INTROGRESSIVE STATUS, POPULATION GENETIC STRUCTURE, PHYLOGEOGRAPHIC HISTORY AND INDIVIDUAL-LEVEL RESOURCE SPECIALIZATION OF THE GUADALUPE BASS *MICROPTERUS TRECULII*

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Doctor of PHILOSOPHY

by

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San Marcos, Texas
December 2012

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ACKNOWLEDGEMENTS

I thank my committee members for their efforts and guidance towards the completion of my research and dissertation. Many members of Dr. Bonner's lab contributed many hours of their time, for which I am extremely grateful. I thank Dr. Tim Bonner, Megan Bean, Josh Perkin, Zach Shattuck, Casey Williams, Tom Heard, Kristy Kollaus, Clara Folb, and Callie Lyda for their help with field collections and Megan Bean and Callie Lyda for their help in the lab. I also thank Alisa Abuzeineh, Jesse Becker, and Dr. Weston Nowlin for their instruction and advice regarding stable isotope analysis. I thank Dijar Lutz-Carrillo, Tim Birdsong, and Dr. Gary Garrett of Texas Parks & Wildlife Department for their assistance in the planning stages of this research and for valuable information on the history of Guadalupe bass management efforts. I also thank the Texas Parks & Wildlife Department and the Department of Biology and Graduate College of Texas State University-San Marcos for funding. Finally, I thank my family. My wife Megan and son Harlan have been a source of unending support throughout this process.

This manuscript was submitted October 29, 2012.

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Introgression between Guadalupe bass *Micropterus treculii* and introduced smallmouth bass *M. dolomieu* poses a threat to Guadalupe bass within its native range. Restoration efforts include stocking hatchery-reared Guadalupe bass with the effectiveness of this strategy appearing to be dependent on the intensity and duration of stocking. However, changes in introgression levels in other Guadalupe bass populations are not known. Additionally, streams within the range of Guadalupe bass have a complex

geologic and hydrologic history that include stream captures and changing hydrologic connections associated with sea level changes resulting from glacial influences. How these historical and contemporary (e.g. stocking hatchery-reared fish) factors affect the population genetic structure of Guadalupe bass is not known. Because stocking of hatchery-reared fish can result in losses of genetic variation, it is also possible that phenotypic variation, including variation in resource use, might also be altered in these populations. My research focused on addressing three primary objectives: 1) assessing levels of introgression between Guadalupe and smallmouth bass across the range of Guadalupe bass, 2) evaluating historical and contemporary factors affecting the population genetic structure of Guadalupe bass, and 3) assessing individual-level resource specialization as it relates to genetic and morphological variation among populations. The results of these studies provide a greater understanding of the phylogeographic history of the Edwards Plateau region and of the effects of genetic diversity in wild populations on population niche dynamics.

CHAPTER I

RANGE-WIDE SURVEY OF THE INTROGRESSIVE STATUS OF GUADALUPE BASS *MICROPTERUS TRECULII*: IMPLICATIONS FOR CONSERVATION AND MANAGEMENT

Abstract

The translocation of fishes for the purpose of sport fisheries has led to contact between congeners that were historically allopatrically distributed and, in some cases, to introgressive hybridization. Smallmouth bass *Micropterus dolomieu* were introduced within the range of Guadalupe bass *Micropterus treculii* 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 in twelve populations throughout the range of the Guadalupe bass was conducted using fifteen microsatellite loci. Results indicate that introgression is now occurring in four populations 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 depressed genetic variation. The variable success of restoration efforts to prevent extirpation of the Guadalupe bass

suggests that protection of remaining non-introgressed populations should be a priority for the conservation of this species.

Introduction

Homogenization of the ichthyofauna of the United States is occurring, in part, as a result of introductions of nonnative fishes (Rahel 2000). Introgressive hybridization, as a result of species introductions, poses several threats to native species including outbreeding depression and replacement of native species by hybrids (Allendorf et al. 2001). Thus, introgressive hybridization provides one mechanism by which extirpation and extinction of native species can occur (Rhymer and Simberloff 1996) and results in a significant threat to the native ichthyofauna (Williams et al. 1989, Perry et al. 2002).

Sport fish 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, Campton and Johnston 1985, Allendorf and Leary 1988, Veerspoor 1988, Dunham et al. 1992, Koppelman 1994, Gelwick et al. 1995, Avise et al. 1997, Pipas and Bulow 1998, Johnson and Fulton 2004, Cordes et al. 2006, Gunnell et al. 2008). Depending on the dynamics of introgressive hybridization, the outcomes range from bimodal hybrid zones (e.g., Redenbach and Taylor 2003), to maintenance of a hybrid swarm (e.g., Childs et al. 1996), to replacement of the native species by hybrids (e.g., Littrell et al. 2007).

Smallmouth bass *Micropterus dolomieu* is among the most widely introduced fish species in the USA (Rahel 2000) and was stocked within the range of Guadalupe bass

Micropterus treculii beginning in 1958, with intensive stockings beginning in 1974 (Garrett 1991). The Guadalupe bass is native to streams from the Brazos River basin to the San Antonio River basin in Texas and occurs primarily in the Edwards Plateau (Edwards 1980). Guadalupe bass inhabit upland streams in these basins, but is typically absent from reaches where flows are primarily from stenothermal springs (Guillory 1980). 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 one Guadalupe bass population despite restorative stocking efforts conducted over a two-year period from 1994-1995.

The primary objective of this 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), populations in 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), I also evaluate the effects of conservation and restoration efforts on genetic diversity.

Methods

Tissue samples of micropterids were collected from October 2006 through June 2010 and included samples from Guadalupe bass, smallmouth bass, and largemouth bass *Micropterus salmoides*. Fish were collected from 50 sites (Table 1.1) among 12 sub-basins. Collections encompassing the native range of the Guadalupe bass were conducted in the Lampasas, San Gabriel, Concho, San Saba, Llano, Pedernales, Colorado, Guadalupe, and Medina sub-basins (Figure 1.1). Collections to include portions of the Guadalupe bass's range expanded by introductions were conducted in the Nueces, Frio, and Sabinal sub-basins. Based on the proximity of sites and the movement of Guadalupe bass (Perkin et al. 2010) and smallmouth bass (Lyons and Kanehl 2002), samples were grouped by sub-drainage for analyses. Micropterids 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 populations outside of the range of Guadalupe bass, were collected from the Devils River in Val Verde County, Texas, and from Belton Lake in Bell and Coryell counties. Texas Parks and Wildlife Department staff performed the latter collection.

Whole genomic DNA was extracted from fin tissues using a high-salt extraction method modified from Miller (1988) where ammonium acetate was substituted for sodium chloride in the cellular protein precipitation step. Purified DNA was rehydrated in 100 μl low tris-EDTA buffer (10 mM tris, 0.1 mM EDTA, pH 8) and the concentration and purity of DNA evaluated by spectrophotometry at 260 and 280 nm (NanoDrop 2000).

Concentrations of DNA were then adjusted to 50 ng/μl using additional low tris-EDTA buffer.

Samples were genotyped at 15 microsatellite loci in six optimized multiplex reactions (Lma121, Mdo1, TPW012, TPW025, TPW060, TPW062, TPW076, TPW090, TPW096, TPW115, TPW121, TPW123, TPW132, TPW134, TPW154; Neff et al. 1999; Malloy et al. 2000; Lutz-Carrillo et al. 2008). Polymerase chain reactions were performed at 10 μl volumes and consisted of 1 X PCR buffer (20 mMtris-HCl [pH = 8.4], 50 mM KCl), 1.5 - 2.0 mM $MgCl_2$ (Table 1.2), 0.2 mM deoxynucleotidetriphosphates (dNTPs), 0.05 μM CAG tailed (5'-CAGTCGGGCGTCATCA-3') primers, 0.15 - 0.35 μM nontailed primers, 0.20 μM 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), 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 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 EDTA, 7.5 mM bromophenol blue) and analyzed alongside size standards on a LI-COR 4300 DNA analyzer. Resulting gel images were scored and alleles 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 segment of the cytochrome *b* gene was amplified using the primers of Song et al. (1998). Polymerase chain reactions were performed at 10 μl volumes and consisted of 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.20 mM dNTPs, 0.20μM cytochrome *b* forward primer, 0.20 μM cytochrome *b* reverse primer, 0.5 U of Platinum *Taq* DNA

polymerase, and 50 ng of template DNA. Samples were first denatured at 94°C for 1.5 min followed by 39 cycles of denaturation at 94°C for 30 s, annealing at 49°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. A 1 μl subsample of each PCR product was then digested using the restriction endonculease *MboI*. Restriction reactions were performed at 10 μl volumes and consisted of 1 X NEBuffer 3 (100 mM $NaCl_2$, 50 mM tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH = 7.9; New England Biolabs, Ipswich, Massachusetts), 0.24 units *MboI*, and 1.0 μl PCR product. Restriction digest occurred for 1 hr at 37°C followed by 20 min at 65°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.

Basic population genetic characteristics for Guadalupe bass grouped by sub-drainage were calculated in GDA (Lewis and Zaykin 2001) and included allelic richness and expected and observed heterozygosities. Tests for departures from Hardy-Weinberg equilibrium (HWE) were conducted using Arlequin (v 3.5, Excoffier and Lischer 2010) and Bonferroni corrections for multiple tests were applied. Population genetic parameters were estimated for non-introgressed Guadalupe bass as well as non-introgressed Guadalupe bass and Guadalupe bass x smallmouth bass hybrids combined. Tests for recent bottleneck events were conducted for each population using the mode-shift test (Luikart et al. 1998) implemented in Bottleneck (v 1.2, Piry et al. 1999). Populations having undergone recent bottleneck events characteristically exhibit small numbers of low-frequency (≤0.100) alleles, increased numbers of intermediate-frequency

(0.101-0.900) alleles (Luikart et al. 1998), and an allele frequency class mode in the intermediate frequency. Largemouth and smallmouth bass were excluded from the micropterid microsatellite data set for basic population genetic characteristics.

To determine the taxonomic assignment power of each microsatellite locus I used the program WHICHLOCI (Banks et al. 2003). Briefly, for microsatellite data from smallmouth bass collected outside of the range of Guadalupe bass and Guadalupe bass from the purportedly non-introgressed Nueces River population, I used the allele frequency differential method based on 1,000 resampled datasets 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 I used the admixture model implemented in STRUCTURE (v 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 Hardy-Weinberg equilibrium. Simultaneously, individuals are probabilistically assigned to groups (parental species) or jointly to two or more populations in the case of admixture. Each sub-drainage was analyzed separately to minimize interference from underlying genetic population structure. Three independent Markov chain Monte Carlo (MCMC) simulations, with K set from 2 to 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. I followed the method of Vähä and Primmer (2006) to determine the appropriate threshold value of q for classifying hybrid and non-hybrid individuals. Briefly, I 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 (v1.0, Nielsen et al. 2006) using smallmouth bass from Belton Lake and the Devils River and presumed non-introgressed Guadalupe bass from the Nueces river as parental types. I then ran STRUCTURE with the same parameters as outlined above at K = 2. Efficiency and accuracy of assignment as wells as overall performance (Vähä and Primmer 2006) were calculated for threshold-*q* values of 0.01, 0.05, 0.10, and 0.20.

For populations where hybrids were detected, I used the program NEWHYBRIDS (v1.1, Anderson and Thompson 2002) to identify recent hybridization events and assign individuals to genotypic classes (i.e., pure, F_1, F_2, and backcrosses). NEWHYBRIDS uses a Bayesian clustering model to compute, via MCMC, the posterior probabilities of each individual belonging to a particular genotypic class. NEWHYBRIDS was run for 200,000 iterations following a burn-in period of 50,000 steps.

Results

Fifteen microsatellite loci were amplified from 630 micropterids throughout the range of Guadalupe bass and 20 micropterids outside of the range of the Guadalupe bass (17 smallmouth bass from Belton Lake; 3 smallmouth bass from the Devils River). All loci were polymorphic with the number of alleles per locus ranging from 6 to 27. Significant departures from HWE, all resulting from heterozygote deficits, were observed in four populations. Departures occurred at one locus each in the San Gabriel River and Llano River populations, three loci each in the Pedernales River and Guadalupe River

populations when Guadalupe bass x smallmouth bass hybrids were included. When hybrids were excluded, two of the HWE departures in the Guadalupe River population were resolved whereas all other HWE departures remained.

Nine of fifteen loci (TPW096, TPW121, TPW154, Mdo1, Lma121, TPW115, TPW025, TPW132, and TPW012) were retained for species discrimination. Simulations suggest that this marker panel is 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 non-hybrid individuals based on analysis of the 10 simulated datasets in STRUCTURE at K=2. Introgressive hybridization between Guadalupe bass and smallmouth bass was detected in four (i.e., San Saba, Llano, Guadalupe and Medina rivers) of the twelve populations sampled (Table 1.3). Percentages of Guadalupe bass x smallmouth bass hybrids (excluding largemouth bass) were 13.7% in the Guadalupe River population, 3.1% in the San Saba River population, 3.9 % in the Llano River population, and 1.6% in the Medina River population. Guadalupe bass were not detected in the Concho River where they previously occurred, only largemouth bass and smallmouth bass were detected. Guadalupe bass x smallmouth bass hybrids or pure smallmouth bass were not detected in four populations within the Guadalupe bass's native range (i.e., Lampasas, San Gabriel, Pedernales, Colorado rivers) and three introduced populations (Nueces, Frio, and Sabinal rivers). Guadalupe bass x largemouth bass hybrids were detected in the Nueces River population (11%), Medina River population (8.1%), and Pedernales River population (5.1%). Only one smallmouth bass x largemouth bass hybrid was detected and occurred in the Medina River population.

Estimates of smallmouth bass genomic influence among the hybrids ranged from 0.053 to 0.861. Mean (± SE) proportion of smallmouth bass genomic influence among Guadalupe bass x smallmouth bass hybrids was 0.389 (± 0.078) in the Guadalupe River population, 0.176 (± 0.0) in the San Saba River population, 0.053 (± 0.001) in the Llano River population, and 0.123 (± 0.0) in the Medina River population. Among Guadalupe bass x largemouth bass hybrids, mean proportion of largemouth bass genomic influence was 0.451 (± 0.195) in the Nueces River population, 0.283 (± 0.069) in the Medina River population, and 0.278 (± 0.073) in the Pedernales River population.

Among hybrid individuals in the Guadalupe River population, 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 population as well as all hybrids from other populations were not assigned to one of the hybrid genotypic classes.

The smallmouth bass *MboI* haplotype was detected in three of four populations where nuclear introgression was documented, occurring at frequencies of 0.008 in the Guadalupe River population, 0.015 in the Medina River population, and 0.018 in the Llano River population. The smallmouth bass haplotype was also detected in the South Concho River where nuclear markers suggested only smallmouth bass and largemouth bass were present. Within the Guadalupe River population, only one introgressed individual possessed a smallmouth bass haplotype and was collected at the Canyon Lake tailrace.

The mode-shift test indicated none of the populations have undergone a recent bottleneck event (Figure 1.2). All populations examined had allele frequency class modes in the low-frequency class whereas bottlenecked populations would exhibit a frequency class mode in the intermediate frequency range. Mode-shift tests were not conducted for the Frio River population or the Sabinal River population due to small sample sizes.

