

Status of an Introgressed Guadalupe Bass Population in a Central Texas Stream

BRADLEY M. LITTRELL*

Department of Biology, Texas State University at San Marcos,
601 University Drive, San Marcos, Texas 78666, USA

DIJAR J. LUTZ-CARRILLO

Texas Parks and Wildlife Department, A. E. Wood Laboratory,
507 Staples Road, San Marcos, Texas 78666, USA

TIMOTHY H. BONNER

Department of Biology, Texas State University at San Marcos,
601 University Drive, San Marcos, Texas 78666, USA

LORAIN T. FRIES

Texas Parks and Wildlife Department, A. E. Wood Laboratory,
507 Staples Road, San Marcos, Texas 78666, USA

Abstract.—Introductions of nonnative smallmouth bass *Micropterus dolomieu* into central Texas streams resulted in introgressive hybridization with an endemic allopatric congener, the Guadalupe bass *M. treculii*. The objectives of this study were to use the variation at 14 microsatellite DNA loci to examine the frequencies of Guadalupe bass, smallmouth bass, and their hybrids 10 years after supplemental stockings of Guadalupe bass in the Blanco River. Genetic analysis identified 40% of individuals as smallmouth bass, 51% as smallmouth bass \times Guadalupe bass hybrids, and 9% as other *Micropterus* hybrids. Pure Guadalupe bass were not collected. Despite supplemental stocking, the frequency of pure Guadalupe bass appears to have declined in the Blanco River in the past 10 years. These results emphasize the need for a current survey of the genetic status of the species throughout its native range.

Intentional stocking of nonnative sport fishes accounts for the majority of introduced fishes in North America (Rahel 2000, 2002). Although nonnative sportfish stockings are accepted and viable management options used by many federal and state agencies to enhance fishing opportunities (Heidinger 1993; Li and Moyle 1993), some stockings are detrimental to resident fishes. When the introduced species is an allopatric congener of a resident fish, weakly developed reproductive isolating mechanisms can increase the likelihood of hybridization and introgression (Hubbs 1955). Consequently, genetic contamination has occurred in several native fishes as a result of intentional sportfish stockings (Busack and Gall 1981; Whitmore 1983; Garrett 1988; Verspoor 1988, Turner et al. 1991).

In central Texas, smallmouth bass *Micropterus dolomieu* were stocked extensively beginning in 1974 (Garrett 1991). However, several streams in the drainages of the Brazos, Colorado, Guadalupe, and

San Antonio rivers of central Texas supported an endemic allopatric congener, the Guadalupe bass *M. treculii* (Hubbs et al. 1991). In the mid-1970s, morphometric evidence of hybridization was found between smallmouth bass and Guadalupe bass in the Guadalupe River drainage (Edwards 1979), and hybridization and introgression were later verified by protein electrophoresis (Whitmore and Butler 1982; Whitmore 1983). By 1990, smallmouth bass \times Guadalupe bass hybrids were common (e.g., the incidence of hybridization was more than 45% in some areas) in central Texas streams (Garrett 1991). Genetic contamination and habitat modifications to lotic environments resulted in a decline in abundance and distribution of Guadalupe bass (Edwards 1978; Edwards 1980; Morizot et al. 1991). Consequently, the Guadalupe bass is listed by several authors and agencies as a species of conservation concern (Johnson 1987; USDI 1989; Hubbs et al. 1991; Warren et al. 2000). In an effort to protect and restore Guadalupe bass populations, smallmouth bass stockings were eliminated within the native range of Guadalupe bass by 1990. In addition, hatchery-produced Guadalupe bass were stocked in two central Texas streams to

