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### Rangewide Survey of the Introgressive Status of Guadalupe Bass: Implications for Conservation and Management

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ARTICLE

## Rangewide Survey of the Introgressive Status of Guadalupe Bass: Implications for Conservation and Management

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### Abstract

The stocking of fishes outside of their native range for the purpose of sport fisheries can lead to secondary contact and introgression between species that were historically allopatrically distributed. Smallmouth Bass *Micropterus dolomieu* were introduced within the range of Guadalupe Bass *M. treculii* in central Texas and introgressive hybridization subsequently occurred. One recent survey of temporal changes in introgression in the Blanco River found that introgression had increased and that Guadalupe Bass had been extirpated. Thus, a survey of changes in introgression across the range of the Guadalupe Bass was conducted in 12 subbasins in the Brazos, Colorado, Guadalupe–San Antonio, and Nueces drainages in Texas using 15 microsatellite loci. The results indicate that introgression is now occurring in four subbasins but no longer occurring in the Lampasas and San Gabriel rivers, where rates were previously 6% and 46%, respectively. Additionally, we found no evidence that stocking of hatchery-reared individuals in the Guadalupe and Nueces rivers has led to severely depressed genetic variation. The variable success of restoration efforts to prevent extirpation of the Guadalupe Bass suggests that protection of the remaining nonintrogressed populations should be a priority for the conservation of this species.

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Freshwater sport fishes are among the most widely introduced fish species in North America (Rahel 2000), and stocking of sport fish often results in previously allopatric species coming into secondary contact. As a result, sport fish introductions account for several known incidences of introgressive hybridization in fishes (e.g., Whitmore 1983; Koppelman 1994; Pipas and Bulow 1998; Johnson and Fulton 2004; Cordes et al. 2006). Introgressive hybridization is one mechanism by which extirpation and extinction of native species can occur (Rhymer and Simberloff 1996) and is a significant threat to many na-

tive fishes in North America (Williams et al. 1989; Perry et al. 2002). The dynamics of introgression can result in multiple outcomes, including bimodal hybrid zones, hybrid swarms, and replacement of native parental species by hybrids. For example, hybridization between Dolly Varden *Salvelinus malma* and Bull Trout *S. confluentus* resulted in a bimodal hybrid zone in which both parental species co-occurred in greater abundance than the hybrids between the two species (Redenbach and Taylor 2003), whereas Childs et al. (1996) found that Pecos Pupfish *Cyprinodon pecosensis* severely declined and Pecos

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Pupfish × Sheepshead Minnow *C. variegatus* hybrids became predominant. Littrell et al. (2007) found that Guadalupe Bass *Micropterus treculii* had been replaced by hybrids between Guadalupe Bass and Smallmouth Bass *Micropterus dolomieu* but that pure Smallmouth Bass also remained. The outcome of hybridization can be affected by behavior, hybrid fitness, and environmental factors, which can determine directionality of introgression and ultimately lead to extirpations.

The Guadalupe Bass is endemic to central Texas and is native to streams from the Brazos River basin to the Guadalupe–San Antonio River basin in central Texas and occurs primarily in the Edwards Plateau (Edwards 1980). Guadalupe Bass inhabit upland streams in these basins but are typically absent from reaches where flows are primarily from stenothermal springs (Guillory 1980). The Guadalupe Bass is currently considered a species of conservation concern by several authors (Hubbs et al. 2008; Jelks et al. 2008) due to declines associated with habitat loss (Edwards 1978) and hybridization (Edwards 1979). Smallmouth Bass *Micropterus dolomieu* are among the most widely introduced fish species in the USA (Rahel 2000) and were stocked within the range of Guadalupe Bass *Micropterus treculii* beginning in 1958, with intensive stockings beginning in 1974 (Garrett 1991). Hybridization between Guadalupe Bass and introduced Smallmouth Bass was first reported by Edwards (1979), and introgression was subsequently documented by Whitmore (1983). Although several studies (Whitmore and Butler 1982; Whitmore 1983; Garrett 1991; Morizot et al. 1991) have collectively examined the introgressive status of Guadalupe Bass across its range, one study (Littrell et al. 2007) has examined temporal changes in introgression, showing the apparent extirpation of Guadalupe Bass from the Blanco River, Texas, despite restorative stocking efforts in 1994–1995.

