Shoal Bass Hybridization in the Chattahoochee River Basin near Atlanta, Georgia

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Abstract: The shoal bass (Micropterus cataractae) is a sportfish endemic to the Apalachicola-Chattahoochee-Flint Basin of the southeastern United States. Introgression with several non-native congeners poses a pertinent threat to shoal bass conservation, particularly in the altered habitats of the Chattahoochee River. Our primary objective was to characterize hybridization in shoal bass populations near Atlanta, Georgia, including a population inhabiting Big Creek and another in the main stem Chattahoochee River below Morgan Falls Dam (MFD). A secondary objective was to examine the accuracy of phenotypic identifications below MFD based on a simplified suite of characters examined in the field. Fish were genotyped with 16 microsatellite DNA markers, and results demonstrated that at least four black bass species were involved in introgressive hybridization. Of 62 fish genotyped from Big Creek, 27% were pure shoal bass and 65% represented either F_1 hybrids of shoal bass \times smallmouth bass (*M. dolomieu*) or unidirectional backcrosses towards shoal bass. Of 29 fish genotyped below MFD and downstream at Cochran Shoals, 45% were pure shoal bass. Six hybrid shoal bass included both F₁ hybrids and backcrosses with non-natives including Alabama bass (M. henshalli), spotted bass (M. punctulatus), and smallmouth bass. Shoal bass alleles comprised only 21% of the overall genomic composition in Big Creek and 31% below MFD (when combined with Cochran Shoals). Phenotypic identification below MFD resulted in an overall correct classification rate of 86% when discerning pure shoal bass from all other non-natives and hybrids. Results suggest that although these two shoal bass populations feature some of the highest introgression rates documented, only a fleeting opportunity may exist to conserve pure shoal bass in both populations. Continued supplemental stocking of pure shoal bass below MFD appears warranted to thwart increased admixture among multiple black bass taxa, and a similar stocking program could benefit the Big Creek population. Further, selective removal of non-natives and hybrids, which appears to be practical with phenotypic identification, may provide increased benefits towards conserving genetic integrity of these shoal bass populations.

Key words: introgression, Micropterus cataractae, non-native species, supplemental stocking

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The shoal bass (*Micropterus cataractae*) is a sportfish endemic to the Apalachicola-Chattahoochee-Flint (ACF) Basin of the southeastern United States (Williams and Burgess 1999). Unlike largemouth bass (*M. salmoides*), spotted bass (*M. punctulatus*), and other widely distributed black bass species, shoal bass are not known to persist within impoundments or other lentic habitats (Williams and Burgess 1999, Sammons et al. 2015). Shoal bass inhabit medium-to-large streams and are typically encountered near shoal habitats that feature increased flow velocities and large, rocky substrates (Taylor and Peterson 2014, Sammons et al. 2015). Several robust shoal bass populations, like those in the upper and lower Flint River, Georgia, have gained regional and national attention among angling groups (Taylor and Peterson 2014, Sammons et al. 2015). However, the species is considered vulnerable

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to extinction within its native range because of continued range loss (Jelks et al. 2008, Taylor and Peterson 2014). The shoal bass is listed in the 2015 Georgia State Wildlife Action Plan as a High Priority Species and a Species of Special Concern, but the species is not currently state- or federally-listed as threatened or endangered (Albanese et al. 2015).

Habitat degradation, fragmentation, and introgression with non-native congeners are all considered threats to shoal bass conservation (Taylor and Peterson 2014, Sammons et al. 2015), but introgressive hybridization with non-native congeners appears to be the most pertinent threat (Alvarez et al. 2015, Dakin et al. 2015, Tringali et al. 2015a). Hybridization is a natural phenomenon among sympatric black bass species, but when species are introduced outside their native ranges into artificial sympatry, weak reproductive barriers often result in widespread introgressive hybridization (Koppelman 2015). Long-term outcomes of introgressive hybridization are usually uncertain because they are influenced by a number of factors including selective pressures, migration, and competitive interactions (Allendorf et al. 2001, Koppelman 2015). Habitat alteration can magnify the threat of hybridization by compromising reproductive boundaries and potentially increasing relative fitness of hybrid individuals (Bangs et al. 2017). For example, dam-related alterations to riverine habitats appear to facilitate hybridization among fish species (Pringle et al. 2000, Bangs et al. 2017) as do alterations to water clarity or habitat availability (Seehausen et al. 1997, Behm et al. 2010). Severe cases of hybridization among black bass species have resulted in loss of fitness of native species (i.e., outbreeding depression; Goldberg et al. 2005) and hybrid swarms that resulted in loss of pure native species (Avise et al. 1997, Pierce and Van Den Avyle 1997, Allendorf et al. 2001).

