	---
Offspring3	SR032	---	---	---	---	---	---

Appendix 3 Pure samples of Alabama bass (ALB), largemouth bass (LMB), Coosa bass (CSB), shoal bass (SHB), smallmouth bass (SMB), and spotted bass (SPB) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE.

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CSB	SHB	SMB	SPB
ALB	MhAL15_T01	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0.001	0.001
ALB	MhAL15_T03	Tallapoosa River, AL, 2015	0.997	0.001	0	0.001	0.001	0.001
ALB	MhAL15_T04	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0
ALB	MhAL15_T05	Tallapoosa River, AL, 2015	0.994	0.001	0.002	0	0	0.002
ALB	MhAL15_T06	Tallapoosa River, AL, 2015	0.994	0.001	0.002	0	0	0.002
ALB	MhAL15_T07	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T08	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T09	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0.001
ALB	MhAL15_T10	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T12	Tallapoosa River, AL, 2015	0.998	0.001	0	0	0	0
ALB	MhAL15_T13	Tallapoosa River, AL, 2015	0.994	0.001	0.004	0	0	0.001
ALB	MhAL15_T14	Tallapoosa River, AL, 2015	0.996	0.001	0	0.001	0.001	0.001
ALB	MhAL15_T15	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0.001
ALB	MhAL15_T16	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T17	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T19	Tallapoosa River, AL, 2015	0.996	0.001	0.001	0.001	0.001	0.001
ALB	MhAL15_T20	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0.001	0.001	0.001
ALB	MhAL15_T23	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MhAL15_T25	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0.001
ALB	MhAL15_T26	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0.001
ALB	MhAL15_T27	Tallapoosa River, AL, 2015	0.997	0.001	0	0	0	0
ALB	MhAL15_T28	Tallapoosa River, AL, 2015	0.994	0.001	0.002	0	0	0.002
ALB	MhTLR001	Tallapoosa River, AL, 2016	0.994	0.001	0.002	0	0	0.002
ALB	MhTLR002	Tallapoosa River, AL, 2016	0.998	0.001	0	0	0	0
ALB	MhTLR003	Tallapoosa River, AL, 2016	0.994	0.001	0.002	0.001	0.001	0.002
ALB	MhTLR005	Tallapoosa River, AL, 2016	0.997	0.001	0	0	0	0.001
ALB	MhTLR006	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0.001	0.001	0
ALB	MhTLR010	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0.001
ALB	MhTLR011	Tallapoosa River, AL, 2016	0.996	0.002	0	0.001	0.001	0.001
ALB	MhTLR012	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0
ALB	MhTLR013	Tallapoosa River, AL, 2016	0.997	0.001	0	0	0.001	0.001
ALB	MhTLR014	Tallapoosa River, AL, 2016	0.99	0.001	0.004	0	0	0.004
LMB	LDB2590A	D'Olive Bay, Mobile Delta, AL	0.001	0.995	0	0.001	0.001	0.001
LMB	LDB2592A	D'Olive Bay, Mobile Delta, AL	0.001	0.995	0	0.001	0.001	0.001
LMB	LDB2637A	D'Olive Bay, Mobile Delta, AL	0.001	0.95	0	0.047	0.001	0.001
LMB	LDBCANOT11832A	Big Bayou Canot, Mobile-Tensaw, AL	0.017	0.976	0.003	0.001	0.001	0.002
LMB	LDBCANOT11834A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0.995	0	0.001	0.001	0.001
LMB	LDBCANOT11837A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0.995	0	0.001	0.001	0.002
LMB	LDBCANOT11838A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0.995	0	0.001	0.001	0.002
LMB	LDBCANOT2617A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0.995	0	0.001	0.001	0.001
LMB	LDBSIPS114A	Sipsey River, Tombigbee, AL	0.001	0.95	0	0.047	0.001	0.001
LMB	LDBTN11853A	Tensaw Lake, Mobile Delta, Tensaw, AL	0.001	0.995	0	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CSB	SHB	SMB	SPB
LMB	LFLAL01	Florida ASF	0.001	0.995	0	0.001	0.001	0.002
LMB	LFLAL01II	Florida ASF	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLAL02	Florida ASF	0.001	0.995	0	0.001	0.001	0.002
LMB	LFLAL02II	Florida ASF	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLF04	Florida Bass Conservation	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLF11	Florida Bass Conservation	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLM10A	Florida Bass Conservation	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLM12A	Florida Bass Conservation	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLM13A	Florida Bass Conservation	0.001	0.995	0	0.001	0.001	0.001
LMB	LFLM15A	Florida Bass Conservation	0.017	0.976	0.003	0.001	0.001	0.001
LMB	LNB11A	Northern--ASF, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNB12A	Northern--ASF, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNB13A	Northern--ASF, IL	0.001	0.996	0	0.001	0.001	0.001
LMB	LNB19A	Northern--ASF, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNBMATT02A	Lake Mattoon, Little Wabash, IL	0.001	0.995	0	0.001	0.001	0.002
LMB	LNBMATT04A	Lake Mattoon, Little Wabash, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNBMATT05A	Lake Mattoon, Little Wabash, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNBMATT06A	Lake Mattoon, Little Wabash, IL	0.001	0.995	0	0.001	0.001	0.001
LMB	LNBSL01A	Sugar Lake, MN	0.001	0.996	0	0.001	0.001	0.001
LMB	LNBSL04A	Sugar Lake, MN	0.001	0.995	0	0.001	0.001	0.001
LMB	LNBSL08A	Sugar Lake, MN	0.001	0.995	0	0.001	0.001	0.002
LMB	LNBSL10A	Sugar Lake, MN	0.001	0.995	0	0.001	0.001	0.001
CSB	REBigW_S1_02	Big Willis Creek, Coosa River, AL	0.001	0	0.998	0	0	0
CSB	REBigW_S1_03	Big Willis Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REBigW_S1_04	Big Willis Creek, Coosa River, AL	0	0.001	0.997	0.001	0.001	0.001
CSB	REBigW_S1_05	Big Willis Creek, Coosa River, AL	0.001	0	0.997	0	0	0
CSB	REBigW_S1_06	Big Willis Creek, Coosa River, AL	0.001	0	0.998	0	0	0
CSB	REBigW_S1_07	Big Willis Creek, Coosa River, AL	0.001	0	0.998	0	0	0
CSB	REBigW_S1_09	Big Willis Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REBigW_S1_10	Big Willis Creek, Coosa River, AL	0.001	0	0.998	0	0	0
CSB	REChoc_S1_01	Choccolocco Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REChoc_S1_03	Choccolocco Creek, Coosa River, AL	0.005	0.001	0.99	0.001	0.001	0.001
CSB	REChoc_S1_04	Choccolocco Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REChoc_S1_07	Choccolocco Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REChoc_S1_09	Choccolocco Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REChoc_S1_08	Choccolocco Creek, Coosa River, AL	0	0	0.997	0.001	0	0
CSB	REChoc_S1_12	Choccolocco Creek, Coosa River, AL	0.001	0.001	0.996	0	0.001	0.001
CSB	REChoc_S1_13	Choccolocco Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REL_Can_S1_01	Little Canoe Creek, Coosa River, AL	0.003	0.002	0.991	0.001	0.001	0.002
CSB	REL_Can_S1_02	Little Canoe Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REL_Can_S1_03	Little Canoe Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REL_Can_S1_04	Little Canoe Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	REL_Can_S1_05	Little Canoe Creek, Coosa River, AL	0.001	0	0.998	0	0	0
CSB	REL_Can_S1_06	Little Canoe Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REL_Can_S1_07	Little Canoe Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	REL_Can_S1_08	Little Canoe Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	RETerra_S1_01	Terrapin Creek, Coosa River, AL	0	0	0.999	0	0	0
CSB	RETerra_S1_02	Terrapin Creek, Coosa River, AL	0	0	0.999	0	0	0

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CSB	SHB	SMB	SPB
CSB	RETerra_S1_03	Terrapin Creek, Coosa River, AL	0.001	0.001	0.992	0.004	0.001	0.001
CSB	RETerra_S1_04	Terrapin Creek, Coosa River, AL	0	0	0.998	0	0	0
CSB	RETerra_S1_05	Terrapin Creek, Coosa River, AL	0	0.001	0.998	0	0	0.001
CSB	RETerra_S1_06	Terrapin Creek, Coosa River, AL	0	0.001	0.996	0.001	0.001	0.001
CSB	RETerra_S1_08	Terrapin Creek, Coosa River, AL	0	0.002	0.994	0.001	0.001	0.001
CSB	RETerra_S1_09	Terrapin Creek, Coosa River, AL	0	0	0.998	0	0	0
SHB	GASHB13_011	Flint River, 2013	0	0.001	0	0.996	0.002	0.001
SHB	GASHB13_014	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_017	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_018	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_019	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_021	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_022	Flint River, 2013	0	0.001	0	0.996	0.002	0.001
SHB	GASHB13_023	Flint River, 2013	0	0.002	0	0.995	0.001	0.001
SHB	GASHB13_024	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_025	Flint River, 2013	0	0.001	0	0.996	0.002	0.001
SHB	GASHB13_027	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_028	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_030	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	GASHB13_031	Flint River, 2013	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17859	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17860	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17861	Flint River, 2014	0	0.002	0	0.995	0.001	0.001
SHB	USFWS17862	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17863	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17864	Flint River, 2014	0	0.001	0	0.996	0.002	0.001
SHB	USFWS17865	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17866	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17867	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17868	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17869	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17870	Flint River, 2014	0	0.002	0	0.996	0.001	0.001
SHB	USFWS17871	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17872	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17874	Flint River, 2014	0	0.002	0	0.995	0.001	0.001
SHB	USFWS17875	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17876	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SHB	USFWS17877	Flint River, 2014	0	0.001	0	0.996	0.001	0.001
SMB	TWRA501	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA500	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.995	0.001
SMB	TWRA502	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA503	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA504	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA505	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.995	0.001
SMB	TWRA506	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA507	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA508	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA509	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CSB	SHB	SMB	SPB
SMB	TWRA510	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA511	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.995	0.002
SMB	TWRA512	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA513	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA514	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA515	Dale Hollow Reservoir, TN	0	0.002	0	0.001	0.995	0.001
SMB	TWRA516	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA517	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA518	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA519	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA520	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA522	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.995	0.001
SMB	TWRA523	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA524	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA525	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA526	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA527	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA528	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA529	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA530	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA531	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA542	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA546	Dale Hollow Reservoir, TN	0	0.002	0	0.001	0.996	0.001
SMB	TWRA547	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA548	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA549	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA550	Dale Hollow Reservoir, TN	0	0.001	0	0.002	0.996	0.001
SMB	TWRA551	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA552	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SMB	TWRA553	Dale Hollow Reservoir, TN	0	0.001	0	0.001	0.996	0.001
SPB	MpTN15_02	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_03	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_04	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_05	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_06	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.995
SPB	MpTN15_07	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_08	Kentucky Lake, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTN15_09	Kentucky Lake, TN, 2015	0.001	0.003	0	0.001	0.001	0.995
SPB	MpTN15_11	Kentucky Lake, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTN15_012	Kentucky Lake, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTN15_014	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.995
SPB	MpTN15_015	Kentucky Lake, TN, 2015	0.001	0.003	0	0.001	0.001	0.994
SPB	MpTN15_016	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTN15_017	Kentucky Lake, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTNSPB_001	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.002	0	0.001	0.001	0.995
SPB	MpTNSPB_002	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.003	0	0.001	0.001	0.994
SPB	MpTNSPB_003	Centerhill Lake, Caney Fork, TN, 2015	0	0.001	0	0.001	0.001	0.996

SPECIES	SAMPLE_NAME	SOURCE	ALB	LMB	CSB	SHB	SMB	SPB
SPB	MpTNSPB_004	Centerhill Lake, Caney Fork, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTNSPB_005	Centerhill Lake, Caney Fork, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTNSPB_006	Centerhill Lake, Caney Fork, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTNSPB_007	Centerhill Lake, Caney Fork, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTNSPB_009	Centerhill Lake, Caney Fork, TN, 2015	0	0.003	0	0.001	0.001	0.994
SPB	MpTNSPB_010	Centerhill Lake, Caney Fork, TN, 2015	0	0.001	0	0.001	0.002	0.995
SPB	MpTNSPB_011	Centerhill Lake, Caney Fork, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTNSPB_012	Centerhill Lake, Caney Fork, TN, 2015	0	0.001	0	0.001	0.001	0.996
SPB	MpTNSPB_013	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.002	0	0.001	0.001	0.994
SPB	MpTNSPB_014	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.002	0	0.001	0.002	0.994
SPB	MpTNSPB_015	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.004	0	0.001	0.001	0.992
SPB	MpTNSPB_016	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.003	0	0.001	0.002	0.993
SPB	MpTNSPB_017	Centerhill Lake, Caney Fork, TN, 2015	0	0.002	0	0.001	0.001	0.996
SPB	MpTNSPB_018	Centerhill Lake, Caney Fork, TN, 2015	0.001	0.003	0	0.001	0.001	0.994
SPB	MpTNSPB_019	Centerhill Lake, Caney Fork, TN, 2015	0	0.002	0	0.001	0.001	0.996

Appendix 4 A total of 1005 black bass from six species (Alabama bass (ALB), Coosa bass (CSB), largemouth bass (LMB), shoal bass (SHB), smallmouth bass (SMB), and spotted bass (SPB)) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. Data was run using STRUCTURE analysis of K = 6.

