

CAN INVASIVENESS OF NATIVE CYPRINIDS BE PREDICTED FROM LIFE
HISTORY TRAITS? A COMPARISON BETWEEN A NATIVE
INVADER AND A REGIONALLY ENDEMIC CYPRINID
AND STATUS OF AN INTROGRESSED GUADALUPE
BASS POPULATION IN A CENTRAL
TEXAS STREAM

THESIS

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CHAPTER 1

CAN INVASIVENESS OF NATIVE CYPRINIDS BE PREDICTED FROM LIFE HISTORY TRAITS? A COMPARISON BETWEEN A NATIVE INVADER AND A REGIONALLY ENDEMIC CYPRINID

ABSTRACT

Anthropogenic alterations to stream environments generally lead to a decrease in occurrence and abundance of native fishes, and successful establishment and increase in occurrence and abundance of non-native fishes, causing a reduction of global biodiversity and extirpations of distinct and local faunas. Under some circumstances however, anthropogenic alterations will improve conditions for certain native fishes that subsequently become more abundant, thus altering biotic interactions and affecting occurrence and abundance of other native taxa. This study tests the hypothesis that common, overabundant native fishes, relative to endemic forms, exhibit life history characteristics similar to highly invasive exotic species by comparing life history attributes (e.g., habitat selection, reproduction, growth, longevity, and diet) between a species known to increase in abundance after alterations (blacktail shiner *Cyprinella venusta*), and a regionally endemic species (Texas shiner *Notropis amabilis*) in a central Texas stream. Results indicate that *C. venusta* exhibits a more ubiquitous distribution among habitats, invests more energy into reproduction, and has a larger size and longer life span when compared to *N. amabilis*. Collectively, life history attributes of the native invader (*C. venusta*) were similar to those of successful exotic invaders and likely indicate why some native fishes are more successful than others in persisting in modified aquatic systems.

INTRODUCTION

Species introductions and their direct and indirect association with native species extirpations decrease regional and global diversity and accelerate the process of biotic homogenization (Vitousek et al. 1997, Cox 1999, McKinney and Lockwood 1999, Van Driesche and Van Driesche 2000, Low 2002). Non-native taxa that are successful at persisting in a new environment or expanding beyond the initial location of introduction (biological invaders) generally possess a suite of common life history and behavior characteristics such as broad diet (Ehrlich 1989), highly efficient foraging (Rehage et al. 2004), wide range in habitat requirements (Cassey 2002, Gido et al. 2003), high reproductive potential (O'Connor 1986, Walser et al. 2000), high dispersal abilities (Rehage and Sih 2004), rapid growth rates (Grotkopp et al. 2002, Gido et al. 2003), and generally have large natural geographic distributions (Ehrlich 1989). Probability of success of these non-native taxa increases in areas where anthropogenic modifications previously impacted the resident biota, although some studies demonstrate that even pristine environments are susceptible to invasions (Baltz and Moyle 1993, Moyle and Light 1996a, Moyle and Light 1996b, Ross et al. 2001).

Biological invasions and subsequent biotic homogenization are particularly evident in freshwater fish assemblages of North America (Rahel 2000, Rahel 2002). However, biological invasions do not always begin with non-native fishes, but with increases in abundance of a few native fishes (Scott and Helfman 2001). Overabundant native species are often ignored as an initial indicator of biotic homogenization and habitat degradation for both fish assemblages and terrestrial plant and animal communities (Garrott et al. 1993) because numerical dominance among native taxa is

variable through time and space, and historical abundance data are often lacking to quantify abundance shifts. Therefore, it is difficult to assess when a species is naturally abundant or overabundant. Some native fishes are becoming more abundant in modified streams and rivers of central North America (Anderson et al. 1983, Cross and Moss 1987, Pfeleger and Grace 1987, Edwards and Contreras-Balderas 1991, Bonner and Wilde 2000) and likely affect rarer native taxa through competition or predation. Ecological consequences of native invaders are the same as those of non-native invaders—regional diversity is reduced and hence biotic homogenization is increased because native invaders become more abundant. Rarer fishes thus become less abundant or extirpated attributed to modifications of habitat concomitant with increases in biotic interactions with native invaders (Scott and Helfman 2001).