The Chattahoochee River Basin within the metropolitan area of Atlanta, Georgia, supports two remnant populations of shoal bass, but dams and urbanization have altered riverine habitats in the basin. Morgan Falls Dam (MFD) was completed as a hydropower dam in 1904, but the completion of Buford Dam upstream in 1956 created hypolimnetic, coldwater releases that led to the elimination of many warmwater fish species in the main stem Chattahoochee River for 77 km downstream of Buford Dam, including 19 km below MFD (Long and Martin 2008). A 4th-order tributary of the Chattahoochee River above MFD, Big Creek supports a shoal bass population within a 2-km reach between Roswell Mill Dam (completed in the 1830s) and its confluence with the cold waters of the main stem Chattahoochee River (Long and Martin 2008, Dakin et al. 2015). Increased impervious surfaces and development associated with urbanization in Big Creek's watershed has led to increased flashiness of streamflow, decreased groundwater recharge, and increased sedimentation (Rose and Peters 2001, Long and Martin 2008). Shoal bass also persisted in low abundance within the cold waters of the main stem Chattahoochee River below MFD, but beginning in 1989, urban runoff created warmer water temperatures below MFD and spurred public interest in reestablishing a shoal bass fishery (Long and Martin 2008, Dakin et al. 2015). From 2003 to 2007, the Georgia Department of Natural Resources (GADNR) and National Park Service stocked fingerling shoal bass in a 14-km reach below MFD to provide additional sport fishing opportunities and restore the shoal bass population to historical levels (Long and Martin 2008, Porta and Long 2015). The stocking program was successful at increasing local abundance of shoal bass (Porta and Long 2015), and in 2011 GADNR resumed annual stocking of fingerlings to supplement natural reproduction (P. O'Rouke, GADNR, personal communication).

The Chattahoochee River Basin near Atlanta also harbors a relatively high richness of introduced congener species, heightening concerns that introgressive hybridization may confound conservation of the two shoal bass populations in the area. Spotted bass, Alabama bass (M. henshalli), and their hybrids are known from areas surrounding MFD, with spotted bass likely originating from introduced populations downstream and Alabama bass likely originating from introduced populations upstream (Williams and Burgess 1999, Baker et al. 2008). Perhaps most alarming was a series of illegal introductions of smallmouth bass (M. dolomieu) by an angler into the Chattahoochee River below MFD from 2004 to 2006, and an apparently concurrent-but-undocumented introduction of smallmouth bass above MFD (Dakin et al. 2015). Phenotypic identification of smallmouth bass and detection of hybrids among shoal bass can be difficult (Taylor and Peterson 2014), and the thermally-depressed waters of the main stem Chattahoochee River might favor smallmouth bass because they naturally occur in cooler waters (Boschung and Mayden 2004). Dakin et al. (2015) performed a pre-stocking genetic assessment of shoal bass hybridization below MFD in 2005 and documented a markedly higher incidence of hybrid individuals (51% of total sample) than found in similar studies in the lower Flint River (18%; Alvarez et al. 2015) and the Chipola River (16%; Tringali et al. 2015a), suggesting introgression of non-native alleles poses an especially pertinent threat to shoal bass conservation in the altered habitats of the Chattahoochee River Basin near Atlanta.

In the present study, our primary objective was to characterize recent hybridization in Big Creek and evaluate post-stocking hybridization in the Chattahoochee River below MFD. The relatively high richness of black bass species known to occur below MFD presented a unique opportunity to address a secondary objective of examining the accuracy of field-based phenotypic identifications using a simplified suite of characteristics. Whether or not phenotypic identification remains accurate among a mixture of several native and introduced black bass species is unknown, but important to assess because managers may wish to perform targeted removals of non-natives and hybrids in the future as a management action to conserve shoal bass.