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	MhAL15_T01	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0.001	0.001
ALB	MhAL15_T02	Tallapoosa River, AL, 2015	0.98	0.017	0.001	0	0	0.001
ALB	MhAL15_T03	Tallapoosa River, AL, 2015	0.997	0	0.001	0.001	0.001	0.001
ALB	MhAL15_T04	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0
ALB	MhAL15_T05	Tallapoosa River, AL, 2015	0.994	0.002	0.001	0	0	0.002
ALB	MhAL15_T06	Tallapoosa River, AL, 2015	0.994	0.002	0.001	0	0	0.002
ALB	MhAL15_T07	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T08	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T09	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T10	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T12	Tallapoosa River, AL, 2015	0.998	0	0.001	0	0	0
ALB	MhAL15_T13	Tallapoosa River, AL, 2015	0.994	0.004	0.001	0	0	0.001
ALB	MhAL15_T14	Tallapoosa River, AL, 2015	0.996	0	0.001	0.001	0.001	0.001
ALB	MhAL15_T15	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T16	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T17	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T18	Tallapoosa River, AL, 2015	0.981	0.017	0.001	0	0.001	0.001
ALB	MhAL15_T19	Tallapoosa River, AL, 2015	0.996	0.001	0.001	0.001	0.001	0.001
ALB	MhAL15_T20	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0.001	0.001	0.001
ALB	MhAL15_T21	Tallapoosa River, AL, 2015	0.977	0.02	0.001	0	0	0.001
ALB	MhAL15_T22	Tallapoosa River, AL, 2015	0.984	0.014	0.001	0	0	0
ALB	MhAL15_T23	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MhAL15_T24	Tallapoosa River, AL, 2015	0.971	0.027	0.001	0	0	0.001
ALB	MhAL15_T25	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T26	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T27	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T28	Tallapoosa River, AL, 2015	0.994	0.002	0.001	0	0	0.002
ALB	MhNHER10_77	Neely Henry Reservoir, AL	0.997	0	0.001	0	0.001	0.001
ALB	MhTLR001	Tallapoosa River, AL, 2016	0.994	0.002	0.001	0	0	0.002
ALB	MhTLR002	Tallapoosa River, AL, 2016	0.998	0	0.001	0	0	0
ALB	MhTLR003	Tallapoosa River, AL, 2016	0.994	0.002	0.001	0.001	0.001	0.002
ALB	MhTLR004	Tallapoosa River, AL, 2016	0.974	0.022	0.001	0.001	0.001	0.001
ALB	MhTLR005	Tallapoosa River, AL, 2016	0.997	0	0.001	0	0	0.001
ALB	MhTLR006	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0.001	0.001	0
ALB	MhTLR007	Tallapoosa River, AL, 2016	0.985	0.012	0.001	0.001	0.001	0.001
ALB	MhTLR008	Tallapoosa River, AL, 2016	0.984	0.014	0.001	0	0	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	MhTLR009	Tallapoosa River, AL, 2016	0.987	0.006	0.001	0	0	0.005
ALB	MhTLR010	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0.001
ALB	MhTLR011	Tallapoosa River, AL, 2016	0.996	0	0.002	0.001	0.001	0.001
ALB	MhTLR012	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0
ALB	MhTLR013	Tallapoosa River, AL, 2016	0.997	0	0.001	0	0.001	0.001
ALB	MhTLR014	Tallapoosa River, AL, 2016	0.99	0.004	0.001	0	0	0.004
ALB	MhTLR015	Tallapoosa River, AL, 2016	0.984	0.011	0.001	0.001	0.001	0.001
ALB	MpMhNC15_01	Lake Norman, NC, 2015	0.996	0	0.001	0.001	0.001	0.001
ALB	MpMhNC15_02	Lake Norman, NC, 2015	0.996	0.002	0.001	0	0	0.001
ALB	MpMhNC15_06	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MpMhNC15_07	Lake Norman, NC, 2015	0.989	0.001	0.004	0.002	0.002	0.003
ALB	MpMhNC15_08	Lake Norman, NC, 2015	0.997	0.001	0.001	0.001	0	0.001
ALB	MpMhNC15_09	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MpMhNC15_10	Lake Norman, NC, 2015	0.984	0.001	0.006	0.002	0.003	0.004
ALB	MpMhNC15_11	Lake Norman, NC, 2015	0.995	0.001	0.001	0.001	0.001	0.001
ALB	MpMhNC15_12	Lake Norman, NC, 2015	0.973	0.001	0.012	0.004	0.004	0.007
ALB	MpMhNC15_13	Lake Norman, NC, 2015	0.997	0	0.001	0	0	0.001
ALB	MpMhNC15_14	Lake Norman, NC, 2015	0.997	0	0.001	0	0	0
ALB	MpMhNC15_15	Lake Norman, NC, 2015	0.967	0.001	0.018	0.005	0.005	0.005
ALB	MpMhNC15_16	Lake Norman, NC, 2015	0.998	0	0.001	0	0	0
ALB	MpMhNC15_17	Lake Norman, NC, 2015	0.996	0.001	0.001	0.001	0.001	0.001
ALB	MpMhNC15_19	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	ReTLR008	Tallapoosa River, AL, 2016	0.997	0	0.001	0	0	0
ALB	CahabaS3_01	Cahaba River	0.953	0.001	0.012	0.004	0.003	0.027
ALB	CahabaS4_01	Cahaba River	0.997	0	0.001	0.001	0.001	0.001
ALB	GA16JUL047	Juliette, GA 2016	0.996	0.002	0.001	0	0.001	0.001
ALB	GMNHTC9212/ALB	Chattahoochee River	0.996	0	0.001	0.001	0.001	0.001
ALB	LMBOMR112	Ocmulgee River	0.997	0.001	0.001	0	0	0
ALB	LMBOMR113	Ocmulgee River	0.98	0.001	0.009	0.003	0.003	0.004
ALB	LMBOMR117	Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	LMBOMR154	Upper Ocmulgee River	0.997	0.001	0.001	0.001	0.001	0.001
ALB	SHBOMR018	Upper Ocmulgee River	0.975	0.001	0.006	0.006	0.007	0.006
ALB	SPBJUL001	Juliette River	0.997	0	0.001	0	0	0
ALB	SPBJUL002	Juliette River	0.992	0.005	0.001	0	0.001	0.001
ALB	SPBOMR001	Lower Ocmulgee River	0.996	0.001	0.001	0	0.001	0.001
ALB	SPBOMR002	Lower Ocmulgee River	0.993	0.003	0.001	0	0	0.002
ALB	SPBOMR004	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0
ALB	SPBOMR005	Lower Ocmulgee River	0.959	0.005	0.009	0.003	0.003	0.02
ALB	SPBOMR006	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR007	Lower Ocmulgee River	0.98	0.001	0.007	0.003	0.007	0.003
ALB	SPBOMR008	Lower Ocmulgee River	0.995	0.002	0.001	0	0	0.002
ALB	SPBOMR010	Lower Ocmulgee River	0.994	0	0.002	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	SPBOMR011	Lower Ocmulgee River	0.979	0.001	0.004	0.002	0.002	0.011
ALB	SPBOMR012	Lower Ocmulgee River	0.976	0	0.001	0.013	0.001	0.009
ALB	SPBOMR014	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0
ALB	SPBOMR016	Lower Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR017	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR018	Upper Ocmulgee River	0.988	0	0.005	0.002	0.002	0.002
ALB	SPBOMR019	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0
ALB	SPBOMR021	Upper Ocmulgee River	0.986	0.01	0.001	0.001	0.001	0.001
ALB	SPBOMR022	Upper Ocmulgee River	0.993	0	0.003	0.001	0.001	0.001
ALB	SPBOMR023	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR025	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR026	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR027	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR028	Upper Ocmulgee River	0.988	0.001	0.003	0.003	0.003	0.003
ALB	SPBOMR029	Upper Ocmulgee River	0.995	0.001	0.001	0.001	0.002	0.001
ALB	SPBOMR031	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR032	Upper Ocmulgee River	0.995	0.002	0.001	0	0	0.001
ALB	SPBOMR033	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR034	Upper Ocmulgee River	0.992	0.004	0.001	0	0	0.002
ALB	SPBOMR035	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR036	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR037	Upper Ocmulgee River	0.982	0	0.008	0.003	0.003	0.003
ALB	SPBOMR039	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR040	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR041	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR042	Upper Ocmulgee River	0.997	0	0.001	0	0	0.001
ALB	SPBOMR043	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR044	Upper Ocmulgee River	0.998	0.001	0.001	0	0	0
ALB	SPBOMR045	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR046	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR047	Upper Ocmulgee River	0.959	0	0.022	0.006	0.006	0.007
ALB	SPBOMR048	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR049	Upper Ocmulgee River	0.997	0.001	0.001	0	0.001	0.001
ALB	SPBOMR050	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR051	Upper Ocmulgee River	0.994	0	0.002	0.001	0.001	0.001
ALB	SPBOMR053	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR054	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR055	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR057	Upper Ocmulgee River	0.991	0.001	0.003	0.001	0.001	0.002
ALB	SPBOMR058	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR059	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR060	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	SPBOMR061	Upper Ocmulgee River	0.97	0.018	0.004	0.002	0.003	0.003
ALB	SPBOMR062	Upper Ocmulgee River	0.996	0.001	0.001	0.001	0.001	0.001
ALB	SPBOMR063	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR064	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR065	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR066	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB/CSB	GA16CLA018	Clarks Hill, GA 2016	0.934	0.061	0.002	0.001	0.001	0.001
ALB/CSB	MhAL15_T11	Tallapoosa River, AL, 2015	0.94	0.057	0.001	0.001	0.001	0.001
ALB/CSB	MhNHER10_76	Neely Henry Reservoir, AL	0.902	0.08	0.003	0.001	0.006	0.008
ALB/CSB	SPBJUL003	Juliette River	0.913	0.085	0.001	0	0.001	0
ALB/CSB	SPBOMR020	Upper Ocmulgee River	0.641	0.291	0.007	0.002	0.006	0.052
ALB/CSB/LMB	GA16CLA020	Clarks Hill, GA 2016	0.849	0.075	0.048	0.007	0.006	0.015
ALB/CSB/SHB	SHBOMR017	Upper Ocmulgee River	0.739	0.148	0.004	0.096	0.005	0.009
ALB/CSB/SHB	SHBOMR019	Upper Ocmulgee River	0.602	0.207	0.002	0.168	0.012	0.009
ALB/CSB/SHB/SPB/LMB	LMBOMR176	Upper Ocmulgee River	0.47	0.249	0.017	0.24	0.007	0.017
ALB/LMB	MpMhNC15_03	Lake Norman, NC, 2015	0.94	0.001	0.03	0.006	0.015	0.007
ALB/LMB	MpMhNC15_04	Lake Norman, NC, 2015	0.937	0	0.039	0.008	0.007	0.009
ALB/LMB	MpMhNC15_05	Lake Norman, NC, 2015	0.833	0	0.145	0.006	0.007	0.01
ALB/LMB	MpMhNC15_20	Lake Norman, NC, 2015	0.879	0	0.1	0.007	0.008	0.006
ALB/LMB	MpMhNC15_21	Lake Norman, NC, 2015	0.919	0.001	0.05	0.006	0.008	0.016
ALB/LMB	SPBOMR038	Upper Ocmulgee River	0.931	0.001	0.044	0.007	0.007	0.011
ALB/SHB	SHBOMR015	Upper Ocmulgee River	0.727	0.036	0.006	0.227	0.002	0.002
ALB/SHB	SPBOMR003	Lower Ocmulgee River	0.727	0	0.002	0.265	0.005	0.001
ALB/SHB	SPBOMR024	Upper Ocmulgee River	0.917	0.002	0.002	0.073	0.002	0.005
ALB/SHB	SPBOMR030	Upper Ocmulgee River	0.493	0.001	0.004	0.499	0.002	0.001
ALB/SHB	SPBOMR052	Upper Ocmulgee River	0.811	0	0.004	0.177	0.006	0.002
ALB/SHB/SMB	SPBOMR009	Lower Ocmulgee River	0.899	0.002	0.001	0.047	0.045	0.006
ALB/SHB/SPB	SHB034	Chattahoochee River, 2017	0.376	0.001	0.004	0.288	0.004	0.327
ALB/SPB	MhNHER10_78	Neely Henry Reservoir, AL	0.859	0.001	0.004	0.005	0.001	0.13
ALB/SPB	MpMhNC15_18	Lake Norman, NC, 2015	0.638	0.001	0.007	0.001	0.002	0.352
ALB/SPB	SPBFR002	Upper Flint River	0.701	0.001	0.008	0.001	0.003	0.286
ALB/SPB	SPBFR003	Upper Flint River	0.498	0.001	0.002	0.011	0.016	0.472
ALB/SPB	SPBFR004	Upper Flint River	0.585	0.001	0.005	0.001	0.001	0.408
ALB/SPB	SPBFR005	Upper Flint River	0.521	0.001	0.008	0.001	0.002	0.467
ALB/SPB	SPBFR006	Upper Flint River	0.581	0.001	0.004	0.002	0.004	0.409
ALB/SPB	SPBFR007	Upper Flint River	0.546	0.001	0.004	0.013	0.022	0.414
ALB/SPB	SPBFR008	Upper Flint River	0.547	0.001	0.003	0.001	0.001	0.447
ALB/SPB	SPBFR009	Upper Flint River	0.358	0.001	0.01	0.016	0.03	0.585
ALB/SPB	SPBFR010	Upper Flint River	0.707	0.001	0.005	0.011	0.028	0.248
ALB/SPB	SPBFR011	Upper Flint River	0.477	0.001	0.007	0.001	0.002	0.512
ALB/SPB	SPBFR012	Upper Flint River	0.462	0.001	0.006	0.02	0.02	0.492
ALB/SPB	SPBFR013	Upper Flint River	0.463	0.001	0.004	0.001	0.001	0.53

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB/SPB	SPBFR014	Upper Flint River	0.508	0.001	0.018	0.001	0.003	0.469
ALB/SPB	SPBFR015	Upper Flint River	0.517	0.012	0.026	0.002	0.002	0.441
ALB/SPB	SPBFR017	Upper Flint River	0.371	0.001	0.004	0.001	0.001	0.623
ALB/SPB	SPBFR018	Upper Flint River	0.718	0.001	0.011	0.001	0.001	0.267
ALB/SPB	SPBFR019	Upper Flint River	0.635	0.001	0.005	0.001	0.002	0.357
ALB/SPB	SPBFR020	Upper Flint River	0.462	0	0.012	0.002	0.002	0.521
ALB/SPB	SPBFR021	Upper Flint River	0.391	0.001	0.003	0.002	0.002	0.601
ALB/SPB	SPBFR023	Upper Flint River	0.337	0.001	0.004	0.001	0.001	0.657
ALB/SPB	SPBFR024	Upper Flint River	0.564	0	0.017	0.002	0.003	0.414
ALB/SPB	SPBFR025	Upper Flint River	0.493	0	0.006	0.019	0.032	0.45
ALB/SPB	SPBFR026	Upper Flint River	0.547	0.001	0.026	0.004	0.005	0.418
ALB/SPB	SPBFR027	Upper Flint River	0.543	0.001	0.003	0.001	0.002	0.45
ALB/SPB	SPBFR029	Upper Flint River	0.537	0.001	0.007	0.001	0.002	0.453
ALB/SPB	SPBFR030	Upper Flint River	0.397	0.001	0.004	0.001	0.001	0.595
ALB/SPB	SPBFR031	Upper Flint River	0.56	0.001	0.004	0.001	0.002	0.432
ALB/SPB	SPBFR032	Upper Flint River	0.602	0.001	0.004	0.001	0.001	0.391
ALB/SPB	SPBFR033	Upper Flint River	0.569	0.001	0.003	0.001	0.001	0.425
ALB/SPB	SPBFR034	Upper Flint River	0.529	0.001	0.008	0.001	0.002	0.459
ALB/SPB	SPBFR035	Upper Flint River	0.502	0.001	0.005	0.001	0.001	0.49
ALB/SPB	SPBFR036	Upper Flint River	0.443	0	0.009	0.001	0.002	0.544
ALB/SPB	SPBFR037	Upper Flint River	0.627	0.001	0.034	0.002	0.002	0.334
ALB/SPB	SPBOMR013	Lower Ocmulgee River	0.944	0.001	0.004	0.002	0.002	0.047
ALB/SPB	SPBOMR015	Lower Ocmulgee River	0.942	0.002	0.005	0.002	0.002	0.048
ALB/SPB/LMB	SPBFR001	Upper Flint River	0.523	0.001	0.152	0.002	0.002	0.32
ALB/SPB/LMB	SPBFR016	Upper Flint River	0.288	0	0.443	0.002	0.003	0.264
ALB/SPB/SHB	SPBFR028	Upper Flint River	0.408	0.001	0.003	0.233	0.006	0.35
ALB/SPB/SHB/SMB	SPBFR022	Upper Flint River	0.244	0.007	0.002	0.515	0.078	0.154
CSB	REBigCan_S1_01	Big Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_02	Big Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_03	Big Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_05	Big Canoe Creek, Coosa River, AL	0.002	0.981	0.007	0.004	0.006	0.001
CSB	REBigCan_S1_06	Big Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REBigCan_S1_07	Big Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_08	Big Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_09	Big Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REBigW_S1_02	Big Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigW_S1_03	Big Willis Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REBigW_S1_04	Big Willis Creek, Coosa River, AL	0	0.997	0.001	0.001	0.001	0.001
CSB	REBigW_S1_05	Big Willis Creek, Coosa River, AL	0.001	0.997	0	0	0	0
CSB	REBigW_S1_06	Big Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigW_S1_07	Big Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REBigW_S1_09	Big Willis Creek, Coosa River, AL	0	0.998	0	0	0	0

