Investigation of Potential Hybridization Among Black Bass Species in Alan Henry Reservoir, Texas

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Abstract: Reports of extensive hybridization among black bass species throughout North America as well as anecdotal information from anglers led to an investigation of the possibility of hybridization between Alabama bass (*M. henshalli*) and both subspecies of largemouth bass (*M. salmoides salmoides and M. s. floridanus*) in Alan Henry Reservoir, Texas. Fish were collected and identified by field staff and then by using genetic markers. Results suggested no hybridization had occurred between Alabama bass and largemouth bass in Alan Henry Reservoir; however, genetic markers did reveal misclassifications when identification was based on morphology.

Key words: Alabama, bass, hybridization, genetic

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When Alan Henry Reservoir, Texas, was impounded in 1993, it retained a native population of northern largemouth bass (*Micropterus salmoides*). Florida largemouth bass (*M. s. floridanus*; hereafter, "largemouth bass," was introduced in 1993 and quickly introgressed throughout the population. In the most recent surveys of Alan Henry Reservoir, largemouth bass alleles were present at a rate of 72% and largemouth bass genotypes were present at a rate of 10%; however, no northern largemouth bass genotypes were recovered. In 1996, the Texas Parks and Wildlife Department introduced 150 adult Alabama bass (*M. henshalli*) from an unspecified location in the Mobile Basin of Alabama to Alan Henry Reservoir to diversify the black bass fishery in the new impoundment. In order to protect this small founder population, a 458-mm minimum length limit and a three-fish daily bag limit was implemented on Alabama bass while the largemouth population was managed with a five-fish daily bag limit of which no more than two fish could be < 458 mm.

Since the introduction of Alabama bass to Alan Henry Reservoir, anglers have reported catching fish believed to be hybrids of Alabama and largemouth bass. Anglers most commonly described fish that exhibited characteristics of largemouth bass and also possessed glossohyal teeth. Bailey and Hubbs (1949) reported that glossohyal teeth were rare in largemouth bass in Florida and South Carolina and also were seldom developed in northern specimens. In contrast, Alabama bass exhibit glossohyal teeth at a high rate (Baker et al. 2008). However, Bailey and Hubbs (1949) suggested that largemouth bass in the southwestern extremity of their natural range may be separable into a subspecies based on the high incidence of glossohyal teeth. This was supported by Edwards (1980) who examined largemouth bass collected from south Texas in the Texas Natural History Collections from Texas and Mexico and found populations in the Nueces and Medina river basins with low to high frequencies of glossohyal teeth.

Harvest regulations are based on the assumption that anglers can distinguish between species. For species with distinct morphological features, identification and compliance with harvest guidelines is straightforward. However, species that lack easily-identifiable features can cause misidentification and subsequent unintentional noncompliance by anglers (Schmetterling and Long 1999). Alabama bass may be differentiated from both subspecies of largemouth bass using a number of meristic and morphological features, but these features are not always obvious and may be incorrectly assessed by anglers. In addition, introgression between the subspecies of largemouth bass create a variety of phenotypes (Bailey and Hubbs 1949) and further hybridization with Alabama bass would only extend this phenotypic range.

Hybridization among black bass species has been commonly reported, particularly when one species is stocked outside its native range into systems containing congeneric species (Childers 1975, Whitmore and Hellier 1988, Morizot et al. 1991, Koppelman 1994, Pierce and Van Den Avyle 1997, Pipas and Bulow 1998, Littrell et al. 2007, Godbout et al. 2009). Therefore the potential exists for Alabama bass to hybridize with both subspecies of largemouth bass in Alan Henry Reservoir. We conducted this study to determine if hybridization was occurring among these species in Alan Henry Reservoir using morphological observations as a primary screening method and genetic markers to verify morphological observations.

Methods

Black bass were collected by pulsed-DC electrofishing throughout Alan Henry Reservoir on 4 May 2010 during daylight hours.

The water body was divided into three sections (upper, middle, and lower), and 15 stations were randomly selected using ArcGIS software within each section. Each station was electrofished for 5 min and an attempt was made to collect all black bass encountered. Collection ceased when either a 100-target fish was reached or all the stations were sampled. All black bass collected were weighed (g) and measured (mm), identified to species by Texas Parks and Wildlife (TPWD) fisheries personnel, and fin clipped for genetic analysis. Fin clips were preserved in 70% EtOH prior to processing.