Methods

Collection and Phenotypic Identification

Black bass were collected in Big Creek on 13 and 15 May 2015 wherein a team of backpack electrofishers sampled 1 km of wadeable shoal habitat immediately downstream of Roswell Mill Dam (Taylor 2017). We used a boat-mounted electrofisher to sample black bass at two sites on the Chattahoochee River on 11 May 2015: one immediately downstream of MFD (latitude 33.96643,

 Table 1. A simplified suite of phenotypic characteristics that are representative of a 'typical' specimen of each black bass species (or species group) that was used to perform field identification of specimens obtained from the Chattahoochee River. Data were compiled from Etnier and Starnes (1993), Williams and Burgess (1999), Boschung and Mayden (2004), Baker et al. (2008), Baker et al. (2013), Freeman et al. (2015), and Tringali et al. (2015b).

Taxon	Blotch patterns	Body coloration	Upper maxilla extends beyond eye	Dorsal fin morphology	Tooth patch on tongue	Second dorsal fin ray count
largemouth bass	variable to non-existent mid-lateral blotching	green dorsal, white ventral	yes	deeply-notched or separate	no	not considered
Alabama bass, spotted bass, or Choctaw bass	distinguishable blotches form a mid-lateral stripe	green dorsal, white ventral; horizontal spotting below mid-lateral stripe	no	connected	yes	not considered
redeye bass, Chattahoochee bass, or Bartram's bass	variable to non-existent mid-lateral blotching	white or orange-to-red margins on posterior fins; silver-white crescent on posterior half of eyelid	no	connected	usually yes	not considered
smallmouth bass	vertically-elongated mid-lateral blotches	brown-to-bronze dorsal, dark grey ventral	no	connected	usually yes ^a	usually 14 (range: 13—15)
shoal bass	vertically-elongated mid-lateral blotches	green-to-gold dorsal, white ventral	no	connected	no	usually 12 (range: 10—13)

a. This trait likely varies regionally, but is consistent with specimens from our study area that likely originated from the Tennessee River system (Lake Blue Ridge; Dakin et al. 2015)

longitude -84.382844) and another approximately 9 km downstream at the upstream extent of Cochran Shoals (33.910727, -84.447561). Fish featuring phenotypic characters fully consistent with largemouth bass were released because largemouth bass are naturally sympatric with shoal bass and are not typically involved in elevated rates of hybridization (Alvarez et al. 2015, Tringali et al. 2015a, Bangs et al. 2017). For all other black bass specimens, we recorded total length (TL; mm), stored fin-clips in individual vials of 95% ethanol, and released specimens near their site of capture. For specimens captured at both Chattahoochee River sites, we also recorded a phenotypic identification and took a photograph prior to release. Phenotypic identification was performed based on a simplified suite of six morphological, meristic, and pigmentation characters (Table 1) that are representative of 'typical' specimens of each species, as compiled from various species descriptions and keys (Table 1; Etnier and Starnes 1993, Williams and Burgess 1999, Boschung and Mayden 2004, Baker et al. 2008, Baker et al. 2013, Freeman et al. 2015, Tringali et al. 2015b). Three biologists identified each specimen in concert until an agreement was reached; the number of soft dorsal rays was only considered when distinguishing smallmouth bass from shoal bass because of the difficulty in distinguishing these species. Specimens with intermediate characteristics of species were identified as hybrids.

Genetic Identification

Genotyping.—Total genomic DNA was isolated from fin-clips using the Puregene DNA Purification Kit (Gentra Systems). Specimens were genotyped using 16 di-nucleotide microsatellite DNA markers that were developed to amplify across *Micropterus* species (*Msaf* 05, 06, 08, 09, 10, 12, 13, 17, 22, 24, 25, 27, 28, 29, 31, and 32; Seyoum et al. 2013). Six multiplex polymerase chain reactions (PCR) amplified microsatellites, using parameters specified by Alvarez et al. (2015).

Taxonomic assignment.-We used a Bayesian clustering approach and reference genotypes to produce proportional assignments of sampled specimens. Reference genotypes for black bass taxa known to occur in the ACF Basin included: largemouth bass (n=62), Alabama bass (n=63), spotted bass (n=69), and smallmouth bass (n=41; for reference localities, see Tringali et al. 2015b). Within the ACF Basin, shoal bass are naturally sympatric with largemouth bass and Chattahoochee bass (M. chattahoochae; formerly considered redeye bass M. coosae; Baker et al. 2013), and low hybridization rates among these species is likely natural (see Alvarez et al. 2015, Tringali et al. 2015a). Chattahoochee bass reference genotypes were unavailable, but we included reference genotypes of a nominal sister taxon, "Bartram's bass" M. sp. cf. cataractae (n = 15; Freeman et al. 2015), to improve our ability to detect Chattahoochee bass alleles. We also included a nominal non-native form, "Choctaw bass" (*M*. sp. cf. *punctulatus*; n = 56), that visually resembles spotted bass and has been introduced into the ACF Basin within the Chipola River, Florida (Tringali et al. 2015b). Shoal bass reference genotypes were obtained from throughout the ACF Basin (*n* = 55; Taylor 2017).