* Corresponding author: blittrell@bio-west.com

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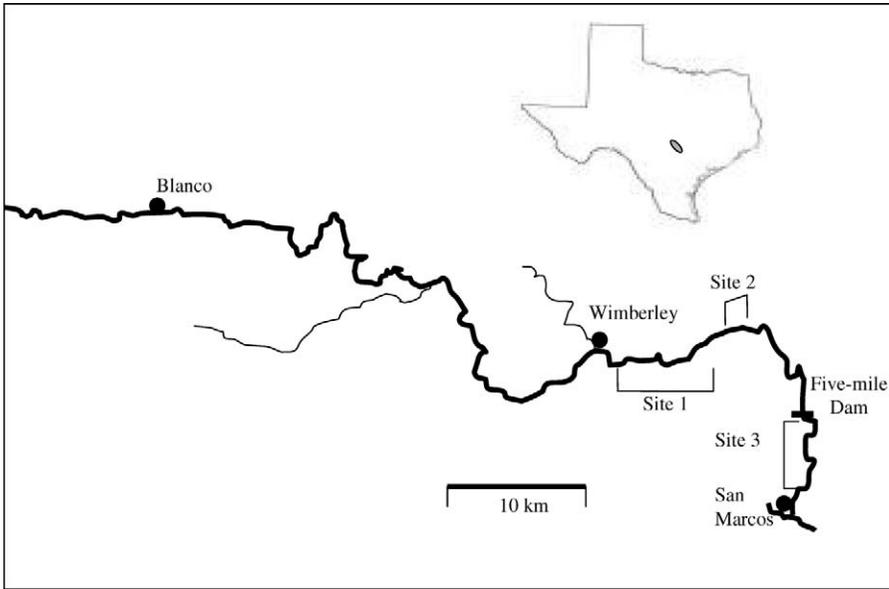


FIGURE 1.—Collection sites for 83 micropterids taken from the Blanco River in Hays County, Texas, during January and February 2005.

restore a dominant Guadalupe bass population and to genetically swamp the smallmouth bass genome (Garrett 1991).

In the Blanco River, a tributary of the San Marcos River (Guadalupe River drainage), 130,000 smallmouth bass were stocked from 1977 through 1980. In 1991, 30% of micropterids ($N=88$) collected from the Blanco and San Marcos rivers were genetically identified as interspecific hybrids between smallmouth bass and Guadalupe bass (Morizot et al. 1991). As part of the restoration effort, 80,000 hatchery-produced Guadalupe bass were stocked in the Blanco River in 1994 and 1995. Hatchery fingerlings were produced from broodstock collected from a population thought to be without introgression in the upper Guadalupe River. In addition, each broodfish was evaluated by means of a minimally invasive biopsy technique (Van Meter 1995) and electrophoresis of diagnostic loci (Morizot et al. 1991). One objective of this study was to examine the effectiveness of supplemental stocking by evaluating the current proportion and genetic influence of Guadalupe bass, smallmouth bass, and their hybrids in the Blanco River. A second objective was to examine the gene flow between our collection sites to identify superior stocking locations for future restoration efforts.

Methods

The Blanco River is a 570-km², limestone-dominated drainage along the eastern edge of the Edwards Plateau in Kendall, Blanco, and Hays counties of central Texas. Bass were collected from three sites on the Blanco River

(Figure 1). Site 1 consisted of an 11-km segment of river immediately downstream from Wimberley, Texas. Site 2 was a 2-km stretch between site 1 and the city of Kyle, Texas. Site 3 comprised a 10-km segment downstream from Five-Mile dam to the confluence with the San Marcos River. Habitats at sites 1 and 2 were dominated by wide shallow runs with bedrock substrate, whereas habitat at site 3 was more heterogeneous with fast riffle and deep pool habitats interspersed over cobble and gravel substrates. Although smallmouth bass and Guadalupe bass have been collected in the upper reaches of the Blanco River (T. H. Bonner, unpublished), the area was not sampled because previous studies (Morizot et al. 1991) collected only largemouth bass *M. salmoides* in this section of the river.

At sites 1 and 3, bass were captured by angling with an assortment of tackle in January and February 2005. Three float trips consisting of three to five anglers in kayaks were conducted at each site, resulting in approximately 75 man-hours of total fishing time per site. At site 2, bass were collected with a boat-mounted electrofisher (Coffelt Model VVP-15) in February 2005. Total length (TL; nearest mm) and weight (g) were measured for each bass collected, and anal fin clips were taken and preserved in a 70% solution of ethanol. All fish were released alive.