To improve techniques for biomonitoring and to better manage riverine fish assemblages against biotic homogenization, there is a need to identify potential native invasive fishes within an assemblage, and to demonstrate that these native invasive fishes can affect rarer fishes. This is especially important in dynamic assemblages where historical abundance data are lacking. To assist with the identification of potential invaders, I predict that native invader fishes will possess life history and behavioral characters similar to those documented for non-native invaders. Herein, I test this predication by comparing habitat use, age and growth, reproductive traits, and diets between a wide-ranging minnow (blacktail shiner *Cyprinella venusta*) and one with a narrow range (Texas shiner *Notropis amabilis*) that co-exist in a modified river in central Texas and occupy similar niches. Therefore, the purpose of this study is to define and

compare life history attributes of a native invader and a regional endemic in an altered river.

The potential native invader, *C. venusta*, is found in streams and rivers of Gulf of Mexico drainages from Florida to Texas (Robison and Buchanan 1988, Page and Burr 1991, Etnier and Starnes 1993). *Cyprinella venusta* is a benthic and drift invertivore (Hale 1962, Hambrick and Hibbs 1977, Goldstein and Simon 1999) and a crevice spawner (Heins 1990). Its abundance has increased in altered systems (Anderson et al. 1983, Walser and Bart 1999), and therefore, it meets two criteria of a native invader (e.g., becomes overabundant in disturbed areas and is naturally wide-ranging; Scott and Helfman 2001). In contrast, the rarer taxon used in this study, *N. amabilis*, is endemic only to streams in the Edwards Plateau region and Rio Grande drainage of Texas and Mexico (Hubbs et al. 1991). Little is reported in the literature about diet, habitat selection, and reproductive traits of *N. amabilis*; however, *N. amabilis* likely is an obligate riverine fish.

MATERIALS AND METHODS

Ten sites within the Blanco River basin (Figure 1-1) were sampled seasonally from October 2003 through August 2005. The Blanco River drains 571 km² of the Guadalupe River drainage, and is typical of many Edwards Plateau streams by having clear hard water, predominately bedrock and gravel substrates, and extreme hydrologic variability. The Blanco River is imperiled by numerous low-head dams, groundwater withdrawals, municipal and agriculture runoff, and wastewater effluents. Despite these impacts, the fish assemblage structure (taxa occurrence and abundance) is similar to other Edwards Plateau streams and consists of a fairly diverse group of endemic and native fishes (T. Bonner, unpublished data). Sites 1-8 were spaced along the Blanco River main stem from near the headwaters to the confluence with the San Marcos River. Sites 9 (Little Blanco River) and 10 (Cypress Creek) were located in the two largest tributaries of the Blanco River.

At each site, available geomorphic units (i.e., riffles, runs, and pools) were sampled with a backpack electrofisher (Smith-Root Model 12-B POW) and seines (1.2 by 4.3 m, mesh size: 3.2 mm; 1.8 by 3.7 m, mesh size: 3.2 mm). For each geomorphic unit, all fish were identified to species, enumerated, and thirty randomly-selected individuals from each species were measured to the nearest millimeter. All fish were released except for voucher specimens, which were exposed to a lethal dose of MS-222 (80 mg/l) and preserved in 10% formalin.

Length (m), width (m), and maximum depth (cm) were measured for each geomorphic unit sampled. Current velocity and depth were measured at 3 to 5 points, depending on stream width, along a cross-stream transect near the center of each

geomorphic unit using a Marsh-McBirney Flo-Mate Model 2000 portable flow meter. Percent substrate type (i.e., bedrock, gravel, cobble, sand, silt, or detritus), percent woody debris, and percent vegetation were visually estimated as a percent of total habitat area (Taylor and Lienesch 1996; Taylor and Warren 2001). Total discharge was calculated, and water quality parameters (temperature, dissolved oxygen, pH, and conductivity) were measured (YSI model 85 and 60) once at each site.

In addition to quarterly sampling, approximately 10 adult *C. venusta* and 10 adult *N. amabilis* were collected monthly from sites 1, 5, and 8 from October 2003 to September 2004. These fish were exposed to a lethal dose of MS-222 (80 mg/l), and preserved in 10% formalin for diet and reproductive analyses. In the laboratory, total length (nearest mm) and weight (nearest 1 mg) of preserved specimens were measured. Using a dissecting microscope, gonads were removed, sexed, and weighed for determination of gonadosomatic indices (GSI; [gonad weight/body weight]*100). For each female, the stage of ovarian development was classified as immature or resting, developing, mature, or spent (Williams and Bonner 2006). Measurements of oocyte diameter were taken from the female with the highest GSI for each species each month. Oocytes were separated from surrounding tissue by vigorous shaking in a vial containing 10% formalin. Once separated, oocytes were distributed in a clear plastic dish and the diameters of 30 randomly-selected developing and mature oocytes were measured to the nearest 0.05 mm along their longest axis using an ocular micrometer and dissecting microscope. To estimate clutch size, total number of mature oocytes were counted in 10 reproductively mature females of each species in April. Fecundity counts were compared using analysis of covariance (ANCOVA) with total length as the