We used Bayesian clustering models in Program Structure v. 2.3.4 (Pritchard et al. 2000) and supervised estimators to determine the number of genetic clusters (K) among all reference genotypes (Puechmaille 2016). Program Structure proportionally assigns individual genotypes to K based on conformance to Hardy-Weinberg and linkage equilibrium, assigning genotypes probabilistically to populations with some degree of uncertainty (Pritchard et

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independent runs each of K=1 through K=15. For each run, we used a burn-in of 20,000 and 200,000 Markov chain Monte Carlo iterations and assumed the admixture model and independent allele frequencies among taxa. We estimated K using a suite of four supervised estimators (MedMeaK, MaxMeaK, MedMedK, and MaxMedK) that disregard 'spurious' clusters that do not obtain a mean or median membership coefficient threshold of ≥ 0.5 within taxonomic groupings (Puechmaille 2016). The maximum value of the estimators obtained across all model runs was adopted as the optimal *K* among the reference genotypes.

Finally, we estimated proportional taxonomic assignments of genotypes collected below MFD using Program Structure, reference genotypes, and the K value identified among reference genotypes. We performed 20 independent model runs using the 'PopFlag' option and the model settings detailed previously to assign the unknown genotypes collected below MFD to taxon based solely on the allele frequencies within reference genotypes (Pritchard et al. 2000). Because we were not interested in incorporating intra-taxon genetic variation in our taxonomic assignments, we only included 20 runs wherein estimated clusters aligned with taxonomic boundaries (i.e., not including runs that instead identified intra-specific variation within reference genotypes; sensu Puechmaille 2016). We then obtained the optimal alignment of independent Program Structure runs using a cluster matching and permutation algorithm in Program Clumpp v. 1.1.2 (Jakobsson and Rosenberg 2007) using the 'LargeKGreedy' algorithm and the G' pairwise matrix similarity statistic from 1000 randomly-sequenced runs. We followed Dakin et al. (2015) to classify individuals as pure (≥0.90 assignment to one respective taxon), backcrossed (approximately 0.75–0.90 assignment to one taxon), or F₁ or later-generation hybrid (remaining individuals). We combined all individual assignments by location to estimate the overall percentage of the genome each taxa comprised in the sample population. Any individual assignments that were suspected to reflect assignment uncertainty were examined for missing allele scores and private alleles not represented in the reference specimens.

Identification Comparison

To determine the error and accuracy associated with phenotypic identification, we compared it with genetic identification using coarse-scale identity categories (pure shoal bass or non-native/ hybrid) that could be rapidly used in the field as a way to target and remove non-native alleles. Phenotypic characters are inappropriate for quantifying individual- and population-level introgression because hybrids do not necessarily feature intermediate phenotypes of parental species (Allendorf et al. 2001, Koppelman

2015). However, phenotypic characteristics can identify black bass species and recent hybrids (i.e., first filial generation; F₁) with some degree of accuracy (see Whitmore 1983, Pipas and Bulow 1998). An error matrix was used to compare phenotypic identifications (i.e., predicted) to genetic identifications (i.e., actual), and we calculated several measures of accuracy (e.g., correct classification rate) and error (e.g., sensitivity and specificity; Fielding and Bell 1997). Specimen photographs, along with genetic identification descriptions, were uploaded to the Figshare online repository and are available at the following address: <https://doi.org/10.6084/m9 .figshare.5633368.v1>.

Results

In Big Creek, we collected 62 black bass specimens (mean TL=154 mm; SD=101 mm). Big Creek specimens were not formally identified using our suite of phenotypic characters; however, all specimens resembled shoal bass except one fish that appeared to be an Alabama bass. In the Chattahoochee River, 9 black bass were collected immediately below MFD (mean TL=315X mm; SD=92 mm) and an additional 20 were collected from Cochran Shoals (mean TL = 297 mm; SD = 99 mm). Field-based phenotypic identifications took approximately 30 to 90 sec per specimen, with fish that resembled smallmouth bass or shoal bass taking the longest to distinguish using dorsal ray counts. Phenotypic identifications below MFD yielded 2 shoal bass, 3 Alabama/spotted/ Choctaw bass, 1 smallmouth bass, and 3 hybrids; whereas, phenotypic identification at Cochran Shoals produced 18 shoal bass, 1 Alabama/spotted/Choctaw bass, and 1 hybrid.