Total genomic DNA was isolated from 3–5 mm³ of anal fin tissue by means of a modified version of the Purgene protocol for fish tissue (Gentra Systems, Inc., Minneapolis, Minnesota). Genomic DNA was quantified by fluorometry (Hoefer DyNA Quant 200) and

adjusted to a concentration of 50 ng DNA extract/ μ L before polymerase chain reaction (PCR). Genotypes were obtained at 14 unlinked microsatellite loci (*Lma10*, *Lma12* [Colbourne et al. 1996]; *Lma120*, *Lma121* [Neff et al. 1999]; *Mdo1*, *Mdo3*, *Mdo5*, *Mdo6*, *Mdo7*, *Mdo10* [Malloy et al. 2000]; *Msal13*, *Msal21*, *Msal25*, and *Lar7* [DeWoody et al. 2000]) for each individual collected. These loci possess varying levels of polymorphism within and among *Micropterus* species (Lutz-Carrillo et al. 2006; D. Lutz-Carrillo, unpublished). Reactions were performed in 10- μ L volumes using a single-locus and six multiplex PCRs with an MJ Research PTC-200 thermocycler (MJ Research, Waltham, Massachusetts). Amplified products were separated by electrophoresis in a 6.5% polyacrylamide gel and detected by infrared label with a NEN 4200 Global IR2 DNA Sequencer (LI-COR Biotechnology, Lincoln, Nebraska). BIONUMERIC (version 4.0, Applied Maths, Kortrijk, Belgium) was used for gel image processing and allele scoring.

The program WHICHLOCI (Banks et al. 2003) was used to assess locus-specific assignment power based on 10 resampled data sets of 10,000 individuals each using the allelic frequency differential method (Shriver et al. 1997). The Bayesian inference algorithm implemented in STRUCTURE (version 2.0; Pritchard et al. 2000) was used to infer the genetic contribution of known species groups to unknown individuals. This program defines the probability of each individual belonging to a group or the joint probabilities that it belongs to two or more groups by clustering multilocus genotypes based on the principles of Hardy–Weinberg and linkage equilibrium; these clustering probabilities approximate genomic proportions. Known species groups consisted of largemouth bass from Lake Kickapoo, Texas ($N = 28$), and the Devils River, Texas ($N = 36$); smallmouth bass from the Devils River, Texas ($N = 26$), and the Possum Kingdom State Fish Hatchery, Texas ($N = 15$); and Guadalupe bass from the upper Guadalupe River ($N = 53$). Allele frequencies based on these populations are assumed to be conserved enough within species to make reliable assignments. Unknowns consisted of individuals collected from the Blanco River. For three independent STRUCTURE runs (50,000 burn-in steps and 300,000 Markov chain–Monte Carlo [MCMC] repetitions) incorporating all of the known genotypes, incorporating no prior population information, and assuming correlated allele frequencies and admixture, $k = 3$ resulted in the correct assignment of all known genotypes to three distinct species groups corresponding to largemouth bass, smallmouth bass, and Guadalupe bass. Clustering probabilities for the known genotypes were arcsine-transformed for normality,

and confidence intervals (CIs) of ± 1.96 SD were used to create classification threshold values (minimum q -values). Unknown genotypes from the Blanco River were then assigned to a species group under an independent run of the $k = 3$ model or partitioned into more than one group (hybrids) if transformed clustering probabilities did not meet threshold cutoff values (the minimum q -values were 0.944 for largemouth bass, 0.965 for Guadalupe bass, and 0.941 for smallmouth bass; results were identical when a threshold of 0.950 was used for all three groups).

Within sites, significant departures from Hardy–Weinberg equilibrium (HWE) were evaluated by means of the procedure of Guo and Thompson (1992), while the likelihood-ratio test of Slatkin and Excoffier (1996) was used to evaluate linkage disequilibrium (LD) between pairs of loci. Among sites, genetic differentiation was assessed by pairwise F_{ST} ; significance levels were evaluated by permutating individuals among samples with the program ARLEQUIN (version 2.000, Schneider et al. 2000). Numbers of effective migrants per generation was estimated with the method of Slatkin (1995). In addition, a Bayesian method, implemented in BAYESASS (version 1.3, Wilson and Rannala 2003), was used to estimate recent migration rates in each direction between sites with genotypes pooled for sites 1 and 2. Default settings were selected after consistent results were obtained for multiple parameter settings.