Fin clips from Alabama bass collected from Neely Henry Reservoir, Alabama, by the Alabama Wildlife and Freshwater Fisheries Department were acquired as reference samples. A subset of microsatellite loci previously shown to produce species-specific polymorphisms among black bass species (Lutz-Carrillo et al. 2008) was screened against the Alabama bass collected from Neely Henry reservoir as well as reference collections of both subspecies of largemouth bass. Based on reliability, allele frequency differences, and amplicon size, three of these microsatellites (*MiSaTPW058*, *MiSaTPW106*, and *MiSaTPW167*) were multiplexed for use in this study.

Genomic DNA was isolated from each fin clip, quantified, and adjusted following the method outlined in Lutz-Carrillo and Dumont (2012). Polymerase chain reactions (PCR) were performed in 10-µL volumes using a Mastercycler ep gradient S thermal cycler (Eppendorf). Reactions consisted of 50 ng template DNA, 1× PCR Buffer (20-mM Tris-HCl pH 8.4, 50-mM KCl; Invitrogen), 2-mM $MgCl_2$ (Invitrogen), 0.2-mM dNTPs, 0.04 µM of each unlabeled 5′-tailed primer, 0.2 µM of each unlabeled non-tailed primer, 0.3 µM of a 25% labeled (IRDye 700 or IRDye 800, LI-COR) fluorescent custom tail (see Lutz-Carrillo et al. 2008), and 0.5 U Platinum *Taq* DNA polymerase (Invitrogen). Cycling parameters were 94 C for 1.5 min, followed by 32 cycles of denaturation at 94 C for 30 s, annealing at 60 C for 30 s, extension at 72 C for 45 s, and a final extension at 72 C for 10 min. Amplicons were evaluated using a NEN 4300 DNA sequencer (LI-COR) and sized using BioNumerics version 6.5 (Applied Maths). The resulting allele frequencies and combinations of species specific alleles within and among loci in each sample were used as estimates of the genetic composition of each fish.

Results

A total of 225 black bass were collected with 0 (5 stations) to 14 (1 station) fish being collected per station; all fish were >100 mm in length (Figure 1). There was substantial overlap in the spatial distribution of Alabama bass and largemouth bass (Figure 2) within the reservoir. Field identification suggested 130 black bass were Alabama bass, 92 were largemouth bass, and 3 fish were hybrids (Table 1). Samples from Neely Henry Reservoir, Alabama, were all identified as Alabama bass based on morphology ($n = 50$). Genetic data generally agreed with the field identification; however, genetic analyses indicated that two Alabama bass were misidentified as largemouth bass (both >530 mm TL), one largemouth bass was identified as an Alabama bass (183 mm TL), and three Alabama bass were misidentified as hybrids in the field (all <200 mm TL). All three field-identified hybrids were resolved as non-introgressed Alabama bass using the three-locus multiplex. A single fish from the Neely Henry Reservoir sample did not contain a genotype compatible with the Alabama bass. These individuals were removed and allele frequencies were established for the Alabama bass from Neely

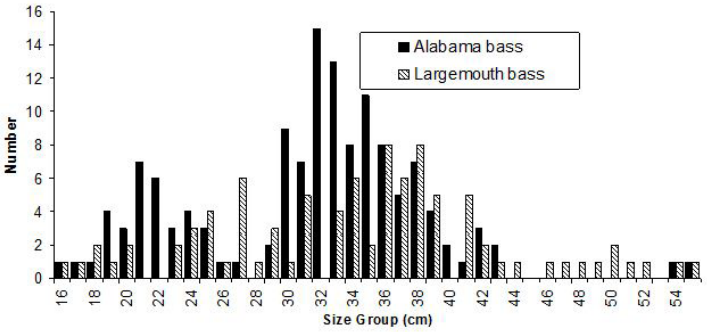

Figure 1. Length-frequency histogram of *Micropterus* spp. collected by electrofishing from Alan Henry Reservoir, Texas, 4 May 2010. Total sample size was 134 Alabama bass and 91 largemouth bass.