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
CSB	REBigW_S1_10	Big Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	RECheaS1_01	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_02	Cheaha Creek, Coosa River, AL, 2011	0.001	0.994	0.001	0.001	0.001	0.001
CSB	RECheaS1_04	Cheaha Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	RECheaS1_05	Cheaha Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	RECheaS1_06	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_07	Cheaha Creek, Coosa River, AL, 2011	0.001	0.994	0.001	0.001	0.001	0.001
CSB	RECheaS1_08	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_09	Cheaha Creek, Coosa River, AL, 2011	0.001	0.997	0	0	0	0
CSB	RECheaS1_10	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REChoc_S1_01	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REChoc_S1_03	Choccolocco Creek, Coosa River, AL	0.005	0.99	0.001	0.001	0.001	0.001
CSB	REChoc_S1_04	Choccolocco Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REChoc_S1_07	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REChoc_S1_08	Choccolocco Creek, Coosa River, AL	0	0.997	0	0.001	0	0
CSB	REChoc_S1_09	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REChoc_S1_12	Choccolocco Creek, Coosa River, AL	0.001	0.996	0.001	0	0.001	0.001
CSB	REChoc_S1_13	Choccolocco Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REHat_S2_08	Hatchet Creek, Coosa River, AL, 2011	0.001	0.991	0.002	0.001	0.002	0.003
CSB	REHat_S2_09	Hatchet Creek, Coosa River, AL, 2011	0.001	0.997	0.001	0	0	0.001
CSB	REHat_S2_10	Hatchet Creek, Coosa River, AL, 2011	0.013	0.977	0.003	0.002	0.002	0.004
CSB	REHat_S2_11	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REHat_S2_12	Hatchet Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REHat_S2_13	Hatchet Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	REHat_S2_14	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REHat_S2_15	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REL_Can_S1_01	Little Canoe Creek, Coosa River, AL	0.003	0.991	0.002	0.001	0.001	0.002
CSB	REL_Can_S1_02	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REL_Can_S1_03	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REL_Can_S1_04	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REL_Can_S1_05	Little Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REL_Can_S1_06	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REL_Can_S1_07	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REL_Can_S1_08	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	RELitW_S3_01	Little Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	RELitW_S3_03	Little Willis Creek, Coosa River, AL	0.005	0.991	0.001	0.001	0.001	0
CSB	RELitW_S3_04	Little Willis Creek, Coosa River, AL	0.001	0.997	0.001	0	0	0.001
CSB	RELitW_S3_05	Little Willis Creek, Coosa River, AL	0.004	0.99	0.002	0.001	0.002	0.002
CSB	RELitW_S3_06	Little Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	RELitW_S3_07	Little Willis Creek, Coosa River, AL	0.001	0.997	0	0	0	0.001
CSB	RELitW_S3_08	Little Willis Creek, Coosa River, AL	0.001	0.997	0	0	0	0.001
CSB	RELR_S1_03	Little River, Coosa River, AL, 2011	0.001	0.997	0.001	0	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
CSB	RETerra_S1_01	Terrapin Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	RETerra_S1_02	Terrapin Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	RETerra_S1_03	Terrapin Creek, Coosa River, AL	0.001	0.992	0.001	0.004	0.001	0.001
CSB	RETerra_S1_04	Terrapin Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	RETerra_S1_05	Terrapin Creek, Coosa River, AL	0	0.998	0.001	0	0	0.001
CSB	RETerra_S1_06	Terrapin Creek, Coosa River, AL	0	0.996	0.001	0.001	0.001	0.001
CSB	RETerra_S1_08	Terrapin Creek, Coosa River, AL	0	0.994	0.002	0.001	0.001	0.001
CSB	RETerra_S1_09	Terrapin Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REWal_S1_02	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0.001
CSB	REWal_S1_03	Walnut Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	REWal_S1_04	Walnut Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REWal_S1_05	Walnut Creek, Coosa River, AL, 2011	0.002	0.995	0.001	0.001	0.001	0
CSB	REWal_S1_07	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REWal_S1_08	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REWal_S1_09	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB/ALB	RELitW_S3_02	Little Willis Creek, Coosa River, AL	0.101	0.893	0.003	0.001	0.001	0.001
CSB/ALB	RELR_S1_01	Little River, Coosa River, AL, 2011	0.29	0.704	0.002	0.001	0.001	0.002
CSB/ALB	RELR_S1_05	Little River, Coosa River, AL, 2011	0.083	0.907	0.007	0.002	0.001	0.001
CSB/ALB	RELR_S1_06	Little River, Coosa River, AL, 2011	0.207	0.751	0.022	0.005	0.004	0.011
CSB/ALB	RELR_S1_07	Little River, Coosa River, AL, 2011	0.346	0.641	0.008	0.001	0.002	0.002
CSB/ALB	REWal_S1_06	Walnut Creek, Coosa River, AL, 2011	0.107	0.889	0.002	0.001	0.001	0.001
CSB/ALB/SMB/SPB	SHB031	Chattahoochee River	0.102	0.761	0.023	0.015	0.056	0.043
CSB/ALB/SPB	RELR_S1_02	Little River, Coosa River, AL, 2011	0.039	0.885	0.019	0.003	0.007	0.046
CSB/SMB	RELR_S1_08	Little River, Coosa River, AL, 2011	0.003	0.926	0.002	0.001	0.067	0.002
CSB/SPB	RELR_S1_04	Little River, Coosa River, AL, 2011	0.007	0.939	0.013	0.003	0.008	0.029
LMB	CCTCB_06	Choctawhatchee River, FL, 2015	0.001	0	0.995	0.001	0.001	0.001
LMB	CCTCB_16	Choctawhatchee River, FL, 2015	0.001	0	0.994	0.001	0.002	0.001
LMB	LDB2590A	D'Olive Bay, Mobile Delta, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDB2592A	D'Olive Bay, Mobile Delta, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDB2637A	D'Olive Bay, Mobile Delta, AL	0.001	0	0.95	0.047	0.001	0.001
LMB	LDBCANOT11832A	Big Bayou Canot, Mobile-Tensaw, AL	0.017	0.003	0.976	0.001	0.001	0.002
LMB	LDBCANOT11834A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDBCANOT11837A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.002
LMB	LDBCANOT11838A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.002
LMB	LDBCANOT2617A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDBSIPS114A	Sipsey River, Tombigbee, AL	0.001	0	0.95	0.047	0.001	0.001
LMB	LDBTN11853A	Tensaw Lake, Mobile Delta, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLAL01	Florida ASF	0.001	0	0.995	0.001	0.001	0.002
LMB	LFLAL01II	Florida ASF	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLAL02	Florida ASF	0.001	0	0.995	0.001	0.001	0.002
LMB	LFLAL02II	Florida ASF	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLF04	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LFLF11	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM10A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM12A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM13A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM15A	Florida Bass Conservation	0.017	0.003	0.976	0.001	0.001	0.001
LMB	LMA028	Lake Martin, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR001	Lower Flint River	0.001	0	0.995	0.001	0.002	0.001
LMB	LMBFR002	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR003	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR004	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR005	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR006	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR007	Lower Flint River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBFR008	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR009	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR010	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR011	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR012	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR013	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR015	Lower Flint River	0.018	0.003	0.975	0.001	0.001	0.002
LMB	LMBFR016	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR017	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR018	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR019	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR020	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR021	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR022	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR023	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR024	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR025	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR026	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR027	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR028	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR029	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR030	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR031	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR032	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR033	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR034	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR035	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR036	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR037	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LMBFR038	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR039	Lower Flint River	0.001	0	0.996	0.001	0.001	0.001
LMB	LMBFR040	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR041	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR042	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR043	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR044	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR045	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR046	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR047	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR048	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR049	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR050	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR051	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR052	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR053	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR054	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR055	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR056	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR057	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR058	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR059	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR060	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR061	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR062	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR063	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR064	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR065	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR066	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR067	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR068	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR069	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR070	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR071	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR072	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR073	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR074	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR075	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR076	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR077	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR078	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR079	Upper Flint River	0.001	0	0.995	0.001	0.001	0.002

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LMBFR080	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR081	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR082	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR083	Upper Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR101	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR106	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR108	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR109	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR111	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR114	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR116	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR118	Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR120	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR121	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR122	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR123	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR124	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR125	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR126	Lower Ocmulgee River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBOMR127	Lower Ocmulgee River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBOMR128	Lower Ocmulgee River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBOMR130	Lower Ocmulgee River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBOMR133	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR134	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR136	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR137	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR138	Lower Ocmulgee River	0.001	0	0.994	0.001	0.002	0.001
LMB	LMBOMR139	Lower Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR140	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR141	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR142	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR143	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR144	Upper Ocmulgee River	0.001	0	0.996	0.001	0.001	0.001
LMB	LMBOMR145	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR146	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR147	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR148	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR149	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR150	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR151	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR152	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR153	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LMBOMR155	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR156	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR157	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR158	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR159	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR160	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR161	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR162	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR163	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR164	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR165	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR166	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR167	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR168	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR169	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR170	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR171	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR172	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR173	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR174	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR175	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR177	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR178	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR179	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBOMR180	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR181	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR182	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR183	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR184	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR185	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR186	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBOMR187	Upper Ocmulgee River	0.001	0	0.995	0.001	0.001	0.002
LMB	LNB11A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNB12A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNB13A	Northern--ASF, IL	0.001	0	0.996	0.001	0.001	0.001
LMB	LNB19A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT02A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.002
LMB	LNBMATT04A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT05A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT06A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBSL01A	Sugar Lake, MN	0.001	0	0.996	0.001	0.001	0.001
LMB	LNBSL04A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LNBSL08A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.002
LMB	LNBSL10A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.001
LMB	SHB029	Chattahoochee River, 2013	0.001	0	0.995	0.001	0.001	0.001
LMB	SPB002	Chattahoochee River, 2013	0.001	0	0.995	0.001	0.001	0.001
LMB	SPB003	Chattahoochee River, 2013	0.001	0	0.995	0.001	0.001	0.001
LMB	SPB004	Chattahoochee River, 2013	0.001	0	0.995	0.001	0.001	0.001
LMB	SPB005	Chattahoochee River, 2013	0.001	0	0.995	0.001	0.001	0.001
LMB	VADGIFSPB001	VADGIF	0.001	0	0.995	0.001	0.001	0.002
LMB/ALTB/BTRB	GMNHTC12317	Micropterus floridanus	0.017	0.345	0.345	0.002	0.002	0.29
LMB/SHB/SMB	LMBOMR102	Ocmulgee River	0.017	0.003	0.93	0.025	0.023	0.002
SHB	GASHB13_011	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_014	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_017	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_018	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_019	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_021	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_022	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_023	Flint River, 2013	0	0	0.002	0.995	0.001	0.001
SHB	GASHB13_024	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_025	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_027	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_028	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_030	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_031	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_001	Flint River, 2016	0	0	0.001	0.996	0.002	0.001
SHB	GASHB16_003	Flint River, 2016	0	0	0.002	0.995	0.001	0.001
SHB	GASHB16_007	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_008	Flint River, 2016	0	0	0.002	0.995	0.001	0.001
SHB	GASHB16_009	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_010	Flint River, 2016	0	0	0.002	0.996	0.001	0.001
SHB	GASHB16_012	Flint River, 2016	0	0	0.003	0.994	0.001	0.001
SHB	GASHB16_013	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_014	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_015	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_016	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_019	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_021	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_022	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_024	Flint River, 2016	0	0	0.002	0.995	0.001	0.001
SHB	GASHB16_025	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_026	Flint River, 2016	0	0	0.001	0.995	0.002	0.001
SHB	GASHB16_027	Flint River, 2016	0	0	0.001	0.996	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	GASHB16_028	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_029	Flint River, 2016	0	0	0.001	0.996	0.002	0.001
SHB	GASHB17(2)_001	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_002	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_003	Chattahoochee River	0	0	0.002	0.996	0.001	0.001
SHB	GASHB17(2)_004	Chattahoochee River	0	0	0.001	0.996	0.002	0.001
SHB	GASHB17(2)_005	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_006	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_007	Chattahoochee River	0	0	0.002	0.995	0.001	0.001
SHB	GASHB17(2)_008	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_009	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_010	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHB17(2)_011	Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	GASHHY12_01	Hybrid Project, 2012	0	0	0.002	0.995	0.002	0.001
SHB	GASHHY12_02	Hybrid Project, 2012	0	0	0.001	0.996	0.002	0.001
SHB	GASHHY12_03	Hybrid Project, 2012	0	0	0.001	0.996	0.001	0.001
SHB	GASHHY12_04	Hybrid Project, 2012	0	0	0.001	0.996	0.001	0.001
SHB	GMNHTC3525	Micropterus cataractae	0	0	0.001	0.996	0.001	0.001
SHB	GMNHTC3532	Micropterus cataractae	0	0	0.001	0.996	0.001	0.001
SHB	LMBOMR103	Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHB001	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB002	Chattahoochee River, 2005	0	0.005	0.001	0.991	0.002	0.001
SHB	SHB003	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB004	Chattahoochee River, 2005	0	0	0.001	0.981	0.013	0.003
SHB	SHB005	Chattahoochee River, 2005	0	0	0.002	0.995	0.001	0.001
SHB	SHB006	Chattahoochee River, 2005	0	0	0.001	0.996	0.002	0.001
SHB	SHB007	Chattahoochee River, 2005	0	0	0.001	0.974	0.021	0.003
SHB	SHB008	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB009	Chattahoochee River, 2005	0	0	0.002	0.996	0.001	0.001
SHB	SHB010	Chattahoochee River, 2005	0	0	0.001	0.982	0.013	0.004
SHB	SHB011	Chattahoochee River, 2005	0	0	0.002	0.995	0.001	0.001
SHB	SHB012	Chattahoochee River, 2005	0	0	0.002	0.996	0.001	0.001
SHB	SHB013	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB014	Chattahoochee River, 2005	0	0	0.001	0.995	0.002	0.001
SHB	SHB015	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB016	Chattahoochee River, 2005	0	0	0.002	0.995	0.001	0.001
SHB	SHB017	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB018	Chattahoochee River, 2005	0	0	0.002	0.996	0.001	0.001
SHB	SHB019	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB020	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB021	Chattahoochee River, 2005	0	0	0.001	0.996	0.002	0.001
SHB	SHB022	Chattahoochee River, 2005	0.001	0	0.001	0.982	0.011	0.005