Results

A total of 83 micropterids (TL, 195–376 mm) were collected from the Blanco River. Of these, 66 were collected by angling from site 1 ($N = 48$) and site 3 ($N = 18$), and 17 were collected by electrofishing from site 2. Although 14 loci were employed, WHICHLOCI indicated that only 3 loci were required to meet accuracy specifications (95% correctly assigned with a log of odds [LOD] ratio ≥ 2) by means of the “leave-one-out” approach for reassignment (Table 1).

Collectively, 40% of individuals were classified as smallmouth bass and 60% were classified as hybrids. Among the hybrid genotypes, smallmouth bass \times Guadalupe bass hybrids were the most abundant (84%), followed by smallmouth bass \times largemouth bass hybrids (10%) and then by multispecies hybrids (6%), which exhibited introgression from all three species. Pure Guadalupe bass were not collected (Table 2). Estimated genomic proportions pooled over all sites were predominantly smallmouth bass (0.83), followed by Guadalupe bass (0.16) and then by largemouth bass (0.01). The mean genomic contribution of Guadalupe bass in the smallmouth bass \times Guadalupe bass hybrids

TABLE 1.—Select microsatellite loci, WHICHLOCI rank (10 resampled data sets, 10,000 samples each), and baseline allele frequencies (allele frequencies ≥ 0.05 in bold italics) for smallmouth bass (SMB), Guadalupe bass (GB), and largemouth bass (LMB). The assignment success using all loci was 99.35 ± 0.74 ; 0.59 ± 0.72 failed to be correctly assigned under the specified stringency threshold (log of odds ratio = 2).

Locus	Rank	Score	% Relative score	SMB	GB	LMB
<i>Mdo1</i>	1	2.58	8.91			
217				0.28	0.00	0.99
220				0.50	0.09	0.01
222				0.00	0.72	0.00
224				0.00	0.16	0.00
226				0.17	0.03	0.00
228				0.04	0.00	0.00
230				0.02	0.00	0.00
<i>Lar7</i>	2	2.57	8.85			
126				0.03	0.07	0.00
128				0.03	0.05	0.00
130				0.09	0.37	0.01
132				0.05	0.01	0.00
135				0.00	0.20	0.00
137				0.13	0.02	0.00
139				0.13	0.03	0.00
142				0.00	0.16	0.00
146				0.00	0.09	0.60
148				0.25	0.00	0.03
150				0.07	0.00	0.02
151				0.11	0.00	0.02
153				0.02	0.00	0.00
155				0.00	0.00	0.03
159				0.08	0.00	0.02
163				0.02	0.00	0.14
167				0.00	0.00	0.01
175				0.01	0.00	0.13
<i>Lma120</i>	3	2.56	8.82			
198				0.08	0.00	0.00
200				0.55	0.00	0.00
202				0.34	0.01	0.04
204				0.04	0.88	0.19
206				0.00	0.05	0.15
208				0.00	0.06	0.25
211				0.00	0.00	0.24
213				0.00	0.00	0.13
Mean (all loci)		2.23	7.69			

was 0.28 (range, 0.033–0.798), whereas that of smallmouth bass was 0.71 (range, 0.183–0.925).

The proportionate genomic contribution of the three species varied among sites. The mean \pm SE smallmouth bass influence was 0.89 ± 0.13) at site 1, 0.82 ± 0.22 at site 2, and 0.66 ± 0.22 at site 3. Guadalupe bass genomic contribution increased in a downstream direction, ranging from 0.09 ± 0.13 at site 1, to 0.16 ± 0.20 at site 2, to 0.33 ± 0.22 at site 3. The genomic contribution of largemouth bass was consistently low at all sites, ranging from 0.01 ± 0.01 to 0.03 ± 0.05 . Genotypes also varied among sites; the percentage of smallmouth bass \times Guadalupe bass hybrids ranged from 40% and 35% at sites 1 and 2, respectively, to 94% at site 3.

TABLE 2.—Genotype of 83 micropterids collected from the Blanco River, Texas, during January and February 2005 at each of three sampling sites. The data are numbers of individuals.