Genetic Identification

Supervised estimators suggested there were K=7 genetic clusters among the reference genotypes, and those clusters aligned well with the taxa included. Taxonomic assignment of Big Creek specimens revealed a few pure shoal bass (n = 17; 27%), but the majority were shoal bass \times smallmouth bass hybrids (n = 40; 65%) that represented both unidirectional backcrosses towards shoal bass (n=31) and F_1 or later-generation hybrids (n=9; Table 2, Figure 1). Four specimens had individual assignments of 11%-40% Alabama bass alleles, yet these hybrids also contained shoal bass and smallmouth bass alleles. Individual assignments featuring Spotted bass alleles were notably rare, though one individual was assigned to 4% spotted bass and 11% Choctaw bass and another individual representing a shoal bass × smallmouth bass × Alabama bass was also estimated to contain 6% Choctaw bass alleles. In the overall population sample at Big Creek, shoal bass alleles comprised 79% of the sample, whereas smallmouth bass comprised 18%, Alabama bass comprised 2%, and Choctaw bass comprised 1%.

Table 2. Genetic classification of black bass specimens sampled in several sites within the Chattahoochee River Basin near Roswell, Georgia, based on genotyping with 16 microsatellite DNA markers. Classifications included pure individuals, backcrossed ("BC") individuals, and F₁ or later-generation (gen.) hybrids. For BC classifications, the taxon comprising the majority of the assignment is listed first. Assignments with "multiple" taxa may have included Choctaw bass and Bartram's bass, mixed with other listed taxa.

Genetic classification	n
Big Creek	
Pure shoal bass	17
BC shoal bass x smallmouth bass	31
BC shoal bass x Choctaw bass	1
F ₁ (or later gen.) shoal bass x smallmouth bass	9
F_{1} (or later gen.) shoal bass x smallmouth bass x Alabama bass	4
Chattahoochee River below Morgan Falls Dam	
Pure shoal bass	1
Pure Alabama bass	1
Pure smallmouth bass	1
BC shoal bass x smallmouth bass	1
F ₁ (or later gen.) shoal bass x smallmouth bass	1
F ₁ (or later gen.) shoal bass x spotted bass	1
F_1 (or later gen.) smallmouth bass x spotted bass	1
F ₁ (or later gen.) Alabama bass x spotted bass	1
F ₁ (or later gen.) Alabama bass x multiple	1
Chattahoochee River at Cochran Shoals	
Pure shoal bass	15
BC shoal bass x Alabama bass	1
BC smallmouth bass x spotted bass	1
F ₁ (or later gen.) shoal bass x Alabama bass	2
F ₁ (or later gen.) shoal bass x multiple	1

Taxonomic assignment of specimens from the Chattahoochee River revealed spatial differences in hybridization. Below MFD, at least four black bass taxa were involved in introgressive hybridization. One pure shoal bass, smallmouth bass, and Alabama bass were recovered (Table 2; Figure 1); each represented 11% of the population sample. The remaining six specimens (67% of sample) were F₁ or later-generation hybrids or backcrosses. Although no pure spotted bass were encountered, spotted bass alleles comprised 15%-49% of individual proportional assignments of hybrids. One aberrant hybrid individual also was estimated to contain 8% Choctaw bass alleles and 2% Bartram's bass alleles. The overall population sample below MFD was comprised of 35% shoal bass alleles, 24% Alabama bass, 24% smallmouth bass, 15% spotted bass, 1% "Bartram's bass," and 1% "Choctaw bass." Downstream at Cochran Shoals, pure shoal bass dominated the sample (n=15; 75%), and shoal bass hybrids with Alabama bass (F1 and backcrosses towards