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	SHB023	Chattahoochee River, 2005	0	0	0.001	0.996	0.001	0.001
SHB	SHB024	Chattahoochee River, 2010	0	0	0.001	0.996	0.002	0.001
SHB	SHB027	Chattahoochee River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	SHB028	Chattahoochee River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC095	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC096	Little Uchee, 2008	0	0	0.001	0.996	0.002	0.001
SHB	SHBLUC097	Little Uchee, 2008	0	0	0.001	0.993	0.004	0.001
SHB	SHBLUC098	Little Uchee, 2008	0	0	0.001	0.996	0.002	0.001
SHB	SHBLUC100	Little Uchee, 2008	0	0	0.001	0.995	0.002	0.001
SHB	SHBLUC102	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC103	Little Uchee, 2008	0	0	0.001	0.996	0.002	0.001
SHB	SHBLUC104	Little Uchee, 2008	0	0	0.001	0.996	0.002	0.001
SHB	SHBLUC105	Little Uchee, 2008	0	0	0.002	0.995	0.002	0.001
SHB	SHBLUC107	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC108	Little Uchee, 2008	0	0	0.002	0.994	0.001	0.001
SHB	SHBLUC109	Little Uchee, 2008	0	0	0.001	0.994	0.004	0.001
SHB	SHBLUC110	Little Uchee, 2008	0	0	0.002	0.993	0.004	0.001
SHB	SHBLUC111	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC112	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC113	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC114	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC115	Little Uchee, 2008	0	0	0.001	0.996	0.002	0.001
SHB	SHBLUC116	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC117	Little Uchee, 2008	0.001	0	0.002	0.994	0.001	0.001
SHB	SHBLUC118	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBLUC119	Little Uchee, 2008	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR005	Lower Ocmulgee River	0	0	0.002	0.994	0.002	0.001
SHB	SHBOMR006	Lower Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR009	Lower Ocmulgee River	0.025	0.004	0.001	0.968	0.001	0.001
SHB	SHBOMR010	Lower Ocmulgee River	0	0.029	0.001	0.96	0.008	0.001
SHB	SHBOMR013	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR014	Upper Ocmulgee River	0	0	0.001	0.996	0.002	0.001
SHB	SHBOMR016	Upper Ocmulgee River	0	0	0.001	0.996	0.002	0.001
SHB	SHBOMR020	Upper Ocmulgee River	0.024	0.004	0.001	0.969	0.001	0.001
SHB	SHBOMR021	Upper Ocmulgee River	0.003	0.001	0.023	0.963	0.001	0.008
SHB	SHBOMR022	Upper Ocmulgee River	0.025	0.003	0.001	0.968	0.001	0.001
SHB	SHBOMR024	Upper Ocmulgee River	0.001	0.002	0.001	0.968	0.006	0.022
SHB	SHBOMR025	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR027	Upper Ocmulgee River	0	0	0.002	0.996	0.001	0.001
SHB	SHBOMR028	Upper Ocmulgee River	0	0.024	0.001	0.968	0.006	0.001
SHB	SHBOMR029	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR031	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	SHBOMR032	Upper Ocmulgee River	0	0	0.001	0.982	0.012	0.003
SHB	SHBOMR033	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR034	Upper Ocmulgee River	0	0.015	0.002	0.964	0.018	0.001
SHB	SHBOMR035	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR036	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR037	Upper Ocmulgee River	0.008	0.02	0.001	0.965	0.001	0.005
SHB	SHBOMR038	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR039	Upper Ocmulgee River	0.001	0.025	0.003	0.96	0.002	0.009
SHB	SHBOMR040	Upper Ocmulgee River	0	0.005	0.001	0.978	0.012	0.004
SHB	SHBOMR041	Upper Ocmulgee River	0	0	0.001	0.996	0.002	0.001
SHB	SHBOMR042	Upper Ocmulgee River	0	0	0.002	0.995	0.002	0.001
SHB	SHBOMR043	Upper Ocmulgee River	0.002	0.017	0.001	0.977	0.001	0.002
SHB	SHBOMR044	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR045	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR046	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR047	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR048	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR049	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR050	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR051	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR052	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR053	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR054	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR055	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR056	Upper Ocmulgee River	0	0	0.001	0.982	0.012	0.004
SHB	SHBOMR057	Upper Ocmulgee River	0	0	0.002	0.995	0.001	0.001
SHB	SHBOMR059	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	SHBOMR060	Upper Ocmulgee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_001	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_002	Hooch, Chattahoochee River	0	0	0.002	0.995	0.001	0.001
SHB	ShGASHB17_003	Hooch, Chattahoochee River	0	0	0.002	0.996	0.001	0.001
SHB	ShGASHB17_004	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_005	Hooch, Chattahoochee River	0.001	0	0.002	0.995	0.001	0.001
SHB	ShGASHB17_006	Hooch, Chattahoochee River	0	0	0.001	0.996	0.002	0.001
SHB	ShGASHB17_007	Hooch, Chattahoochee River	0	0	0.002	0.996	0.001	0.001
SHB	ShGASHB17_008	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_009	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_010	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_011	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_012	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_013	Hooch, Chattahoochee River	0	0	0.001	0.995	0.001	0.001
SHB	ShGASHB17_015	Hooch, Chattahoochee River	0	0	0.002	0.995	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	ShGASHB17_017	Hooch, Chattahoochee River	0.001	0	0.002	0.995	0.001	0.001
SHB	ShGASHB17_018	Hooch, Chattahoochee River	0	0	0.002	0.996	0.001	0.001
SHB	ShGASHB17_019	Hooch, Chattahoochee River	0	0	0.001	0.996	0.002	0.001
SHB	ShGASHB17_020	Hooch, Chattahoochee River	0	0	0.001	0.996	0.002	0.001
SHB	ShGASHB17_021	Hooch, Chattahoochee River	0	0	0.001	0.996	0.001	0.001
SHB	ShGASHB17_022	Hooch, Chattahoochee River	0	0	0.002	0.995	0.001	0.001
SHB	ShGASHB17_023	Hooch, Chattahoochee River	0.001	0.002	0.002	0.967	0.002	0.026
SHB	USFWS17859	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17860	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17861	Flint River, 2014	0	0	0.002	0.995	0.001	0.001
SHB	USFWS17862	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17863	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17864	Flint River, 2014	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17865	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17866	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17867	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17868	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17869	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17870	Flint River, 2014	0	0	0.002	0.996	0.001	0.001
SHB	USFWS17871	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17872	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17874	Flint River, 2014	0	0	0.002	0.995	0.001	0.001
SHB	USFWS17875	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17876	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17877	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17878	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17879	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17880	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17881	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17882	Flint River, 2014	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17927	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17928	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17929	Flint River, 2017	0	0	0.002	0.996	0.001	0.001
SHB	USFWS17930	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17931	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17932	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17933	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17934	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17935	Flint River, 2017	0	0	0.002	0.996	0.001	0.001
SHB	USFWS17936	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17937	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17938	Flint River, 2017	0	0	0.001	0.996	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	USFWS17939	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17940	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17941	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17942	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17943	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17944	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17945	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17946	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17947	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17948	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17949	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17950	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17951	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19381	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19382	Flint River, 2015	0.001	0.025	0.002	0.968	0.001	0.003
SHB	USFWS19383	Flint River, 2015	0.001	0	0.002	0.995	0.001	0.001
SHB	USFWS19384	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19385	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19386	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19387	Flint River, 2015	0	0	0.002	0.995	0.001	0.001
SHB	USFWS19388	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19389	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19392	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19393	Flint River, 2015	0	0	0.002	0.995	0.001	0.001
SHB	USFWS19394	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19395	Flint River, 2015	0.002	0.016	0.001	0.979	0.001	0.001
SHB	USFWS19396	Flint River, 2015	0	0	0.001	0.995	0.002	0.001
SHB	USFWS19397	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19398	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19399	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19400	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19401	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19402	Flint River, 2015	0	0	0.002	0.995	0.001	0.001
SHB	USFWS19403	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19404	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19405	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19406	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19407	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19408	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19409	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19410	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24801	Flint River, 2017	0	0	0.001	0.996	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	USFWS24802	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24803	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24805	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24806	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24807	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24808	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24809	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24811	Flint River, 2017	0	0.015	0.001	0.981	0.001	0.001
SHB	USFWS24812	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24813	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24814	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24815	Flint River, 2017	0	0	0.002	0.995	0.001	0.001
SHB	USFWS24816	Flint River, 2017	0	0	0.002	0.995	0.001	0.001
SHB	USFWS24817	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24818	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24819	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24821	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS24822	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24823	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS24824	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24825	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24826	Flint River, 2017	0	0	0.001	0.995	0.001	0.001
SHB	USFWS24827	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB	USFWS24828	Flint River, 2017	0	0	0.001	0.996	0.002	0.001
SHB	USFWS24830	Flint River, 2017	0	0	0.001	0.996	0.001	0.001
SHB/ALB	GA16OMR097	Ocmulgee River, GA 2016	0.416	0	0.006	0.573	0.003	0.002
SHB/ALB	SHBOMR008	Lower Ocmulgee River	0.335	0.004	0.006	0.647	0.003	0.005
SHB/ALB	SHBOMR012	Upper Ocmulgee River	0.488	0.001	0.003	0.503	0.003	0.003
SHB/ALB	USFWS17873	Flint River, 2014	0.461	0	0.004	0.53	0.003	0.002
SHB/ALB	USFWS24804	Flint River, 2017	0.481	0	0.004	0.51	0.003	0.001
SHB/ALB	USFWS24820	Flint River, 2017	0.106	0.001	0.003	0.886	0.002	0.001
SHB/ALB/SPB	SHB032	Chattahoochee River, 2017	0.289	0.001	0.009	0.398	0.005	0.299
SHB/CSB	SHBOMR001	Lower Ocmulgee River	0.004	0.294	0.012	0.644	0.024	0.022
SHB/CSB	SHBOMR002	Lower Ocmulgee River	0.004	0.294	0.011	0.639	0.031	0.022
SHB/CSB	SHBOMR003	Lower Ocmulgee River	0.001	0.047	0.002	0.937	0.01	0.003
SHB/CSB	SHBOMR004	Lower Ocmulgee River	0.001	0.066	0.001	0.928	0.002	0.002
SHB/CSB	SHBOMR011	Lower Ocmulgee River	0.014	0.02	0.011	0.939	0.004	0.012
SHB/CSB	SHBOMR023	Upper Ocmulgee River	0.002	0.15	0.007	0.813	0.003	0.025
SHB/CSB	SHBOMR061	Upper Ocmulgee River	0.015	0.129	0.012	0.835	0.006	0.003
SHB/LMB	SHB025	Chattahoochee River, 2011	0.002	0	0.106	0.871	0.006	0.015
SHB/LMB	SHBOMR030	Upper Ocmulgee River	0.24	0.001	0.009	0.737	0.011	0.002
SHB/LMB	USFWS24829	Flint River, 2017	0.001	0	0.493	0.502	0.003	0.002

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB/SMB	SHBLUC106	Little Uchee, 2008	0	0	0.001	0.835	0.163	0.001
SHB/SMB	SHBOMR026	Upper Ocmulgee River	0.001	0.006	0.002	0.862	0.099	0.031
SHB/SPB	SHB026	Chattahoochee River, 2013	0.001	0	0.005	0.545	0.005	0.444
SHB/SPB	SHBOMR058	Upper Ocmulgee River	0.001	0.002	0.003	0.941	0.004	0.049
SHB/SPB	ShGASHB17_016	Hooch, Chattahoochee River	0.001	0.001	0.001	0.942	0.002	0.053
SHB/SPB/ALB	ShGASHB17_014	Hooch, Chattahoochee River	0.093	0.004	0.003	0.709	0.035	0.156
SMB	GFEC0001	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0002	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0003	GO Fish Education Center, GA--GA	0.008	0.022	0.001	0.002	0.962	0.005
SMB	GFEC0004	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0005	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0006	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0007	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0008	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0010	GO Fish Education Center, GA--GA	0.01	0.004	0.001	0.002	0.981	0.002
SMB	GFEC0015	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.995	0.001
SMB	GFEC0016	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0017	GO Fish Education Center, GA--TN	0	0	0.001	0.051	0.946	0.001
SMB	GFEC0018	GO Fish Education Center, GA--TN	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0019	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0020	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0021	GO Fish Education Center, GA--TN	0	0	0.002	0.002	0.995	0.001
SMB	GFEC0022	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0023	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0024	GO Fish Education Center, GA--SC	0.001	0	0.003	0.002	0.994	0.001
SMB	GFEC0025	GO Fish Education Center, GA--SC	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0026	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0027	GO Fish Education Center, GA--SC	0.001	0	0.003	0.001	0.994	0.001
SMB	GFEC0028	GO Fish Education Center, GA--SC	0.01	0.003	0.001	0.002	0.983	0.001
SMB	GFEC0029	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0030	GO Fish Education Center, GA--SC	0	0	0.001	0.002	0.995	0.001
SMB	GFEC0031	GO Fish Education Center, GA--SC	0.001	0	0.008	0.001	0.987	0.003
SMB	GFEC0032	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0034	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0035	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0036	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0037	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0038	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0039	GO Fish Education Center, GA--GA	0.042	0.004	0.001	0.001	0.95	0.001
SMB	GFEC0040	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0041	GO Fish Education Center, GA--GA	0.009	0.003	0.001	0.001	0.984	0.001
SMB	GFEC0043	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SMB	GFEC0045	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.994	0.002
SMB	GFEC0046	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	SMBARFLFR_01	ARKSMB Fourche La Fave, 1997	0.001	0	0.003	0.001	0.991	0.004
SMB	SMBARFLFR_02	ARKSMB Fourche La Fave, 1997	0	0	0.001	0.002	0.995	0.001
SMB	SMBARFLFR_03	ARKSMB Fourche La Fave, 1997	0.001	0	0.003	0.001	0.994	0.001
SMB	SMBARFLFR_04	ARKSMB Fourche La Fave, 1997	0.001	0.01	0.002	0.001	0.983	0.002
SMB	SMBARFLFR_05	ARKSMB Fourche La Fave, 1997	0.001	0	0.008	0.001	0.987	0.003
SMB	SMBARFLFR_06	ARKSMB Fourche La Fave, 1997	0	0	0.001	0.002	0.995	0.001
SMB	SMBARFLFR_07	ARKSMB Fourche La Fave, 1997	0.001	0	0.003	0.001	0.994	0.001
SMB	SMBARFLFR_09	ARKSMB Fourche La Fave, 1997	0	0	0.001	0.001	0.996	0.001
SMB	SMBARSFLFR_01	ARKSMB South Fourche La Fave	0	0	0.001	0.001	0.995	0.001
SMB	SMBARSFLFR_02	ARKSMB South Fourche La Fave	0	0	0.001	0.001	0.995	0.001
SMB	SMBARSFLFR_03	ARKSMB South Fourche La Fave	0	0	0.001	0.002	0.995	0.001
SMB	SMBARSFLFR_04	ARKSMB South Fourche La Fave	0	0	0.001	0.001	0.996	0.001
SMB	SMBARSFLFR_05	ARKSMB South Fourche La Fave	0.001	0	0.002	0.001	0.99	0.005
SMB	SMBARSFLFR_06	ARKSMB South Fourche La Fave	0.001	0	0.002	0.001	0.99	0.005
SMB	SMBARSFLFR_07	ARKSMB South Fourche La Fave	0	0	0.001	0.001	0.992	0.004
SMB	SMBARSFLFR_09	ARKSMB South Fourche La Fave	0.002	0.001	0.019	0.004	0.957	0.017
SMB	SMBARSFLFR_10	ARKSMB South Fourche La Fave	0.001	0.001	0.002	0.001	0.972	0.024
SMB	SMBKYLFBC_02	SMB KY, Left Fork Beaver Creek	0.001	0.018	0.015	0.001	0.96	0.005
SMB	SMBKYLFBC_06	SMB KY, Left Fork Beaver Creek	0.002	0.012	0.013	0.001	0.968	0.005
SMB	SMBKYLFBC_09	SMB KY, Left Fork Beaver Creek	0	0	0.001	0.001	0.996	0.001
SMB	TWRA500	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.995	0.001
SMB	TWRA501	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA502	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA503	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA504	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA505	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.995	0.001
SMB	TWRA506	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA507	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA508	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA509	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA510	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA511	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.995	0.002
SMB	TWRA512	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA513	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA514	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA515	Dale Hollow Reservoir, TN	0	0	0.002	0.001	0.995	0.001
SMB	TWRA516	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA517	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA518	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA519	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SMB	TWRA520	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA521	Dale Hollow Reservoir, TN	0	0	0.001	0.052	0.946	0.001
SMB	TWRA522	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.995	0.001
SMB	TWRA523	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA524	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA525	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA526	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA527	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA528	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA529	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA530	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA531	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA541	Dale Hollow Reservoir, TN	0	0.002	0.001	0.002	0.974	0.02
SMB	TWRA542	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA546	Dale Hollow Reservoir, TN	0	0	0.002	0.001	0.996	0.001
SMB	TWRA547	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA548	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA549	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA550	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA551	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA552	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA553	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA555	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA556	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA557	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA558	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA559	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA560	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA561	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA562	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB/ALB	GFEC0009	GO Fish Education Center, GA--GA	0.078	0.001	0.006	0.003	0.909	0.003
SMB/ALB	GFEC0011	GO Fish Education Center, GA--GA	0.354	0.001	0.006	0.004	0.632	0.002
SMB/ALB	GFEC0012	GO Fish Education Center, GA--GA	0.343	0.001	0.003	0.005	0.496	0.152
SMB/ALB	GFEC0014	GO Fish Education Center, GA--GA	0.446	0.002	0.002	0.002	0.547	0.001
SMB/ALB	GFEC0033	GO Fish Education Center, GA--GA	0.076	0.001	0.002	0.003	0.917	0.001
SMB/ALB/CSB	GFEC0013	GO Fish Education Center, GA--GA	0.067	0.017	0.008	0.004	0.884	0.02
SMB/CSB	GFEC0042	GO Fish Education Center, GA--GA	0.002	0.348	0.002	0.001	0.642	0.005
SMB/CSB	GFEC0044	GO Fish Education Center, GA--GA	0.001	0.308	0.002	0.002	0.685	0.003
SMB/CSB	SMBKYLFBC_04	SMB KY, Left Fork Beaver Creek	0.001	0.044	0.003	0.001	0.942	0.009
SMB/SPB	SMBARFLFR_08	ARKSMB Fourche La Fave Rive	0.001	0	0.003	0.002	0.695	0.299
SMB/SPB	SMBARSFLFR_08	ARKSMB South Fourche La Fave	0.001	0	0.003	0.003	0.92	0.073
SMB/SPB	SMBKYLFBC_01	SMBKY, Left Fork Beaver Creek	0.001	0	0.002	0.003	0.787	0.207

