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Genetic Analysis Reveals Dispersal of Florida Bass Haplotypes from Reservoirs to Rivers in Central Texas

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NOTE

Genetic Analysis Reveals Dispersal of Florida Bass Haplotypes from Reservoirs to Rivers in Central Texas

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Abstract

We analyzed the genetic structure of putative largemouth bass *Micropterus salmoides* populations in nonstocked, wadeable streams of central Texas. Mitochondrial D-loop sequences were generated for 69 fish sampled for this project. In addition, 27 largemouth bass and Florida bass *M. floridanus* specimens provided by the Texas Parks and Wildlife Department (TPWD) were sequenced. The TPWD samples represented stock lineages as well as wild fish from outside of the sampling region. Our analyses revealed the presence of both largemouth bass and Florida bass mitochondrial haplotypes at all sampling locations. Haplotypes of the nonnative Florida bass accounted for 26% of all haplotypes. The presence of Florida bass haplotypes at the sampling locations indicates that the influence of stocking reaches far beyond managed reservoirs. The admixture of nonnative genetic material can increase genetic diversity of native populations, but outbreeding depression, competition, and other negative impacts are of concern. Although the stocking of nonnative Florida bass in reservoirs may enhance fishing opportunities, it also has the ability to alter stream systems that are directly connected to stocked reservoirs.

The black basses *Micropterus* spp. currently include nine recognized species (Near and Koppelman 2009), some of which are among the most popular sport fishes in North America. Five species of *Micropterus* occur in Texas: spotted bass

M. punctulatus, largemouth bass *M. salmoides*, Guadalupe bass *M. treculii*, Florida bass *M. floridanus*, and smallmouth bass *M. dolomieu*. The first three of these species are native to Texas, while the latter two are nonnative and are stocked as sport fish. The status of the Florida bass is still under debate. Although many consider the Florida bass to be a valid species (Kassler et al. 2002; Near et al. 2003, 2004; Eschmeyer 2012), species designation has not been completely accepted (Nelson et al. 2004). Here, we side with the former perspective in using a full species designation for the Florida bass.

Largemouth bass and Florida bass are the most extensively managed black basses in Texas. Recent trends in fisheries management have involved a shift to the stocking of Florida bass in lieu of largemouth bass across much of North America, as Florida bass attain a larger maximum size than largemouth bass. These species are closely related and hybridize readily when in natural sympatry (Bailey and Hubbs 1949) and when stocked outside of their native ranges (Gelwick et al. 1995). The results of such stocking events have been variable and debated (Philipp and Whitt 1991; Maceina and Murphy 1992; Philipp 1992), but data from a Texas reservoir indicate that Florida bass introgression into native largemouth bass populations can occur rapidly, with nonnative alleles found in the

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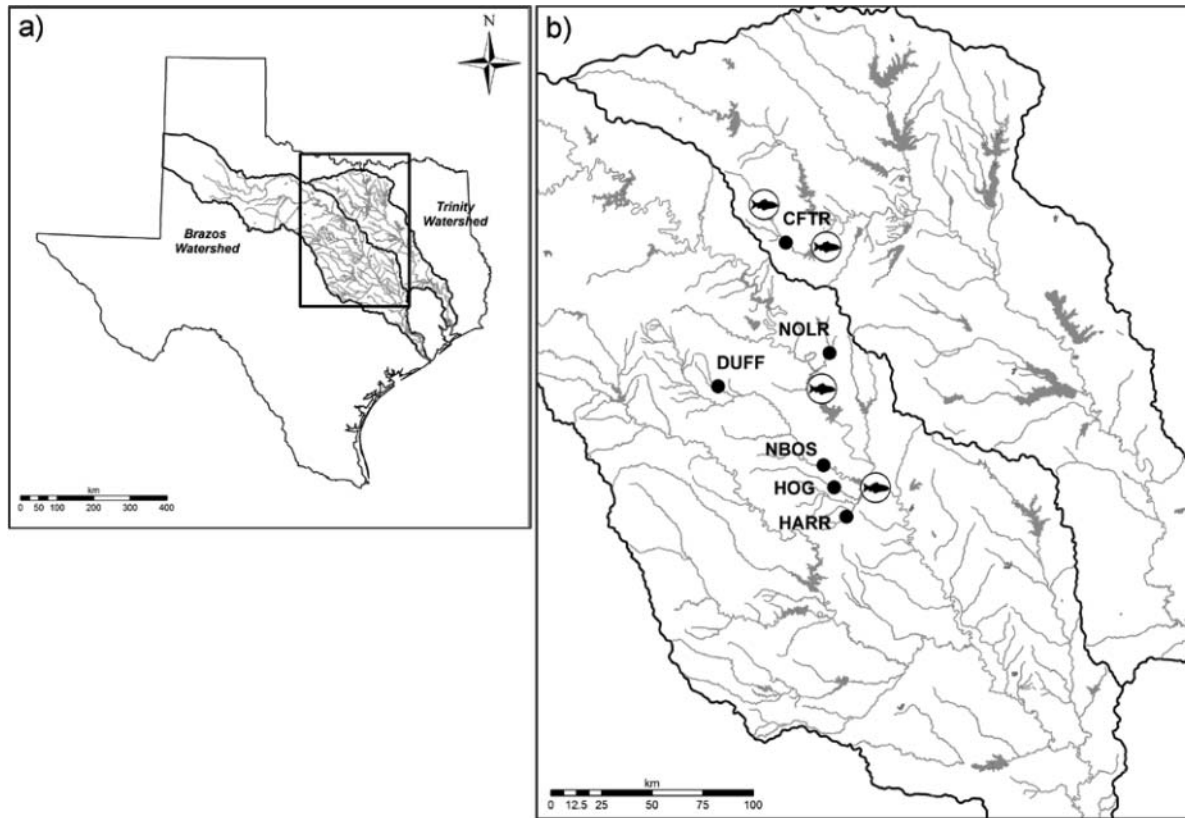