covariate. For growth estimations, modal class progression analysis, Bhattacharya method (FiSAT II, Version 1.2, Food and Agriculture Organization 2005) was used to determine mean lengths and age groups for *N. amabilis* and *C. venusta* based on seasonal length-frequency samples. Mean total lengths, determined in January to account for total annual growth in each age group, were used for growth comparisons. For diet analyses, alimentary tracts were removed from three randomly selected individuals of each species from each collection. Contents from the stomach to the first loop of the intestine were identified to the lowest practical taxon and enumerated using a dissecting microscope. To aid in comparing between species, gut contents were separated into four broad categories (algae, sediment/detritus, terrestrial insects, and aquatic invertebrates) and percent occurrence was calculated for each.

Blanco River habitat data were summarized using principal components (PC) analysis. Qualitative data (i.e., geomorphic units) were represented by dummy variables, and quantitative data were z-score-transformed to reduce the magnitude of differences between variables (Krebs 1999). Principal component scores were examined by season. Associations between fish species abundance and PC axes were assessed using Pearson's product-moment correlation (Zar 1999). Univariate analysis was also used to assess species distributions. Patterns between species abundance and depth and velocity were examined by comparing the relative abundance of each species within depth and velocity increments to that expected by a random distribution. Proportions of each geomorphic unit sampled were calculated (based on areal coverage; m^2) and compared to the relative abundance of each species within the various geomorphic units using Fisher's exact tests.

RESULTS

The Blanco River is a wide and shallow river with predominately bedrock and gravel substrates and small amounts of instream vegetation and woody debris. Among sites, mean stream width (\pm SD) ranged from 8.2 (6.2) to 43.5 (3.5) m and mean depth ranged from 0.3 (0.2) to 0.8 (0.1) m. Mean percent aquatic vegetation and cover (\pm SD) averaged 11.9 (24.3) and 3.2 (8.9), respectively. Among a total of 173,975 m² sampled, geomorphic units ($N = 198$) consisted of runs (58% of total area; mean current velocity \pm SE = 0.34 ± 0.27 m/s), pools (34%; 0.06 ± 0.07 m/s), riffles (6%; 0.59 ± 0.28 m/s), and backwaters (1%; 0.0 ± 0.0 m/s).

The first two PC axes of the ordination analysis explained 33% of the total variation in qualitative and quantitative habitat data. The first PC axis explained 21% of the total variation and described a current velocity, depth, and substrate gradient (Table 1-1). Geomorphic units with high negative loadings on PC I were narrow riffles with higher current velocities whereas those with high positive loadings were deep, slow-moving pools with silt substrates. The second PC axis explained 12% of the total variation and described a current velocity, substrate, and vegetation gradient. Geomorphic units with high negative loadings on PC II were vegetated areas with silt, cobble, and gravel substrates whereas those with high positive loadings were bedrock areas with higher current velocities.

Habitat use

A total of 3,308 *N. amabilis* and 11,917 *C. venusta* was collected from the Blanco River and its tributaries. Abundances of *N. amabilis* were positively associated with PC I

(i.e., deep pools and silt substrates) in fall, and negatively associated with PC II (i.e., vegetated areas) in winter (Table 1-2). Abundances of *C. venusta* were not associated with PC I across seasons, and were positively associated with PC II (i.e., swiftly flowing bedrock runs) in fall, spring, and summer. Comparatively, *N. amabilis* were more abundant in deeper pools and runs, especially in the fall and winter, whereas *C. venusta* were most abundant in swift runs in the spring and summer (Figure 1-2).

Among depth and current velocity gradients, *N. amabilis* were more abundant than expected in deeper depths and relatively slow current velocities whereas *C. venusta* were more widely distributed among depth and current velocity gradients (Figure 1-3). Among available geomorphic units available ($N = 198$), *Notropis amabilis* abundance differed (Fisher's exact test; $P = 0.05$) from expected (i.e., geomorphic units available) with highest relative abundance in runs and pools (Figure 1-4). *Cyprinella venusta* abundance did not differ from expected (Fisher's Exact test, $P = 0.10$). The ubiquitous distribution of *C. venusta* among depths, current velocities, and geomorphic units contributed to a skewed pattern in co-occurrence with *N. amabilis*. *Cyprinella venusta* were captured in 86% of the geomorphic units sampled with 47% of those units containing *N. amabilis*. In contrast, *N. amabilis* were captured in 41% of the geomorphic units sampled with 99% of those units also containing *C. venusta*.