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SMB/SPB	SMBKYLFBC_03	SMBKY, Left Fork Beaver Creek	0.001	0.008	0.006	0.02	0.838	0.127
SMB/SPB	SMBKYLFBC_08	SMBKY, Left Fork Beaver Creek	0.001	0.001	0.004	0.001	0.888	0.105
SMB/SPB	SMBKYLFBC_10	SMBKY, Left Fork Beaver Creek	0.001	0	0.011	0.003	0.751	0.234
SMB/SPB	SMBKYLFBC_11	SMBKY, Left Fork Beaver Creek	0	0	0.001	0.003	0.875	0.121
SMB/SPB/ALB	TWRA563	Dale Hollow Reservoir TN 2017	0.142	0.002	0.007	0.004	0.565	0.281
SMB/SPB/CSB	SMBKYLFBC_05	SMB KY, Left Fork Beaver Creek	0.003	0.025	0.004	0.002	0.759	0.207
SPB	LMBFR014	Lower Flint River	0.001	0	0.031	0.004	0.004	0.96
SPB	MpTN15_012	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTN15_013	Kentucky Lake, TN, 2015	0	0	0.002	0.022	0.02	0.956
SPB	MpTN15_014	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.995
SPB	MpTN15_015	Kentucky Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.994
SPB	MpTN15_016	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_017	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_018	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_019	Kentucky Lake, TN, 2015	0.001	0	0.006	0.002	0.002	0.99
SPB	MpTN15_02	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_020	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_03	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_04	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_05	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_06	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.995
SPB	MpTN15_07	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_08	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTN15_09	Kentucky Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.995
SPB	MpTN15_10	Kentucky Lake, TN, 2015	0.001	0	0.011	0.002	0.002	0.983
SPB	MpTN15_11	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_001	Centerhill Lake, TN, 2015	0.001	0	0.002	0.001	0.001	0.995
SPB	MpTNSPB_002	Centerhill Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.994
SPB	MpTNSPB_003	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_004	Centerhill Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_005A	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_006	Centerhill Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_007	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_009	Centerhill Lake, TN, 2015	0	0	0.003	0.001	0.001	0.994
SPB	MpTNSPB_010	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.002	0.995
SPB	MpTNSPB_011	Centerhill Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_012	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_013	Centerhill Lake, TN, 2015	0.001	0	0.002	0.001	0.001	0.994
SPB	MpTNSPB_014	Centerhill Lake, TN, 2015	0.001	0	0.002	0.001	0.002	0.994
SPB	MpTNSPB_015	Centerhill Lake, TN, 2015	0.001	0	0.004	0.001	0.001	0.992
SPB	MpTNSPB_016	Centerhill Lake, TN, 2015	0.001	0	0.003	0.001	0.002	0.993
SPB	MpTNSPB_017	Centerhill Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SPB	MpTNSPB_018	Centerhill Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.994
SPB	MpTNSPB_019	Centerhill Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_020	Centerhill Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.994
SPB	MpTNSPB_021	Centerhill Lake, TN, 2015	0.001	0	0.006	0.002	0.002	0.989
SPB	MpTNSPB_022	Centerhill Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.994
SPB	MpTNSPB_023	Centerhill Lake, TN, 2015	0.001	0	0.01	0.003	0.003	0.983
SPB	MpTNSPB_024	Centerhill Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_025	Centerhill Lake, TN, 2015	0.001	0	0.025	0.004	0.004	0.966
SPB	MpTNSPB_026	Centerhill Lake, TN, 2015	0.001	0	0.005	0.002	0.002	0.99
SPB	MpTNSPB_027	Centerhill Lake, TN, 2015	0.001	0	0.008	0.002	0.002	0.987
SPB	MpTNSPB_029	Centerhill Lake, TN, 2015	0.001	0	0.006	0.002	0.002	0.988
SPB	MpTNSPB_030	Centerhill Lake, TN, 2015	0.001	0	0.002	0.001	0.001	0.995
SPB	SPBCH343	Center Hill Lake, TN 2010	0.001	0	0.003	0.001	0.001	0.994
SPB	SPBCH344	Center Hill Lake, TN 2010	0.001	0	0.003	0.001	0.001	0.994
SPB	SPBCH345	Center Hill Lake, TN 2010	0	0	0.001	0.001	0.001	0.996
SPB	SPBCH346	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	SPBCH347	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	SPBCH348	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	SPBCH349	Center Hill Lake, TN 2010	0	0	0.001	0.001	0.001	0.997
SPB	TWRA532	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA533	Dale Hollow Reservoir TN 2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA534	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA535	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA536	Dale Hollow Reservoir TN 2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA537	Dale Hollow Reservoir TN 2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA538	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA539	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA540	Dale Hollow Reservoir TN 2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA543	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA544	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA545	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA554	Dale Hollow Reservoir TN 2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA564	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA565	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA566	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA567	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.997
SPB	TWRA568	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.003	0.003	0.99
SPB	TWRA569	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.002	0.994
SPB	TWRA570	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.002	0.994
SPB	TWRA571	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.002	0.002	0.992
SPB	TWRA572	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA573	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SPB	TWRA574	Watauga Reservoir TN TWRA2017	0.001	0	0.004	0.003	0.003	0.99
SPB	TWRA575	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA576	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA577	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.002	0.002	0.993
SPB	TWRA578	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA579	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.002	0.002	0.993
SPB	TWRA580	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.995
SPB	TWRA581	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA582	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA583	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA584	Watauga Reservoir TN TWRA2017	0	0	0.001	0.002	0.002	0.993
SPB	TWRA585	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.002	0.002	0.992
SPB	TWRA586	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA587	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA588	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.002	0.002	0.994
SPB	TWRA589	Watauga Reservoir TN TWRA2017	0.001	0	0.001	0.001	0.001	0.996
SPB	TWRA590	Watauga Reservoir TN TWRA2017	0.001	0	0.001	0.001	0.001	0.996
SPB	TWRA591	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA592	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.995
SPB	TWRA593	Watauga Reservoir TN TWRA2017	0	0	0.003	0.002	0.002	0.993
SPB	TWRA594	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA595	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA596	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.995
SPB	TWRA597	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.994
SPB	TWRA598	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.001	0.001	0.993
SPB	TWRA599	Watauga Reservoir TN TWRA2017	0.003	0.019	0.001	0.001	0.007	0.968
SPB	TWRA600	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.002	0.002	0.993
SPB	TWRA601	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA602	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA603	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA604	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA605	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA606	Watauga Reservoir TN TWRA2017	0.001	0	0.004	0.001	0.001	0.993
SPB	TWRA607	Watauga Reservoir TN TWRA2017	0	0	0.001	0.011	0.012	0.975
SPB	TWRA609	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA610	Watauga Reservoir TN TWRA2017	0.001	0	0.004	0.001	0.001	0.992
SPB	TWRA611	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.001	0.002	0.993
SPB	TWRA612	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.001	0.001	0.994
SPB	TWRA613	Watauga Reservoir TN TWRA2017	0.001	0	0.003	0.001	0.001	0.994
SPB/ALB	MpTN15_01	Kentucky Lake, TN, 2015	0.211	0.001	0.006	0.001	0.001	0.78
SPB/ALB	SHB030	Chattahoochee River, 2011	0.11	0.001	0.015	0.002	0.002	0.87
SPB/ALB	SPB001	Chattahoochee River, 2011	0.109	0.001	0.016	0.002	0.002	0.87

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SPB/ALB/SHB	SHB033	Chattahoochee River, 2017	0.274	0.001	0.007	0.167	0.003	0.55
SPB/LMB	MpTNSPB_008	Centerhill Lake, TN, 2015	0.002	0	0.093	0.005	0.005	0.894
SPB/SMB	TWRA608	Watauga Reservoir TN TWRA2017	0.001	0.002	0.002	0.001	0.046	0.948

Appendix 5 Reference genotypes were sorted and retained for future uses based on Q-values from six species (n=70 for each species; Alabama bass (ALB), Coosa bass (CSB), largemouth bass (LMB), shoal bass (SHB), smallmouth bass (SMB), and spotted bass (SPB)). The results showed Q-value from STRUCTURE. Data was run using STRUCTURE analysis of K = 6.

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	MhAL15_T12	Tallapoosa River, AL, 2015	0.998	0	0.001	0	0	0
ALB	MhTLR002	Tallapoosa River, AL, 2016	0.998	0	0.001	0	0	0
ALB	MpMhNC15_16	Lake Norman, NC, 2015	0.998	0	0.001	0	0	0
ALB	SPBOMR016	Lower Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR031	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR044	Upper Ocmulgee River	0.998	0.001	0.001	0	0	0
ALB	SPBOMR064	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB	SPBOMR066	Upper Ocmulgee River	0.998	0	0.001	0	0	0
ALB	MhAL15_T01	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0.001	0.001
ALB	MhAL15_T03	Tallapoosa River, AL, 2015	0.997	0	0.001	0.001	0.001	0.001
ALB	MhAL15_T04	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0
ALB	MhAL15_T07	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T08	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T09	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T10	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T15	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T16	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T17	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhAL15_T20	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0.001	0.001	0.001
ALB	MhAL15_T23	Tallapoosa River, AL, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MhAL15_T25	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T26	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0.001
ALB	MhAL15_T27	Tallapoosa River, AL, 2015	0.997	0	0.001	0	0	0
ALB	MhTLR005	Tallapoosa River, AL, 2016	0.997	0	0.001	0	0	0.001
ALB	MhTLR006	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0.001	0.001	0
ALB	MhTLR010	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0.001
ALB	MhTLR012	Tallapoosa River, AL, 2016	0.997	0.001	0.001	0	0	0
ALB	MhTLR013	Tallapoosa River, AL, 2016	0.997	0	0.001	0	0.001	0.001
ALB	MhNHER10_77	Neely Henry Reservoir, AL	0.997	0	0.001	0	0.001	0.001
ALB	MpMhNC15_06	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MpMhNC15_08	Lake Norman, NC, 2015	0.997	0.001	0.001	0.001	0	0.001
ALB	MpMhNC15_09	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	MpMhNC15_13	Lake Norman, NC, 2015	0.997	0	0.001	0	0	0.001
ALB	MpMhNC15_14	Lake Norman, NC, 2015	0.997	0	0.001	0	0	0

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
ALB	MpMhNC15_19	Lake Norman, NC, 2015	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR004	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0
ALB	SPBOMR006	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR014	Lower Ocmulgee River	0.997	0	0.001	0.001	0.001	0
ALB	SPBOMR017	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR019	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0
ALB	SPBOMR023	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR025	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR026	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR027	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR033	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR035	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR036	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR039	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR040	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR041	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR042	Upper Ocmulgee River	0.997	0	0.001	0	0	0.001
ALB	SPBOMR043	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR045	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR046	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR048	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR049	Upper Ocmulgee River	0.997	0.001	0.001	0	0.001	0.001
ALB	SPBOMR050	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR053	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR054	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR055	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR058	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBOMR059	Upper Ocmulgee River	0.997	0	0.001	0	0.001	0.001
ALB	SPBOMR060	Upper Ocmulgee River	0.997	0	0.001	0.001	0.001	0.001
ALB	SPBOMR063	Upper Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	SPBOMR065	Upper Ocmulgee River	0.997	0	0.001	0	0	0
ALB	SPBJUL001	Juliette River	0.997	0	0.001	0	0	0
ALB	CahabaS4_01	Cahaba River	0.997	0	0.001	0.001	0.001	0.001
ALB	LMBOMR112	Ocmulgee River	0.997	0.001	0.001	0	0	0
ALB	LMBOMR117	Ocmulgee River	0.997	0.001	0.001	0	0	0.001
ALB	LMBOMR154	Upper Ocmulgee River	0.997	0.001	0.001	0.001	0.001	0.001
CSB	REBigCan_S1_06	Big Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REBigCan_S1_09	Big Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REBigW_S1_03	Big Willis Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	RECheaS1_04	Cheaha Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REChoc_S1_04	Choccolocco Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REChoc_S1_13	Choccolocco Creek, Coosa River, AL	0	0.999	0	0	0	0