FIGURE 1. Maps of (a) Texas, showing the locations of the Trinity River and Brazos River watersheds; and (b) the Trinity and Brazos River tributaries, displaying sampling sites (black dots) and known Florida bass stocking locations (fish symbols; HOG = Hog Creek; HARR = Harris Creek; DUFF = Duffau Creek; NBOS = North Bosque River; NOLR = Nolan River; CFTR = Clear Fork [Trinity River]).

majority of largemouth bass in less than 4 years (Maceina et al. 1988).

While the stocking of nonnative fish species occurs worldwide, often little is known about the long-term impacts of such actions. Generally, fisheries with high numbers of introduced species appear to be less stable than those with mostly native fauna (Moyle 1986). Negative impacts of the stocking and release of baitfish are documented for many North American species, including the red shiner *Cyprinella lutrensis* (Walters et al. 2008; Blum et al. 2010) and kokanee *Oncorhynchus nerka* (Morgan et al. 1978). Impacts of the large-scale introductions of largemouth bass and Florida bass include the muddling of historical species boundaries (Philipp et al. 1983) and obscuring of the native range of largemouth bass (Boschung and Mayden 2004). Data on largemouth bass and Florida bass introductions are abundant, yet relatively little is known about watershed-scale impacts of fish stocking in Texas.

We analyzed mitochondrial D-loop sequences of putative largemouth bass populations in wadeable streams from the Brazos and Trinity River watersheds in central Texas and tested for the presence of Florida bass haplotypes. These streams represent nonstocked locations that are connected to stocked reservoirs by varying distances. We compared mitochondrial haplotypes of largemouth bass and Florida bass found in the

Trinity and Brazos River drainages with haplotypes found in populations from Illinois and Florida and in Texas Parks and Wildlife Department (TPWD) stocks. Our results illustrate the geographic extent to which Florida bass haplotypes have spread from known stocking locations and show that the stocking of nonnative fish into reservoirs has impacts on aquatic communities that are distant to the actual stocking location.

METHODS

Study sites and sampling techniques.—Samples of putative largemouth bass were collected at five sites within the Brazos River system and at one site in the Trinity River system (Figure 1). The Brazos River locations include Hog Creek, Harris Creek, Duffau Creek, North Bosque River, and Nolan River; the Trinity River site lies on Clear Fork. All sampling locations are tributaries of stocked reservoirs, spanning distances of 5–80 km from stocking sites.

Specimens were collected by using a backpack electrofisher (Smith-Root Model LR-24) and seine nets (4.6 × 1.8 m or 1.8 × 1.8 m) during the course of long-term fish community monitoring projects (Pease et al. 2011; Stanley et al. 2012). All fish were initially identified as native largemouth bass. At the time of sampling, we had no reason to believe that the

stocking of distant reservoirs was influencing sampling locations on wadeable streams, and there were no apparent morphological differences among sampled fish. Florida bass and largemouth bass are difficult to distinguish in the field by using simple morphological traits, especially for juveniles, as there is significant overlap in their phenotypes (Kassler et al. 2002). Small fish were retained, while larger specimens were identified, fin clipped, and released. Fin clips were stored in 99.8% ethanol. In addition to field samples, reference samples of Florida bass and largemouth bass lineages stocked in Texas were obtained from TPWD (D. Lutz-Carrillo, TPWD, A. E. Wood Laboratory). The TPWD also provided samples of wild-caught largemouth bass from Texas and Illinois and of wild Florida bass captured in Florida. The TPWD samples served as a baseline for comparing the haplotypes of our black bass samples with the haplotypes of known stocked lineages and nonnative wild lineages.