Discussion

Introgressive hybridization between Guadalupe bass and smallmouth bass occurred in four of 12 populations and 11 of 50 sites examined throughout the range of Guadalupe bass. The percentage of hybrids within each population was relatively low (< 10%) in three of the populations but was 13.7 % in the Guadalupe River drainage. Additionally, mean proportion of smallmouth bass allelic influence in hybrids was greatest in the Guadalupe River population. Among individuals of hybrid ancestry in the Guadalupe River, the mtRFLP haplotypes were biased towards the Guadalupe bass haplotype, with the smallmouth bass mtRFLP haplotype occurring in only one of fourteen individuals. However, whether this bias is due to a greater frequency of male smallmouth bass mating with female Guadalupe bass than vice versa or to asymmetric hybrid viability (Bolnick et al. 2008) is uncertain. The Guadalupe River population was the only population where both non-introgressed Guadalupe bass and smallmouth bass were documented to occur together as well as the only population where F_1 hybrids were detected. Stocking of hatchery-reared Guadalupe bass occurred within this sub-basin in 16 of the years between 1992 and 2010 at a mean of 61,787 Guadalupe bass per stocking

event. In the Blanco (Littrell et al. 2007) and Concho rivers, where pure smallmouth bass have recently been found, non-introgressed Guadalupe bass are no longer present. 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 a Guadalupe bass population that might have otherwise been extirpated.

In populations where Guadalupe bass and Guadalupe bass x smallmouth bass hybrids were found but pure smallmouth bass were absent (i.e., San Saba, Llano, and Medina rivers), the percentage of hybrids within each population was less than 4%. Additionally, the mean proportional contribution of smallmouth bass to each hybrid genome was lower than in populations where pure smallmouth bass individuals were present. The Guadalupe and Concho rivers were among the most intensively stocked with smallmouth bass and are the two populations where pure smallmouth bass were detected. This intense stocking is a likely cause of the higher rates of introgression in the Guadalupe River population and extirpation of Guadalupe bass from the Concho River as propagule pressure can be a strong determinant of levels of introgression (Bennett et al. 2010). Additionally, the presence of smallmouth bass and F_1 hybrids in the Guadalupe River population suggests that continuing hybridization between the parental species likely contributes to the higher proportional contributions of smallmouth bass in introgressed individuals.

Since the survey of introgression between Guadalupe bass and smallmouth bass by Garrett (1991), changes in levels of introgression have varied among populations. In this 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 populations, as is the proportional influence of smallmouth bass on the genomes of hybrids. This likely reflects the persistence of a few smallmouth bass alleles from relatively unsuccessful introductions of smallmouth bass. While earlier rates of introgression in the Lampasas and San Gabriel rivers were reported at 6% and 46%, respectively (Garrett 1991), no evidence of genetic influence by smallmouth bass was found in either population. This occurred despite no active efforts to restore Guadalupe bass via supplemental stockings or to eradicate smallmouth bass in these populations. The Guadalupe River population has also experienced a decline in hybridization rates from 30% (Garrett 1991) to 13.7%. Among sites within the Guadalupe drainage, Canyon Lake and the Canyon Lake tailrace had the highest percentage of hybrids. This is likely due to intense propagule pressure of smallmouth bass at this site, as well as the favorable habitat for smallmouth bass within the reservoir, and below the dam. Additionally, the effects of hypolimnetic releases from the reservoir alter the habitat in the tailrace making it less likely to be suitable for Guadalupe bass (Edwards 1978).

Guadalupe bass were not collected in the Concho River and appear to possibly be extirpated from the system. Littrell et al. (2007) found both smallmouth bass and Guadalupe bass x smallmouth bass hybrids in the Blanco River, which suggests that directional introgression might have been the cause of extirpation in that system. In the Concho River, however, only smallmouth bass and largemouth bass were collected with no evidence of genetic influence by Guadalupe bass. In this case, the loss of Guadalupe bass may have been due to interspecific competition or changes in local habitat conditions, rather than the effects of introgression alone.

Among populations with departures from HWE at any locus, two of four exhibited hybridization between Guadalupe bass and smallmouth bass. These sub-basins were also sampled at a large number of sites relative to populations that did not show departures from HWE. Thus, departures from HW expectations are possibly the result of either hybridization (Berrebi et al. 2000) or underlying genetic structure (García De León et al. 1997) among sampling sites. Exclusion of hybrids from the dataset did result in two of the HWE departures in the Guadalupe River population being resolved. However, departures from HWE remained in the three other populations suggesting that hybridization and underlying genetic structure likely both contribute to observed HWE departures.

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, Danway et al. 2011). In the Guadalupe River, where hatchery stockings occurred from 1992 to 2000 and from 2004 to 2010, and in the Nueces River where a refuge population was established from 2,000 hatchery-reared fingerlings produced from an unknown number of broodstock, I expected to observe some deficit of genetic variation. Yet no genetic signatures of a genetic bottleneck were resolved by the mode-shift analysis. Although no bottlenecks were detected, the introduced Nueces River population did exhibit a lower proportion of alleles in the low frequency class compared to its original source population in the Guadalupe River, indicating that a reduction in genetic diversity occurred during the establishment of this population. Despite the large number of individuals stocked into the Guadalupe

River, the number of alleles per locus was within the range of other non-stocked populations. Explanations for the lack of depauperate genetic diversity in the Guadalupe River population include sufficient genetic diversity among hatchery broodstock, low survival of hatchery reared individuals, or hatchery reared individuals making up a small proportion of the population. However, declining levels of introgression over time in this population suggest that hatchery offspring survived at substantial rates, indicating that sufficient genetic diversity among hatchery broodstock is the most likely explanation. Within all other sampled populations there was no evidence for a genetic bottleneck or substantial deficit of genetic variation relative to the other populations.

Introgressed and non-introgressed populations of Guadalupe bass remain within its native range. The protection of native non-introgressed populations should be the conservation priority as introgressed populations are of much less conservation value (Allendorf et al. 2001). Previous efforts to restore Guadalupe bass populations 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 population to near 10% occurred only with persistent stocking over an 18-year period. Currently, stocking of Guadalupe bass is occurring in the South Llano River within the Llano sub-drainage. This population differs from the Blanco River and Guadalupe River populations 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 could focus on populations with similar attributes (e.g., San Saba and Medina rivers).

Many members of the genus *Micropterus* have a limited range (Near et al. 2003) and occur allopatrically. As such, prezygotic reproductive isolating mechanisms strong enough to prevent hybridization are less likely to be in place (Hewitt 1989) when anthropogenic introductions bring these species into contact. As a result, hybridization between micropterids is not uncommon (Edwards 1979, Avise et al. 1997, Pipas and Bulow 1998). 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 micropterids.

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Table 1.1. Collection localities and sample sizes for 50 sites sampled for Guadalupe bass and mean allelic diversity (A) for Guadalupe bass both with and without hybrids included.

Site	Brazos Drainage	Latitude	Longitude	*N*	A (GB)	A (GB + GB x SMB)
	Lampasas Sub-basin				4.07	4.07
1	Lampasas at CR 2313	31.118969	-98.056487	1		
2	Sulphur Creek	31.085512	-98.050967	39		
	San Gabriel Sub-basin				4.13	4.13
3	San Gabriel at CR 366	30.629248	-97.473401	1		
4	San Gabriel at HWY 29	30.645684	-97.584944	2		
5	San Gabriel at Georgetown	30.652201	-97.664165	1		
6	San Gabriel at CR 100	30.645204	-97.584193	21		
7	Brushy Creek at CR 685	30.526131	-97.566500	2		
8	South Fork San Gabriel	30.620716	-97.860925	7		
9	North Fork San Gabriel	30.703142	-97.877302	4		
	Colorado Drainage					
	Concho Sub-basin				-	-
10	South Concho at HWY 277	31.187243	-100.501079	6		
	San Saba Sub-basin				3.40	3.53
11	San Saba at RR 1311	30.912848	-99.493067	3		
12	San Saba at HWY 87	31.004189	-99.269006	29		
	Llano Sub-basin				5.47	5.80
13	South Llano at HWY 377	30.362359	-99.889112	4		
14	South Llano at TTU Campus	30.470444	-99.785106	52		
15	Llano at RR385	30.589030	-99.597470	9		
16	Llano at RR1871	30.657785	-99.324270	10		
17	Johnson Fork Creek	30.451800	-99.673344	10		
	Pedernales Sub-basin				5.00	5.00
18	Pedernales at Alfred Petsch RD	30.209652	-99.004594	12		
19	Pedernales at Boos LN	30.221806	-98.902316	41		
20	Pedernales at RR 1320	30.272003	-98.544831	6		
21	Pedernales at Jung LN	30.223772	-98.740697	1		
22	Pedernales at State Park	30.338287	-98.252449	10		
23	Live Oak Creek	30.280092	-98.937622	1		
24	Barons Creek	30.236780	-98.843431	22		
25	Cypress Creek	30.383316	-98.190608	10		
26	North Grape Creek	30.345260	-98.502452	15		
	Colorado Sub-basin				6.00	6.00
27	Colorado at HWY 290	29.705723	-96.536608	40		
	Guadalupe Drainage					
	Guadalupe Sub-basin				6.80	8.27
28	Guadalupe and Johnson Creek Confluence	30.073959	-99.247406	12		
29	Guadalupe at HWY27	29.964620	-98.897338	6		
30	Guadalupe at Sisterdale	29.957073	-98.717458	9		
31	Guadalupe at Spring Branch	29.882458	-98.448014	11		
32	South Fork Guadalupe at HWY 39	29.968468	-99.443178	4		
33	North Fork Guadalupe at Mayhugh Crossing	30.073556	-99.355972	8		
34	North Fork Guadalupe at Benson Crossing	30.052667	-99.452000	11		
35	Johnson Creek at Byas Spring Rd	30.145971	-99.337939	39		
36	Johnson Creek at 2222 W	30.167531	-99.362626	5		
37	Canyon Lake	29.896020	-98.273563	17		
38	Canyon lake Tailrace	29.862902	-98.165760	1		
	San Antonio Drainage					
	Medina Sub-basin				5.93	5.93
39	Medina at CR 477	29.371517	-98.896394	3		
40	Medina at CR 4779	29.357987	-98.893626	3		
41	Medina at Rio Medina	29.441933	-98.896930	1		
42	Medina at English Crossing	29.680950	-98.976613	9		
43	Medina at Bandera City Park	29.723464	-99.067883	50		
	Nueces Drainage					
	Nueces Sub-basin				3.27	3.27
44	Nueces at HWY 83 S of Uvalde	29.066542	-99.849930	13		
45	Nueces at HWY 55	29.397506	-100.000520	4		
46	Nueces at RR 335	29.885155	-100.020561	1		
47	Nueces at Barksdale	29.721005	-100.033307	24		
	Frio Sub-basin				1.87	1.87
48	Frio at RM 1050	29.603947	-99.737577	1		
49	Frio at John Davis RD	29.694535	-99.756903	1		
	Sabinal Sub-basin				1.93	1.93
50	Sabinal at Ranch Road 187	29.516624	-99.508978	4		

Table 1.2. Polymerase chain reaction conditions for six microsatellite multiplexes used to amplify 15 microsatellite loci in Guadalupe, smallmouth, and largemouth bass.

Multiplex	Locus	MgCl (μM)	Annealing Temperature (˚C)	Cycles	Primer Concentration (μM)
MPX1	TPW060	1.5	60.0	25x	0.05
	TPW062				0.35
MPX2	TPW076	2.0	60.0	25x	0.15
	TPW123				0.20
	TPW096				0.15
MPX3	TPW154	1.5	63.4	27x	0.20
	TPW121				0.20
MPX4	TPW115	2.0	63.4	31x	0.15
	Lma121				0.20
	Mdo1				0.15
MPX5	TPW134	2.0	59.0	26x	0.10
	TPW132				0.20
	TPW025				0.30
MPX6	TPW090	1.5	61.6	29x	0.20
	TPW012				0.15

Table 1.3. Number of individuals from each sampling site assigned to each parental or hybrid genotype based on results from analyses of nine microsatellite loci in STRUCTURE. Numbers in bold indicate the percentage of each genotype by sub-drainage including only individuals with genomic contributions from Guadalupe or smallmouth bass.

	Guadalupe	Smallmouth	Largemouth	GB x SMB	GB x LMB	SMB x LMB
Brazos Drainage						
Lampasas Sub-basin	**100.0**	**0.0**		**0.0**	**0.0**	**0.0**
Lampasas at CR 2313	1	-	-	-	-	-
Sulphur Creek	38	-	1	-	-	-
San Gabriel Sub-basin	**100.0**	**0.0**		**0.0**	**0.0**	**0.0**
San Gabriel at CR 366	-	-	1	-	-	-
San Gabriel at HWY 29	2	-	-	-	-	-
San Gabriel at Georgetown	1	-	-	-	-	-
San Gabriel at CR 100	21	-	-	-	-	-
Brushy Creek	2	-	-	-	-	-
South Fork San Gabriel	2	-	5	-	-	-
North Fork San Gabriel	1	-	3	-	-	-
Colorado Drainage						
Concho Sub-basin	**0.0**	**100.0**		**0.0**	**0.0**	**0.0**
South Concho at HWY 277	-	3	3	-	-	-
San Saba Sub-basin	**96.9**	**0.0**		**3.1**	**0.0**	**0.0**
San Saba at RR 1311	3	-	-	-	-	-
San Saba at HWY 87	28	-	-	1	-	-
Llano Sub-basin	**96.1**	**0.0**		**3.9**	**0.0**	**0.0**
South Llano at HWY 377	3	-	-	1	-	-
South Llano at TTU Campus	46	-	5	1	-	-
Llano at RR 385	5	-	3	1	-	-
Llano at RR 1871	10	-	-	0	-	-
Johnson Fork Creek	10	-	-	-	-	-
Pedernales Sub-basin	**94.9**	**0.0**		**0.0**	**5.1**	**0.0**
Pedernales at Alfred Petsch RD	6	-	6	-	-	-
Pedernales at Boos LN	38	-	2	-	1	-
Pedernales at RR 1320	1	-	4	-	1	-
Pedernales at Jung LN	1	-	-	-	-	-
Pedernales at State Park	9	-	-	-	1	-
Live Oak Creek	1	-	-	-	-	-
Barons Creek	18	-	4	-	-	-
Cypress Creek	9	-	-	-	1	-
North Grape Creek	11	-	3	-	1	-
Colorado Sub-basin	**100.0**	**0.0**		**0.0**	**0.0**	**0.0**
Colorado at HWY 290	40	-	-	-	-	-
Guadalupe Drainage						
Guadalupe Sub-basin	**85.3**	**1.0**		**13.7**	**0.0**	**0.0**
Guadalupe at Johnson Creek Confluence	10	-	1	1	-	-
Guadalupe at HWY27	5	-	-	1	-	-
Guadalupe at Sisterdale	7	-	-	2	-	-
Guadalupe at Spring Branch	11	-	-	-	-	-
South Fork Guadalupe at HWY 39	-	-	4	-	-	-
North Fork Guadalupe at Maylmugh Crossing	6	-	2	-	-	-
North Fork Guadalupe at Benson Crossing	2	-	8	1	-	-
Johnson Creek at Byas Spring Rd	34	-	5	-	-	-
Johnson Creek at 2222 W	4	-	1	-	-	-
Canyon Lake	8	1	-	8	-	-
Canyon lake Tailrace	-	-	-	1	-	-
San Antonio Drainage						
Medina Sub-basin	**88.7**	**0.0**		**1.6**	**8.1**	**1.6**
Medina at CR 477	-	-	-	-	3	-
Medina at CR 4779	1	-	1	-	1	-
Medina at Rio Medina	-	-	1	-	-	-
Medina at English Crossing	6	-	1	1	1	-
Medina at Bandera City Park	48	-	1	-	-	1
Nueces Drainage						
Nueces Sub-basin	**88.9**	**0.0**		**0.0**	**11.1**	**0.0**
Nueces at HWY 83 S of Uvalde	3	-	6	-	4	-
Nueces at HWY 55	4	-	-	-	-	-
Nueces at RR 335	1	-	-	-	-	-
Nueces at Barksdale	24	-	-	-	-	-
Frio Sub-basin	**100.0**	**0.0**		**0.0**	**0.0**	**0.0**
Frio at RM 1050	1	-	-	-	-	-
Frio at John Davis RD	1	-	-	-	-	-
Sabinal Sub-basin	**100.0**	**0.0**		**0.0**	**0.0**	**0.0**
Sabinal at RR 187	3	-	1	-	-	-