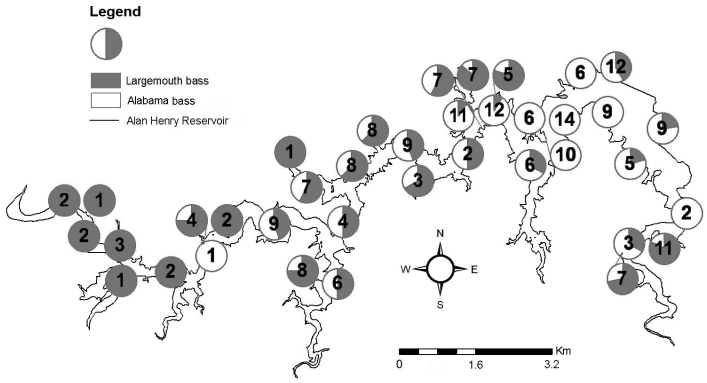

Figure 2. Proportion of largemouth bass and Alabama bass (based on genetic analyses) collected at each sampling location. Gray segments indicate largemouth bass and white segments indicate Alabama bass. The number in the center of the circle indicates the total number of all bass collected at each station. Five stations where no fish were collected were omitted from this figure.

Table 1. Number of black bass classified as Alabama bass, largemouth bass, or a hybrid bass by morphological characteristics in the field and number verified by genetic analysis from Alan Henry Reservoir, Texas.

	Field classification	Genetic classification
Alabama bass	130	134
Hybrid bass	3	0
Largemouth bass	92	91

Table 2. Allele frequencies for multiplexed loci in Alabama bass (ALB) from Neely Henry Reservoir, Alabama, (NH) and Alan Henry Reservoir, Texas, (AH) and largemouth bass (LMB) in AH.

Locus	Allele	ALB (NH)	ALB (AH)	LMB (AH)
MiSaTPW106	369	0.064	0.097	0.000
	371	0.904	0.903	0.000
	373	0.032	0.000	0.911
	375	0.000	0.000	0.089
MiSaTPW167	194	1.000	1.000	0.000
	213	0.000	0.000	0.694
	215	0.000	0.000	0.306
MiSaTPW58	141	0.000	0.000	0.050
	159	0.000	0.000	0.044
	163	1.000	1.000	0.000
	167	0.000	0.000	0.906

Henry Reservoir ($n = 49$), Alabama bass from Alan Henry Reservoir ($n = 129$), and largemouth bass from Alan Henry Reservoir ($n = 90$) (Table 2).

Allele frequencies for Alabama bass from Neely Henry Reservoir and Alan Henry Reservoir were similar, with two loci completely fixed and one locus nearly fixed for alleles differentially sized from largemouth bass (Table 2). Using either the Alabama bass population alone or combined Alabama bass genotypes from Neely Henry and Alan Henry Reservoir as references for the Alabama bass, no hybrids were detected in Alan Henry Reservoir. In the Alan Henry Reservoir collection, the single individual removed from the Alabama bass group was consistent at all three loci with largemouth bass, both individuals removed from largemouth bass were consistent at all three loci with Alabama bass, and all three individuals identified as suspected hybrids were consistent at all three loci with Alabama bass. In addition, the single individual removed from the Neely Henry group was consistent at all three loci with largemouth bass.

Overall in Alan Henry Reservoir, TPWD field staff identified 96.3% of Alabama bass correctly and 98.9% of largemouth bass correctly. All misclassified Alabama bass lacked glossohyal teeth and all misclassified largemouth bass were < 200 mm with one exhibiting a glossohyal tooth patch. Reorganizing the samples to reflect these misclassifications, we evaluated a total of 134 Alabama bass and 91 largemouth bass at three loci each without detecting alleles from one species within the genetic background of the other. Assuming alleles do exist in non-native genotypes at a rate of 0.01 in this population, the binomial probability of not detecting one in a sample of 1350 alleles (225 fish, 3 loci each) is 1×10^{-6} assuming a Poisson distribution.