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
CSB	REHat_S2_13	Hatchet Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	REL_Can_S1_02	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REL_Can_S1_03	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	REL_Can_S1_04	Little Canoe Creek, Coosa River, AL	0	0.999	0	0	0	0
CSB	RETerra_S1_01	Terrapin Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	RETerra_S1_02	Terrapin Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	REWal_S1_03	Walnut Creek, Coosa River, AL, 2011	0	0.999	0	0	0	0
CSB	REBigCan_S1_01	Big Canoe Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_02	Big Canoe Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_03	Big Canoe Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_07	Big Canoe Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigCan_S1_08	Big Canoe Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigW_S1_02	Big Willis Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigW_S1_06	Big Willis Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigW_S1_07	Big Willis Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigW_S1_09	Big Willis Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REBigW_S1_10	Big Willis Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_01	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_05	Cheaha Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	RECheaS1_06	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_08	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	RECheaS1_10	Cheaha Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REChoc_S1_01	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REChoc_S1_07	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REChoc_S1_09	Choccolocco Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REHat_S2_11	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REHat_S2_12	Hatchet Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REHat_S2_14	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REHat_S2_15	Hatchet Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REL_Can_S1_05	Little Canoe Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	REL_Can_S1_06	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REL_Can_S1_07	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	REL_Can_S1_08	Little Canoe Creek, Coosa River, AL	0	0.998	0	0	0	0
CSB	RELitW_S3_01	Little Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	RELitW_S3_06	Little Willis Creek, Coosa River, AL	0.001	0.998	0	0	0	0
CSB	RETerra_S1_04	Terrapin Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	RETerra_S1_05	Terrapin Creek, Coosa River, AL, 2011	0	0.998	0.001	0	0	0.001
CSB	RETerra_S1_09	Terrapin Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REWal_S1_02	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0.001
CSB	REWal_S1_04	Walnut Creek, Coosa River, AL, 2011	0	0.998	0	0	0	0
CSB	REWal_S1_07	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REWal_S1_08	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
CSB	REWal_S1_09	Walnut Creek, Coosa River, AL, 2011	0.001	0.998	0	0	0	0
CSB	REBigW_S1_04	Big Willis Creek, Coosa River, AL, 2011	0	0.997	0.001	0.001	0.001	0.001
CSB	REBigW_S1_05	Big Willis Creek, Coosa River, AL, 2011	0.001	0.997	0	0	0	0
CSB	RECheaS1_09	Cheaha Creek, Coosa River, AL, 2011	0.001	0.997	0	0	0	0
CSB	REChoc_S1_08	Choccolocco Creek, Coosa River, AL	0	0.997	0	0.001	0	0
CSB	REHat_S2_09	Hatchet Creek, Coosa River, AL, 2011	0.001	0.997	0.001	0	0	0.001
CSB	RELitW_S3_04	Little Willis Creek, Coosa River, AL	0.001	0.997	0.001	0	0	0.001
CSB	RELitW_S3_07	Little Willis Creek, Coosa River, AL	0.001	0.997	0	0	0	0.001
CSB	RELitW_S3_08	Little Willis Creek, Coosa River, AL	0.001	0.997	0	0	0	0.001
CSB	RELR_S1_03	Little River, Coosa River, AL, 2011	0.001	0.997	0.001	0	0.001	0.001
CSB	REChoc_S1_12	Choccolocco Creek, Coosa River, AL	0.001	0.996	0.001	0	0.001	0.001
CSB	RETerra_S1_06	Terrapin Creek, Coosa River, AL, 2011	0	0.996	0.001	0.001	0.001	0.001
CSB	REWal_S1_05	Walnut Creek, Coosa River, AL, 2011	0.002	0.995	0.001	0.001	0.001	0
CSB	RECheaS1_02	Cheaha Creek, Coosa River, AL, 2011	0.001	0.994	0.001	0.001	0.001	0.001
CSB	RECheaS1_07	Cheaha Creek, Coosa River, AL, 2011	0.001	0.994	0.001	0.001	0.001	0.001
CSB	RETerra_S1_08	Terrapin Creek, Coosa River, AL, 2011	0	0.994	0.002	0.001	0.001	0.001
CSB	RETerra_S1_03	Terrapin Creek, Coosa River, AL, 2011	0.001	0.992	0.001	0.004	0.001	0.001
CSB	REHat_S2_08	Hatchet Creek, Coosa River, AL, 2011	0.001	0.991	0.002	0.001	0.002	0.003
CSB	REL_Can_S1_01	Little Canoe Creek, Coosa River, AL	0.003	0.991	0.002	0.001	0.001	0.002
CSB	RELitW_S3_03	Little Willis Creek, Coosa River, AL	0.005	0.991	0.001	0.001	0.001	0
CSB	REChoc_S1_03	Choccolocco Creek, Coosa River, AL	0.005	0.99	0.001	0.001	0.001	0.001
CSB	RELitW_S3_05	Little Willis Creek, Coosa River, AL	0.004	0.99	0.002	0.001	0.002	0.002
LMB	LNB13A	Northern--ASF, IL	0.001	0	0.996	0.001	0.001	0.001
LMB	LNBSL01A	Sugar Lake, MN	0.001	0	0.996	0.001	0.001	0.001
LMB	LMBFR039	Lower Flint River	0.001	0	0.996	0.001	0.001	0.001
LMB	LMBOMR144	Upper Ocmulgee River	0.001	0	0.996	0.001	0.001	0.001
LMB	LDB2590A	D'Olive Bay, Mobile Delta, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDB2592A	D'Olive Bay, Mobile Delta, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDBCANOT11834A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDBCANOT11837A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.002
LMB	LDBCANOT11838A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.002
LMB	LDBCANOT2617A	Big Bayou Canot, Mobile-Tensaw, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LDBTN11853A	Tensaw Lake, Mobile Delta, Tensaw, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLAL01	Florida ASF	0.001	0	0.995	0.001	0.001	0.002
LMB	LFLAL01II	Florida ASF	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLAL02	Florida ASF	0.001	0	0.995	0.001	0.001	0.002
LMB	LFLAL02II	Florida ASF	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLF04	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLF11	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM10A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM12A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001
LMB	LFLM13A	Florida Bass Conservation	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LNB11A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNB12A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNB19A	Northern--ASF, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT02A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.002
LMB	LNBMATT04A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT05A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBMATT06A	Lake Mattoon, Little Wabash, IL	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBSL04A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.001
LMB	LNBSL08A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.002
LMB	LNBSL10A	Sugar Lake, MN	0.001	0	0.995	0.001	0.001	0.001
LMB	LMA028	Lake Martin, AL	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR001	Lower Flint River	0.001	0	0.995	0.001	0.002	0.001
LMB	LMBFR002	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR003	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR004	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR005	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR006	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR008	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR009	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR010	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR011	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR012	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR013	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR016	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR017	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR018	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR019	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR020	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR021	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR022	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR023	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR024	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR025	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR026	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR027	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR028	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR029	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR030	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR031	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR032	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR033	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR034	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
LMB	LMBFR035	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR036	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR037	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR038	Lower Flint River	0.001	0	0.995	0.001	0.001	0.002
LMB	LMBFR040	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR041	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR042	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
LMB	LMBFR043	Lower Flint River	0.001	0	0.995	0.001	0.001	0.001
SHB	GASHB13_011	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_014	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_017	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_018	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_019	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_021	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_022	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_024	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_025	Flint River, 2013	0	0	0.001	0.996	0.002	0.001
SHB	GASHB13_027	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_028	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_030	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	GASHB13_031	Flint River, 2013	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17859	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17860	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17862	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17863	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17864	Flint River, 2014	0	0	0.001	0.996	0.002	0.001
SHB	USFWS17865	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17866	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17867	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17868	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17869	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17870	Flint River, 2014	0	0	0.002	0.996	0.001	0.001
SHB	USFWS17871	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17872	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17875	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17876	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17877	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17878	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17879	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17880	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17881	Flint River, 2014	0	0	0.001	0.996	0.001	0.001
SHB	USFWS17882	Flint River, 2014	0	0	0.001	0.996	0.002	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SHB	USFWS19381	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19384	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19385	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19386	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19388	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19389	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19392	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19394	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19397	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19398	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19399	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19400	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19401	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19403	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19404	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19405	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19406	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19407	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19408	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	USFWS19409	Flint River, 2015	0	0	0.001	0.996	0.002	0.001
SHB	USFWS19410	Flint River, 2015	0	0	0.001	0.996	0.001	0.001
SHB	GMNHTC3525	Micropterus cataractae	0	0	0.001	0.996	0.001	0.001
SHB	GMNHTC3532	Micropterus cataractae	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_001	Flint River, 2016	0	0	0.001	0.996	0.002	0.001
SHB	GASHB16_007	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_009	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_010	Flint River, 2016	0	0	0.002	0.996	0.001	0.001
SHB	GASHB16_013	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_014	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_015	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_016	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_019	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_021	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_022	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_025	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SHB	GASHB16_027	Flint River, 2016	0	0	0.001	0.996	0.001	0.001
SMB	TWRA501	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA502	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA503	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA504	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA506	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA507	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SMB	TWRA508	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA509	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA510	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA512	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA513	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA514	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA516	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA517	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA518	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA519	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA520	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA523	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA524	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA525	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA526	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA527	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA528	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA529	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA530	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA531	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA542	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA546	Dale Hollow Reservoir, TN	0	0	0.002	0.001	0.996	0.001
SMB	TWRA547	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA548	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA549	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA550	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA551	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA552	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA553	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA555	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA556	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA557	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA558	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA559	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA560	Dale Hollow Reservoir, TN	0	0	0.001	0.001	0.996	0.001
SMB	TWRA561	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	TWRA562	Dale Hollow Reservoir, TN	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0001	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0002	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0004	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0005	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0006	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SMB	GFEC0007	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0008	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0016	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0018	GO Fish Education Center, GA--TN	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0019	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0020	GO Fish Education Center, GA--TN	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0022	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0023	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0025	GO Fish Education Center, GA--SC	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0026	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0029	GO Fish Education Center, GA--SC	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0032	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0034	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0035	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0036	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0037	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0038	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	GFEC0040	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0043	GO Fish Education Center, GA--GA	0	0	0.001	0.002	0.996	0.001
SMB	GFEC0046	GO Fish Education Center, GA--GA	0	0	0.001	0.001	0.996	0.001
SMB	SMBARFLFR_09	ARKSMB Fourche La Fave River, 1997	0	0	0.001	0.001	0.996	0.001
SMB	SMBARSFLFR_04	ARKSMB South Fourche La Fave, 1997	0	0	0.001	0.001	0.996	0.001
SPB	TWRA567	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.997
SPB	SPBCH349	Center Hill Lake, TN 2010	0	0	0.001	0.001	0.001	0.997
SPB	MpTN15_012	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTN15_016	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_017	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_018	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_02	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_020	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_03	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_04	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_05	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_07	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_08	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTN15_11	Kentucky Lake, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_003	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_004	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_005A	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_006	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_007	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_011	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.002	0.001	0.001	0.996

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SPB	MpTNSPB_012	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	MpTNSPB_017	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_019	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.002	0.001	0.001	0.996
SPB	MpTNSPB_024	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.001	0.996
SPB	TWRA532	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA533	Dale Hollow Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA534	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA535	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA536	Dale Hollow Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA537	Dale Hollow Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA538	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA539	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA540	Dale Hollow Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA543	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA544	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA545	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA554	Dale Hollow Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA564	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA565	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA576	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA578	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA581	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA583	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA586	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA587	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA589	Watauga Reservoir TN TWRA2017	0.001	0	0.001	0.001	0.001	0.996
SPB	TWRA590	Watauga Reservoir TN TWRA2017	0.001	0	0.001	0.001	0.001	0.996
SPB	TWRA591	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA594	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA595	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA601	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA603	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.996
SPB	TWRA604	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	TWRA605	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.996
SPB	TWRA609	Watauga Reservoir TN TWRA2017	0	0	0.001	0.001	0.001	0.996
SPB	SPBCH345	Center Hill Lake, TN 2010	0	0	0.001	0.001	0.001	0.996
SPB	SPBCH346	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	SPBCH347	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	SPBCH348	Center Hill Lake, TN 2010	0	0	0.002	0.001	0.001	0.996
SPB	MpTN15_014	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.995
SPB	MpTN15_06	Kentucky Lake, TN, 2015	0	0	0.002	0.001	0.001	0.995
SPB	MpTN15_09	Kentucky Lake, TN, 2015	0.001	0	0.003	0.001	0.001	0.995

SPECIES	SAMPLE_NAME	SOURCE	ALB	CSB	LMB	SHB	SMB	SPB
SPB	MpTNSPB_001	Centerhill Lake, Caney Fork, TN, 2015	0.001	0	0.002	0.001	0.001	0.995
SPB	MpTNSPB_010	Centerhill Lake, Caney Fork, TN, 2015	0	0	0.001	0.001	0.002	0.995
SPB	MpTNSPB_030	Centerhill Lake, Caney Fork, TN, 2015	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA566	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA572	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA573	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA575	Watauga Reservoir TN TWRA2017	0.001	0	0.002	0.001	0.001	0.995
SPB	TWRA580	Watauga Reservoir TN TWRA2017	0	0	0.002	0.001	0.001	0.995

Appendix 6 Samples of redeye bass (CHB = Cahaba bass; CSB = Coosa bass; TLPB = Tallapoosa bass; WRB = Warrior bass) were genotyped with 64 fixed SNPs. The results showed Q-value from STRUCTURE. STRUCTURE analyses were run along with pure samples of Alabama bass (ALB).

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
CHB/ALB/TLPB	CahabaS3_02	Cahaba River 2011	0.332	0.623	0.007	0.036	0.003
CHB/TLPB	CahabaS3_03	Cahaba River 2011	0.005	0.925	0.007	0.06	0.004
CHB/ALB/CSB	CahabaS3_04	Cahaba River 2011	0.201	0.4	0.368	0.017	0.014
CHB/ALB/CSB	CahabaS3_05	Cahaba River 2011	0.203	0.385	0.383	0.016	0.014
CHB/ALB	CHB001	Cahaba ADCNR 2010	0.059	0.922	0.004	0.011	0.004
CHB/ALB/CSB	CHB002	Cahaba ADCNR 2010	0.051	0.785	0.149	0.013	0.002
CHB	CHB003	Cahaba ADCNR 2010	0.001	0.989	0.003	0.005	0.002
CHB	CHB004	Cahaba ADCNR 2010	0.001	0.988	0.004	0.005	0.001
CHB/ALB/CSB	CHB005	Cahaba ADCNR 2010	0.026	0.893	0.056	0.009	0.015
CHB	CHB006	Cahaba ADCNR 2010	0.002	0.981	0.009	0.006	0.002
CHB	CHB008	Cahaba ADCNR 2010	0.001	0.969	0.023	0.005	0.002
CHB/ALB	CHB009	Cahaba ADCNR 2010	0.097	0.888	0.008	0.004	0.002
CHB	CHBCFC001	Caffee Creek	0.013	0.953	0.004	0.007	0.023
CHB/TLPB/CSB	CHBCFC002	Caffee Creek	0.002	0.885	0.021	0.091	0.002
CHB	CHBCFC003	Caffee Creek	0.001	0.984	0.005	0.008	0.002
CHB/CSB	CHBCFC004	Caffee Creek	0.001	0.931	0.059	0.008	0.001
CHB	CHBCFC005	Caffee Creek	0.001	0.976	0.007	0.013	0.002
CHB	CHBCFC006	Caffee Creek	0.001	0.981	0.01	0.007	0.001
CHB/TLPB/CSB	CHBCFC007	Caffee Creek	0.001	0.452	0.048	0.497	0.002
CHB	CHBCFC008	Caffee Creek	0.006	0.973	0.005	0.012	0.004
CHB	CHBCFC009	Caffee Creek	0.001	0.987	0.007	0.005	0.001
CHB	CHBCFC010	Caffee Creek	0.001	0.978	0.005	0.014	0.002
CHB/CSB	CHBCFC011	Caffee Creek	0.001	0.913	0.078	0.006	0.002
CHB	CHBCFC012	Caffee Creek	0.001	0.987	0.006	0.004	0.002
CHB	CHBCFC013	Caffee Creek	0.004	0.983	0.008	0.004	0.002
CHB	CHBLCR001	Little Cahaba River	0.001	0.986	0.007	0.004	0.002
CHB	CHBLCR002	Little Cahaba River	0.001	0.97	0.009	0.018	0.002
CHB	CHBLCR003	Little Cahaba River	0.001	0.979	0.014	0.005	0.001
CHB/ALB/CSB/TLPB	CHBLCR004	Little Cahaba River	0.384	0.372	0.209	0.033	0.002
CHB	CHBLCR005	Little Cahaba River	0.001	0.978	0.012	0.008	0.001
CHB/ALB/CSB	CHBLCR006	Little Cahaba River	0.498	0.456	0.031	0.012	0.003
CHB/TLPB	CHBLCR007	Little Cahaba River	0.001	0.954	0.01	0.034	0.002
CHB	CHBLCR008	Little Cahaba River	0.025	0.956	0.011	0.006	0.002
CHB	CHBLCR009	Little Cahaba River	0.001	0.959	0.02	0.018	0.002