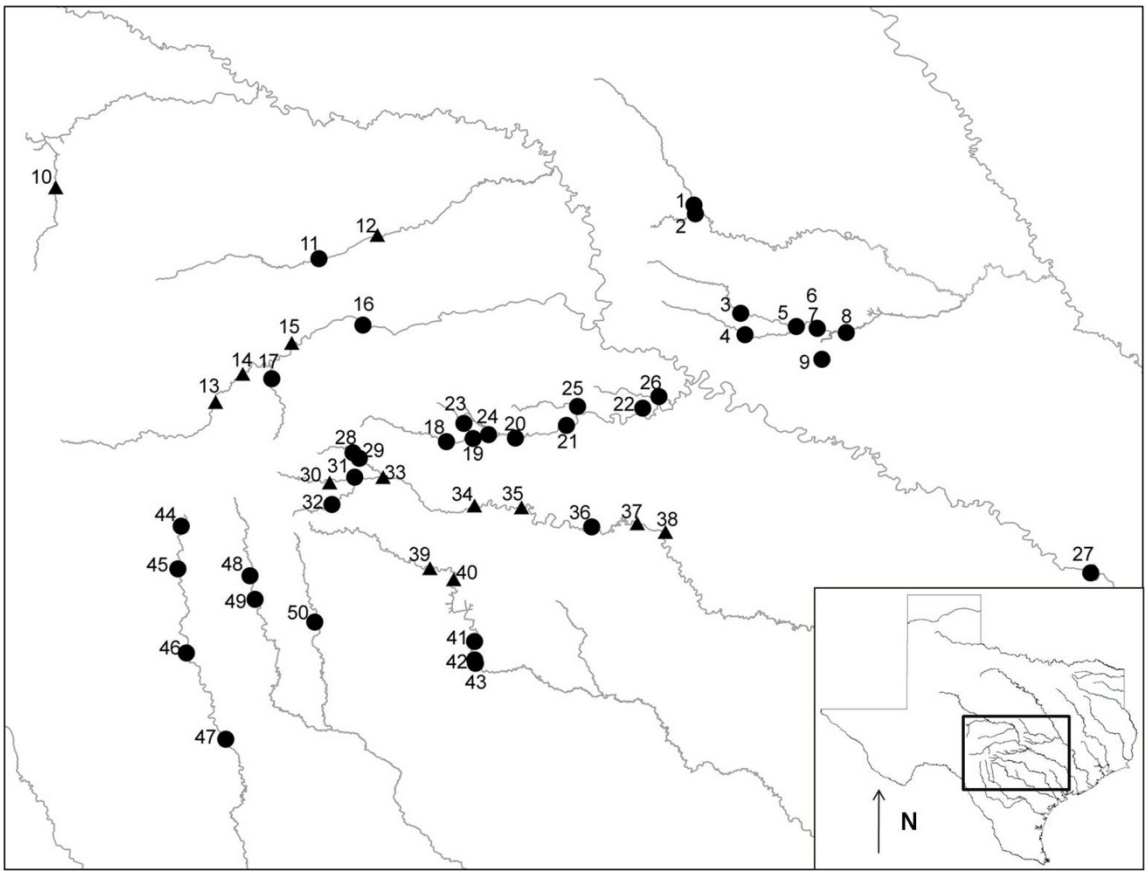

Figure 1.1. Map of localities sampled for Guadalupe bass in this study. Site numbers correspond to those given in Table 1.1. Circles indicate sites where no genetic influence of smallmouth bass was found and triangles indicate sites where genetic influence of smallmouth bass was found.

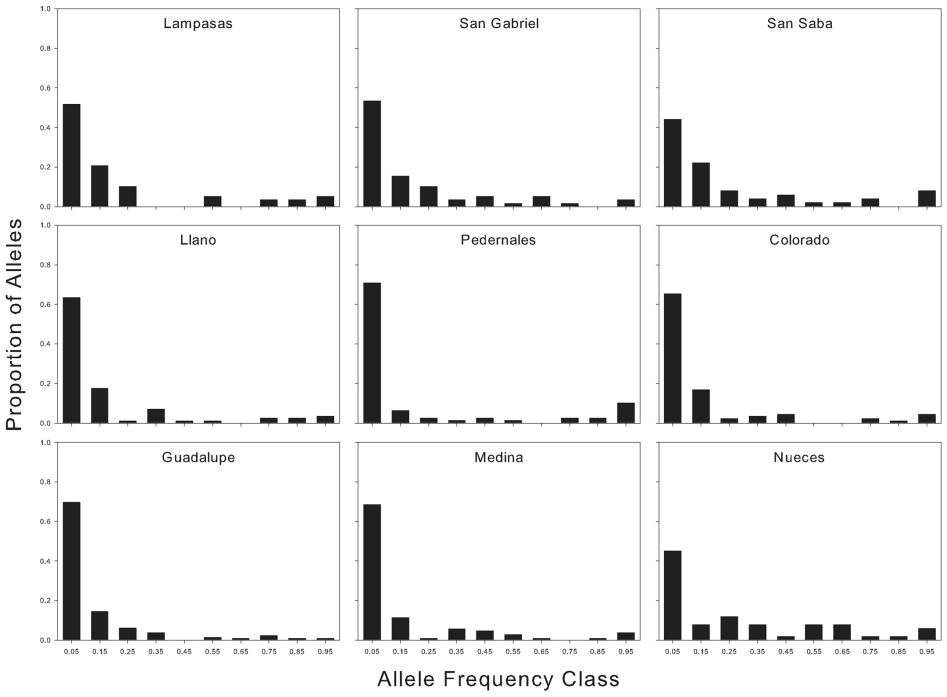

Figure 1.2. Allele frequency distributions from nine Guadalupe bass populations for mode-shift tests of possible recent bottleneck events.

CHAPTER II

POPULATION GENETIC STRUCTURE AND PHYLOGEOGRAPHIC HISTORY OF THE GUADALUPE BASS *MICROPTERUS TRECULII*

Abstract

The Guadalupe bass *Micropterus treculii* occurs primarily in upland streams of the Edwards Plateau region of Texas, an area with high levels of endemism. I examined patterns of genetic diversity in nine Guadalupe bass populations and evaluated the geographic distribution of genetic variation within the species. Microsatellites and mtDNA sequences revealed differing patterns of genetic structure. The incongruence between microsatellites and mtDNA is a result of ancient hybridization giving rise to a distinct mtDNA lineage that is more genetically similar to largemouth bass *M. salmoides* and was observed in the Guadalupe/San Antonio and Nueces River drainages. Population genetic structure was largely influenced by contemporary hydrologic connections with many populations within subdrainages forming their own groups in Bayesian clustering analysis and spatial analysis of molecular variance. While population genetic structure was primarily influenced by patterns of hydrologic connectivity, evidence from both microsatellites and mtDNA suggests stream capture might have led to movement of Guadalupe bass between Colorado and Guadalupe River drainages. Because genetic variation in Guadalupe bass is structured at the subdrainage scale and

patterns of genetic variation are not strictly congruent with patterns in hydrologic connectivity, populations within subdrainages should be considered as management units in future conservation and restoration efforts.

Introduction

Patterns of genetic structure in freshwater organisms are often shaped by geological and climatological histories (Avise 1992) as well as by life-history traits of species (Turner et al. 1996). Natural phenomena such as sea level changes and stream captures that reconfigure hydrological connections have the potential to both create vicariance events as well as reunite previously isolated populations and influence the evolutionary histories of North American freshwater fishes. For example, fluctuations in sea level during the Late Miocene and Pliocene are associated with allopatric speciation in the genus *Micropterus* (Near et al. 2003), and Schonhuth et al. (2011) noted the effects of repeated stream captures on the phylogeographic structure of the Mexican stoneroller, *Campostoma ornatum*. Contemporary factors such as migration (Silva et al. 2011) and human activities including translocations (Sonstebo et al. 2008) can also affect genetic structure and typically have a homogenizing effect.

The Edwards Plateau is an area with high levels of endemism (Bowles and Arsuffi 1993) and the karst terrain of the Edwards Plateau is comprised largely of Cretaceous limestone that forms the Edwards-Trinity aquifer system. Although geographic patterns of genetic variation often reflect present day drainage patterns in organisms that inhabit these stream systems (Avise et al. 1987), evidence that stream captures have played an important role in transferring lineages among drainages is

reflected in the phylogeographic patterns of several taxa (Richardson and Gold 1995, Schonhuth et al. 2012) within the Edwards Plateau region.

The Guadalupe bass *Micropterus treculii* occurs in streams on the Edwards Plateau in the Brazos, Colorado, Guadalupe/San Antonio, and Nueces River drainages as well as in the Gulf Coast Plains in the Colorado River drainage. The Guadalupe bass inhabits streams fed by springs arising from the Edwards-Trinity aquifer system, but typically occurs in eurythermal downstream reaches rather than the stenothermal springs themselves. Guadalupe bass are capable of long distance movements (Perkin et al. 2010) and in conjunction with their long life span (Edwards 1980) have the potential for movement among subdrainages. Additionally, the status of Guadalupe bass as a sport fish and restoration efforts to alleviate the effects of introgression with introduced smallmouth bass, *M. dolomieu*, have led to translocations of Guadalupe bass among drainages. Movement of fish among drainages can result in not only reductions in genetic diversity within populations (Nock et al. 2011) but can also alter patterns of genetic structure among populations (Lajbner et al. 2011, Salminen et al. 2012). Thus, shallow divergences and shared mtDNA haplotypes among populations might not only reflect recent range expansions (Bohlen et al. 2007) but possibly also human-aided transfers of fish among drainages.

Studies of genetic structure of aquatic organisms in the region have focused on spring associated species whereas the present study focuses on a species that inhabits lower reaches of the streams and has a greater potential for movement among streams. In this study, I examined geographic patterns of genetic variation, using microsatellite and mtDNA analyses, to identify historical and contemporary factors affecting the genetic

structure of Guadalupe bass. Specifically, I evaluated the concordance of patterns of genetic structure to hydrologic connectivity as well as examined patterns of genetic diversity in Guadalupe bass populations as they related to the stocking and translocation of fish across drainages. The results of this study will be useful in identifying processes responsible for the genetic structure not only of Guadalupe bass but also for other freshwater species in the region. Additionally, these results will be useful in guiding conservation and restoration efforts for the Guadalupe bass.

Methods

Guadalupe bass were collected from nine distinct populations (Table 2.1) comprising the species' native and introduced ranges by electrofishing, seining, and angling. A small fin clip was taken from either the pectoral or caudal fin of each individual and stored in 70% ethanol at room temperature. Individuals identified as hybrids in previous analyses (see Chapter 1) were excluded from analyses to preclude smallmouth bass and largemouth bass *M. salmoides* alleles from influencing inferences of the genetic structure of Guadalupe bass.

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

Samples were genotyped at 15 microsatellite loci in six optimized multiplex reactions (Lma121, Mdo1, TPW012, TPW025, TPW060, TPW062, TPW076, TPW090, TPW096, TPW115, TPW121, TPW123, TPW132, TPW134, TPW154; Neff et al. 1999; Malloy et al. 2000; Lutz-Carrillo et al. 2008). Polymerase chain reactions were performed at 10 μl volumes and consisted of 1 X PCR buffer (20 mMtris-HCl [pH = 8.4], 50 mM KCl), 1.5 - 2.0 mM $MgCl_2$, 0.2 mM deoxynucleotidetriphosphates (dNTPs), 0.05 μM CAG tailed (5'-CAGTCGGGCGTCATCA-3') primers, 0.15 - 0.35 μM nontailed primers, 0.20 μM 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), 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 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 EDTA, 7.5 mM bromophenol blue) and analyzed alongside size standards on a LI-COR 4300 DNA analyzer. Resulting gel images were scored and alleles assigned to band classes using BioNumerics version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium).

Sequence data were obtained from a subset of Guadalupe bass for a 1,105 base-pair (bp) portion of the mitochondrial cytochrome *b* gene (CYTB) and a 431-432 bp portion of the mitochondrial control region (CR). Initial amplification of the mitochondrial genes was performed via polymerase chain reaction using the M-13 tailed primers MTR-CYTB-F (M13-21) 5'-ATGGCTTGAAAAACCATCGTTG-3' and MTR-CYTB-R (M13-27) 5'-TCCGGCATCCAGTTTACAAGAC-3' for CYTB and MTR-CNTR-F (M13-27) 5'-CACCCCTAGCTCCCAAAGCTA-3' and MTR-CNTR-R (M13-

21) 5'-TGAAGTAGGAACCAAATGCCAG-3' for CR. Reactions were performed at 15 µl volumes and consisted of 1 X PCR buffer (20 mM tris-HCL [pH = 8.4], 50 mM KCl); 1.5 mM MgCl; 0.2 mM deoxynucleotide triphosphates; 0.20 µM each of forward and reverse primers; 0.75 units of Platinum Taq DNA polymerase (Invirogen); and 50 ng of template DNA. Samples were first denatured at 94°C for 1.5 min followed by 39 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 15 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Resultant PCR products were purified using ExoSAP-IT (Affymetrix) following the manufacturers recommended protocols. Purified PCR products were then cycle sequenced with M13 primers (M13-21: TGTAAAACGACGGCCAGT, M13-27: CAGGAAACAGCTATGAC) using Big Dye Terminators and analyzed on an Applied Biosystems 3730xl DNA Genetic Analyzer. Sequences were aligned in Geneious (Biomatters, Auckland, New Zealand) using the MUSCLE alignment algorithm (Edgar 2004).

Guadalupe bass population genetic characteristics for all microsatellite loci were estimated in Arlequin (v 3.5, Excoffier and Lischer 2010) and FSTAT (v 2.9.3, Goudet 2002) and included observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (k), and number of private alleles per population (P_a). Allelic richness was estimated using rarefaction to account for differences in sample sizes among populations. Tests for departures from Hardy-Weinberg equilibrium (HWE) were conducted using Arlequin and Bonferroni corrections for multiple tests were applied. Pairwise F_{ST} values were calculated and significance assessed using 1,000 non-parametric bootstrap replicates and Bonferonni corrections for multiple comparisons were applied. The Bayesian clustering algorithm implemented in STRUCTURE (v 2.3, Pritchard et al. 2000) was

employed to infer population structure from the microsatellite dataset with no a priori consideration of population from which an individual was collected. STRUCTURE probabilistically assigns each individual to one of a predetermined number of populations (*K*) by determining population groupings that minimize deviations from HWE and linkage equilibrium. The admixture model was run with *K* values ranging from 2 to 11 for 300,000 iterations following a burn-in of 50,000 iterations. The appropriate *K* value was determined following Evanno et al. (2005).