Age and Growth

Based on length-frequency analyses, *N. amabilis* had a shorter life span than *C. venusta*. The *N. amabilis* population consisted of four year classes (2002 through 2005) from October 2003 through July 2005. Year classes 2002 and 2003 persisted into their

third summer (age 2) and likely died before October of the third year (Figure 1-5). During reproduction, breeding individuals consisted primarily of age-1 fish: year class 2003 in July 2004 and year class 2004 in July 2005. Age-0 fish (year classes 2004 and 2005) were first captured in the July collections. The *C. venusta* population consisted of six year classes (2000 through 2005). Year classes 2000 and 2001 persisted into their fifth summer (age 4) and likely died before October of the fifth year. During reproduction, breeding individuals consisted primarily of age-1 and age-2 fish, and to a lesser extent, age-3 fish. Age-0 fish (year classes 2004 and 2005) were first captured in the July collections.

Total lengths and consequently growth by age group were similar between *N. amabilis* and *C. venusta* (Table 1-3). Maximum mean total length (± 1 SD) was 44.4 (10.8) for age-0 and 60.5 (2.2) for age -1 *N. amabilis*. The largest *N. amabilis* taken was an age-2, 70 mm fish. Maximum mean total length (± 1 SD) was 45.0 (10.4) for age-0 and 66.0 (7.0) for age-1 *C. venusta*. The largest *C. venusta* taken was an age-4, 120 mm fish.

Reproductive Traits

A total of 353 *N. amabilis* and 361 *C. venusta* was analyzed for reproductive condition. In both fishes, oocyte diameters exhibited a trimodal distribution suggesting development of multiple cohorts throughout the spawning season (Figure 1-6). For *N. amabilis*, mature ovaries were present in three of fourteen females collected in October 2003 (Table 1-4). All ovaries were classified as immature or resting in individuals collected in November and December 2003. Gonadal recrudescence in *N. amabilis* began

as early as January 2004 when 24% of females collected contained developing ovaries. Mature ovaries first appeared in February (17% of females). By April, 75% of female *N. amabilis* contained mature ovaries. From May through July, mature ovaries were found in 25 to 31% of females. Reproduction peaked again in August and September 2004 when 86% and 71% of females, respectively, contained mature ovaries. Clutch size ranged from 102 to 286 with a mean (\pm SD) of 174.5 (62.2). For *C. venusta*, all females collected from October 2003 through March 2004 were classified as immature or resting. Gonadal development began in April when 23% of females contained mature ovaries. Percentage of mature ovaries increased from May through July 2004 and by August and September 100% of female *C. venusta* ($n = 25$) contained mature ovaries. With total length as a covariate, estimates of clutch size were significantly smaller than those of *N. amabilis* ($F_{1,17} = 23.3$, $P < 0.001$) (Figure 1-7), and ranged from 84 to 343 with a mean (\pm SD) of 195.8 (92.2).

Spawning periodicity, as indicated by mean female GSI values, corresponded with trends in ovarian development. Mean female GSI values for *N. amabilis* were $<3\%$ from October 2003 through January 2004, increased to 3.8% in February and 5.9% in March, and peaked in April (GSI = 7.0%). Mean GSI values then decreased by early summer to 3.8% in July before a second smaller peak in August (GSI = 5.2%) and September (GSI = 4.6%) (Figure 1-8). For *C. venusta*, mean female GSI values were low from October through March (GSI range = 1.6 - 2.7%), rose sharply in April (GSI = 5.9%), were high from May through August (GSI range = 6.6 - 7.4%), and peaked in September (GSI = 7.7%) (Figure 1-8). Comparatively, *N. amabilis* spawned larger clutches of eggs relative to total length, and exhibited an extended spawning season

(February through September) compared to that of *C. venusta* (April through September). However, increased longevity (4 years vs. 2 years) and larger maximum sizes (120 mm vs. 70 mm) of *C. venusta* result in higher lifetime reproductive potential.

Diet

Alimentary tracts of 36 individuals of each species were examined for diet analysis. Food items were present in 31 (86%) *N. amabilis* guts examined. Common food items were aquatic insects (71%) and algae (61%) (Figure 1-9). Among aquatic insects, ephemeropterans were the most abundant (31% of insects) followed by Trichoptera (26%), Diptera (22%), Coleoptera (12%), and Lepidoptera (4%) (Figure 1-10). Terrestrial insects were found in 6% of guts and comprised 5% of all insect taxa identified. Sediment and detritus, perhaps incidentally ingested with other food items, were found in 13% of all *N. amabilis* examined.