Figure 1. Proportional taxonomic assignment of 62 black bass specimens from Big Creek and 29 from the Chattahoochee River below Morgan Falls Dam (MFD), Georgia, based on genotyping with 16 microsatellite DNA markers. Inset (a) illustrates assignment of reference genotypes to seven genetic clusters (largemouth bass, "LMB"; Alabama bass, "ALB"; spotted bass, "SPB"; Choctaw bass, "CTB"; smallmouth bass, "SMB"; Bartram's bass, "BAR"; and shoal bass, "SHB"). Inset (b) depicts the proportional assignment of Big Creek specimens. Inset (c) depicts the proportional assignment of 29 specimens from the Chattahoochee River (1–9 from below MFD; 10–29 from Cochran Shoals), wherein sample numbers correspond with photographs available online at <https://doi.org/10.6084/m9.figshare.5633368.v1>.

shoal bass) were second-most common (n=3; 15%). One hybrid specimen estimated to contain mostly smallmouth bass (45%) and shoal bass (40%) alleles was also estimated to contain 9% Choctaw bass alleles and 2% Bartram's bass alleles. The overall population sample at Cochran Shoals was comprised of 84% shoal bass alleles, 7% Alabama bass, 6% smallmouth bass, 1% spotted bass, and 1% "Choctaw bass." When specimens from below MFD and Cochran Shoals were combined, the overall population sample was comprised of 69% shoal bass alleles, 12% smallmouth bass, 12% Alabama bass, 6% spotted bass, and 1% "Choctaw bass."

Because Choctaw bass and Bartram's bass were not previously known from this area, we investigated the allele scores of the four specimens assigned to either or both taxa. One specimen from Big Creek had missing allele scores for *Msa* 12, but this locus was largely fixed across all taxa examined in this study. None of the four specimens contained private alleles among the reference genotypes, and allele scores for these four specimens aligned with Choctaw bass and Bartram's bass reference alleles at some loci. A determination of whether these specimens actually represent hybrids containing Choctaw bass, Bartram's bass, or a different black bass taxon not included in the reference genotypes (i.e., native Chattahoochee bass) is beyond the scope of this study, but of these, only Chattahoochee bass is known from this system.

Identification Comparison

Comparisons between genetic identifications and phenotypic identifications at the two Chattahoochee River sites suggested that phenotypic identifications provided a reasonably accurate means of distinguishing pure shoal bass from non-natives and hybrids. The resulting error matrix revealed 16 fish were correctly field-identified as pure shoal bass, 9 fish were correctly field-identified as non-natives or hybrids, 4 fish were incorrectly field-identified as pure shoal bass but were actually non-natives or hybrids (specifically, these 4 specimens were non-native backcrosses toward shoal bass), and 0 fish were incorrectly field-identified as non-natives or hybrids but were actually pure shoal bass. Field-identification produced an overall correct classification rate of 86%, with a sensitivity of 100% (i.e., probability that pure shoal bass were correctly identified by phenotype) and a specificity of 69% (i.e., the probability that non-natives and hybrids were correctly identified by phenotype). A complementary conceptualization of these results is that the phenotypic identification method produced a false positive rate of 31% and a false negative rate of 0%. Phenotypic identification of shoal bass produced a positive predictive power of 80% (i.e., the probability that phenotypic identifications of pure shoal bass were accurate) and phenotypic identification of non-natives and hybrids produced a negative predictive power of 100% (i.e., the probability that phenotypic identifications of non-natives and hybrids were accurate). The 20 specimens identified as pure shoal bass based on phenotypic traits were estimated to contain 88% shoal bass alleles. If field-identified non-natives and hybrids had been removed, the non-shoal bass alleles in the sample would have decreased by 63% (i.e., from a non-shoal bass genomic composition of 31% to 12%) with no pure shoal bass removed. Photographs taken during phenotypic identification illustrated the traits of a genetically confirmed pure shoal bass, shoal bass × smallmouth bass F₁ hybrid, and pure smallmouth bass (Figure 2). One of the specimens (#4 in Figure 2) that was estimated to contain Choctaw bass and Bartram's bass alleles was phenotypically identified as a hybrid; however, the photograph of this specimen depicted some phenotypic traits typical of native Chattahoochee bass (or non-native redeye bass or Bartram's bass), including a silver-white crescent on the posterior half of the eyelid and a prominent white margin on the anal fin (Figure 3).



Figure 2. Photographs capturing the phenotypic traits of genetically confirmed (a) pure shoal bass, (b) F₁ smallmouth x shoal bass hybrid, and (c) pure smallmouth bass from the Chattahoochee River below Morgan Falls Dam, Georgia.