Spatial analysis of molecular variance (SAMOVA v 1.0; Dupanloup et al. 2002) was used to assess maximally differentiated groupings of sampled populations based on microsatellite data and mtDNA sequence data analyzed separately. SAMOVA partitions populations into a pre-defined number of groups such that among-group differentiation (F_{CT}) is maximized, and the most likely number of groups can be determined as the value of *K* that maximizes F_{CT}. SAMOVA was performed for the number of defined groups (*K*) ranging from 2 to 9.

Nucleotide substitution models used in maximum-likelihood (ML) and Bayesian phylogenetic analyses of sequence data were selected in jModeltest (Posada 2008) for each gene region based on the Akaike information criterion (AIC). Largemouth bass was used as an outgroup for phylogenetic analyses. The GTR+G model selected for CYTB and the HKY+I model was selected for the CR and used in subsequent ML and Bayesian phylogenetic analyses. ML analyses were performed using PhyML 3.0 (Guindon et al. 2010) with the starting tree determined by BIONJ analysis and nearest neighbor interchange branch swapping. Support for nodes was estimated using 1,000 bootstrap replicates and a 50% consensus tree was constructed. Bayesian analyses were performed

using MrBayes (v 3.1.2, Huelsenbeck and Ronquist 2001) with 4 heated chains each run for 1,000,000 generations and sampled every 1000 generations after a burn-in of 100,000 generations. Sampled topologies after the burn-in were used to construct a 50% consensus tree with posterior probabilities used to assess node support. Additionally, a parsimony haplotype network was constructed in TCS (v 1.21, Clement et al. 2000) to explore relationships among haplotypes with shallow levels of divergence.

Bayesian estimates of mutation-scaled effective population size (Θ) were estimated using the program Migrate-n (v 3.2.17, Beerli and Felsenstein 2001). Uniform priors were used for both Θ and M and the SLICE algorithm was used to sample prior distributions and generate posterior distributions. Four heated chains (temperatures 1, 1.5, 3, and 10,000) were run for 5.0×10^6 steps with sampling every 100 steps following a burn-in period of 5.0×10^4 steps.

Results

A total of 494 Guadalupe bass were genotyped at 15 microsatellite loci. One locus, Lma121, exhibited a departure from HWE in the Guadalupe River population that was due to a heterozygote deficiency. Mean allelic richness for each population across all loci ranged from 3.06 to 5.74 (Table 2.1) with the lowest allelic richness occurring in the Nueces subdrainage. Number of private alleles per population ranged from 0 to 14 with the San Saba population being the only population with no private alleles.

The Bayesian clustering method of Pritchard et al. (2000) resolved five groups (Figure 2.1) of populations based on analysis of microsatellite data. The Lampasas and San Gabriel populations in the Brazos River drainage formed one group, the San Saba,

Llano, and Lower Colorado populations formed a second group in the Colorado River drainage, the Guadalupe and Nueces populations formed a third group, and the Pedernales and Medina populations each formed their own separate groups. While most individuals had high assignment probabilities to the geographic group from which they were collected, many individuals in the Guadalupe population were jointly assigned to both the Guadalupe/Nueces group and to the Colorado drainage group (excluding the Pedernales population).

Twelve and sixteen unique haplotypes were detected for the CR (Table 2.2) and CYTB (Table 2.3), respectively. Estimates of Θ from Migrate-n based on mtDNA sequences ranged from 0.0004 in the San Saba population to 0.0095 in the San Gabriel population. The estimate of Θ for the introduced Nueces population was 0.0005, whereas the mean Θ estimate for native populations was 0.0024. Phylogenetic analyses of mtDNA sequence data resolved two distinct groups of haplotypes for both CR and CYTB (Figures 2.2 – 2.5). In both CR and CYTB, one haplotype group (CR: MTR-CR12; CYTB: MTR-CB14, MTR-CB15, and MTR-CB16) was found in only the Guadalupe, Medina, and Nueces populations, whereas haplotypes from the second group were found across all populations. For CR, private haplotypes were present in the San Gabriel (n=1), San Saba (n=2), Pedernales (n=1), Colorado (n=1), and Guadalupe (n=1) populations. For CYTB, private haplotypes were present in the Lampasas (n=1), Llano (n=5), Guadalupe (n=2), and Medina (n=1) populations. Relationships among haplotypes within the two groups were not well resolved for either CR or CYTB in phylogenetic analyses.

The haplotype networks for CR (Figure 2.6) and CYTB (Figure 2.7) each consisted of two disconnected groups. One group included individuals from all populations while the second group included individuals only from the Guadalupe, Medina, and Nueces populations. The haplotype networks also reveal a pattern of greater frequency haplotypes shared across drainages and lower frequency haplotypes typically occurring in a single subdrainage or two subdrainages within the same drainage. Four haplotypes (CR01, CR02, CR08, and CR12) were found across multiple drainages while two haplotypes (CR03 and CR04) were found in two subdrainages both within the Brazos River drainage. CR01 and CR02 were the most abundant haplotypes and occurred across all drainages in which Guadalupe bass is native while CR08 was found in two drainages. Although CR12 was also found in two drainages (i.e., Guadalupe/San Antonio and Nueces River drainages) the Nueces population was established by the stocking of fish originating from the Guadalupe/San Antonio River drainage. CR haplotypes unique to the Brazos River drainage were closely related to CR02, and CR haplotypes unique to the Colorado River drainage were closely related to CR01. Six haplotypes in the CYTB haplotype network (CB01, CB02, CB03, CB04, CB06, and CB14) were found across multiple drainages and CB12 was found in two subdrainages within the Colorado River drainage. Haplotypes CB01 and CB04 were found across all drainages in which Guadalupe bass is native and CB02, CB03, and CB06 were each found in two drainages. Like haplotype CR12, CB14 was found in the Guadalupe/San Antonio River drainage as well as the Nueces River drainage. CYTB haplotypes unique to the Brazos River drainage were closely related to CB02 whereas haplotypes unique to the Colorado River drainage were closely related to both CB01 and CB02. Five CYTB haplotypes unique to

the Colorado River drainage were found only in the Llano subdrainage. Two of the haplotypes were most closely related to CB01, one haplotype was most closely related to CB02, and two haplotypes were each equally related to CB01 and CB02.

Contrasting patterns of population structure were identified by SAMOVA for microsatellite and mtDNA data. For the microsatellite data, the optimal group structure was identified as K=7 with F_{CT}=0.164 and genetic variation was partitioned as 16.4% among groups, 2.9% among populations within groups, and 80.7% within populations. At K=7, the Lampasas and San Gabriel populations comprised one group, the Llano and Colorado populations comprised a second group, and each of the remaining groups were comprised of a single population. For the mtDNA data, an optimal group structure was identified as K=4 with ϕ_{CT}=0.741 and genetic variation was partitioned as 74.2 % among groups, 0.6% among populations within groups, and 25.2% within populations. At K=4, the Lampasas, San Gabriel, San Saba, Llano, Pedernales, and Colorado populations form a single group while the Guadalupe, Medina, and Nueces populations are each partitioned to their own group.

Discussion

Clustering analyses based on microsatellite data indicate that genetic structuring of Guadalupe bass is largely affected by major drainage connections. Groups defined in this analysis consisted of a single population or multiple populations from within the same drainage with the exception of the Guadalupe and Nueces populations belonging to the same group. In this case, the Nueces population is an introduced population and was derived from hatchery stock collected from the Guadalupe River and grouping of these

two populations together is expected as introduced populations often cluster with the population from which they originated (Danway et al. 2011).

Pairwise F_{ST} values (Table 2.4) reflect this same influence of drainage connection on population structure. The mean pairwise F_{ST} value for within drainage comparisons was 0.11 compared to 0.22 for among-drainage comparisons. Among the within-drainage comparisons, the Pedernales population was the most distinct based on multiple analyses. The mean pairwise F_{ST} value of the Pedernales population to other populations within the Colorado River drainage was 0.18 while the mean pairwise F_{ST} value of other populations within their respective drainages ranged from 0.06 to 0.11. This pattern of greater within drainage pairwise F_{ST} values for the Pedernales population is congruent with the results of the Bayesian clustering analyses in which the Pedernales forms its own cluster. Likewise, the Medina and Guadalupe populations each form a separate group in Bayesian clustering analysis. However, the pairwise F_{ST} value between these two populations is much lower (F_{ST}=0.097) than within-drainage F_{ST} values for the Pedernales population despite the more distant hydrologic connection between the Guadalupe and Medina rivers near the Gulf of Mexico compared to the nearer hydrologic connections of the Pedernales River within the Colorado River drainage.

Discordance among results of SAMOVA for microsatellites and mtDNA is associated with the occurrence of one CR haplotype and three CYTB haplotypes more genetically similar to those of largemouth bass than to other Guadalupe bass haplotypes in the Guadalupe, Medina, and Nueces populations. Near et al. (2004) found that alleles for the mitochondrial NADH subunit 2 gene were derived from two distinct lineages as the result of ancient hybridization with an ancestor of largemouth bass whereas alleles of

the nuclear genes were all derived from a single lineage more genetically similar to those of spotted bass *M. punctulatus*. Because of the deep divergence between these two lineages of haplotypes, partitioning of genetic variation among groups in SAMOVA was inflated compared to the genetic variation partitioned among groups for microsatellites. SAMOVA of microsatellites indicated stronger genetic structuring at the subdrainage level than did Bayesian clustering analysis with a greater number of groups represented by a single population. As with the Bayesian clustering analysis, the Lampasas and San Gabriel populations formed a group within the Brazos River drainage and had a relatively low F_{ST} value of 0.057.

While phylogenetic analyses of mtDNA data were largely unresolved, parsimony haplotype networks displayed a pattern of closely related haplotypes often occurring within the same subdrainage or drainage. The effects of contemporary drainage connections on the genetic structure of aquatic organisms are well known (Avise et al. 1987). However, other factors including translocations of individuals by humans (Sonstebo et al. 2008) and stream captures (Burridge et al. 2006, Schonhuth et al. 2012) can also influence geographic patterns of genetic diversity and structure. For mtDNA, lineages were not always confined to subdrainages or drainages. For example, the CYTB allele CB05 is found only in the Lampasas population in the Brazos River drainage but is most closely related to alleles found only in the Colorado and Guadalupe/San Antonio River drainages. Two possible explanations for this pattern include incomplete lineage sorting and anthropogenic translocations of fish among populations. While incomplete lineage sorting among drainages could explain the observed pattern, the close relation of the rare CB05 haplotype to the abundant CB02 haplotype that is not found in the Brazos

River drainage and distant relationship of CB05 to other haplotypes in the Brazos River drainage suggest that this haplotype plausibly might be introduced in the Lampasas population. Although no translocations of Guadalupe bass by private individuals have been recorded, game fish are among the most widely introduced species (Rahel 2000) and illegal transfers of fish across drainages have been documented for other *Micropterus* species (Oswald 2007, Johnson et al. 2009).

Evidence from Bayesian clustering analysis of microsatellites as well as multiple alleles shared between the Llano population in the Colorado River drainage and the Guadalupe population suggests stream captures have led to transfers of Guadalupe bass between the Colorado and Guadalupe River drainages and has had a marked influence on the contemporary genetic structure of Guadalupe bass. Additionally, pairwise F_{ST} values between the Guadalupe population and the San Saba and Llano populations are lower than other among-drainage F_{ST} estimates and are comparable to within-drainage pairwise F_{ST} estimates. Geologic (Woodruff and Abbot 1979) evidence of stream captures has been documented within the range of the Guadalupe bass and Schonhuth et al. (2012) documented similar patterns of shared lineages among the Guadalupe and Colorado River drainages for *Dionda* species.

Estimates of genetic diversity, including allelic richness (k), H_e, and Θ, were depressed in the introduced population of Guadalupe bass in the Nueces River. Reductions in genetic diversity are common when new populations are established from a limited number of individuals (Grapputo et al. 2006, Danway et al. 2011). The Nueces population was established by the stocking of 2,000 individuals; however, these individuals were fingerlings produced in a hatchery setting from a small number of

broodstock. Despite the reduction in genetic diversity relative to the source population in the Guadalupe subdrainage, levels of genetic diversity for the Nueces population are similar to levels of genetic diversity found in some native populations (e.g., Lampasas and San Saba populations). Stocking of hatchery-reared individuals has also occurred in other drainages as a part of restoration efforts for Guadalupe bass to mitigate the effects of introgressive hybridization with introduced smallmouth bass. For example, nearly one million Guadalupe bass fingerlings were stocked in the Guadalupe subdrainage between 1992 and 2010. Despite the potential for stocking of hatchery reared fish to reduce genetic diversity (Nock et al. 2011), the Guadalupe population has maintained high levels of genetic diversity and is one of the most diverse populations examined. Additionally, 80.7% of genetic variation was partitioned within populations in SAMOVA indicating that, overall, individual Guadalupe bass populations are genetically diverse.

Near et al. (2003) proposed a model of allopatric speciation, as a result of sea level fluctuations, in the genus *Micropterus* primarily during the Miocene and Pliocene, and intraspecific lineage diversification occurring during the Pleistocene. During this period of lineage diversification, sea level changes and stream captures allowed for dispersal of fish among drainages (Conner and Suttkus 1986). Divergences among mitochondrial sequences within the two lineages (as a result of ancient hybridization) were shallow; however, genetic structuring among populations was still evident. Geographic patterns of genetic diversity in Guadalupe bass demonstrate the effect of hydrologic connections on the population genetic structure of aquatic organisms in the Edwards Plateau region of Texas, but also highlight the importance of stream captures in the movement of organisms across drainages, which are also know for other aquatic taxa

in the region (Schonhuth et al. 2012). Given the patterns of genetic diversity observed for Guadalupe bass, populations within subdrainages would serve as ideal management units as they reflect the scale at which Guadalupe bass populations are genetically structured and occur within easily defined geographic areas. With population genetic structuring occurring at the subdrainage level, future restoration efforts should seek to prevent erosion of these patterns by avoiding the translocation of fish among drainages and subdrainages where possible.

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Table 2.1. Population genetic characteristics for nine populations of Guadalupe bass. Estimates from microsatellites include number of alleles per locus (k), observed heterozygosity (H_o), expected heterozygosity (H_e), and number of private alleles (P_a). Estimates of mutation-scaled effective population size (Θ) were calculated from mtDNA sequences of the control region and cytochrome *b*.

	$N_{(Msats)}$	k	H_o	H_e	P_a	$N_{(mtDNA)}$	Θ
Lampasas	39	3.71	0.39	0.43	4	14	0.0011
San Gabriel	26	3.99	0.48	0.48	1	11	0.0095
San Saba	31	3.33	0.54	0.53	0	13	0.0004
Llano	95	4.50	0.48	0.48	4	38	0.0019
Pedernales	94	3.62	0.48	0.48	6	25	0.0008
Colorado	38	5.48	0.43	0.49	7	12	0.0020
Guadalupe	84	5.74	0.56	0.61	10	27	0.0024
Medina	33	5.01	0.51	0.48	14	11	0.0008
Nueces	54	3.06	0.40	0.41	2	15	0.0005

Table 2.2. Frequencies of mitochondrial control region haplotypes from Guadalupe bass sampled from nine populations.