The primary objective of our study was to assess the introgressive status of Guadalupe Bass across its range and to

evaluate temporal changes in introgression using a suite of microsatellite loci. Because propagule pressure can impact the directionality and ultimate outcome of introgression in populations (Bennett et al. 2010), samples from rivers that received the greatest intensity stocking of Smallmouth Bass should exhibit the highest rates of introgression. Given that the stocking of hatchery-reared individuals and the creation of refuge populations can lead to reduced genetic diversity within populations (Osborne et al. 2006), we also evaluate the effects of conservation and restoration efforts on genetic diversity.

## METHODS

Tissue samples of three species of black bass (genus *Micropterus*) were collected from October 2006 through June 2010: Guadalupe Bass, Smallmouth Bass, and Largemouth Bass *M. salmoides*. Fish were collected from 50 sites (see Bean 2012 for more detailed site information) among 12 river subbasins (Table 1) encompassing the range of the Guadalupe Bass in the Lampasas, San Gabriel, Concho, San Saba, Llano, Pedernales, Colorado, Guadalupe, and Medina, Nueces, Frio, and Sabinal subbasins (Figure 1). Based on the proximity of sites with subbasins, population genetic structuring largely at the subbasin level (Bean 2012), and the movement of Guadalupe Bass (Perkin et al. 2010) and Smallmouth Bass (Lyons and Kanehl 2002), samples were grouped by subbasin for analyses. Fish were collected using a combination of sampling gears, including backpack electrofishing, boat electrofishing, seining, and angling. Tissue samples consisted of fin clips taken from either pectoral or caudal fins and preserved in 70% ethanol at room temperature. Additional samples of Smallmouth Bass, from two localities outside of the range of Guadalupe Bass, were collected from the Devils River in Val Verde County, Texas, and from

TABLE 1. Geographic coordinates for the midpoint of the sampled area for each subbasin; sample sizes ( $N$ ) of all black bass species collected; and observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and mean allelic richness ( $k$ ) for nonintrogressed Guadalupe Bass in 12 subbasins across its range. Population genetic parameters were not estimated for samples from the Concho, Frio, and Sabinal rivers due to small sample sizes or the absence of nonintrogressed Guadalupe Bass.

Subbasin	Latitude	Longitude	$N$	$H_O$	$H_E$	$k$
1. Lampasas	31.085512	−98.050967	40	0.39	0.43	3.71
2. San Gabriel	30.652201	−97.664165	38	0.48	0.48	3.99
3. Concho	31.187243	−100.501079	6			
4. San Saba	31.004189	−99.269006	32	0.54	0.53	3.33
5. Llano	30.589030	−99.597470	85	0.48	0.48	4.50
6. Pedernales	30.272003	−98.544831	118	0.48	0.48	3.62
7. Colorado	29.705723	−96.536608	40	0.43	0.49	5.48
8. Guadalupe	29.964620	−98.897338	123	0.56	0.61	5.74
9. Medina	29.357987	−98.893626	66	0.51	0.48	5.01
10. Nueces	29.885155	−100.020561	42	0.40	0.41	3.06
11. Frio	29.603947	−99.737577	2			
12. Sabinal	29.516624	−99.508978	4			