Figure 3. Photograph of a hybrid specimen (#4 in Figure 1) that appeared to contain Choctaw bass and Bartram's bass alleles based on genetic assignment, but phenotypic characters corresponded with native Chattahoochee Bass, including a silver-white crescent on the posterior half of the eyelid and a prominent white margin on the anal fin.

Discussion

The results of this study confirmed that the two shoal bass populations in the Chattahoochee River Basin near Atlanta, Georgia, contained abnormally high percentages of non-native and hybrid individuals (73% Big Creek; 45% total between MFD and Cochran Shoals) and abnormally high genomic compositions of non-native alleles (21% Big Creek; 31% total between MFD and Cochran Shoals). Noticeably lower percentages of hybrid individuals (16%-18%) and overall genomic composition of non-native alleles (1%-7%) have been documented in shoal bass populations of the lower Flint River and the Chipola River (Alvarez et al. 2015, Tringali et al. 2015a). Our results also verified that at least four black bass taxa are involved in introgressive hybridization below MFD, including native shoal bass and non-native smallmouth bass, Alabama bass, and spotted bass. Additional taxa, including non-native "Choctaw bass," non-native "Bartram's bass," and native Chattahoochee bass may have also been involved in hybridization. Phenotypic characteristics suggested that some of the fish with aberrant genetic assignments may have represented hybrids with native Chattahoochee bass, but we were unable to arrive at an exact determination because we lacked Chattahoochee bass reference genotypes in our genetic assignment. Despite ongoing introgression in both populations, pure shoal bass were identified in both populations and the majority of backcrossing was unidirectional towards shoal bass, indicating that fleeting opportunity exists to conserve the genetic integrity of both shoal bass populations.

The high amounts of hybridization observed in Big Creek between shoal bass and smallmouth bass was unexpected. In a 2005 sample, Dakin et al. (2015) observed 2 pure smallmouth bass and 1 shoal bass \times smallmouth bass F₁ hybrid along with 25 pure shoal bass. At that time, Dakin et al. (2015) suggested that the Big Creek shoal bass population could be more easily restored to genetic purity because of its presumed isolation from other black bass populations and its restriction to a small, 2-km stream reach. Ten years later, our results demonstrated that the invasion of smallmouth bass into Big Creek had a disproportionately large effect on the genetic integrity of shoal bass, perhaps because the shoal bass population in Big Creek is characterized by low adult abundance and suffers from variable recruitment that is likely linked to flashy hydrology (Taylor 2017). The relative lack of spotted bass alleles in Big Creek suggests that MFD may delineate the species' upstream invasion extent; whereas, Alabama bass alleles have apparently dispersed downstream from Lake Lanier into the Big Creek population and farther downstream to Cochran Shoals (Williams and Burgess 1999). Regardless of the exact mechanisms influencing introgression in the Big Creek population, the diminished number of pure shoal bass may represent the imminent loss of unique genetic diversity harbored in the Big Creek population (see Dakin et al. 2015) if conservation actions are not taken. Further study of the invasion of smallmouth bass and subsequent hybridization in Big Creek could provide novel insights into the mechanisms that are causing shoal bass population declines in tributary streams throughout the fragmented Chattahoochee River Basin (Taylor and Peterson 2014, Sammons et al. 2015).

Spatial differences in the distribution of shoal bass versus non-natives and hybrids were evident in the main stem Chattahoochee River. Immediately below MFD, shoal bass had the lowest genomic composition of all three areas sampled in this study. Despite a low sample size below MFD, our results indicated that more hybrid individuals occurred in the altered habitats immediately below the dam than farther downstream at Cochran Shoals. Pure shoal bass dominated the Cochran Shoals sample; however, we focused our sampling efforts on shoal habitats which may have biased our sample towards overrepresentation of shoal bass in this area. The 2005 samples by Dakin et al. (2015) also captured the spatial trend of increased non-natives and hybrids below MFD compared to Cochran Shoals, although the authors reported a higher percentage (33%) of pure smallmouth bass sampled below MFD than we estimated. More thorough sampling of all available habitats is warranted to better characterize hybridization between MFD and Cochran Shoals in the Chattahoochee River.