Food items were present in 33 (92%) *C. venusta* guts examined. Aquatic insects were the most common food item (82% of stomachs) (Figure 1-9). Algae were found in 73% of fish and sediment or detritus were present in 21%. No terrestrial insects were observed in stomachs of *C. venusta*. The most abundant aquatic insects included trichopterans (38% of insects identified), dipterans (32%), and ephemeropterans (23%) (Figure 1-10). Lepidoptera and Coleoptera occurred in low abundance and comprised a combined 7% of all insect taxa identified. Comparatively, aquatic insects dominated the diets of both species; however, algae and sediment/detritus were more common in diets of *C. venusta*, and terrestrial insects were more common in *N. amabilis*.

DISCUSSION

Cyprinella venusta, relative to the regionally endemic *N. amabilis*, was more broadly distributed among available habitats in the Blanco River drainage, had a longer life span, reached larger maximum sizes, and exhibited higher lifetime reproductive potential. Collectively, these traits are common among invasive fish species (Sakai et al. 2001, Cassey 2002, Gido et al. 2003). Habitat plasticity and high reproductive potential are traits thought to promote invasiveness of western mosquitofish *Gambusia affinis* and Mozambique tilapia *Oreochromis mossambicus* (Ehrlich 1989, Peterson et al. 2004); and these traits, as well as large size, are thought to enable the spread of common carp *Cyprinus carpio*, perhaps the most widespread invasive fish species in the world (Koehn 2004). In addition, Marchetti et al. (2004) found that physiological tolerance and maximum size were important factors in predicting spread and integration of invasive species in California watersheds. Invasive fish in Mediterranean streams of the Iberian Peninsula were characterized by large size, long longevity, late maturity, high fecundity, and short reproductive spans compared to native species (Vila-Gispert et al. 2005). Iberian streams, like those of the Great Plains region of central North America including the Blanco River, exhibit strong seasonal and interannual variation in flow, and thus endemic fishes in these areas have evolved primarily the opposite suite of traits (short lived, small size, and longer reproductive seasons) to deal with the naturally variable environment. However, this natural variability has been reduced by water regulation resulting in conditions which favor invasive species (Vila-Gispert et al. 2005). Similar trends in invasive species benefiting from a reduction in natural variability resulting from

anthropogenic alterations are well documented (Moyle 1986, Baltz and Moyle 1993, Moyle and Light 1996a, Moyle and Light 1996b).

Another important life history difference between the species studied lies in their specific spawning strategies. *Cyprinella venusta*, like other members of the genus *Cyprinella*, is a crevice spawner which attaches its eggs to crevices in the substrate whereas members of the genus *Notropis* are classified as broadcast spawners, with few exceptions, who simply scatter eggs over the substrate. In the Great Plains region, anthropogenic flow modification resulting from dams and reduced stream flows tend to decrease the natural variability of stream systems and seem to benefit substrate spawning cyprinids. Trends of increasing abundance of substrate spawning cyprinids have been documented in several Great Plains drainages as a result of flow alterations. Abundance of *Cyprinella lutrensis* (a close relative of *C. venusta*) has increased in the Canadian River, Texas after intense flow alterations resulting from dam construction (Bonner and Wilde 2000). Similarly, increasing abundance of *P. vigilax*, an egg clusterer (Johnston and Page 1992), has been documented in the lower Rio Grande, Texas as a result of flow modifications (Edwards and Contreras-Balderas 1991). In addition, flow alterations resulting from a hydropower dam on the Brazos River, Texas have resulted in much higher abundance of *C. venusta* and *C. lutrensis* downstream from Possum Kingdom Reservoir when compared to sites unimpacted by the reservoir (Anderson et al. 1983).

Changes in the abundance of native species resulting from anthropogenic alterations, such as those listed above, represent the initial native invasion stage of the homogenization process as described by Scott and Helfman (2001). Recognizing native invaders and their potential influence on other less-abundant native species is critical in

understanding the complex relationship between assemblage structure and anthropogenic disturbance and thus assessing impacts of stream alterations. Results of this study suggest that native invaders, relative to endemic native taxa, have traits similar to those of exotic invasive species (habitat generalist, long life span, large size, and high reproductive potential) and, at least in rivers and streams of the Great Plains region, are commonly substrate spawners. These traits will aid in identifying potential native invaders in other systems, thus providing a more comprehensive measure of stream degradation. In addition, incorporating the native invader concept into current and future biomonitoring techniques (i.e., IBI, Biological Condition Gradient) will allow more accurate assessments of stream condition.