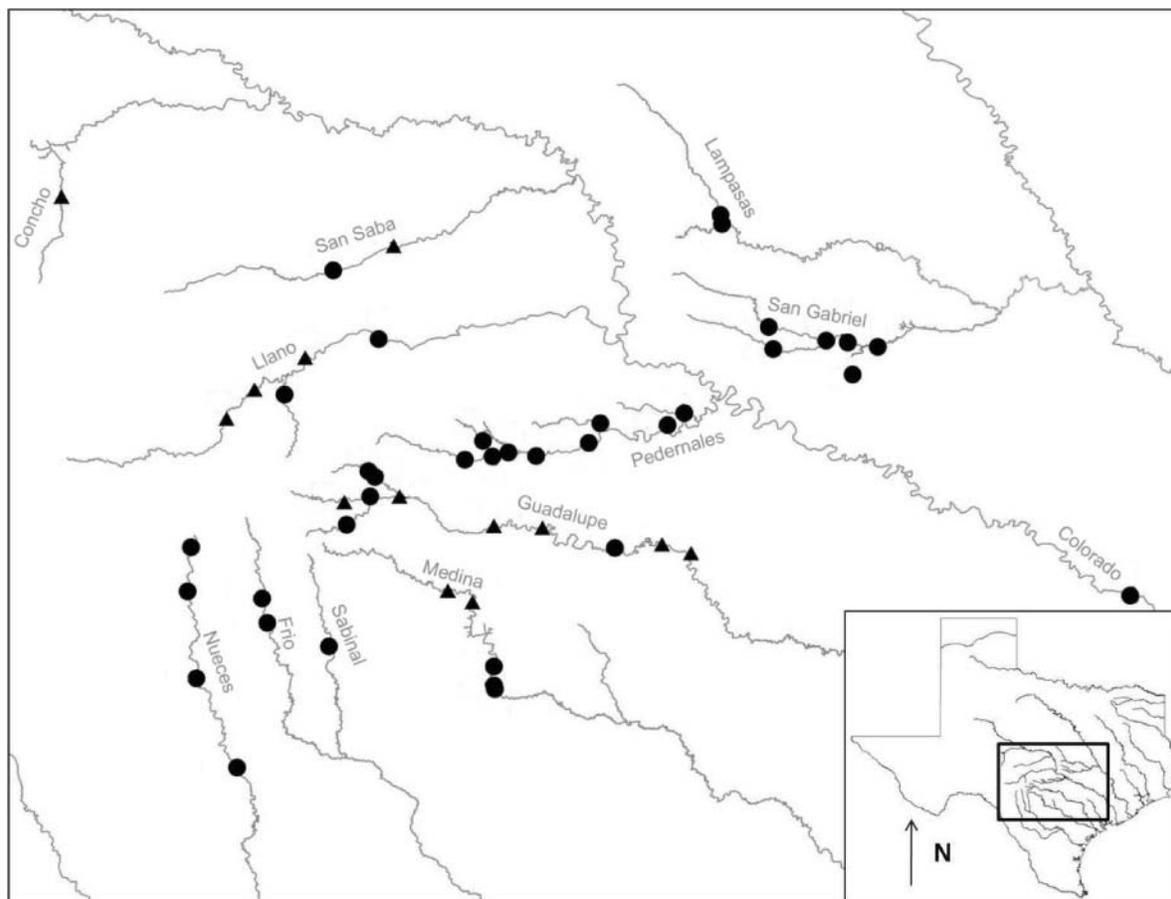


FIGURE 1. Texas localities sampled that encompass the native and introduced range of Guadalupe Bass. Circles indicate sites where no genetic influence of Smallmouth Bass was found, and triangles indicate sites where genetic influence of Smallmouth Bass was found.

Belton Lake in Bell and Coryell counties. The later collection was performed by Texas Parks and Wildlife Department staff.

Whole genomic DNA was extracted from fin tissues using a high-salt extraction method modified from Miller et al. (1988), where ammonium acetate was substituted for sodium chloride in the cellular protein precipitation step. Purified DNA was rehydrated in 100  $\mu$ L of low-tris-EDTA buffer (10 mM of tris, 0.1 mM of EDTA, pH = 8) and the concentration and purity of DNA evaluated by spectrophotometry at 260 and 280 nm with a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Concentrations of DNA were then adjusted to 50 ng/ $\mu$ L using additional low tris-EDTA buffer.

Samples were genotyped at 15 microsatellite loci (*Lma121*, *Mdo1*, *TPW012*, *TPW025*, *TPW060*, *TPW062*, *TPW076*, *TPW090*, *TPW096*, *TPW115*, *TPW121*, *TPW123*, *TPW132*, *TPW134*, *TPW154*) in six optimized multiplex reactions (Neff et al. 1999; Malloy et al. 2000; Lutz-Carrillo et al. 2008). Polymerase chain reactions (PCRs) were performed at 10- $\mu$ L volumes and consisted of 1  $\times$  PCR buffer (20 mM tris-HCl [pH = 8.4], 50 mM of KCl), 1.5–2.0 mM of MgCl<sub>2</sub> (Table 2), 0.2 mM of deoxynucleotide triphosphates (dNTPs), 0.05  $\mu$ M of

CAG-tailed (5'-CAGTCGGGCGTCATCA-3') primers, 0.15–0.35  $\mu$ M of nontailed primers, 0.20  $\mu$ M of a 25% labeled CAG sequence (Lutz-Carrillo et al. 2008; IRDye 700 or IRDye 800 label; LI-COR, Lincoln, Nebraska), 0.5 units (U) of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, California), and 50 ng of template DNA. Samples were first denatured at 94°C for 1.5 min followed by 25–31 cycles of denaturation at 94°C for 30 s, annealing at 59.0–63.4°C (Table 2) for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. Amplicons were denatured in a formamide stop solution (2.5 mM of EDTA, 7.5 mM of bromophenol blue) and analyzed alongside size standards on a LI-COR 4300 DNA analyzer. Resulting gel images were scored and alleles were assigned to band classes using BioNumerics version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium).