DNA methods.—Genomic DNA was extracted from frozen or ethanol-preserved tissue by using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, California) in accordance with the manufacturer's protocol for tissue samples. For amplification and sequencing of the mitochondrial D-loop control region, we used the primers CR-F (5'-GGATT TTAACCCYCACCMCT-3') and CR-R (5'-TTCTAGGGCTCA TCTTAACATCTTC-3') with an M13-41 adapter (Husemann et al., in press). The PCR products were tested on a 1% agarose gel stained with Gel Red (0.1 × , Biotium) and photographed. The PCR products were purified using solid-phase reversible immobilization (DeAngelis et al. 1995) with carboxylated

magnetic beads (Bangs Laboratories) and a 96-ring solid-phase reversible immobilization plate (Agencourt). The purified PCR products were sequenced by the Yale University Sequencing Facility (New Haven, Connecticut). Sequences were inspected, trimmed, and aligned using Geneious version 5.0.3 (Drummond et al. 2006). Median joining networks were constructed under default conditions by using Network (Bandelt et al. 1999).

RESULTS

In total, 69 sequences trimmed to 883 bp in length were obtained from the fish sampled for this project. An additional 27 sequences were generated from TPWD samples: 10 Florida bass from the stock lineage, 8 largemouth bass from the stock lineage, 4 wild-caught Florida bass, 3 wild-caught largemouth bass from Texas, and 2 wild-caught largemouth bass from Illinois. All sequences were submitted to GenBank (accession numbers JN979571-JN979602 and JN979661-JN979724). Within the 69 samples obtained from our sampling effort, 51 haplotypes were found, 16 of which corresponded to Florida bass. Three single-base insertion-deletion events were found. None of the insertion-deletion events was unique, and none of them resulted in the creation of a new haplotype. The median joining network, which included regional and TPWD samples, showed two distinct groups corresponding to largemouth bass and Florida bass (Figure 2). Florida bass haplotypes were found at all sampling locations, with no appreciable pattern related to the distance from known stocking sites. Due to the uniparental inheritance of mitochondria, only maternal lineages are sampled

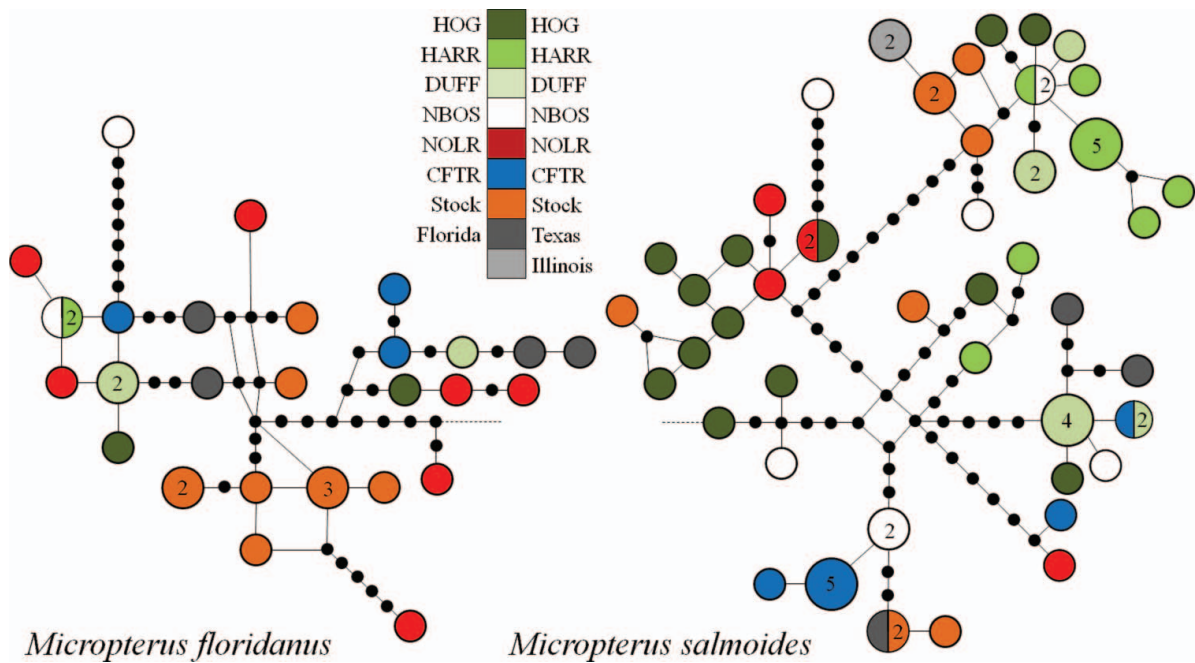


FIGURE 2. Median joining network of Florida bass *Micropterus floridanus* and largemouth bass *M. salmoides* haplotypes. Black dots represent intermediate unsampled (inferred) haplotypes, and circles represent sampled haplotypes, with circle size proportional to sample size (shown inside circle for all $n > 1$). Color corresponds to sample origin (location codes are defined in Figure 1). The dotted line between Florida bass and largemouth bass networks represents a gap of 30 mutational steps.

and hybrid individuals cannot be differentiated from nonhybrids. At this time, we cannot determine whether our samples represent a hybridizing group of largemouth bass and Florida bass or two distinct co-occurring species.