Information reported herein for *N. amabilis* provides the first detailed life history description for this species endemic to central Texas streams and tributaries of the Rio Grande. *Notropis amabilis* generally were associated with flowing pools and deep runs and avoided shallow high-velocity riffles and lentic backwater areas. Individuals exhibited relatively fast initial growth over a life span of two years, and produced multiple cohorts of eggs during a protracted spawning season with reproductive peaks in spring and again in late summer. Gut contents suggest that *N. amabilis* is an invertevore drift predator (Goldstein and Simon 1999) feeding primarily in the water column on aquatic insects.

Information gathered on *C. venusta* provides confirmation of life history attributes previously described from other regions, and supplies more specific regionalized data from Edwards Plateau drainages. In the Blanco River, *C. venusta* exhibit a ubiquitous distribution among habitats characteristic of a habitat generalist. Similarly, Baker and

Ross (1981) noted that *C. venusta* showed a less distinct pattern of resource utilization when compared to other stream cyprinids suggesting a broad ecological niche. This study confirmed that *C. venusta* have a life span of up to four years (Ross 2000), and although growth rates were higher here than reported in Mississippi (Ross 2000), growth rates of stream fish are highly variable and are influenced by local abiotic and biotic factors. In Mississippi, as in this study, female blacktail shiners were reproductive from April through September (Heins and Dorsett 1986). Oocyte diameters were smaller and clutch size estimates were slightly lower in this study compared to fish collected in Mississippi. However, wide geographical variation exists in ova size among populations of *C. venusta* (Ross 2000, Machado et al. 2002), and given that fecundity is directly associated with fish size, lower clutch size estimates are likely a result of smaller average length. Food items identified in this study were generally similar to those found in previous studies (Hale 1962, Hambrick and Hibbs 1977), with insects and algae being the most common items.

Anthropogenic modifications to the stream environment (i.e., reduced stream flow, dam construction) will likely increase with continued development in the Blanco River watershed resulting in an overall decrease in the natural variability of the system and thus likely favoring species with invasive tendencies such as *C. venusta*. Such flow alterations, along with increased biotic interactions (i.e., competition) with overabundant *C. venusta* could negatively impact endemic species (*N. amabilis*), and result in significant changes in assemblage structure. Based on the native invader concept, these changes will be somewhat predictable; however, continued monitoring is crucial to confirm these changes and to further explore the mechanisms behind them.

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FIGURES

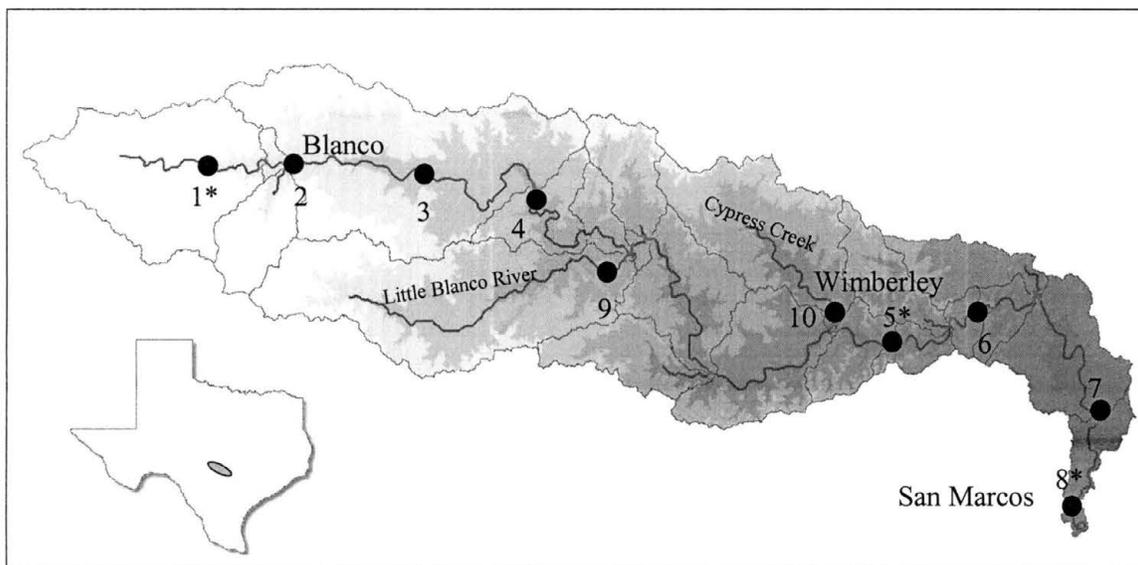


Figure 1-1. Map of study sites within the Blanco River drainage (asterisks denote sites of monthly collections).