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
CHB/TLPB	CHBLCR010	Little Cahaba River	0.001	0.962	0.007	0.028	0.002
CHB	CHBLCR011	Little Cahaba River	0.001	0.978	0.011	0.008	0.002
CHB	CHBLCR012	Little Cahaba River	0.001	0.982	0.007	0.005	0.005
CHB/ALB/CSB/TLPB	CHBLCR013	Little Cahaba River	0.426	0.511	0.039	0.021	0.003
CHB/CSB/TLPB	CHBLCR014	Little Cahaba River	0.003	0.762	0.174	0.058	0.003
CHB	CHBLCR015	Little Cahaba River	0.001	0.98	0.008	0.01	0.001
CHB	CHBLCR016	Little Cahaba River	0.001	0.977	0.015	0.006	0.002
CHB/CSB/WRB	CHBLCR017	Little Cahaba River	0.001	0.838	0.077	0.019	0.065
CHB	CHBLCR018	Little Cahaba River	0.002	0.967	0.011	0.016	0.003
CHB	CHBLCR019	Little Cahaba River	0.003	0.963	0.008	0.007	0.018
CHB/ALB/CSB/TLPB	CHBLCR020	Little Cahaba River	0.366	0.264	0.172	0.192	0.006
CHB/TLPB	LtCahabaS1_01	Little Cahaba River 2011	0.001	0.932	0.011	0.044	0.013
CHB	LtCahabaS1_02	Little Cahaba River 2011	0.001	0.974	0.005	0.017	0.003
CHB/TLPB	LtCahabaS1_03	Little Cahaba River 2011	0.003	0.918	0.019	0.058	0.002
CHB	LtCahabaS1_04	Little Cahaba River 2011	0.001	0.986	0.003	0.007	0.003
CHB/CSB/TLPB/WRB	LtCahabaS1_05	Little Cahaba River 2011	0.009	0.674	0.267	0.029	0.021
CHB/CSB/TLPB	LtCahabaS1_06	Little Cahaba River 2011	0.001	0.86	0.064	0.073	0.002
CHB	LtCahabaS1_07	Little Cahaba River 2011	0.013	0.961	0.014	0.009	0.003
CHB/CSB/TLPB	LtCahabaS1_08	Little Cahaba River 2011	0.003	0.681	0.213	0.1	0.003
CHB	LtCahabaS1_09	Little Cahaba River 2011	0.001	0.985	0.006	0.007	0.001
CSB	REBigCan_S1_01	Big Canoe Creek, Coosa R, AL 2011	0.001	0.01	0.985	0.003	0.002
CSB	REBigCan_S1_02	Big Canoe Creek, Coosa R, AL 2011	0.001	0.015	0.974	0.008	0.003
CSB	REBigCan_S1_03	Big Canoe Creek, Coosa R, AL 2011	0.001	0.006	0.976	0.016	0.001
CSB/WRB	REBigCan_S1_05	Big Canoe Creek, Coosa R, AL 2011	0.003	0.017	0.919	0.016	0.045
CSB	REBigCan_S1_06	Big Canoe Creek, Coosa R, AL 2011	0.001	0.007	0.987	0.004	0.001
CSB	REBigCan_S1_07	Big Canoe Creek, Coosa R, AL 2011	0.001	0.008	0.984	0.005	0.002
CSB	REBigCan_S1_08	Big Canoe Creek, Coosa R, AL 2011	0.001	0.011	0.984	0.003	0.002
CSB	REBigCan_S1_09	Big Canoe Creek, Coosa R, AL 2011	0.001	0.005	0.99	0.003	0.001
CSB	REBigW_S1_02	Big Willis Creek, Coosa R, AL, 2011	0.001	0.006	0.985	0.007	0.002
CSB	REBigW_S1_03	Big Willis Creek, Coosa R, AL, 2011	0.001	0.009	0.985	0.004	0.001
CSB/TLPB	REBigW_S1_04	Big Willis Creek, Coosa R, AL, 2011	0.001	0.007	0.938	0.053	0.002
CSB/CHB/TLPB	REBigW_S1_05	Big Willis Creek, Coosa R, AL, 2011	0.001	0.145	0.771	0.08	0.003
CSB/TLPB	REBigW_S1_06	Big Willis Creek, Coosa R, AL, 2011	0.001	0.007	0.968	0.023	0.001
CSB/TLPB	REBigW_S1_07	Big Willis Creek, Coosa R, AL, 2011	0.001	0.007	0.968	0.023	0.002
CSB	REBigW_S1_09	Big Willis Creek, Coosa R, AL, 2011	0.001	0.007	0.983	0.009	0.001
CSB/TLPB	REBigW_S1_10	Big Willis Creek, Coosa R, AL, 2011	0.001	0.009	0.928	0.06	0.002
CSB	RECheaS1_01	Cheaha Creek, Coosa River, AL, 2011	0.001	0.017	0.967	0.011	0.004
CSB	RECheaS1_02	Cheaha Creek, Coosa River, AL, 2011	0.001	0.011	0.981	0.003	0.003
CSB	RECheaS1_04	Cheaha Creek, Coosa River, AL, 2011	0.001	0.005	0.99	0.003	0.001
CSB	RECheaS1_05	Cheaha Creek, Coosa River, AL, 2011	0.001	0.007	0.988	0.003	0.002
CSB	RECheaS1_06	Cheaha Creek, Coosa River, AL, 2011	0.001	0.006	0.984	0.007	0.002
CSB	RECheaS1_07	Cheaha Creek, Coosa River, AL, 2011	0.001	0.011	0.983	0.003	0.002

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
CSB	RECheaS1_08	Cheaha Creek, Coosa River, AL, 2011	0.001	0.014	0.974	0.01	0.002
CSB	RECheaS1_09	Cheaha Creek, Coosa River, AL, 2011	0.001	0.02	0.966	0.009	0.004
CSB	RECheaS1_10	Cheaha Creek, Coosa River, AL, 2011	0.001	0.005	0.985	0.006	0.004
CSB	REChoc_S1_01	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.004	0.989	0.005	0.001
CSB/CHB/TLPB	REChoc_S1_03	Choccolocco Creek, Coosa R, AL, 2011	0.004	0.065	0.903	0.021	0.008
CSB	REChoc_S1_04	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.005	0.99	0.003	0.002
CSB	REChoc_S1_07	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.004	0.989	0.005	0.001
CSB	REChoc_S1_08	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.006	0.988	0.003	0.002
CSB	REChoc_S1_09	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.003	0.99	0.005	0.001
CSB	REChoc_S1_12	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.006	0.988	0.003	0.001
CSB	REChoc_S1_13	Choccolocco Creek, Coosa R, AL, 2011	0.001	0.004	0.991	0.003	0.001
CSB/CHB/TLPB	REHat_S2_08	Hatchet Creek, Coosa River, AL, 2011	0.001	0.038	0.929	0.022	0.009
CSB	REHat_S2_09	Hatchet Creek, Coosa River, AL, 2011	0.001	0.006	0.987	0.003	0.002
CSB	REHat_S2_10	Hatchet Creek, Coosa River, AL, 2011	0.024	0.008	0.962	0.004	0.003
CSB	REHat_S2_11	Hatchet Creek, Coosa River, AL, 2011	0.001	0.005	0.986	0.007	0.001
CSB	REHat_S2_12	Hatchet Creek, Coosa River, AL, 2011	0.001	0.006	0.975	0.017	0.001
CSB	REHat_S2_13	Hatchet Creek, Coosa River, AL, 2011	0.001	0.005	0.99	0.003	0.001
CSB	REHat_S2_14	Hatchet Creek, Coosa River, AL, 2011	0.001	0.004	0.988	0.006	0.001
CSB	REHat_S2_15	Hatchet Creek, Coosa River, AL, 2011	0.001	0.004	0.989	0.005	0.002
CSB	REL_Can_S1_01	Little Canoe Creek, Coosa R, AL, 2011	0.005	0.007	0.982	0.003	0.004
CSB	REL_Can_S1_02	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.006	0.988	0.003	0.001
CSB	REL_Can_S1_03	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.008	0.987	0.004	0.001
CSB	REL_Can_S1_04	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.01	0.984	0.004	0.001
CSB	REL_Can_S1_05	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.01	0.984	0.003	0.002
CSB	REL_Can_S1_06	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.004	0.991	0.003	0.001
CSB	REL_Can_S1_07	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.005	0.991	0.003	0.001
CSB	REL_Can_S1_08	Little Canoe Creek, Coosa R, AL, 2011	0.001	0.006	0.989	0.003	0.001
CSB	RELitW_S3_01	Little Willis Creek, Coosa R, AL, 2011	0.001	0.009	0.979	0.01	0.002
CSB/ALB/CHB	RELitW_S3_02	Little Willis Creek, Coosa R, AL, 2011	0.103	0.139	0.745	0.01	0.003
CSB/CHB	RELitW_S3_03	Little Willis Creek, Coosa R, AL, 2011	0.005	0.033	0.952	0.007	0.003
CSB	RELitW_S3_04	Little Willis Creek, Coosa R, AL, 2011	0.001	0.013	0.98	0.005	0.002
CSB	RELitW_S3_05	Little Willis Creek, Coosa R, AL, 2011	0.004	0.007	0.97	0.005	0.014
CSB	RELitW_S3_06	Little Willis Creek, Coosa R, AL, 2011	0.001	0.009	0.982	0.006	0.002
CSB	RELitW_S3_07	Little Willis Creek, Coosa R, AL, 2011	0.001	0.01	0.974	0.01	0.005
CSB/CHB	RELitW_S3_08	Little Willis Creek, Coosa R, AL, 2011	0.001	0.032	0.959	0.006	0.002
CSB/ALB	RELR_S1_01	Little River, Coosa River, AL, 2011	0.292	0.014	0.683	0.004	0.007
CSB/ALB/CHB	RELR_S1_02	Little River, Coosa River, AL, 2011	0.128	0.029	0.812	0.009	0.022
CSB	RELR_S1_03	Little River, Coosa River, AL, 2011	0.001	0.006	0.986	0.006	0.001
CSB/CHB	RELR_S1_04	Little River, Coosa River, AL, 2011	0.002	0.317	0.658	0.006	0.016
CSB	RELR_S1_05	Little River, Coosa River, AL, 2011	0.097	0.009	0.889	0.004	0.002
CSB/ALB/CHB	RELR_S1_06	Little River, Coosa River, AL, 2011	0.246	0.07	0.677	0.006	0.002
CSB/ALB	RELR_S1_07	Little River, Coosa River, AL, 2011	0.351	0.009	0.61	0.007	0.023

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
CSB/ALB	RELR_S1_08	Little River, Coosa River, AL, 2011	0.074	0.009	0.891	0.009	0.016
CSB	RETerra_S1_01	Terrapin Creek, Coosa R, AL, 2011	0.001	0.005	0.99	0.003	0.001
CSB	RETerra_S1_02	Terrapin Creek, Coosa R, AL, 2011	0.001	0.008	0.986	0.004	0.001
CSB	RETerra_S1_03	Terrapin Creek, Coosa R, AL, 2011	0.001	0.007	0.969	0.019	0.003
CSB	RETerra_S1_04	Terrapin Creek, Coosa R, AL, 2011	0.001	0.005	0.987	0.006	0.001
CSB	RETerra_S1_05	Terrapin Creek, Coosa, AL, 2011	0.001	0.005	0.985	0.007	0.001
CSB	RETerra_S1_06	Terrapin Creek, Coosa R, AL, 2011	0.001	0.004	0.985	0.009	0.001
CSB/TLPB	RETerra_S1_08	Terrapin Creek, Coosa R, AL, 2011	0.001	0.003	0.942	0.052	0.001
CSB	RETerra_S1_09	Terrapin Creek, Coosa R, AL, 2011	0.001	0.006	0.989	0.003	0.001
CSB	REWal_S1_02	Walnut Creek, Coosa River, AL, 2011	0.001	0.103	0.866	0.028	0.003
CSB	REWal_S1_03	Walnut Creek, Coosa River, AL, 2011	0.001	0.007	0.988	0.003	0.001
CSB	REWal_S1_04	Walnut Creek, Coosa River, AL, 2011	0.001	0.005	0.99	0.003	0.001
CSB	REWal_S1_05	Walnut Creek, Coosa River, AL, 2011	0.003	0.005	0.986	0.003	0.003
CSB/ALB/TLPB	REWal_S1_06	Walnut Creek, Coosa River, AL, 2011	0.112	0.018	0.825	0.042	0.003
CSB	REWal_S1_07	Walnut Creek, Coosa River, AL, 2011	0.001	0.006	0.974	0.018	0.001
CSB	REWal_S1_08	Walnut Creek, Coosa River, AL, 2011	0.001	0.006	0.984	0.008	0.001
CSB/CHB	REWal_S1_09	Walnut Creek, Coosa River, AL, 2011	0.001	0.096	0.874	0.025	0.003
TLPB/ALB/CHB	SHB031	Chattahoochee River	0.156	0.056	0.036	0.749	0.003
TLPB	RECrokS1_03	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.005	0.005	0.989	0.001
TLPB	RECrokS1_04	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.007	0.005	0.986	0.001
TLPB	RECrokS1_05	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.006	0.007	0.984	0.002
TLPB	RECrokS1_06	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.005	0.005	0.987	0.002
TLPB	RECrokS1_07	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.005	0.004	0.989	0.002
TLPB	RECrokS1_08	Crooked Creek, Tallapoosa , AL, 2011	0.001	0.005	0.004	0.989	0.001
TLPB	RECrokS1_09	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.007	0.005	0.987	0.001
TLPB	RECrokS1_10	Crooked Creek, Tallapoosa, AL, 2011	0.001	0.008	0.004	0.987	0.001
TLPB	REEnita_S1_01	Enitachopco Creek, Tallapoosa, AL, 2011	0.001	0.012	0.005	0.98	0.001
TLPB/CHB	REEnita_S1_02	Enitachopco Creek, Tallapoosa, AL, 2011	0.002	0.038	0.004	0.947	0.009
TLPB	REEnita_S1_03	Enitachopco Creek, Tallapoosa, AL, 2011	0.001	0.009	0.005	0.984	0.001
TLPB	REEnita_S1_04	Enitachopco Creek, Tallapoosa, AL, 2011	0.001	0.005	0.003	0.99	0.002
TLPB/ALB	REEnita_S2_05	Enitachopco Creek, Tallapoosa, AL, 2011	0.094	0.005	0.004	0.886	0.011
TLPB	REEnita_S2_06	Enitachopco Creek, Tallapoosa, AL, 2011	0.001	0.006	0.003	0.988	0.002
TLPB	REEnita_S2_07	Enitachopco Creek, Tallapoosa, AL, 2011	0.001	0.005	0.004	0.988	0.002
TLPB/ALB	REEnita_S2_08	Enitachopco Creek, Tallapoosa, AL, 2011	0.586	0.01	0.003	0.39	0.011
TLPB	REHB_S1_01	Horseshoe Bend Creek, Tallapoosa, AL	0.002	0.007	0.004	0.985	0.001
TLPB/ALB	REHB_S1_02	Horseshoe Bend Creek, Tallapoosa, AL	0.106	0.006	0.005	0.882	0.001
TLPB	REHB_S1_03	Horseshoe Bend Creek, Tallapoosa, AL	0.001	0.005	0.005	0.987	0.002
TLPB/ALB	REHB_S1_04	Horseshoe Bend Creek, Tallapoosa, AL	0.17	0.006	0.004	0.819	0.002
TLPB/CHB	REHB_S1_05	Horseshoe Bend Creek, Tallapoosa, AL	0.008	0.185	0.01	0.785	0.012
TLPB	REHB_S1_06	Horseshoe Bend Creek, Tallapoosa, AL	0.004	0.007	0.004	0.98	0.004
TLPB/ALB/CHB/WRB	REHB_S1_07	Horseshoe Bend Creek, Tallapoosa, AL	0.047	0.047	0.005	0.877	0.025
TLPB	REHB_S1_09	Horseshoe Bend Creek, Tallapoosa, AL	0.009	0.006	0.006	0.976	0.003