The direction of introgression was evaluated using a mitochondrial PCR-RFLP. A 1,120 base pair (bp) segment of the cytochrome *b* gene was amplified using the primers of Song et al. (1998). Polymerase chain reactions were performed at 10- $\mu$ L volumes and consisted of 1  $\times$  PCR buffer, 1.5 mM of MgCl<sub>2</sub>, 0.20 mM of dNTPs, 0.20  $\mu$ M of cytochrome *b* forward primer,

TABLE 2. Polymerase chain reaction conditions for the six microsatellite multiplexes used to amplify 15 microsatellite loci in Guadalupe, Smallmouth, and Largemouth Bass.

Multiplex	Locus	MgCl ( $\mu\text{M}$ )	Annealing temperature ( $^{\circ}\text{C}$ )	Cycles	Primer concentration ( $\mu\text{M}$ )
MPX1	<i>TPW060</i>	1.5	60.0	25	0.05
	<i>TPW062</i>				0.35
MPX2	<i>TPW076</i>	2.0	60.0	25	0.15
	<i>TPW123</i>				0.20
	<i>TPW096</i>				0.15
MPX3	<i>TPW154</i>	1.5	63.4	27	0.20
	<i>TPW121</i>				0.20
MPX4	<i>TPW115</i>	2.0	63.4	31	0.15
	<i>Lma121</i>				0.20
	<i>Mdo1</i>				0.15
MPX5	<i>TPW134</i>	2.0	59.0	26	0.10
	<i>TPW132</i>				0.20
	<i>TPW025</i>				0.30
MPX6	<i>TPW090</i>	1.5	61.6	29	0.20
	<i>TPW012</i>				0.15

0.20  $\mu\text{M}$  of cytochrome *b* reverse primer, 0.5 U of Platinum *Taq* DNA polymerase, and 50 ng of template DNA. Samples were first denatured at  $94^{\circ}\text{C}$  for 1.5 min followed by 39 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $49^{\circ}\text{C}$  for 30 s, extension at  $72^{\circ}\text{C}$  for 1 min, and a final extension at  $72^{\circ}\text{C}$  for 10 min. A 1- $\mu\text{L}$  subsample of each PCR product was then digested using the restriction endonuclease *MboI*. Restriction reactions were performed at 10- $\mu\text{L}$  volumes and consisted of  $1\times$  NEBuffer 3 (100 mM of  $\text{NaCl}_2$ , 50 mM of tris-HCl, 10 mM of  $\text{MgCl}_2$ , 1 mM of dithiothreitol, pH = 7.9; New England Biolabs, Ipswich, Massachusetts), 0.24 units of *MboI*, and 1.0  $\mu\text{L}$  of PCR product. Restriction digest occurred for 1 h at  $37^{\circ}\text{C}$  followed by 20 min at  $65^{\circ}\text{C}$  to inactivate the enzyme. Restriction fragments were separated by electrophoresis alongside a size standard in a 1% agarose gel stained with ethidium bromide and then visualized under ultraviolet light. Genotypes were then manually assigned for each individual based on a visual evaluation of banding patterns.

We used MICRO-CHECKER (v2.2.3, Van Oosterhout et al. 2004) to test for null alleles, stuttering, and large-allele dropout in the microsatellite data. Basic population genetic characteristics for Guadalupe Bass grouped by subbasin included allelic richness, expected heterozygosities, and observed heterozygosities, which were calculated in GDA (version 1.1; Lewis and Zaykin 2001) and FSTAT (version 2.9.3; Goudet 2002). Allelic richness (*k*) was estimated using rarefaction to account for differences in sample sizes among subbasins. Tests for departures from Hardy–Weinberg equilibrium (HWE) were conducted for each locus in each sample using Arlequin (version 3.5; Excoffier et al. 2010) and sequential Bonferroni corrections for multiple tests were applied. Population genetic parameters were estimated for nonintrogressed Guadalupe Bass to prevent inflation

of genetic diversity estimates by the presence of Smallmouth Bass alleles. Tests for recent bottleneck events were conducted for each subbasin using the mode-shift test (Luikart et al. 1998) implemented in Bottleneck (version 1.2; Piry et al. 1999). Populations having undergone recent bottleneck events characteristically exhibit small numbers of low-frequency alleles ( $\leq 0.100$ ) and a majority of alleles with intermediate frequencies (0.101–0.900; Luikart et al. 1998).