	CR01	CR02	CR03	CR04	CR05	CR06	CR07	CR08	CR09	CR10	CR11	CR12
Lampasas	2	1	7	4	-	-	-	-	-	-	-	-
San Gabriel	5	-	1	2	3	-	-	-	-	-	-	-
San Saba	8	-	-	-	-	2	3	-	-	-	-	-
Llano	22	9	-	-	-	-	-	6	-	-	-	-
Pedernales	1	23	-	-	-	-	-	-	1	-	-	-
Colorado	7	4	-	-	-	-	-	-	-	1	-	-
Guadalupe	9	3	-	-	-	-	-	1	-	-	2	12
Medina	3	-	-	-	-	-	-	-	-	-	-	8
Nueces	2	-	-	-	-	-	-	-	-	-	-	13

Table 2.3. Frequencies of cytochrome *b* haplotypes from Guadalupe bass sampled from nine populations.

	CB01	CB02	CB03	CB04	CB05	CB06	CB07	CB08	CB09	CB10	CB11	CB12	CB13	CB14	CB15	CB16
Lampasas	1	-	12	-	1	-	-	-	-	-	-	-	-	-	-	-
San Gabriel	3	-	5	2	-	-	-	-	-	-	-	-	-	-	-	-
San Saba	11	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Llano	15	7	3	3	-	4	1	1	1	1	2	-	-	-	-	-
Pedernales	1	23	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Colorado	4	4	-	1	-	-	-	-	-	-	-	3	-	-	-	-
Guadalupe	5	3	-	3	-	1	-	-	-	-	-	-	2	11	2	-
Medina	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
Nueces	-	1	-	-	-	-	-	-	-	-	-	-	-	13	-	-

Table 2.4. Pairwise F_{ST} values for nine populations of Guadalupe bass based on 15 microsatellite loci.

	Lampasas	San Gabriel	San Saba	Llano	Pedernales	Colorado	Guadalupe	Medina
San Gabriel	0.057	-						
San Saba	0.272	0.175	-					
Llano	0.270	0.173	0.031	-				
Pedernales	0.379	0.291	0.165	0.183	-			
Colorado	0.281	0.203	0.073	0.062	0.191	-		
Guadalupe	0.223	0.153	0.054	0.068	0.170	0.102	-	
Medina	0.373	0.294	0.184	0.178	0.311	0.234	0.097	-
Nueces	0.313	0.248	0.164	0.181	0.338	0.235	0.100	0.183

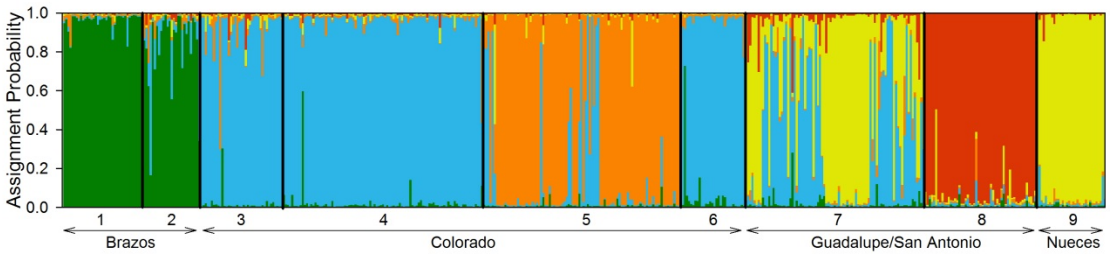

Figure 2.1. Estimated population structure from Bayesian clustering analyses of microsatellite data in STRUCTURE. Each individual is represented by a vertical line divided into *K*=5 segments representing the proportional group assignment probability. Black vertical lines separate sampled subdrainages and numbers below the x-axis identify populations. 1 = Lampasas, 2 = San Gabriel, 3 = San Saba, 4 = Llano, 5 = Pedernales, 6 = Colorado, 7 = Guadalupe, 8 = Medina, 9 = Nueces.

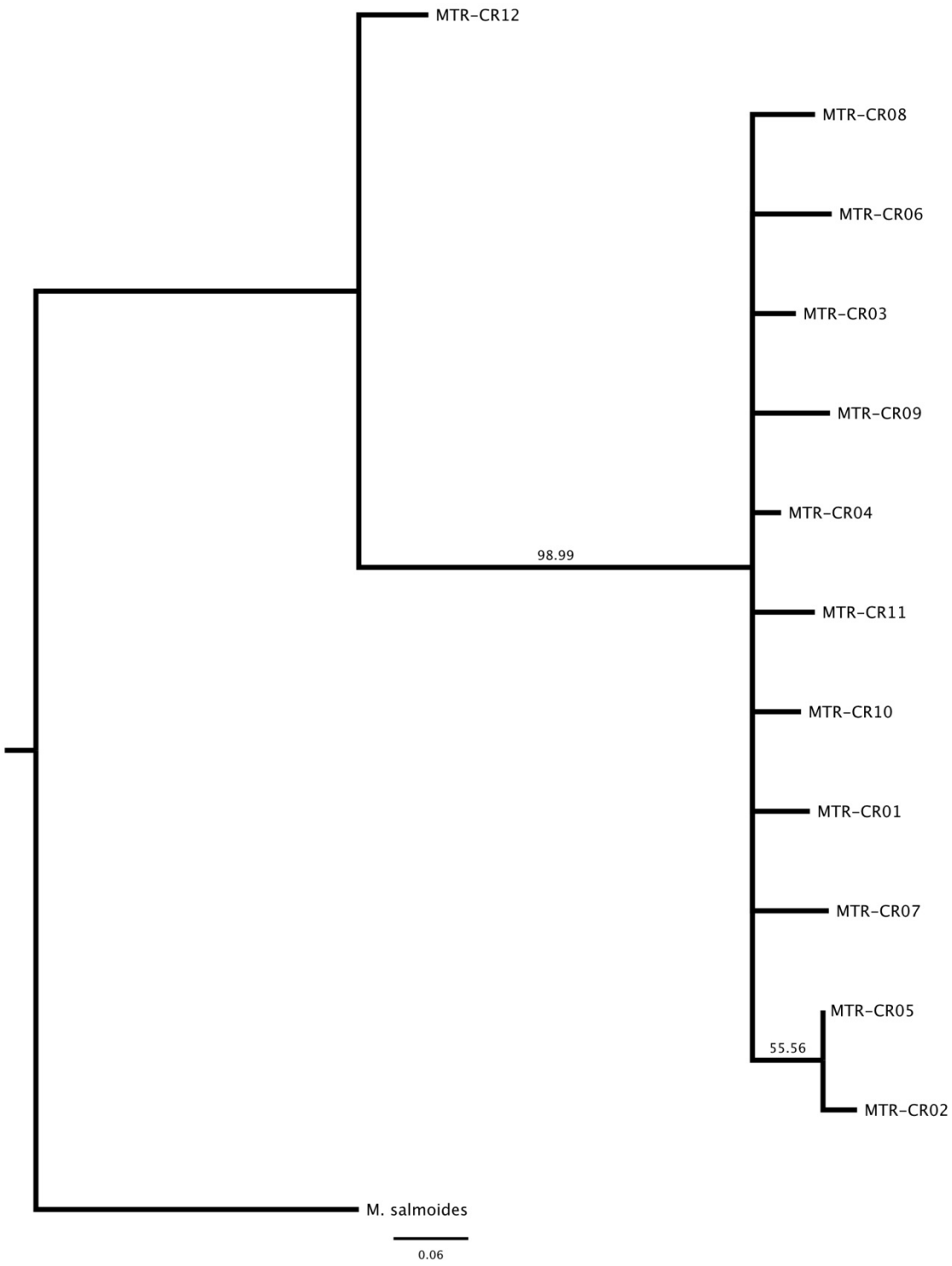

Figure 2.2. Tree resulting from maximum likelihood analysis of the control region dataset. Numbers above branches represent bootstrap support values.

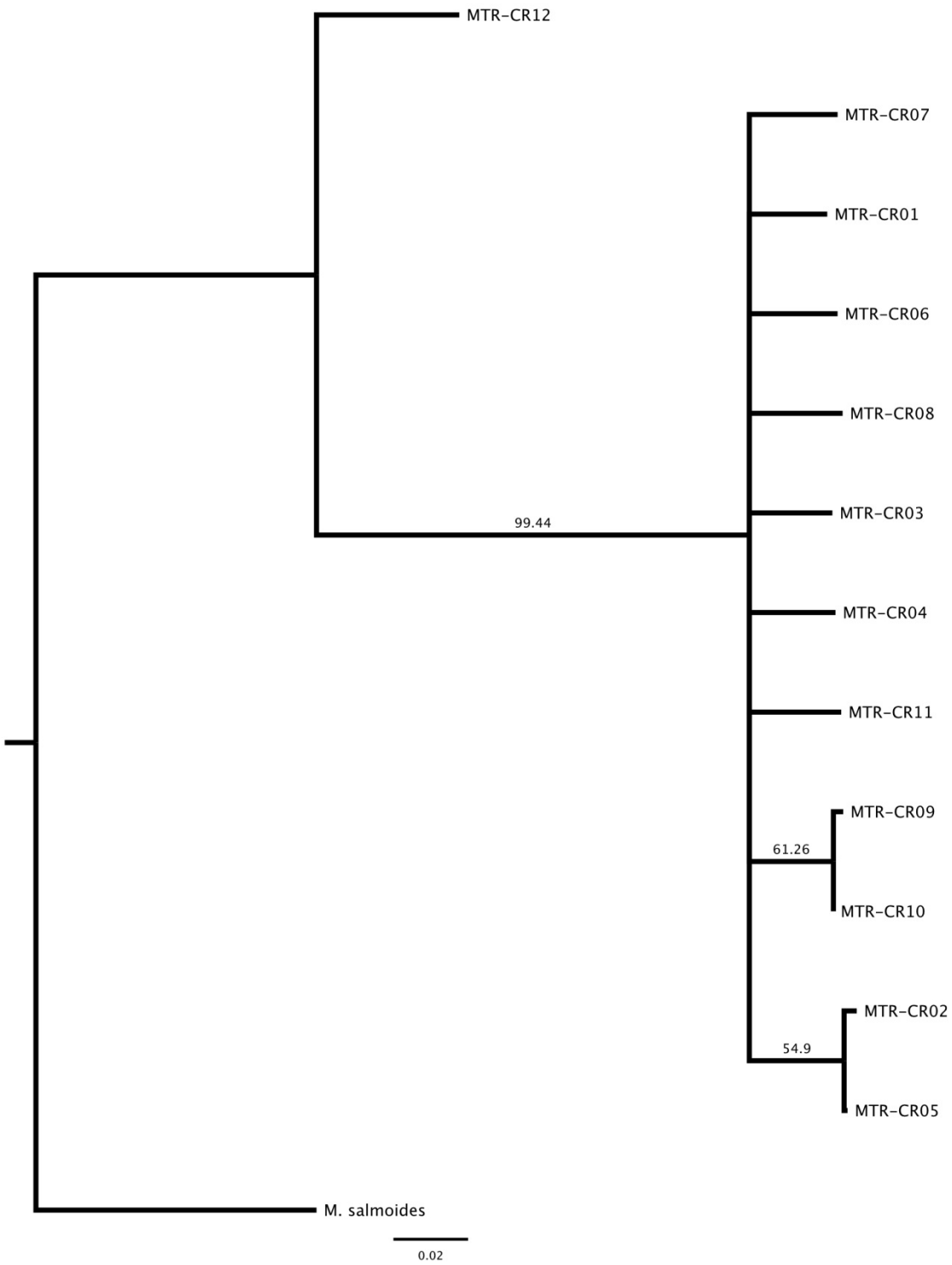

Figure 2.3. Tree resulting from Bayesian analysis of the control region dataset. Numbers above branches represent posterior probability support estimates.

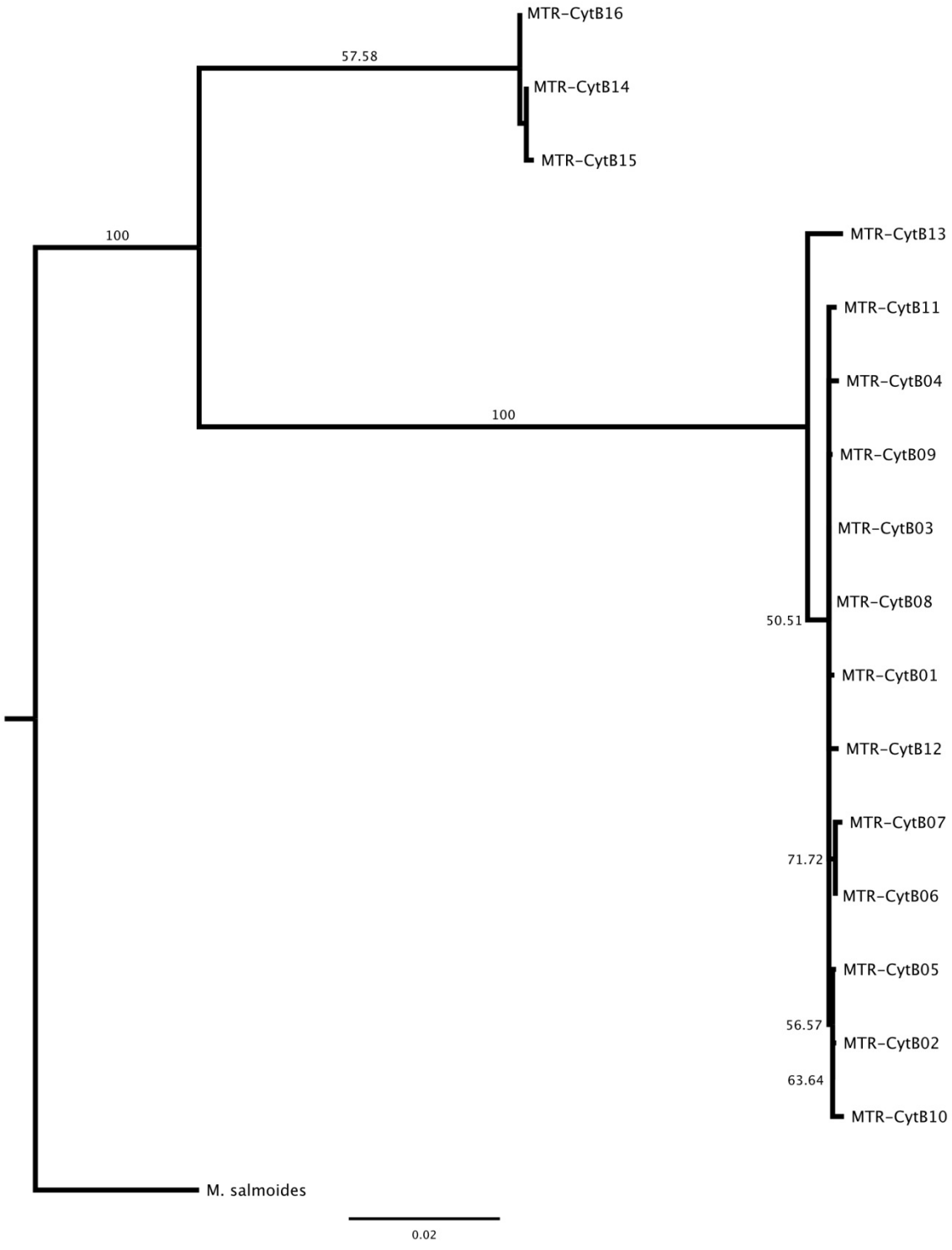

Figure 2.4. Tree resulting from maximum likelihood analysis of the cytochrome *b* dataset. Numbers above branches represent bootstrap support values.