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
TLPB	REMadi_S1_01	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.009	0.006	0.982	0.002
TLPB	REMadi_S1_02	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.007	0.005	0.985	0.002
TLPB	REMadi_S1_03	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.009	0.009	0.98	0.002
TLPB	REMadi_S1_04	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.01	0.007	0.98	0.001
TLPB	REMadi_S1_05	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.005	0.004	0.989	0.001
TLPB	REMadi_S1_06	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.007	0.007	0.984	0.002
TLPB	REMadi_S1_07	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.005	0.005	0.988	0.001
TLPB	REMadi_S1_08	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.016	0.016	0.965	0.002
TLPB	REMadi_S1_09	Mad Indian Creek, Tallapoosa, AL, 2011	0.002	0.01	0.008	0.978	0.001
TLPB	REMadi_S1_10	Mad Indian Creek, Tallapoosa, AL, 2011	0.001	0.006	0.005	0.987	0.001
TLPB	REPI_S1_01	Price Island Creek, Tallapoosa, AL, 2011	0.008	0.01	0.032	0.945	0.006
TLPB	REPI_S1_02(2)	Price Island Creek, Tallapoosa, AL, 2011	0.008	0.006	0.003	0.981	0.002
TLPB/CHB/CSB	REPI_S1_03(2)	Price Island Creek, Tallapoosa, AL, 2011	0.005	0.106	0.079	0.799	0.011
TLPB	REPI_S1_04	Price Island Creek, Tallapoosa, AL, 2011	0.002	0.011	0.007	0.978	0.002
TLPB	REPI_S1_05	Price Island Creek, Tallapoosa, AL, 2011	0.001	0.005	0.005	0.987	0.001
TLPB	REPI_S1_06(2)	Price Island Creek, Tallapoosa, AL, 2011	0.001	0.005	0.005	0.987	0.001
TLPB/ALB/CHB	REShol_S1_01	Shoal Creek, Tallapoosa River, AL, 2011	0.524	0.021	0.008	0.445	0.002
TLPB	REShol_S1_02	Shoal Creek, Tallapoosa River, AL, 2011	0.005	0.007	0.006	0.976	0.007
TLPB/ALB	REShol_S1_03	Shoal Creek, Tallapoosa River, AL, 2011	0.089	0.009	0.006	0.891	0.005
TLPB	REShol_S1_04	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.006	0.005	0.986	0.001
TLPB/CHB/CSB	REShol_S1_06	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.01	0.013	0.975	0.001
TLPB	REShol_S2_01	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.061	0.031	0.901	0.006
TLPB	REShol_S2_02	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.012	0.004	0.982	0.001
TLPB	REShol_S2_03	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.007	0.005	0.987	0.001
TLPB	REShol_S2_04	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.005	0.003	0.99	0.001
TLPB	REShol_S2_05	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.008	0.006	0.983	0.002
TLPB	REShol_S2_06	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.007	0.004	0.987	0.001
TLPB/CHB/CSB	REShol_S2_07	Shoal Creek, Tallapoosa River, AL, 2011	0.001	0.128	0.075	0.793	0.004
TLPB/ALB	REWAD_S1_01	Wadley Creek, Tallapoosa, AL, 2011	0.042	0.009	0.005	0.941	0.003
TLPB	REWAD_S1_02	Wadley Creek, Tallapoosa, AL, 2011	0.004	0.007	0.004	0.983	0.002
TLPB	REWAD_S1_03(2)	Wadley Creek, Tallapoosa, AL, 2011	0.001	0.004	0.003	0.989	0.002
TLPB	REWAD_S1_04(2)	Wadley Creek, Tallapoosa, AL, 2011	0.005	0.005	0.004	0.98	0.007
TLPB	ReTLR001	Tallapoosa River, AL (2016)	0.011	0.016	0.005	0.965	0.004
TLPB/ALB	ReTLR002	Tallapoosa River, AL (2016)	0.045	0.005	0.003	0.943	0.005
TLPB/CHB	ReTLR003	Tallapoosa River, AL (2016)	0.015	0.138	0.017	0.82	0.01
TLPB	ReTLR004	Tallapoosa River, AL (2016)	0.002	0.007	0.003	0.983	0.005
TLPB	ReTLR005	Tallapoosa River, AL (2016)	0.009	0.006	0.004	0.977	0.005
TLPB	ReTLR006	Tallapoosa River, AL (2016)	0.066	0.004	0.003	0.923	0.003
TLPB	ReTLR007	Tallapoosa River, AL (2016)	0.001	0.006	0.008	0.984	0.002
TLPB	ReTLR009	Tallapoosa River, AL (2016)	0.001	0.008	0.004	0.985	0.002
TLPB	ReTLR010	Tallapoosa River, AL (2016)	0.022	0.012	0.008	0.956	0.001
TLPB	ReTLR011	Tallapoosa River, AL (2016)	0.002	0.008	0.004	0.984	0.002

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
TLPB	ReTLR012	Tallapoosa River, AL (2016)	0.005	0.015	0.006	0.954	0.02
TLPB/ALB	ReTLR013	Tallapoosa River, AL (2016)	0.034	0.005	0.003	0.948	0.009
TLPB	ReTLR014	Tallapoosa River, AL (2016)	0.002	0.016	0.004	0.975	0.002
TLPB	ReTLR015	Tallapoosa River, AL (2016)	0.002	0.01	0.004	0.978	0.006
TLPB/CHB	ReTLR016	Tallapoosa River, AL (2016)	0.005	0.029	0.005	0.956	0.005
TLPB	ReTLR017	Tallapoosa River, AL (2016)	0.001	0.005	0.004	0.989	0.001
TLPB	ReTLR018	Tallapoosa River, AL (2016)	0.007	0.014	0.026	0.937	0.016
TLPB/ALB	ReTLR019	Tallapoosa River, AL (2016)	0.108	0.005	0.007	0.877	0.002
TLPB	ReTLR020	Tallapoosa River, AL (2016)	0.012	0.007	0.003	0.972	0.006
TLPB/CHB	ReTLR021	Tallapoosa River, AL (2016)	0.001	0.105	0.004	0.888	0.003
TLPB	ReTLR022	Tallapoosa River, AL (2016)	0.003	0.011	0.004	0.956	0.027
TLPB	ReTLR023	Tallapoosa River, AL (2016)	0.001	0.008	0.006	0.984	0.002
TLPB	ReTLR024	Tallapoosa River, AL (2016)	0.002	0.006	0.003	0.983	0.007
TLPB	ReTLR025	Tallapoosa River, AL (2016)	0.06	0.008	0.005	0.925	0.002
TLPB/ALB/CHB	ReTLR026	Tallapoosa River, AL (2016)	0.272	0.031	0.005	0.687	0.005
TLPB	ReTLR027	Tallapoosa River, AL (2016)	0.005	0.006	0.002	0.985	0.002
TLPB/ALB	ReTLR028	Tallapoosa River, AL (2016)	0.319	0.006	0.004	0.669	0.002
TLPB	ReTLR029	Tallapoosa River, AL (2016)	0.001	0.009	0.005	0.984	0.001
TLPB	ReTLR030	Tallapoosa River, AL (2016)	0.005	0.007	0.004	0.976	0.008
TLPB/ALB	ReTLR031	Tallapoosa River, AL (2016)	0.07	0.007	0.005	0.916	0.003
TLPB/ALB	ReTLR032	Tallapoosa River, AL (2016)	0.295	0.014	0.008	0.682	0.002
TLPB	ReTLR033	Tallapoosa River, AL (2016)	0.001	0.029	0.003	0.965	0.002
TLPB	ReTLR034	Tallapoosa River, AL (2016)	0.002	0.004	0.002	0.988	0.003
WRB	WRBBBF001	Blackburn Fork	0.023	0.002	0.001	0.001	0.973
WRB	WRBBBF002	Blackburn Fork	0.009	0.005	0.003	0.001	0.982
WRB/ALB	WRBBBF003	Blackburn Fork	0.242	0.006	0.003	0.004	0.745
WRB	WRBBBF004	Blackburn Fork	0.001	0.002	0.002	0.002	0.995
WRB/ALB	WRBBBF005	Blackburn Fork	0.091	0.002	0.002	0.005	0.901
WRB/ALB	WRBBBF006	Blackburn Fork	0.121	0.005	0.004	0.002	0.868
WRB/CHB/CSB	WRBBBF007	Blackburn Fork	0.002	0.053	0.022	0.006	0.917
WRB/ALB	WRBBBF008	Blackburn Fork	0.098	0.01	0.012	0.002	0.879
WRB	WRBBBF009	Blackburn Fork	0.023	0.001	0.001	0.001	0.973
WRB/ALB	WRBBBF010	Blackburn Fork	0.09	0.002	0.002	0.002	0.905
WRB/ALB	WRBBBF011	Blackburn Fork	0.248	0.002	0.002	0.002	0.746
WRB	WRBBBF012	Blackburn Fork	0.001	0.002	0.002	0.002	0.992
WRB/ALB	WRBBBF013	Blackburn Fork	0.294	0.001	0.002	0.002	0.701
WRB/ALB	WRBBBF014	Blackburn Fork	0.233	0.003	0.002	0.002	0.759
WRB/ALB	WRBBBF015	Blackburn Fork	0.12	0.001	0.001	0.001	0.876
WRB	WRBBBF016	Blackburn Fork	0.001	0.001	0.001	0.001	0.994
WRB/ALB	WRBBBF017	Blackburn Fork	0.35	0.004	0.004	0.003	0.639
WRB/ALB	WRBBBF018	Blackburn Fork	0.362	0.002	0.001	0.003	0.632
WRB/ALB	WRBBBF019	Blackburn Fork	0.164	0.003	0.002	0.007	0.824

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
WRB/ALB	WRBBBF020	Blackburn Fork	0.107	0.002	0.002	0.002	0.886
WRB	WRBBBF021	Blackburn Fork	0.001	0.002	0.001	0.001	0.995
WRB	WRBBBF022	Blackburn Fork	0.004	0.006	0.003	0.004	0.983
WRB	WRBBBF023	Blackburn Fork	0.001	0.001	0.001	0.001	0.995
WRB/ALB	WRBBBF024	Blackburn Fork	0.103	0.002	0.002	0.002	0.891
WRB/ALB	WRBBBF025	Blackburn Fork	0.335	0.001	0.001	0.002	0.661
WRB	WRBBLC001	Blue Creek	0.012	0.005	0.003	0.003	0.976
WRB	WRBBLC002	Blue Creek	0.004	0.001	0.002	0.001	0.992
WRB/ALB	WRBBLC003	Blue Creek	0.245	0.001	0.001	0.001	0.75
WRB	WRBBLC004	Blue Creek	0.001	0.002	0.002	0.002	0.993
WRB	WRBBLC005	Blue Creek	0.001	0.008	0.011	0.006	0.975
WRB	WRBBLC006	Blue Creek	0.001	0.002	0.003	0.002	0.992
WRB	WRBBLC007	Blue Creek	0.006	0.003	0.003	0.004	0.984
WRB	WRBBLC008	Blue Creek	0.002	0.005	0.004	0.003	0.986
WRB	WRBBLC009	Blue Creek	0.016	0.007	0.008	0.006	0.963
WRB	WRBBLC010	Blue Creek	0.001	0.002	0.001	0.001	0.995
WRB	WRBBLC011	Blue Creek	0.008	0.001	0.001	0.001	0.989
WRB	WRBBLC012	Blue Creek	0.001	0.003	0.004	0.002	0.989
WRB	WRBBLC013	Blue Creek	0.013	0.002	0.002	0.002	0.982
WRB	WRBBOC001	Border Creek	0.001	0.003	0.005	0.003	0.987
WRB	WRBBOC002	Border Creek	0.002	0.005	0.007	0.004	0.982
WRB	WRBBOC003	Border Creek	0.001	0.004	0.005	0.004	0.986
WRB	WRBBOC004	Border Creek	0.003	0.008	0.008	0.005	0.977
WRB	WRBBOC005	Border Creek	0.001	0.002	0.003	0.003	0.992
WRB	WRBBOC006	Border Creek	0.001	0.001	0.002	0.001	0.995
WRB	WRBBOC007	Border Creek	0.001	0.005	0.003	0.004	0.987
WRB	WRBBOC008	Border Creek	0.001	0.007	0.006	0.005	0.981
WRB	WRBBOC010	Border Creek	0.001	0.009	0.009	0.007	0.974
WRB	WRBSSF001	Sipsey Fork	0.003	0.003	0.004	0.002	0.988
WRB/CSB	WRBSSF002	Sipsey Fork	0.001	0.017	0.028	0.015	0.94
WRB	WRBSSF003	Sipsey Fork	0.001	0.001	0.001	0.001	0.995
WRB	WRBSSF004	Sipsey Fork	0.003	0.008	0.009	0.007	0.974
WRB/ALB	WRBSSF005	Sipsey Fork	0.097	0.001	0.001	0.002	0.899
WRB	WRBSSF006	Sipsey Fork	0.001	0.002	0.002	0.003	0.992
WRB	WRBSSF007	Sipsey Fork	0.004	0.003	0.003	0.004	0.986
WRB/CSB/TLPSB/CHB	WRBSSF008	Sipsey Fork	0.002	0.028	0.024	0.027	0.919
WRB	WRBSSF009	Sipsey Fork	0.004	0.018	0.006	0.004	0.967
WRB	WRBSSF010	Sipsey Fork	0.001	0.001	0.001	0.001	0.995
WRB/ALB	WRBSSF011	Sipsey Fork	0.1	0.002	0.002	0.003	0.893
WRB/CHB/CSB/TLPB	WRBTKC001	Turkey Creek	0.001	0.379	0.041	0.079	0.5
WRB/CHB/CSB/TLPB	WRBTKC002	Turkey Creek	0.001	0.128	0.463	0.111	0.298
WRB/CHB/CSB	WRBTKC003	Turkey Creek	0.001	0.063	0.453	0.007	0.476

SPECIES	SAMPLE_NAME	SOURCE	ALB	CHB	CSB	TLPB	WRB
WRB/CSB	WRBTKC004	Turkey Creek	0.001	0.008	0.55	0.007	0.434
WRB/CHB/CSB	WRBTKC005	Turkey Creek	0.001	0.138	0.423	0.008	0.43
WRB/CHB/CSB	WRBTKC006	Turkey Creek	0.001	0.603	0.078	0.017	0.301
WRB/CHB/CSB	WRBTKC007	Turkey Creek	0.001	0.088	0.425	0.01	0.476
WRB/CHB/CSB/TLPB	WRBTKC008	Turkey Creek	0.001	0.048	0.053	0.465	0.433
WRB/CHB/CSB/TLPB	WRBTKC009	Turkey Creek	0.005	0.303	0.131	0.021	0.54