To determine the taxonomic assignment power of each microsatellite locus we used the program WHICHLOCI (Banks et al. 2003). Briefly, for microsatellite data from Smallmouth Bass collected outside of the range of Guadalupe Bass and purportedly nonintrogressed Guadalupe Bass from the Nueces River subbasin, we used the allele frequency differential method based on 1,000 resampled data sets of 10,000 individuals each to identify microsatellites with locus-specific assignment power  $>99\%$ . To estimate the admixture proportion (*q*) of each individual's genome contributed by each of the (*K*) parental species we used the admixture model implemented in STRUCTURE (version 2.3; Pritchard et al. 2000) to partition each multilocus genotype. The Bayesian inference algorithm implemented in STRUCTURE creates groups (*K*) under the criteria of minimizing within-group linkage disequilibrium and departures from HWE. Simultaneously, individuals are probabilistically assigned to groups (parental species) or jointly to two or more populations in the case of admixture. Each subbasin was analyzed separately to minimize interference from underlying genetic population structure. Three independent Markov chain–Monte Carlo (MCMC) simulations, with *K* set to 2–4, were performed for 300,000 iterations following a burn-in period of 50,000 steps. The results from each run were then compared to ensure that the MCMC simulations converged around similar

values for all runs. We followed the method of Vähä and Primmer (2006) to determine the appropriate threshold value of  $q$  for classifying hybrid and nonhybrid individuals. Briefly, we simulated 10 populations of 100 individuals each of Guadalupe Bass, Smallmouth Bass,  $F_1$  hybrids,  $F_2$  hybrids, backcrosses to Guadalupe Bass, and backcrosses to Smallmouth Bass (600 individuals total per simulated population) in the program HYBRIDLAB (version 1.0; Nielsen et al. 2006); parental types were Smallmouth Bass from Belton Lake and Devils River and presumed nonintrogressed Guadalupe Bass from the Nueces River. We then ran STRUCTURE with the same parameters as outlined above at  $K = 2$ . Efficiency and accuracy of assignment as well as overall performance (Vähä and Primmer 2006) were calculated for threshold  $q$ -values of 0.01, 0.05, 0.10, and 0.20.

For samples where hybrids were detected, we used the program NEWHYBRIDS (version 1.1; Anderson and Thompson 2002) to identify recent hybridization events and assign individuals to genotypic classes (i.e., pure,  $F_1$ ,  $F_2$ , and backcrosses). This program, which uses a Bayesian clustering model to compute, via MCMC, the posterior probabilities of each individual belonging to a particular genotypic class, was run for 200,000 iterations following a burn-in period of 50,000 steps. A chi-square test was applied to determine directionality of introgression of cytochrome *b* *MboI* haplotypes among hybrids for samples where sufficient numbers of hybrids were detected.

## RESULTS

Fifteen microsatellite loci were amplified from 630 black bass sampled throughout the range of Guadalupe Bass and 20 Smallmouth Bass outside of the range of the Guadalupe Bass (17 from Belton Lake and 3 from the Devils River). All loci were polymorphic, the number of alleles per locus ranging from 6 to 27. Results from analyses in MICRO-CHECKER did not detect evidence for scoring errors. Significant departures from HWE, all resulting from heterozygote deficits, were observed

in four samples. Departures occurred at one locus each in the San Gabriel River and Llano River samples, three loci each in the Pedernales River and Guadalupe River samples when Guadalupe Bass  $\times$  Smallmouth Bass hybrids were included. When hybrids were excluded, two of the HWE departures in the Guadalupe River sample were resolved, whereas all other HWE departures remained.