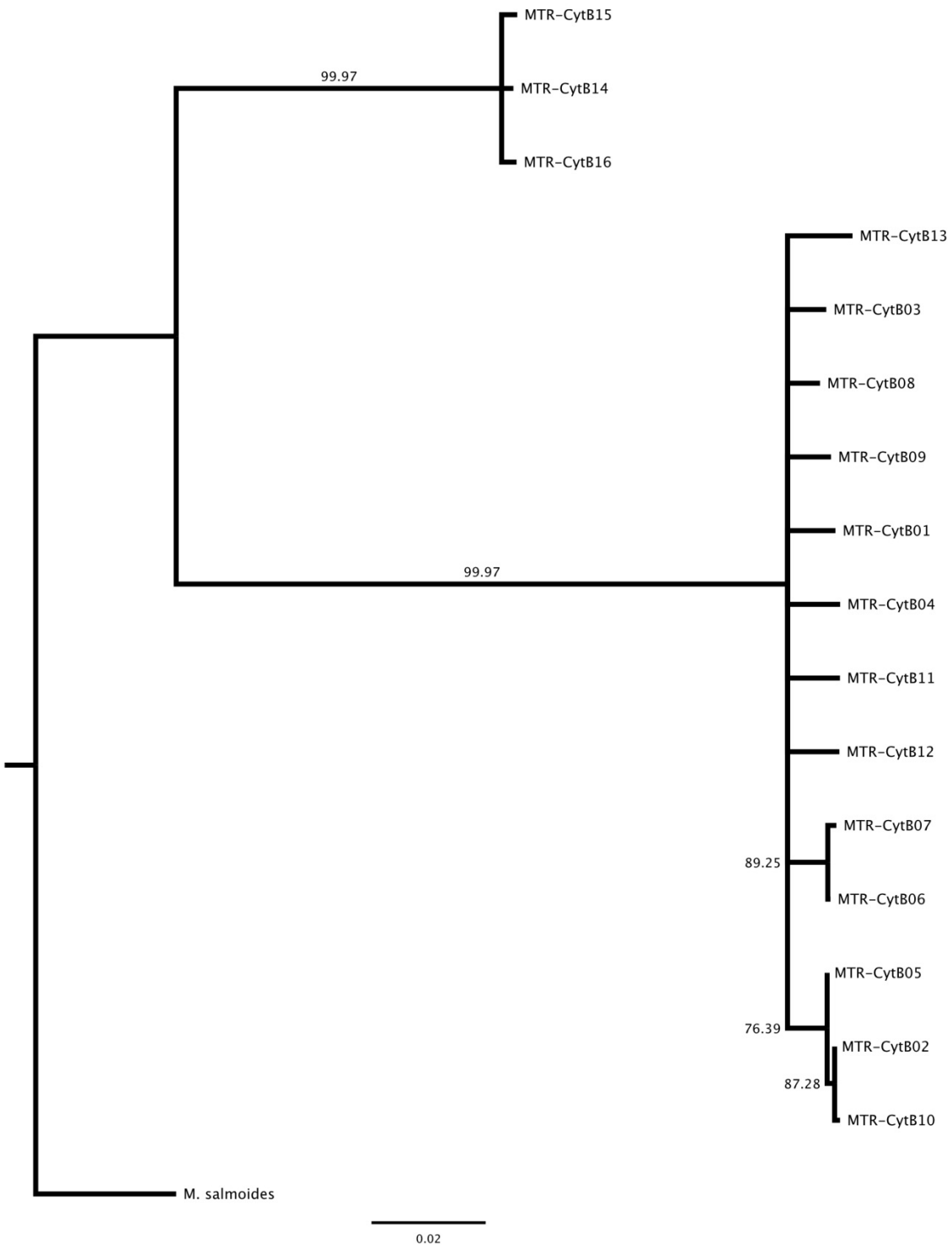

Figure 2.5. Tree resulting from Bayesian analysis of the cytochrome *b* dataset. Numbers above branches represent posterior probability support estimates.

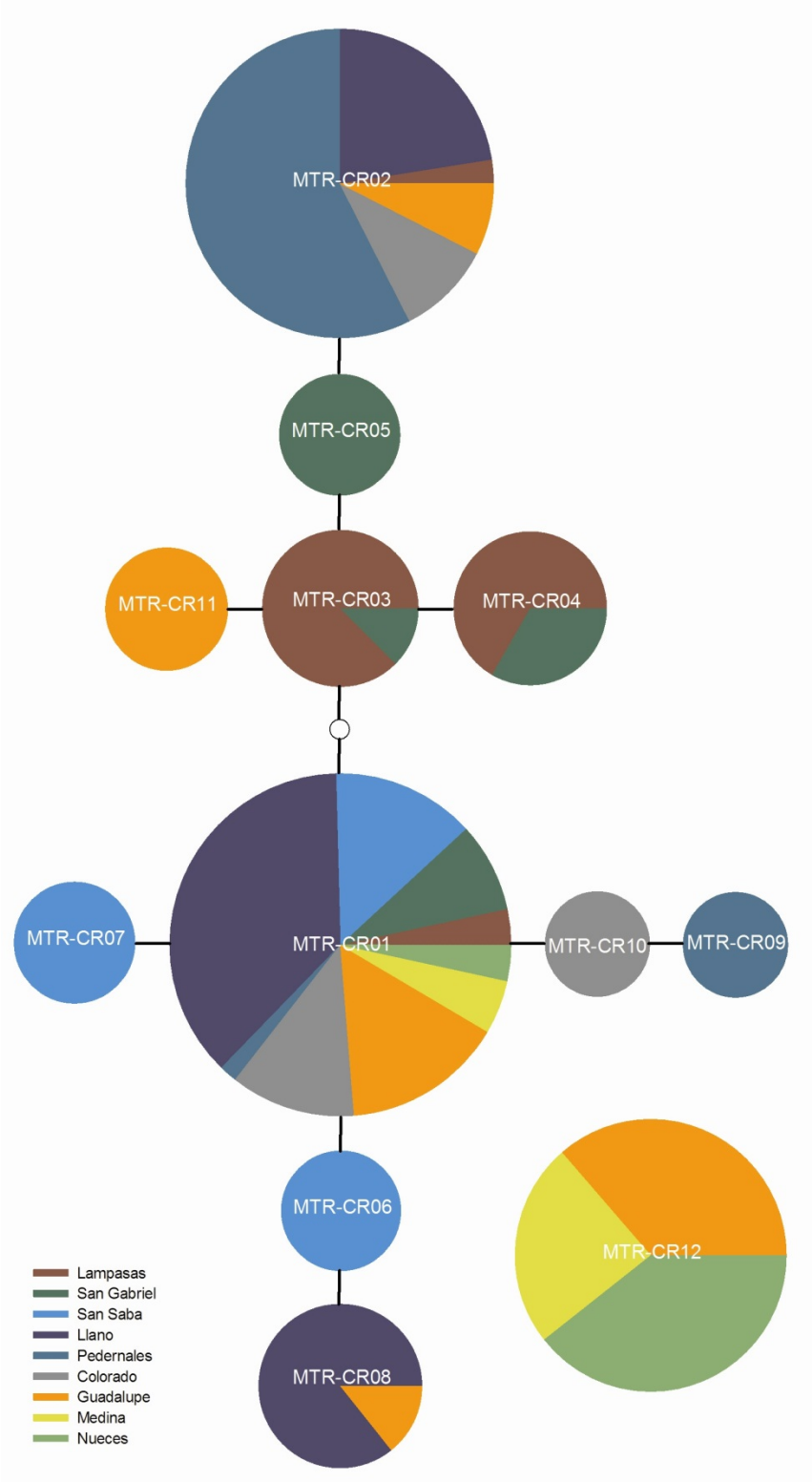

Figure 2.6. Parsimony haplotype network for Guadalupe bass control region haplotypes. Circles represent haplotypes and are scaled to their observed frequency. Bars connecting circles represent single step mutations and empty circles represent missing haplotypes.

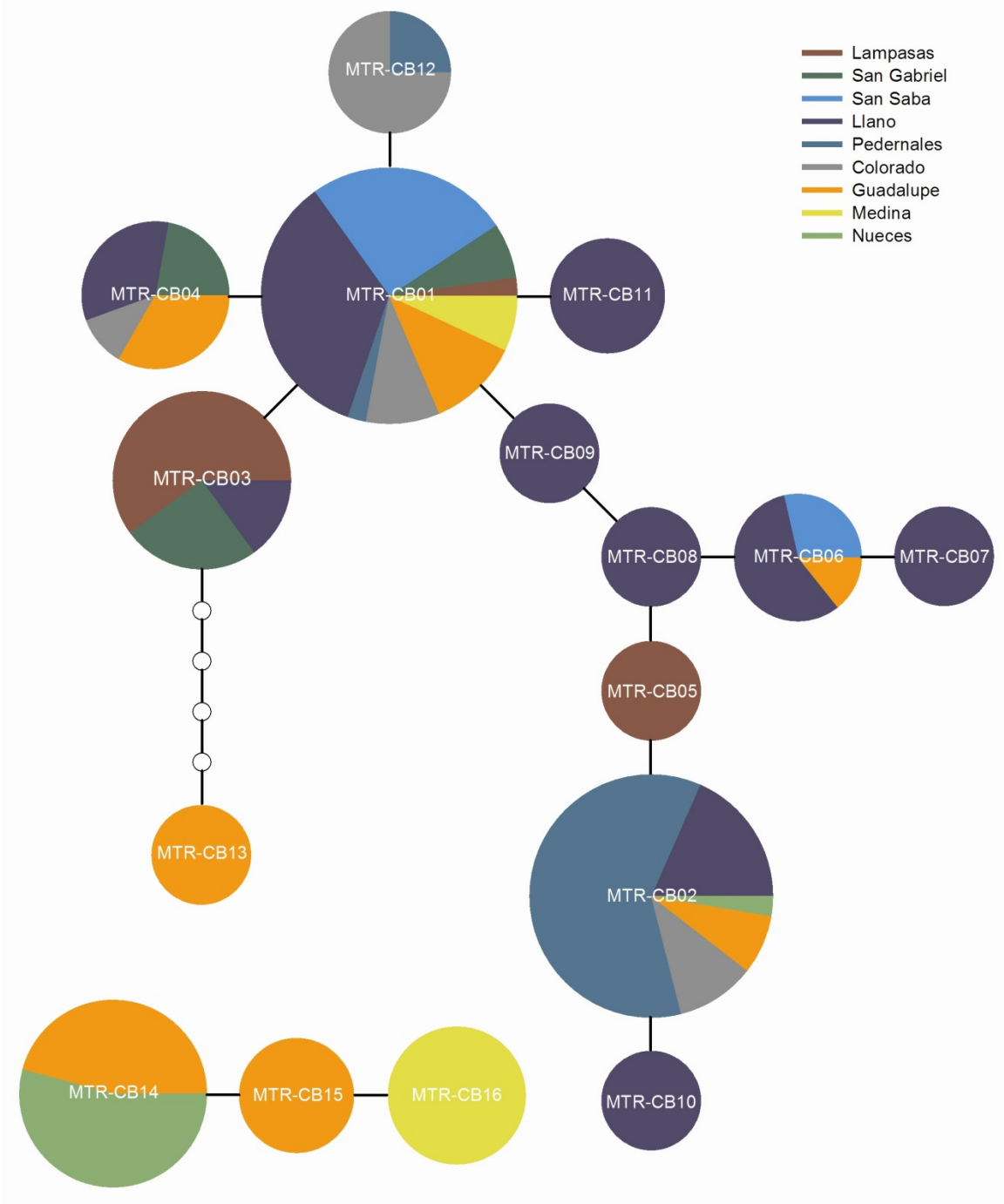

Figure 2.7. Parsimony haplotype network for Guadalupe bass cytochrome *b* haplotypes. Circles represent haplotypes and are scaled to their observed frequency. Bars connecting circles represent single step mutations and empty circles represent missing haplotypes.

CHAPTER III

INDIVIDUAL-LEVEL RESOURCE SPECIALIZATION IN THE GUADALUPE BASS *MICROPTERUS TRECULII*

Abstract

Individual specialization is related to genetic diversity when genetic diversity is manipulated experimentally; however, whether naturally occurring levels of genetic variation are associated with individual specialization in wild populations is not known. Individual specialization was examined in nine populations of Guadalupe bass and variation in specialization was compared to population niche width, trophic diversity, and genetic and morphological variation. Individual specialization was detected in and varied among Guadalupe bass populations, but was not related to genetic diversity. Individual specialization was, however, related to trophic diversity that was, in turn, correlated with variation in jaw length. The results of this study indicate that individual specialization can result from individuals within a population specializing at different trophic levels without an overall increase in population total niche width.

Introduction

A population's niche width is a result of the combined effects of intraspecific competition driving niche expansion and constraints imposed by interspecific

competition (Roughgarden 1972). While populations of species considered to be generalists are often treated as consisting of homogenous generalist individuals, populations that use a wide range of resources often consist of individuals that use a limited subset of resources when compared to the population as a whole (Bolnick et al. 2003). Such intrapopulation variation is referred to as individual specialization when it is not associated with polymorphism such as sexual dimorphism, ontogenetic shifts, or distinct morphotypes. Individual specialization is one pathway by which populations can increase their niche width (Van Valen 1965) and can have important ecological (Beaudoin et al. 1999, Quevado et al. 2009, Duffy 2010) and evolutionary (Knudsen et al. 2009) consequences. For instance, density of *Daphnia dentifera* that utilize both epilimnetic and hypolimnetic habitats is correlated with density of the congener *D. pulicaria*, whereas density of *D. dentifera* that utilize only epilimnetic habitats is not (Duffy 2010). Additionally, Knudsen et al. (2009) suggested that consistent among-individual niche differences that are correlated with morphology provide the intrapopulation variation upon which disruptive selection can act.

Individual specialization can arise from ecological release from interspecific competition (Van Valen 1965) or as a result of intraspecific competition (Svanbäck and Bolnick 2007). When individual specialization occurs, resource use by individuals is often correlated with existing variation in morphology (Svanbäck and Bolnick 2007) and might reflect variation in resource use efficiency associated with morphological traits (Bolnick et al. 2003). For example, foraging efficiency in bluegill *Lepomis macrochirus* is related to pectoral fin length in open water versus structurally diverse habitats and individual foraging strategies reflect morphological variation among individuals

(Ehlinger 1990). Additionally, the degree to which morphology and diet are correlated increases with competition (Svanbäck and Bolnick 2007).