DISCUSSION

The largemouth bass has a long and nearly continuous stocking history in the state of Texas, but recent stocking efforts have transitioned from largemouth bass to Florida bass. The Florida bass is widely considered a better sport fish because it attains a greater maximum size, which strongly influences the stocking of this species across much of North America. Although stocked reservoirs are commonly studied (e.g., Maceina et al. 1988), the impact of fish stocking on stream networks that are connected to these reservoirs remains largely unknown. Our sampling locations represent nonstocked, wadeable streams, all of which are connected to stocked reservoirs at varying distances. Understanding the genetic impact of stocking is particularly important, as largemouth bass and Florida bass readily hybridize (Bailey and Hubbs 1949; Gelwick et al. 1995) and outbreeding depression has been documented across lineages of largemouth bass (Philipp et al. 2002; Goldberg et al. 2005).

Of the 69 fish sampled for this project, 51 fish had haplotypes corresponding to largemouth bass and 18 fish (26%) had haplotypes that corresponded to Florida bass. The largest proportion of Florida bass haplotypes occurred in the Nolan River, a tributary that drains directly into Lake Whitney. In contrast, the four remaining sample sites within the Brazos River drainage are tributaries of Lake Waco and had a lower incidence of Florida bass haplotypes. Both Lake Whitney and Lake Waco have been stocked with largemouth bass and Florida bass over the past 40 years (www.tpwd.state.tx.us; accessed 10 November 2011). All stocking events since the 1980s have involved the stocking of Florida bass. Lake Whitney received nearly 10 times the number of Florida bass fingerlings than did Lake Waco since this transition, and the higher stocking effort may explain the larger proportion of Florida bass haplotypes in populations upstream.

Our results suggest that the stocking of Florida bass has a significant influence on fish populations far beyond the stocked reservoirs. We found Florida bass haplotypes in Duffau Creek, which is situated more than 80 km upstream from the closest documented stocking location. Although Florida bass and largemouth bass haplotypes are distinct, we were not able to genetically differentiate between hybrids and pure lineages by using the D-loop marker, and morphological species delimitation was not possible because the majority of the sampled individuals were juveniles. Therefore, the origin of fish containing Florida bass mitochondrial DNA cannot be definitively pinpointed. Florida bass alleles in fish sampled outside of the reservoirs presumably arose from one of two sources: (1) direct dispersal of stocked fish and their descendants from the reservoirs into surrounding streams or (2) hybridization with and introgression into native largemouth bass populations. Further

studies using nuclear markers will help to distinguish between these scenarios or combinations thereof.

Fifty-one different haplotypes were recovered from our sample of 69 fish, indicating high levels of genetic diversity in largemouth bass and Florida bass in central Texas. While high genetic diversity is often a sign of healthy populations, changes in the genetic makeup of populations due to hybridization may reduce genetic diversity over the long term and can even lead to extinction (Rhymer and Simberloff 1996). Hybridization due to anthropogenic translocation is considered a major threat to fish species worldwide (Allendorf et al. 2001) and may lead to outbreeding depression, which has been shown to occur in mixed lineages of largemouth bass and Florida bass (Philipp et al. 2002; Goldberg et al. 2005).

The spread of nonnative alleles into native populations is a common theme in fisheries and wildlife management, particularly with regard to heavily managed species such as the largemouth bass (Gelwick et al. 1995; Johnson and Fulton 1999) and salmonids (Apostolidis et al. 2008; Campos et al. 2008; Dawnay et al. 2011). The presence of Florida bass haplotypes in all sampled largemouth bass populations in central Texas highlights the large-scale impacts that fish stocking can have on the genetic makeup of fish populations. While it is evident that largemouth bass in central Texas have high genetic diversity, the portions of diversity that are attributable to natural versus anthropogenic sources are unknown. Further studies using nuclear markers would be useful for differentiating hybrids from pure lineages of Florida bass and largemouth bass in Texas. Understanding the extent of Florida bass dispersal and hybridization with largemouth bass would elucidate the mechanisms of introgression and would shed light on the impacts of Florida bass stocking on native largemouth bass populations in central Texas.

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