Nine of 15 loci (*TPW096*, *TPW121*, *TPW154*, *Mdo1*, *Lma121*, *TPW115*, *TPW025*, *TPW132*, and *TPW012*) were retained for species discrimination. Simulations in WHICHLOCI suggested that this marker panel to be sufficient to assign individuals to species-specific groups with an accuracy  $>99\%$ . A threshold value of  $q = 0.05$  was determined to be the most appropriate value for categorization of hybrid and nonhybrid individuals based on analysis in STRUCTURE of the 10 data sets simulated in HYBRIDLAB. Introgressive hybridization between Guadalupe Bass and Smallmouth Bass was detected in 4 (i.e., San Saba, Llano, Guadalupe and Medina rivers; Table 3) of the 12 subbasins sampled, based on admixture proportions estimated in STRUCTURE. Percentages of individuals identified as Guadalupe Bass  $\times$  Smallmouth Bass hybrids (excluding Largemouth Bass) were 13.7% in the Guadalupe River, 3.1% in the San Saba River, 3.9% in the Llano River, and 1.6% in the Medina River samples. Guadalupe Bass were not detected in the Concho River where they previously occurred; only Largemouth Bass and Smallmouth Bass were detected. Guadalupe Bass  $\times$  Smallmouth Bass hybrids or pure Smallmouth Bass were not detected in four subbasins (Lampasas, San Gabriel, Pedernales, and Colorado rivers) within the Guadalupe Bass's native range and three subbasins where Guadalupe Bass were introduced (Nueces, Frio, and Sabinal rivers). Guadalupe Bass  $\times$  Largemouth Bass hybrids were detected in the Nueces River (11%), Medina River (8.1%), and Pedernales River samples (5.1%). Only one Smallmouth Bass  $\times$  largemouth Bass hybrid was detected and occurred in the Medina River subbasin.

TABLE 3. Percentage of black bass individuals from 12 Texas subbasins assigned to each parental or hybrid genotype based on the results of analyses of nine microsatellite loci in STRUCTURE version 2.3 (Pritchard et al. 2000); GB = Guadalupe Bass, SMB = Smallmouth Bass, LMB = Largemouth Bass.

Subbasin	GB	SMB	GB $\times$ SMB	GB $\times$ LMB	SMB $\times$ LMB
Lampasas	100.0	0.0	0.0	0.0	0.0
San Gabriel	100.0	0.0	0.0	0.0	0.0
Concho	0.0	100.0	0.0	0.0	0.0
San Saba	96.9	0.0	3.1	0.0	0.0
Llano	96.1	0.0	3.9	0.0	0.0
Pedernales	94.9	0.0	0.0	5.1	0.0
Colorado	100.0	0.0	0.0	0.0	0.0
Guadalupe	85.3	1.0	13.7	0.0	0.0
Medina	88.7	0.0	1.6	8.1	1.6
Nueces	88.9	0.0	0.0	11.1	0.0
Frio	100.0	0.0	0.0	0.0	0.0
Sabinal	100.0	0.0	0.0	0.0	0.0

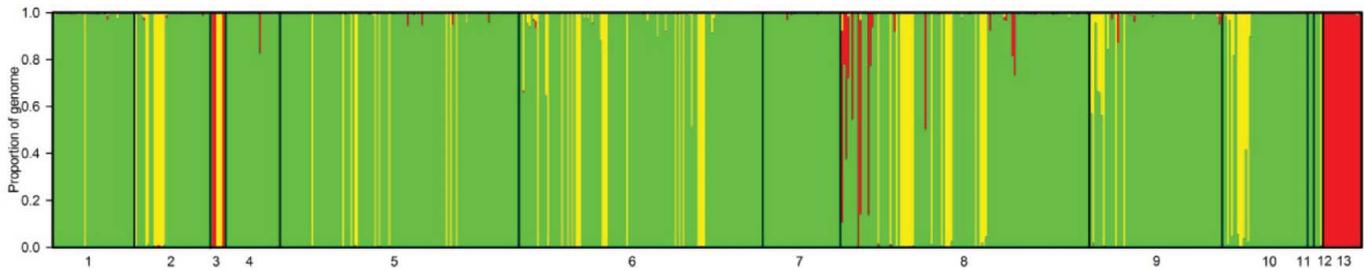


FIGURE 2. Bayesian inference of admixture proportions between Guadalupe (green), Smallmouth (red), and Largemouth (yellow) Bass as determined with STRUCTURE version 2.3 (Pritchard et al. 2000). Each vertical bar represents an individual; the proportions of each bar represented by the different colors indicate the admixture proportions attributed to the different parental species. The numbers along the bottom of figure denote subbasins and correspond to those in Table 1. [Figure available online in color.]

Smallmouth Bass admixture proportions estimated in STRUCTURE ranged from 0.053 to 0.861 (Figure 2) among the individual hybrids. Mean  $\pm$  SE Smallmouth Bass admixture proportions among Guadalupe Bass  $\times$  Smallmouth Bass hybrids was  $0.389 \pm 0.078$  in the Guadalupe River sample,  $0.176 \pm 0.0$  in the San Saba River sample,  $0.053 \pm 0.001$  in the Llano River sample, and  $0.123 \pm 0.0$  in the Medina River sample. Among Guadalupe Bass  $\times$  Largemouth Bass hybrids, mean Largemouth Bass admixture proportion was  $0.451 \pm 0.195$  in the Nueces River sample,  $0.283 \pm 0.069$  in the Medina River sample, and  $0.278 \pm 0.073$  in the Pedernales River sample.