Genetic diversity can affect processes at scales ranging from populations to ecosystem level effects (Hughes et al. 2008). The interactions between genetic diversity, fitness, and population size are of great interest in ecology, evolutionary biology, and conservation, and fitness and population size can be related to individual specialization (Bolnick et al. 2003). Individuals that are resource specialists can have higher fitness than generalist individuals (Robinson et al. 1996) and populations composed of specialists are expected to maintain larger population sizes (Van Valen 1965). Additionally, among-individual variation in resource use increases with population genetic diversity in captive flour beetles *Triboleum castaneum* (Agashe and Bolnick 2010). However, whether intrapopulation levels of genetic diversity are related to individual specialization in wild populations is not known.

Bolnick et al. (2002) suggested that stable isotope ratios are useful for estimating individual specialization as they provide a long-term estimate of an individual's diet as turnover of stable isotope ratios is relatively slow (Fry and Arnold 1982) and are less likely to provide overestimates of individual specialization than gut content data. Stable isotopes also provide the benefit that estimates of resource use are obtained even among individuals with empty stomachs. The percentage of individuals with empty stomachs in *Micropterus* is up to 50% (Lewis et al. 1974). Because populations comprised of specialist individuals should have isotopic variances greater than that of populations comprised of generalist individuals, variances of stable isotope signatures can be used to assess the degree of individual specialization in populations (Araújo et al. 2007).

Given that genetic diversity and individual resource specialization have implications that reach beyond populations and affect communities and ecosystem processes, the objectives of this study were to 1) determine whether individual specialization in diet occurs in Guadalupe bass *Micropterus treculii* populations, 2) test the hypothesis that individual diet specialization is correlated with intrapopulation genetic diversity, and 3) identify correlations between resource use and morphological variation. Specifically, I used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes, a suite of 15 microsatellite markers, and morphological data for nine populations of Guadalupe bass with varying levels of genetic diversity to assess these objectives.

Methods

Guadalupe bass and potential prey items were sampled from nine distinct populations (Table 3.1) across the range of Guadalupe bass. Fish were collected at each site using a combination of seining and electrofishing, whereas macroinvertebrates were collected using a combination of kick nets, Hess samplers, and dip nets. Kick nets and Hess samplers were used to collect macroinvertebrates in riffle habitats whereas dip nets were used in habitats with lower current velocities including pools, submerged aquatic vegetation, and terrestrial vegetation along the stream edge. Fish and large invertebrates (i.e., crayfish) were stored on ice in the field while smaller invertebrates were stored in 70% ethanol.

In the lab, each Guadalupe bass was photographed, a small fin clip was taken from either the pectoral or caudal fin of each individual and stored in 70% ethanol for genetic analyses, gut contents removed and preserved in 70% ethanol, and a 5 mm x 5

mm x 20 mm piece of epaxial muscle tissue removed for stable isotope analysis. Other fish considered as potential prey items were identified to species and a sample of epaxial muscle tissue removed for stable isotope analysis. Individuals not large enough to provide a sample of epaxial muscle tissue for stable isotope analysis were eviscerated and the remainder of the body used for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. Macroinvertebrates were identified to the lowest feasible taxonomic level (order or family) for stable isotope analysis. Guadalupe bass gut contents were analyzed similarly to environmental samples of fish and macroinvertebrates in that they were identified to the same taxonomic level.

All samples used for stable isotope analysis were dried at 60°C for 72 hours and then ground into a fine powder using a mortar and pestle. For each sample, 1 mg ± 0.2 mg of dried tissue was analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC-Davis Stable Isotope Facility. Isotopic values are reported in δ notation where:

$$\delta R = \left(\frac{R_{sample}}{R_{reference}} - 1 \right) \times 100$$

and R is the ratio $^{13}C:^{12}C$ or $^{15}N:^{14}N$ and isotopic references are Pee Dee Belemnite or atmospheric nitrogen, respectively.

Whole genomic DNA was extracted from fin tissues using a high-salt extraction method modified from Miller (1988) where ammonium acetate was substituted for sodium chloride in the cellular protein precipitation step. Purified DNA was rehydrated in 100 μl low tris-EDTA buffer (10 mM tris, 0.1 mM EDTA, pH 8) and the concentration and purity of DNA evaluated by spectrophotometry at 260 and 280 nm (NanoDrop 2000).

Concentrations of DNA were then adjusted to 50 ng/μl using additional low tris-EDTA buffer. Samples were genotyped at 15 microsatellite loci in six optimized multiplex reactions (Lma121, Mdo1, TPW012, TPW025, TPW060, TPW062, TPW076, TPW090, TPW096, TPW115, TPW121, TPW123, TPW132, TPW134, TPW154; Neff et al. 1999; Malloy et al. 2000; Lutz-Carrillo et al. 2008). Polymerase chain reactions were performed at 10 μl volumes and consisted of 1 X PCR buffer (20 mMtris-HCl [pH = 8.4], 50 mM KCl), 1.5 - 2.0 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 0.05 μM CAG tailed (5'-CAGTCGGGCGTCATCA-3') primers, 0.15 - 0.35 μM nontailed primers, 0.20 μM 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), 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 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 EDTA, 7.5 mM bromophenol blue) and analyzed alongside size standards on a LI-COR 4300 DNA analyzer. Resulting gel images were scored and alleles assigned to band classes using BioNumerics (v5.0, Applied Maths, Sint-Martens-Latem, Belgium).

Expected heterozygosity (H_e) averaged across all microsatellite loci was used as a measure of genetic diversity for Guadalupe bass populations and was calculated in Arlequin (v 3.5, Excoffier and Lischer 2010). Landmarks for morphological analysis were digitized from photographs of each individual using the program tpsDIG2 (Rohlf 2008). Metrics calculated from landmarks included standard length, relative jaw length, and relative body depth (Hubbs and Lagler 1964).

Individual specialization (WIC/TNW) was estimated using the program VarIso (Araújo et al. 2007). WIC/TNW ranges from 0 to 1 with high levels of individual specialization occurring near 0 and low levels of specialization occurring near 1. VarIso estimates individual specialization for a population from the variance of $\delta^{13}C$ values of the population of interest as well as $\delta^{13}C$ values of prey items. Diets of populations with varying degrees of individual specialization are simulated by varying the number of draws per individual among populations from $\delta^{13}C$ values of food items from the real population. The variances of $\delta^{13}C$ for simulated populations were plotted versus the WIC/TNW metric of individual specialization and a quadratic regression was fit to the data in SIGMAPLOT (v11.0, Systat Software, inc., San Jose, CA). Estimates of individual specialization for each population were then determined by solving the resulting quadratic equation for each population using the observed variances of $\delta^{13}C$.

To determine if population niche widths were related to individual specialization, genetic diversity, or morphological variation, estimates of population total niche width (SEA_B) were estimated using the SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) package in R. The population niche width metrics generated in SIBER are similar to the total area of the convex hull surrounding the data points (TA) metrics of Layman et al. (2007) but do not have the same sensitivity to sample size. The TA metric can only increase in area as sample size increases, whereas SEA_B can increase or decrease as the ellipses encompass a set percentage of the data points. Range of $\delta^{15}N$ was calculated for each population of Guadalupe bass as a measure of trophic diversity.

Relationships among individual specialization, population niche width, trophic diversity, genetic diversity, gut contents, and variances of natural log transformed

morphological data were assessed using Pearson's product-moment correlation. Because gut contents were highly variable when identified to the taxonomic level of family, food types were summed in four categories that reflect different foraging modes (Water surface invertebrates, benthic invertebrates, terrestrial invertebrates, and fish) to determine if specialization was correlated with inclusion or exclusion of any of the food types. Correlations were carried out using SIGMAPLOT.

Results

Diets of Guadalupe bass were variable within all populations (Table 3.2) and were comprised largely of larval Ephemeroptera, adult Hemiptera (Vellidae and Corixidae), and fish. Eight of the 25 categories of food items occurred in over half of the populations and eight categories were found in a single population. Food types that were found in a single population fell into two broader categories, benthic macroinvertebrates that were also rare in environmental samples and terrestrial invertebrates. Cyprinidae and Zygoptera were found in gut contents in all populations and frequencies ranged from 2 to 50% and 1 to 24%, respectively. Mean number of food types per population was 9.89 and ranged from 7 to 14. Populations with the greatest number of food types generally included terrestrial insects and aquatic insects that utilize habitats on the water surface, whereas populations with the fewest food types lacked one or both of these broader categories of food types. A total of 34 stomachs (18%) were empty and ranged from 1 to 9 among populations.

While both genetic diversity and morphological variation differed among populations, morphological variation fluctuated more widely among populations. Genetic

diversity (H_e) varied among populations from 0.258 to 0.513, whereas variances of morphological characters differed among populations by as much as an order of magnitude for each. The correlation between Var[Length] and Var[log(depth)] was marginally significant ($r = 0.592$, $p = 0.092$), however, neither variable was correlated with Var[log(jaw)]. Population niche widths (SEA_B) differed among populations (Figure 3.1) and were greatest in the Lampasas and San Gabriel river populations and smallest in the Llano and Colorado River populations.

Mean WIC/TNW across all populations was 0.393. Estimates of individual specialization (Figure 3.2; WIC/TNW) ranged from 0.03 in the Medina River population to 0.84 in the Llano River population and specialization was not related to the occurrence of any of the four categories of food types representing different foraging modes. Additionally, WIC/TNW did not appear to be related to the presence of the introduced congener smallmouth bass *Micropterus dolomieu* or hybridization between the two species (see Chapter 1).

Genetic diversity (H_e) was not correlated with individual specialization (WIC/TNW; Figure 3.3), population niche width (SEA_B), and $\delta^{15}N$ range (Table 3.3). WIC/TNW was correlated with δ^{15} N range ($r = -0.810$, $p = 0.008$; Figure 3.4). Because individual specialization is highest at WIC/TNW values near 0 and lowest at values near 1, the negative correlation coefficient indicates that high levels of individual specialization are associated with greater $\delta^{15}N$ ranges. Although WIC/TNW and SEA_B were not correlated with morphological characters, a marginally significant ($r = 0.584$, $p = 0.098$) correlation between $\delta^{15}N$ range and Var[log(jaw)] was detected.

Discussion

The prediction that populations with greater levels of genetic diversity would exhibit greater individual specialization was not supported. In this study, genetic diversity was not correlated with individual specialization or either of the other trophic niche parameters (SEA_B and $\delta^{15}N$ range) examined. The ability to utilize novel resources is related to genetic variation (Bergerson and Wool 1986), and individual specialization increased with genetic diversity (Agashe and Bolnick 2010) when genetic diversity was experimentally manipulated by combining different laboratory strains of *T. castaneum*. In these instances, however, study organisms were raised in homogeneous habitats, whereas Guadalupe bass in the present study occurred in wild populations where environmental variability can lead to phenotypic plasticity. Other species of *Micropterus* exhibit a great degree of trophic plasticity (Almeida et al. 2012). Thus, specialization is likely able to occur even at low levels of genetic diversity.

While individual specialization was not correlated with genetic diversity or population niche width, it was correlated with a measure of trophic diversity ($\delta^{15}N$ range; $r = -0.810$, $p = 0.008$). Assuming $\delta^{15}N$ fractionation of 3.4‰ per trophic level (Post 2002), differences in trophic levels of individuals within populations varied from as little as less than one half of one trophic level to nearly two trophic levels between the highest and lowest feeding individuals of a given population. Because SEA_B estimates of population niche width are a function of variation in both $\delta^{15}N$ and $\delta^{13}C$ (Jackson et al. 2011), the change in trophic diversity with no corresponding change in population niche width indicates that populations exhibiting high levels of individual specialization are feeding at a wider range of trophic levels, whereas populations exhibiting no to low

levels of individual specialization might be utilizing a wider range of food types constrained to fewer trophic levels.

Variation in jaw morphology is often correlated with intraspecific differences in food resource use (Svanbäck and Eklöv 2002, Cucherousset et al. 2011). In addition to the correlation found between individual specialization and trophic diversity, a marginally significant correlation ($r = 0.584$, $p = 0.098$) between variation in jaw length and trophic diversity was found. While neither individual specialization nor trophic diversity was related to genetic diversity, changes in trophic niche characteristics can result from behavioral plasticity (Almeida et al. 2012) and phenotypic plasticity in morphological traits associated with feeding can occur as a result of diet differences (Wintzer and Motta 2005). Variation in trophic diversity could also result from the combined effects of ontogenetic niche shifts and intrapopulation variation in size or age structure. In this study, however, variation in fish length was not related to trophic diversity, suggesting that the variation in jaw length that was correlated with variation in trophic diversity is due to phenotypic plasticity and not to ontogenetic shifts.

Competition is another important factor affecting individual specialization and niche evolution (Bolnick et al. 2003, Agashe and Bolnick 2010). Intraspecific competition can have contrasting effects on individual specialization depending on the initial preferences of individuals for various prey types (Araújo et al. 2011). One limitation of this study is that estimates of intraspecific competition were not evaluated. Because of this, is not clear whether the observed relationships between individual specialization and trophic diversity and trophic diversity and variation in jaw length are driven by competition or whether some other environmental parameter is the cause of

these relationships and plasticity in morphological characters. Another reason for caution in the interpretation of the results of this study is the extremely high estimates of individual specialization in some of the populations. Because WIC/TNW estimates are based on $\delta^{13}C$ values of prey items detected in the population, they may be inflated if variation in $\delta^{13}C$ is underestimated because of prey items not detected (Araújo et al. 2007). For example, crayfish (Cambaridae) are known to be an important food resource for Guadalupe bass (Edwards 1980) but were not detected in four of the nine populations examined. While WIC/TNW estimates might be inflated for some populations, these populations also have the greatest $\delta^{15}N$ ranges, indicating that the relationship between individual specialization and trophic diversity would likely remain if WIC/TNW estimates could be corrected.

While the pattern of trophic diversity increasing with individual specialization is consistent with expectations, the lack of a correlation between genetic diversity with these two niche characteristics is inconsistent with initial predictions. Although genetic diversity has been shown to be important in facilitating individual specialization (Agashe and Bolnick 2010), specialization can also result from plasticity (Svanbäck and Bolnick 2007). The effect of morphological variation on individual specialization, in conjunction with the lack of a detected effect of genetic variation, further supports that plasticity is largely responsible for intrapopulation niche variation.

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Table 3.1. Geographic coordinates of sampling sites, genetic diversity (H_e), individual specialization (WIC/TNW), population niche width (SEA_B), and trophic diversity ($\delta^{15}N$ Range) estimates for nine populations of Guadalupe bass.

Population	Latitude	Longitude	*N*	H_e	WIC/TNW	SAE_B	N Range
Lampasas River	31.085512	-98.050967	20	0.33	0.67	3.89	1.54
San Gabriel River	30.645204	-97.584193	21	0.35	0.05	1.87	3.85
San Saba River	31.004189	-99.269006	23	0.36	0.08	3.52	6.22
Llano River	30.470444	-99.785106	19	0.40	0.84	0.79	1.20
Pedernales River	30.221806	-98.902316	18	0.26	0.30	1.98	3.97
Colorado River	29.705723	-96.536608	20	0.46	0.14	0.86	2.58
Guadalupe River	30.145971	-99.337939	25	0.51	0.74	1.11	2.48
Medina River	29.723464	-99.067883	20	0.46	0.03	1.71	4.32
Nueces River	29.721005	-100.033307	25	0.35	0.69	2.50	1.68

Table 3.2. Proportions of food items (by numerical abundance) identified in the gut contents of Guadalupe bass from nine populations. Invertebrates listed are aquatic forms unless otherwise indicated.