The two shoal bass populations examined in this study face an uncertain future because of widespread habitat alteration and hybridization with non-native congeners. How habitat alterations in the area may select for native or non-native genomes is unclear, but differences in optimum temperatures for growth and reproduction can play an important role in black bass species replacements (Whitledge et al. 2006). We speculate that smallmouth bass may have an adaptive advantage over native species in the thermally depressed waters of the main stem Chattahoochee River both above and below MFD. If smallmouth bass become established in the main stem Chattahoochee River above MFD, this could create increased propagule pressure into Big Creek and drive species swamping towards the non-native smallmouth bass genome. Below MFD downstream to Cochran Shoals, smallmouth bass abundance is likely higher than that reflected in our data because we specifically targeted shoal habitats for sampling, potentially biasing our sample to include relatively more shoal bass than non-natives and associated hybrids. Standardized 30-min. electrofishing sampling conducted by GADNR indicates smallmouth bass abundance may be increasing over time in the main stem Chattahoochee River (P. Snellings and C. Looney, GADNR, personal communication). In 2007, no smallmouth bass were recorded below MFD and at Cochran Shoals, whereas more recent samples from 2015 to 2017 yielded up to 2 smallmouth bass below MFD and up to 13 at Cochran Shoals per year. Whether admixed individuals will experience some degree of outbreeding depression is unknown, but black bass hybrids often have reduced fitness that could favor pure parental genomes (Goldberg et al. 2005). However, a hybrid swarm could still form if hybrid offspring begin to outnumber pure parental individuals (Avise et al. 1997, Pierce and Van Den Avyle 1997,

Allendorf et al. 2001). Further, we postulate that the extraordinarily high number of black bass species introduced into the study area may further weaken reproductive boundaries among species and encourage localized loss of the pure shoal bass genome. The rate at which hybrid incidence might increase if stocking were ceased is unknown, but continued supplemental stocking of pure shoal bass in the main stem Chattahoochee River below MFD may delay or thwart the onset of hybrid swarming. A similar supplemental stocking program to promote a pure, diverse shoal bass population in Big Creek may be warranted given the high incidence of hybrid individuals. Consideration of the population structure within shoal bass could help identify suitable brood stock source locations for such management actions (Taylor 2017).

Phenotypic identification may provide a practical means for removing non-natives and their associated hybrids in the Chattahoochee River without an appreciable risk of removing pure shoal bass. The phenotypic characters used took only a short time (approximately 30-90 sec) to examine in the field and provided an 86% overall correct classification rate that would have resulted in removal of 63% of the non-native alleles in our sample. Despite these promising results, phenotypic identification is not a substitute for genetic approaches in the quantification of hybridization among black bass or in the identification of suitable shoal bass brood stock, because the error associated with identifying hybrids can be quite high (Whitmore 1983, Koppelman 2015). A few factors could have bolstered our ability to accurately discern shoal bass from non-natives and hybrids. First, stocking of pure shoal bass into the study area likely limited admixture, making pure shoal bass easier to discern from F₁ hybrids. If additional admixing occurs beyond the levels we encountered, phenotypic identifications would be expected to become less accurate (Allendorf et al. 2001). Second, there was a relative lack of smallmouth bass within our small sample, which are difficult to discern from shoal bass (Taylor and Peterson 2014). If more smallmouth bass and their associated hybrids were included in future studies, correct classification rate may be lower than reported because of likely increases to false positive and false negative rates. If phenotypic identification is used for targeted removal of non-natives and hybrids, we suggest that researchers periodically use genetic identification to groundtruth phenotypic identifications, because accuracy of phenotypic identifications will likely vary based on biologist experience, number of taxa involved in hybridization, and the degree of admixture.

A number of management options exist regarding the shoal bass population below MFD, each representing a tradeoff of management effort needed and desired outcome. One option is to discontinue all management efforts in the study area and allocate resources towards conservation of less-jeopardized shoal bass populations. A second option would be to continue supplemental stocking of shoal bass below MFD. Although stocking does not ensure long-term conservation of a pure population, fingerlings reared with broodstock screened against non-native alleles may help overwhelm the gene pool with native alleles. For example, supplemental stocking of native Guadalupe bass (M. treculii) reduced hybridization rates with non-native smallmouth bass by up to 9% per year (Fleming et al. 2015). A third option would be to couple supplemental stocking with targeted removal of non-natives and associated hybrids. Our results suggest removal based on phenotypic identifications is practical, and stocking coincident with removal of non-natives and hybrids non-native alleles could help expedite the removal of non-native alleles from the shoal bass populations. However, managers may wish to consider the spatial and temporal scales at which non-native removal efforts would be effective for local shoal bass conservation because several source populations of non-native congeners are established upstream and downstream of the shoal bass populations we studied.

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