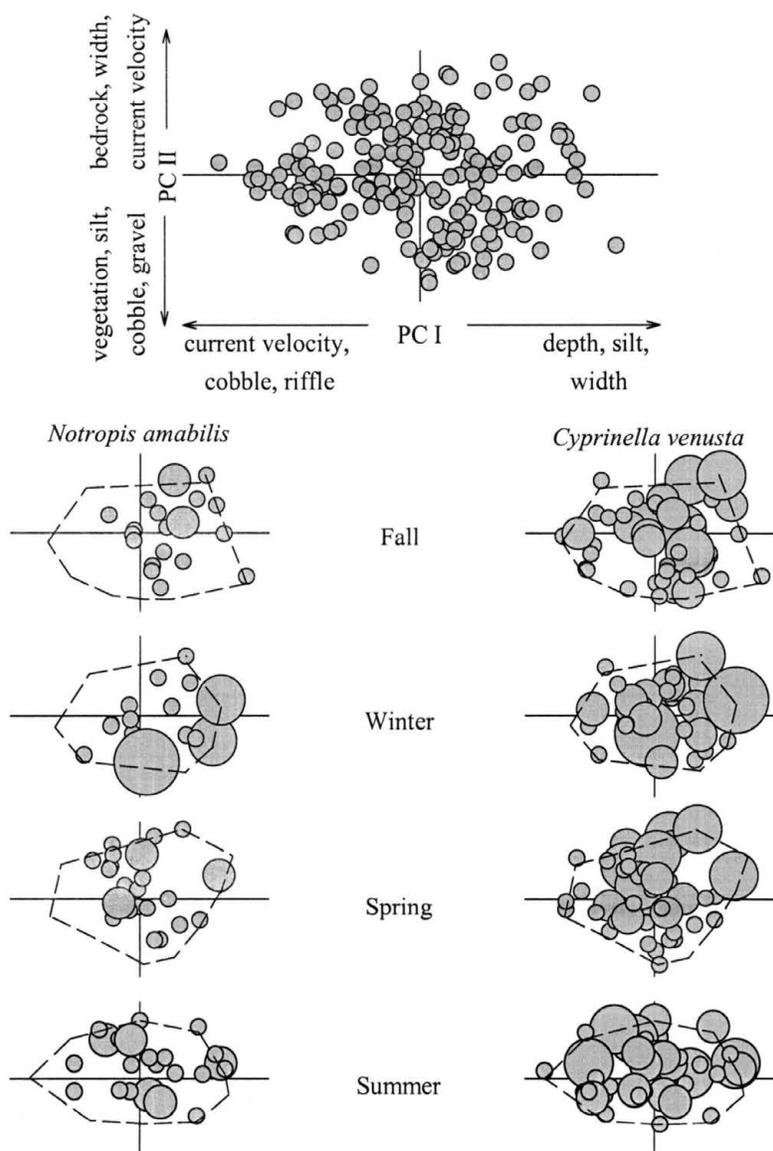


Figure 1-2. Relationships between occurrence and abundance of *N. amabilis* and *C. venusta* and PC axes one and two. Upper graph represents all geomorphic units sampled plotted on PC axes one and two. Lower graphs represent seasonal occurrence (circles) and abundance (size of circles) of the designated species. Dashed line encloses all geomorphic units sampled over a two-year period for each season.