	Lampasas	San Gabriel	San Saba	Llano	Pedernales	Colorado	Guadalupe	Medina	Nueces
Gerridae	0.04	-	-	0.25	-	0.52	0.15	0.10	0.75
Corixidae	-	-	-	0.05	-	-	-	-	0.08
Baetidae	-	0.51	0.05	-	0.83	-	-	0.02	-
Isonychiidae	0.20	-	0.05	-	0.06	-	0.02	0.24	-
Heptageniidae	0.04	0.03	-	-	-	-	0.70	0.25	-
Ephemeridae	-	-	-	-	-	0.02	-	-	-
Ephemeroptera (Adult)	-	-	-	-	-	-	-	0.03	-
Perlidae	-	-	-	-	-	0.02	-	-	-
Trichoptera	0.04	0.22	0.03	-	0.04	0.13	-	0.05	0.01
Chironomidae	-	-	-	-	-	-	-	-	0.05
Diptera (Adult)	0.04	-	-	-	-	-	-	0.02	-
Naucoridae	-	0.01	-	-	-	0.02	-	0.02	0.01
Megaloptera	0.08	-	0.03	-	-	0.04	0.02	0.05	0.01
Anisoptera	0.04	0.01	0.16	-	0.01	-	0.01	0.13	-
Zygoptera	0.08	0.02	0.24	0.05	0.01	0.08	0.03	0.02	0.02
Cambaridae	0.08	-	-	0.05	0.03	-	0.05	-	0.01
Cyprinidae	0.04	0.20	0.32	0.50	0.02	0.10	0.02	0.05	0.03
Poeciliidae	0.12	-	-	0.05	-	-	-	0.03	-
Centrarchidae	-	-	0.05	-	-	0.02	-	-	0.01
Ictaluridae	0.16	-	0.03	-	-	-	-	0.02	0.01
Coleoptera (Terrestrial)	-	-	-	0.05	-	0.02	-	-	-
Cicadidae (Terrestrial)	0.04	-	-	-	-	-	-	-	-
Arachnida (Terrestrial)	-	-	0.03	-	-	-	-	-	-
Acrididae (Terrestrial)	-	-	-	-	-	0.04	-	-	-
Isopoda (Terrestrial)	-	-	-	-	-	-	0.01	-	-

Table 3.3. Pearson product moment correlations of population trophic niche parameters with genetic diversity and morphological characters. ** indicates $p < 0.05$ and * indicates $p < 0.10$.

	SAE_B	$\delta^{15}N$ Range	H_e	Var[Length]	Var[Log(Jaw)]	Var[Log(Depth)]
WIC/TNW	-0.08	-0.810**	0.058	-0.16	-0.412	-0.528
SAE_B	-	0.283	-0.554	0.359	0.455	-0.123
$\delta^{15}N$ Range	-	-	-0.13	0.014	0.584*	0.219

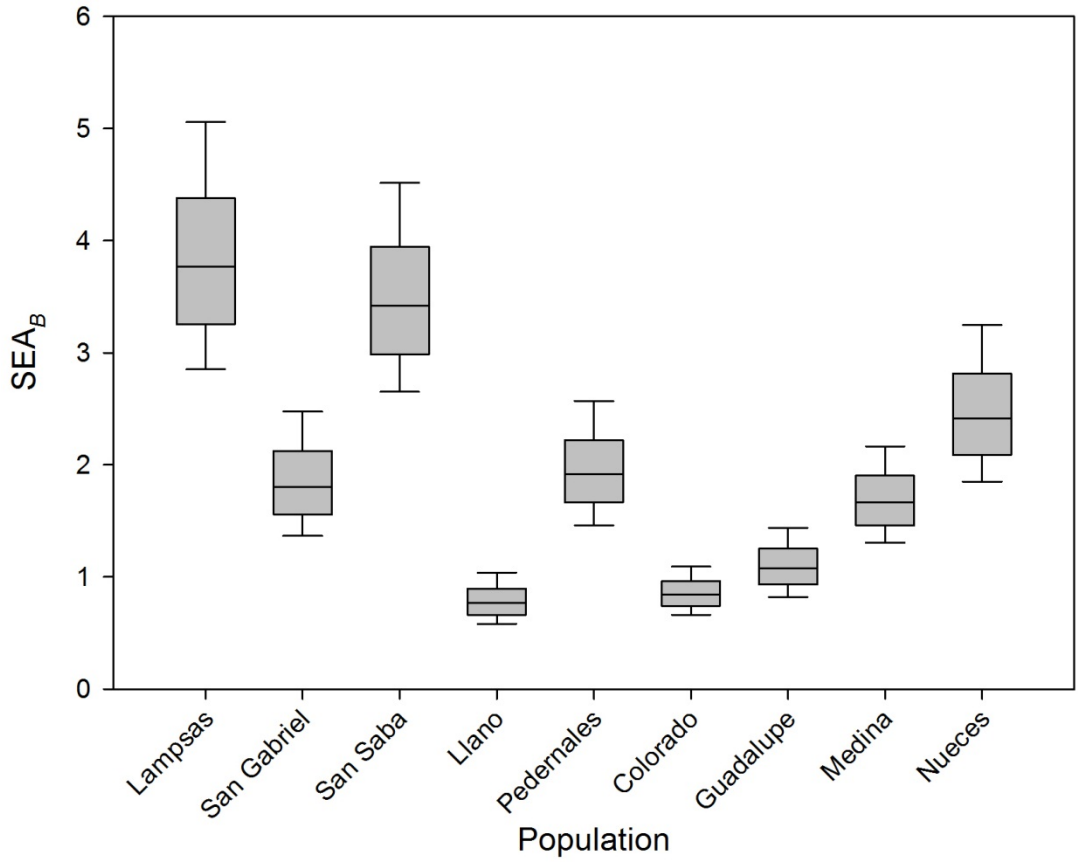

Figure 3.1. Median and distribution of estimated population niche widths represented by box plots of Bayesian estimates of standard ellipse area (SEA_B) for each of nine sampled Guadalupe bass populations.

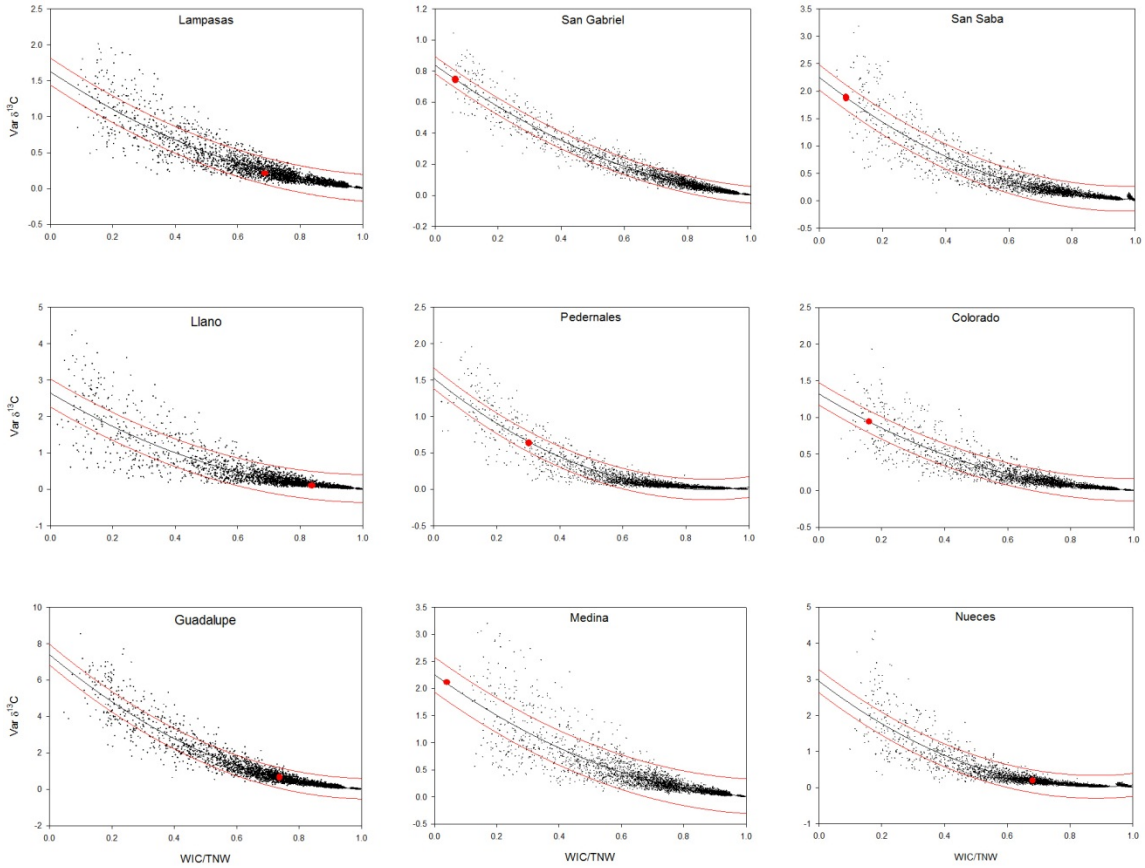

Figure 3.2. Plots of quadratic regressions of variance of $\delta^{13}C$ with WIC/TNW for populations simulated in VarIso (Araújo et al. 2007) with 95% confidence intervals. Red dots indicate where observed population $\delta^{13}C$ values lie on the regression.

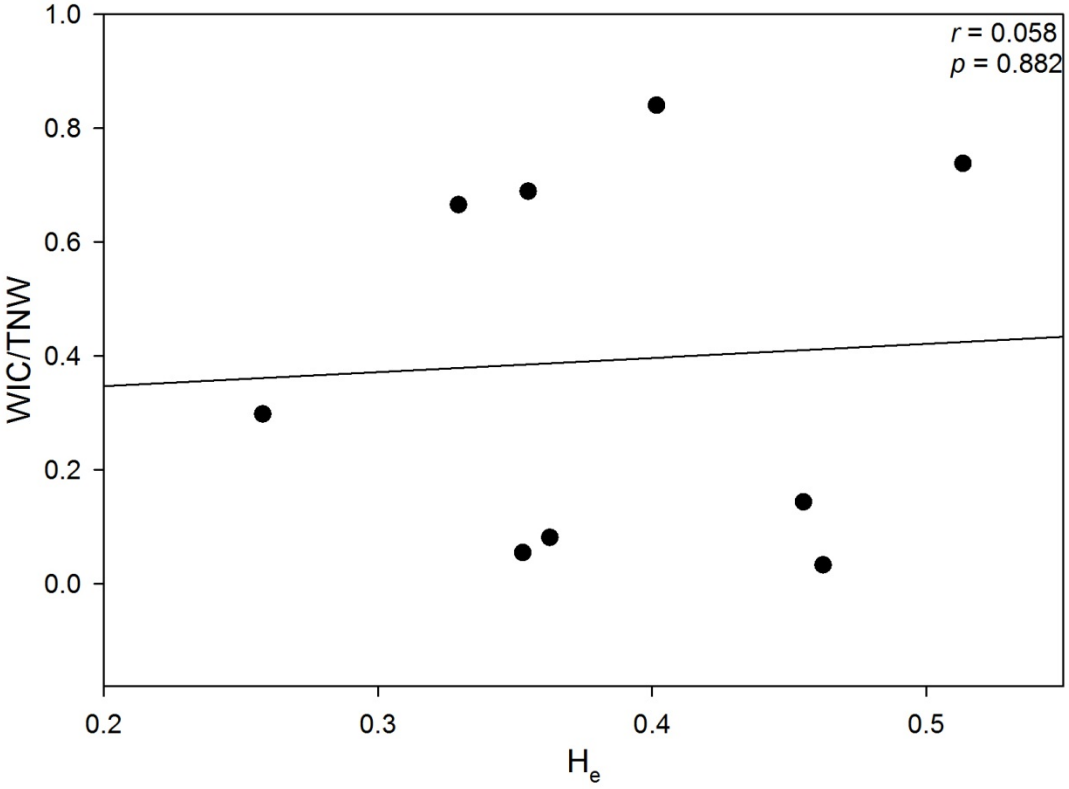

Figure 3.3. Plot of the correlation between genetic diversity (H_e) and individual specialization WIC/TNW. WIC/TNW ranges from 0 to 1 with greater levels of specialization occurring near 0 and lower levels of specialization occurring near 1.

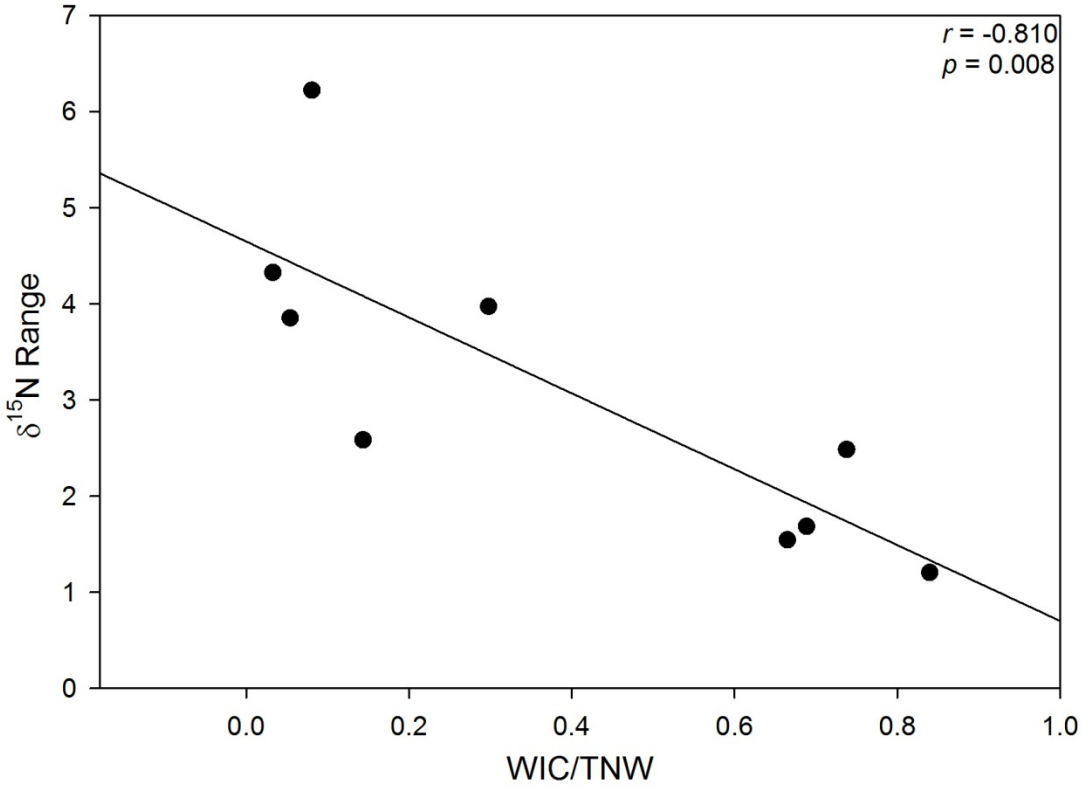

Figure 3.4. Plot of the correlation between trophic diversity ($\delta^{15}N$ range) and individual specialization (WIC/TNW).

VITA

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