Genotype	Site 1	Site 2	Site 3
Smallmouth bass	24	8	1
Guadalupe bass			
Smallmouth bass \times Guadalupe bass	19	6	17
Smallmouth bass \times largemouth bass	4	1	
Smallmouth bass \times largemouth bass \times Guadalupe bass	1	2	

Leaving out largemouth bass-related genotypes, no significant departures from HWE were observed at sites 1 or 2, and only 2 of 14 loci at site 3 showed significant deviations ($\alpha = 0.05$). Significant departures from linkage equilibrium were observed at 17, 12, and 11 of 91 pairwise locus combinations at sites 1, 2, and 3, respectively ($\alpha = 0.05$). Estimates of genetic differentiation among sites indicated that sites 1 and 2 were not significantly different ($F_{ST} = 0.01$, $P = 0.126$), but both were significantly different from site 3 ($F_{ST} = 0.07$ and 0.06 , $P < 0.001$ and 0.001). Slatkin's (1995) estimate of gene flow indicated that the number of effective migrants per generation between sites 1 and 2 ($M' = 22.3$) was substantially greater than the number of migrants exchanged between sites 1 and 3 ($M' = 3.3$) or sites 2 and 3 ($M' = 3.9$). Due to the close proximity and genetic similarity of sites 1 and 2, these sites were pooled for analysis of migration rates. Migration rate 95% CIs based on the collected genotypes were narrower (less than 0.5) than priors assuming noninformative allele frequencies, suggesting that enough genetic variation was present to reliably detect migration rates. Results indicated asymmetric migration, predominantly downstream (Figure 2).

Discussion

Guadalupe bass previously have been collected throughout the Blanco River basin; however, the failure to detect Guadalupe bass genotypes in this study suggests possible genetic extirpation of the species in the Blanco River. We attribute this apparent extirpation to introgression with smallmouth bass despite supplemental stocking of Guadalupe bass. While the lingering LD may be a result of this stocking effort or previous admixture events, the attainment of HWE within sites suggests relatively nonassortative mating between resident Guadalupe bass, smallmouth bass, and their hybrids over multiple generations. Before the supplemental stocking, a total of 218 micropterids, excluding largemouth bass, were collected from similar areas of the Blanco River from 1991 through 1993 (Morizot et al. 1991; Farquhar 1995). In those studies, hybrids

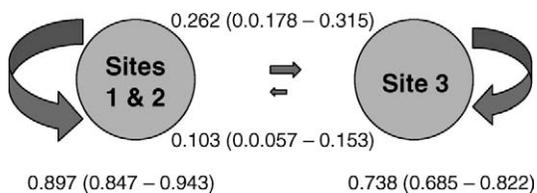


FIGURE 2.—Estimated migration rates (95% confidence intervals in parentheses) among collection sites on the Blanco River using the Bayesian method implemented in BAYESASS (Wilson and Rannala 2003).

comprised 75% of the fish captured, followed by smallmouth bass (17%) and Guadalupe bass (8%). In 2005, 10 years after supplemental stocking, Guadalupe bass were not found, smallmouth bass increased to 40%, and hybrids comprised 60% of the bass assemblage. If Guadalupe bass were still present at a rate of 8%, the probability of obtaining at least one in a random sample of 83 fish is 0.999, assuming a random sample was obtained. Thus, our results suggest that if this fish is not extirpated in the Blanco River, it is severely reduced in number. Consequently, the three supplemental stockings totaling 80,000 Guadalupe bass (two in May 1994 and one in July 1995) were not successful in shifting genetic influence from smallmouth bass toward Guadalupe bass.

The proportions of various hybrid genotypes documented here were similar to those of Morizot et al. (1991) and were dominated by smallmouth bass \times Guadalupe bass hybrids. Multispecies hybrids (smallmouth bass \times Guadalupe bass \times largemouth bass) were identified in low proportions in both studies. In addition, our results identified five individuals as smallmouth bass \times largemouth bass hybrids. Although hybrids between these two species are rare in sympatric populations, they were documented in the San Gabriel, Guadalupe, and Medina rivers (Garrett 1991) and in Squaw Creek Reservoir, Texas, after introduction of nonnative smallmouth bass (Whitmore and Hellier 1988).