Discussion

Rates of hybrid inviability have been shown to accumulate at remarkably slow rates among centrarchids (Bolnick and Near 2005) and all attempted pair-wise *Micropterus* crosses have produced viable progeny under laboratory conditions (summarized in Near et al. 2003). Largemouth bass in particular have been documented to hybridize in the wild with smallmouth bass (Whitmore and Hellier 1988), Guadalupe bass (Littrel et al. 2007), and spotted bass (Godbout et al. 2009), and has produced viable progeny in the laboratory with other genera within the family Centrarchidae (Philipp et al. 1983, Parker et al. 1985). In addition, the Alabama bass has been shown to hybridize with redeye bass *M. coosae* when introduced outside of its native range (Barwick et al. 2006) and is suspected to hybridize with redeye bass where their native ranges overlap (Kassler et al. 2002). Thus, anecdotal information from anglers reporting hybrids between the Alabama bass and largemouth lineage in Alan Henry Reservoir were considered possible. Given that we set out to document the extent of introgression throughout the reservoir, a finding of no hybridization was somewhat surprising.

The lack of hybridization, on the surface, suggests that concerns over anglers misidentifying black bass species for harvest may be eased since mosaic genotypes of these two lineages appear to be absent from the population. However, genotype data suggest that a small number of fish were still misclassified by phenotype in the field. Most of these misclassified fish carried morphological features typically associated with the alternate lineage such as the presence or absence of glossohyal teeth, but the number of fish identified in the field as hybrids may also be the result of expectation bias. Expectation bias has been documented in the behavioral (Rosenthal 1964, Kaptchuk 2003, Finn 2006) and physical sciences (Gotfryd and Hansell 1985, Jeng 2006) with the expectations of investigators leading them to misinterpret data, especially when perceptual data is ambiguous. Because TPWD field personnel were focused on looking for hybrid bass, that expectation may have led them to identify hybrid bass in the population when they were not present. Given that we have now documented a lack of hybridization between these lineages in Alan Henry Reservoir it is hoped that anglers will no longer conclude that Alabama × largemouth bass hybrids exist in the reservoir.

The Alabama bass is in the midst of a taxonomic revision. While previously viewed as a subspecies (*M. punctulatus henshalli*) of the spotted bass (Hubbs and Bailey 1940), phylogenetic work has suggested species status is more appropriate (Kassler et al. 2002, Baker et al. 2008). Morphometric analysis suggests Alabama bass may be the sister species of largemouth bass (Harbaugh 1994). However, phylogenetic work suggest the Alabama bass is a sister taxa to the red-eye bass (Kassler et al. 2002) with a distant node connecting

the Alabama and spotted bass to their most recent common ancestor (MRCA ~ 11 mya; Near et al. 2003). Thus, while the spotted bass and redeye bass readily hybridize with other black bass outside of their native range despite showing some spatial isolation from co-occurring black bass, it is unclear how the ecology and behavior of these taxa relate to the Alabama bass. While closely related by descent to the redeye bass, Alabama bass exist in environments more similar to the spotted bass and are able to thrive in low velocity lotic systems and reservoirs (Warren 2009). In fact, at the two-dimensional geographic level there was substantial overlap in the spatial distribution of Alabama bass and largemouth bass in Alan Henry Reservoir. Given that these species occupy the same physical space, and viewing reservoirs as "disturbed habitats" which Hubbs (1955) suggested could promote hybridization, it was even more surprising to find an absence of hybridization. However, this may be more applicable to allopatric species or to "disturbed" systems that constrict the preferred available habitat for one of the species. Alabama bass and largemouth bass on the other hand exist as sympatric species in several basins. Thus, even if these species do not attain distinct physical or temporal isolation during the spawning period, other pre-zygotic isolating mechanisms, such as species recognition and subsequent assortative mating, may be developed.

Within Alan Henry Reservoir, the current Alabama bass population has increased to levels that no longer require the restrictive regulations imposed to protect the initial cohort stocked in 1996. Combined with the fact that misclassifications are still likely to occur in the absence of hybrid genotypes, a change in harvest regulations has been proposed allowing a five fish daily bag limit with no minimum length limit and no more than two fish under 458 mm for Alabama bass and the largemouth lineage in aggregate. This should address angler concerns relating to species identification and allow them to bring smaller Alabama bass to tournament weigh-ins without penalty.

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