Among hybrid individuals in the Guadalupe River subbasin, one individual was identified as a  $F_1$  hybrid, six individuals were identified as  $F_2$  hybrids, four individuals were identified as backcrosses to Guadalupe Bass, and one individual was identified as a backcross to Smallmouth Bass based on genotypic class assignments in NEWHYBRIDS. Two hybrids from the Guadalupe River sample as well as all hybrids from other samples were not assigned to one of the hybrid genotypic classes.

The Smallmouth Bass *MboI* haplotype was detected in three of four samples where nuclear introgression was documented, occurring at frequencies of 0.008 in the Guadalupe River sample, 0.015 in the Medina River sample, and 0.018 in the Llano River sample. The Smallmouth Bass haplotype also was detected in the Concho River where nuclear markers suggested only Smallmouth Bass and Largemouth Bass were present. Within the Guadalupe River subbasin, directionality of *MboI* introgression was significantly biased towards Guadalupe Bass ( $\chi^2 = 10.29$ ,  $P < 0.01$ ). Only one hybrid individual among the 14 detected in the Guadalupe subbasin possessed a Smallmouth Bass haplotype, and it was collected at the Canyon Lake tailrace.

The mode-shift tests in BOTTLENECK indicated that among all Guadalupe Bass samples tested, recent bottleneck events have not occurred. All samples examined had allele-frequency-class modes in the low-frequency class, whereas samples from bottlenecked populations would exhibit a frequency class mode in the intermediate frequency range. Mode-shift tests were not conducted for the Frio River or Sabinal River samples due to small sample sizes.

## DISCUSSION

Our results indicate that introgressive hybridization between Guadalupe and Smallmouth Bass is occurring in 4 of 12 subbasins examined in this study. The percentage of hybrids within each subbasin was low ( $<4\%$ ) in the three subbasins where hybrids were detected but pure Smallmouth Bass were not. Pure Smallmouth Bass were found in the Concho and Guadalupe subbasins. Guadalupe Bass were not detected and appear to be extirpated from the Concho subbasin and the percentage of introgressed individuals was greatest in the Guadalupe subbasin. These two systems received the greatest intensity of Smallmouth Bass stocking of the subbasins in this study. Similarly, Bennett et al. (2010) found that propagule pressure affected rates of introgression between Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* and introduced Rainbow Trout *O. mykiss*, and Marie et al. (2012) found a linear relationship between number of stocking events and rates of hybridization between domestic and wild Brook Trout *Salvelinus fontinalis*. Hybrid individuals in the Guadalupe subbasin also had the greatest admixture proportions attributed to Smallmouth Bass (mean admixture proportion = 0.389). Intense propagule pressure was common to subbasins with the highest introgression rates and where Guadalupe Bass are extirpated. However, introgression was not detected in the Lampasas subbasin where propagule pressure also was high, and Guadalupe Bass have been extirpated from the Blanco River (Littrell et al. 2007) where propagule pressure was low. Thus, other factors, such as suitability of habitat for Smallmouth Bass, have likely played an important role in the persistence of Smallmouth Bass and subsequent introgression.

Garrett (1991) examined hybridization between Guadalupe and Smallmouth bass using allozyme markers across the range of Guadalupe Bass. Since that survey, patterns of change in levels of introgression have varied among subbasins. In our study introgression was detected in the San Saba, Llano, and Medina rivers, where it previously was undetected. However, rates of introgression are low in these samples, as is the proportional influence of Smallmouth Bass on the genomes of hybrids. This probably reflects the persistence of a few Smallmouth Bass alleles from relatively unsuccessful introductions of Smallmouth

Bass. While earlier rates of introgression were reported at 6% in the Lampasas River and 46% in the San Gabriel River (Garrett 1991), no evidence of genetic influence by Smallmouth Bass was found in either subbasin. This occurred despite no active efforts to restore Guadalupe Bass via supplemental stockings or to eradicate Smallmouth Bass in these localities. The Guadalupe River subbasin has also experienced a decline in hybridization rates from 30% (Garrett 1991) to 13.7%, though, this change occurred with persistent stocking of Guadalupe Bass over an 18-year period. Considering the extirpation of Guadalupe Bass from streams where pure Smallmouth Bass occur and the persistence of hybrids despite the stocking of Guadalupe Bass, it is possible that the stocking of Guadalupe Bass has contributed to the persistence of Guadalupe Bass in this subbasin that might have otherwise been extirpated.