The moderate genetic differentiation between the upstream sites and site 3 was possibly a result of habitat variation, physical distance, or structural impediments limiting gene flow. Sites 1 and 2 exhibited higher proportions of smallmouth bass and lower proportions of hybrids than site 3. Similar longitudinal trends in species composition were also noted by Morizot et al. (1991). Guadalupe bass are most often found in high-velocity habitats, especially immediately downstream of riffles, whereas smallmouth bass use pool habitats to a greater extent (Farquhar 1995). Lower portions of the Blanco River (including site 3) are characterized by more heterogeneous habitats with a large number of riffles, whereas sites 1 and 2 are dominated by long

stretches of run habitats, possibly contributing to higher abundance of smallmouth bass.

Considering gene flow among the sampled sites, restoration efforts in the Blanco River should predictably concentrate on sites near the upstream end of the introgressed areas above physical impediments to migration. Accounting for the rate and direction of gene flow at specific locations might enhance the efficiency of stocking events intended to restore the genetic integrity of a species within its native range. Donor sites that promote wide dispersal and limited immigration would be optimal, allowing for a minimum number of stockings that, once established, would maintain their genetic integrity and annually influence the surrounding sites.

Introgressive hybridization with smallmouth bass is not only a problem in the Blanco River but also has been noted from several localities throughout the range of the Guadalupe bass. Despite this widespread introgression, pure populations are thought to exist in several locations (Garrett 1991). However, given continued introgression in the Blanco River, a current genetic analysis of other populations is warranted to assess conservation status of the species.

Allendorf et al. (2001) noted that hybridized populations are of little conservation value if pure populations exist. However with uncommon taxa or where pure populations are rare, conservation of introgressed populations may be vital to preserving the species (Dowling and Childs 1992). Eradicating introgressed populations and restocking hatchery-raised fish has been proposed for recovery of introgressed populations of cutthroat trout *Oncorhynchus clarkii* (Allendorf and Leary 1988). However, eradication in an entire river system such as the Blanco River would be difficult if not impossible to accomplish without severe negative impacts to the rest of the aquatic community. Furthermore, total eradication of introgressed populations might cause the loss of localized genetic variation, and given that hatchery stock are likely to exhibit reduced genetic diversity, this can lead to genetic homogenization at the population and species levels (Allendorf and Leary 1988; Ryman and Laikre 1991; Dowling and Childs 1992). Selective removal of the introduced species and hybrid individuals that are morphologically similar to the introduced species is one method proposed to conserve localized genetic variation while decreasing proportions of introduced alleles (Dowling and Childs 1992). Nonselective removal of a large portion of the introgressed population, followed by hatchery supplementation, has resulted in recovery of an introgressed population of Leon Springs pupfish *Cyprinodon bovinus* (Echelle and Echelle 1997; Echelle et al.

2004). Although eradication of smallmouth bass in the Blanco River is unlikely, removal of smallmouth bass and morphologically similar hybrids could reduce proportions of smallmouth bass alleles in the population and thereby increase the efficiency of supplemental stockings.

Although stocking of hatchery-raised Guadalupe bass fingerlings was unsuccessful in the Blanco River, this strategy appears to have been successful elsewhere. Localized reduction in the number of hybrids has been noted in Johnson Creek, a tributary to the Guadalupe River, after a series of stockings averaging more than 32,000 fingerlings per year from 1992 to 2005 (Gary Garrett, Texas Parks and Wildlife Department, personal communication). Results from Johnson Creek indicate that persistent stockings can positively influence hybridization levels after several years (Koppelman and Garrett 2002). However, the effects of persistent stockings on a population already dominated by introduced alleles, such as that of the Blanco River, are unknown.

Hybridization with congeners is not exclusive to the Guadalupe bass. The popularity of bass species as sport fish resulted in widespread introductions outside their native ranges. With the exception of the Suwanee bass *M. notius*, hybridization has been documented between each of the recognized *Micropterus* species and at least one other member of the genus (Kassler et al. 2002). Given that four of the seven species are considered rare owing to their small native ranges (Koppelman and Garrett 2002), interspecific hybridization and introgression severely threaten the genetic integrity of species in this genus. The development of prestocking evaluations (Pipas and Bulow 1998) to assess the potential impacts to native species before introductions, along with detailed management plans to aid in conserving the genetic integrity of resident populations (Echelle 1991), will be vital to conserving species diversity within this genus.

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