The outcomes of hybridization for native species largely depend on the dynamics of hybridization. In Tennessee, local declines and replacement of Smallmouth Bass by introduced Redeye Bass *Micropterus coosae* and their hybrids typically occurred when hybrid swarms were formed (Pipas and Bulow 1998). Conversely, Gunnell et al. (2008) found that introgression of mtDNA haplotypes among hybrids of Yellowstone Cutthroat Trout *O. clarkii bouvieri* × Rainbow Trout was slightly directional towards Yellowstone Cutthroat Trout and that hybrids composed only a small percentage of the population. Outcomes of introgression between Guadalupe and Smallmouth Bass varied among subbasins. In the Guadalupe River, both parental species and hybrids were found. In the San Saba, Llano, and Medina subbasins, introgression appears to be directional and favoring Guadalupe Bass. Introgressed individuals from these samples had very small admixture proportions derived from Smallmouth Bass, and mtDNA haplotypes did not indicate directional introgression towards Smallmouth Bass. In fact, the direction of mtDNA introgression in the Guadalupe subbasin was significantly biased towards Guadalupe Bass. In contrast to this pattern, we detected no influence of Guadalupe Bass in the Concho subbasin, and Littrell et al. (2007) found only hybrids and Smallmouth Bass in the Blanco River.

Genetic bottlenecks often occur when large numbers of hatchery-reared individuals are released into wild populations (Osborne et al. 2006; Drauch and Rhodes 2007; Kitada et al. 2009) and when relatively small numbers of individuals are used to establish new populations (Grapputo et al. 2006; Dawnay et al. 2011). In the Guadalupe subbasin (where hatchery stockings occurred from 1992 to 2000 and from 2004 to 2010) and in the Nueces subbasin (where a refuge population was established from 2,000 hatchery-reared fingerlings produced from an unknown number of broodstock), we expected to observe some deficit of genetic variation. In the Guadalupe subbasin, there was no evidence for a genetic bottleneck and allelic richness was comparable to other native Guadalupe Bass samples. Explanations for the lack of reduced genetic diversity in the Guadalupe River sample include sufficient genetic diversity among hatchery broodstock, low survival of hatchery-reared individuals, or

hatchery fish making up a small proportion of the individuals in the Guadalupe River subbasin. However, declining levels of introgression over time in the Guadalupe River suggest that hatchery offspring survived at substantial rates, indicating that sufficient genetic diversity among hatchery broodstock is the most likely explanation. Although no bottlenecks were detected in the Nueces sample, allelic richness was lower in this sample than in the Guadalupe subbasin (Table 1) from which the refuge population in the Nueces River was derived. The reduction in allelic richness in the Nueces subbasin was expected given that probably only a subsample of the alleles from a source population would be present in a newly established population; however, the lack of a genetic bottleneck is surprising considering the small number of individuals initially stocked in the Nueces River.

Given that introgressive hybridization can lead to extirpations (Rhymer and Simberloff 1996), limiting the translocation of species outside of their native range will likely be one of the most important conservation strategies for Guadalupe Bass, as well as other endemic black bass species. Because both introgressed and nonintrogressed Guadalupe Bass populations remain and population genetic structuring is largely at the subbasin scale (Bean 2012), the protection of native nonintrogressed populations should be the conservation priority because introgressed populations are of much less conservation value (Allendorf et al. 2001). Previous efforts to restore Guadalupe Bass within their native range have varied from unsuccessful (Littrell et al. 2007) to somewhat successful. However, the reduction in rates of introgression in the Guadalupe River subbasin to near 10% occurred only with persistent stocking over an 18-year period. Currently, stocking of Guadalupe Bass from nonintrogressed broodstock from the Llano subbasin is occurring in the South Llano River within the Llano River subbasin. Patterns of introgression in the Llano subbasin differ from the Blanco River and Guadalupe River subbasins in that introgression rates are relatively low and the direction of the introgression that is occurring favors the persistence of Guadalupe Bass. If restoration efforts in the South Llano River are successful, future restoration efforts should focus on subbasins with similar patterns of introgression (e.g., San Saba and Medina rivers).

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