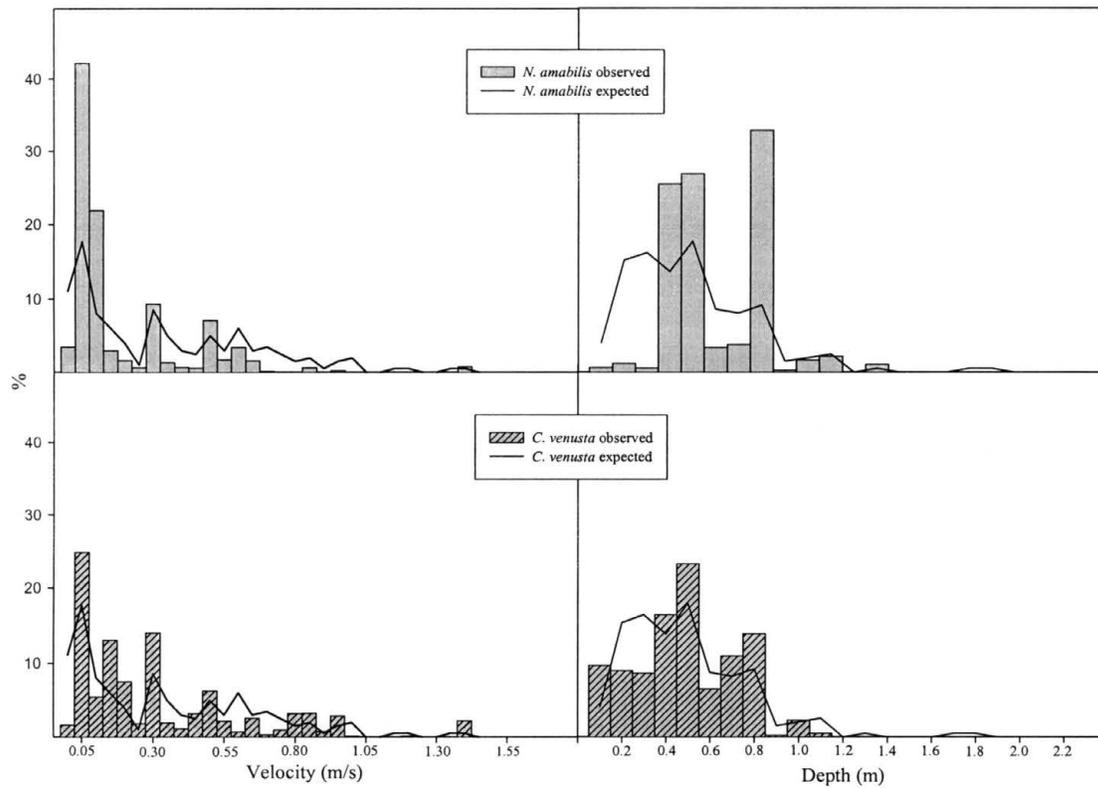


Figure 1-3. Observed versus expected abundance distributions for *N. amabilis* and *C. venusta* relative to velocity (m/s) and depth (m). Expected values assume a random distribution among available habitats.

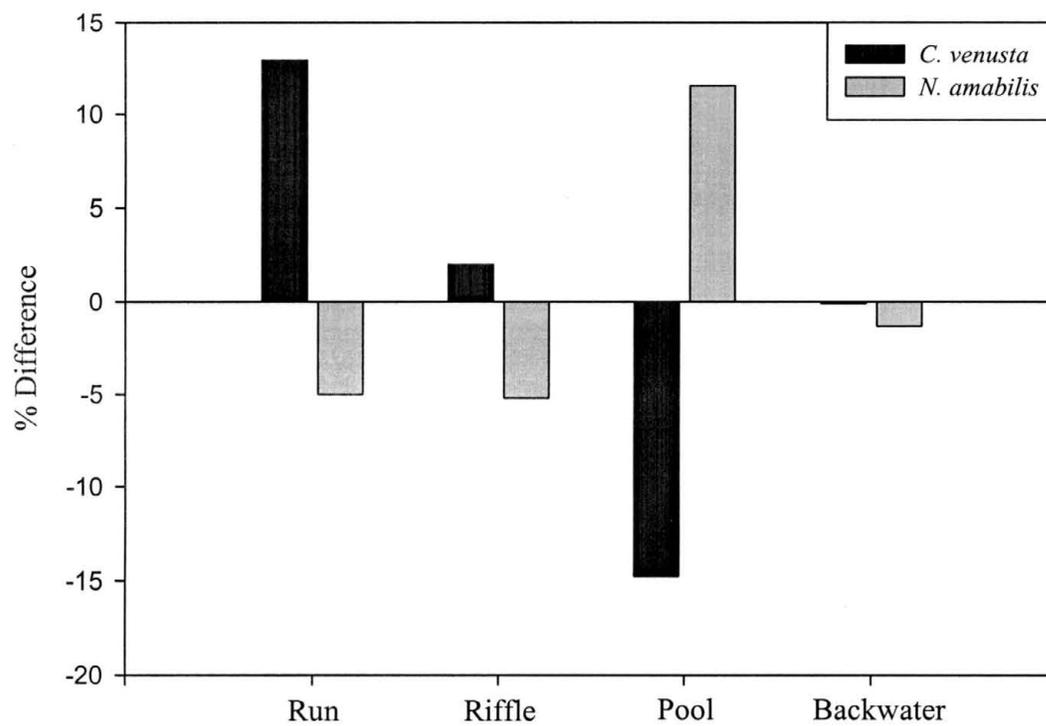


Figure 1-4. Percent deviation from expected values for relative abundance of *C. venusta* and *N. amabilis* collected from the various geomorphic units sampled.

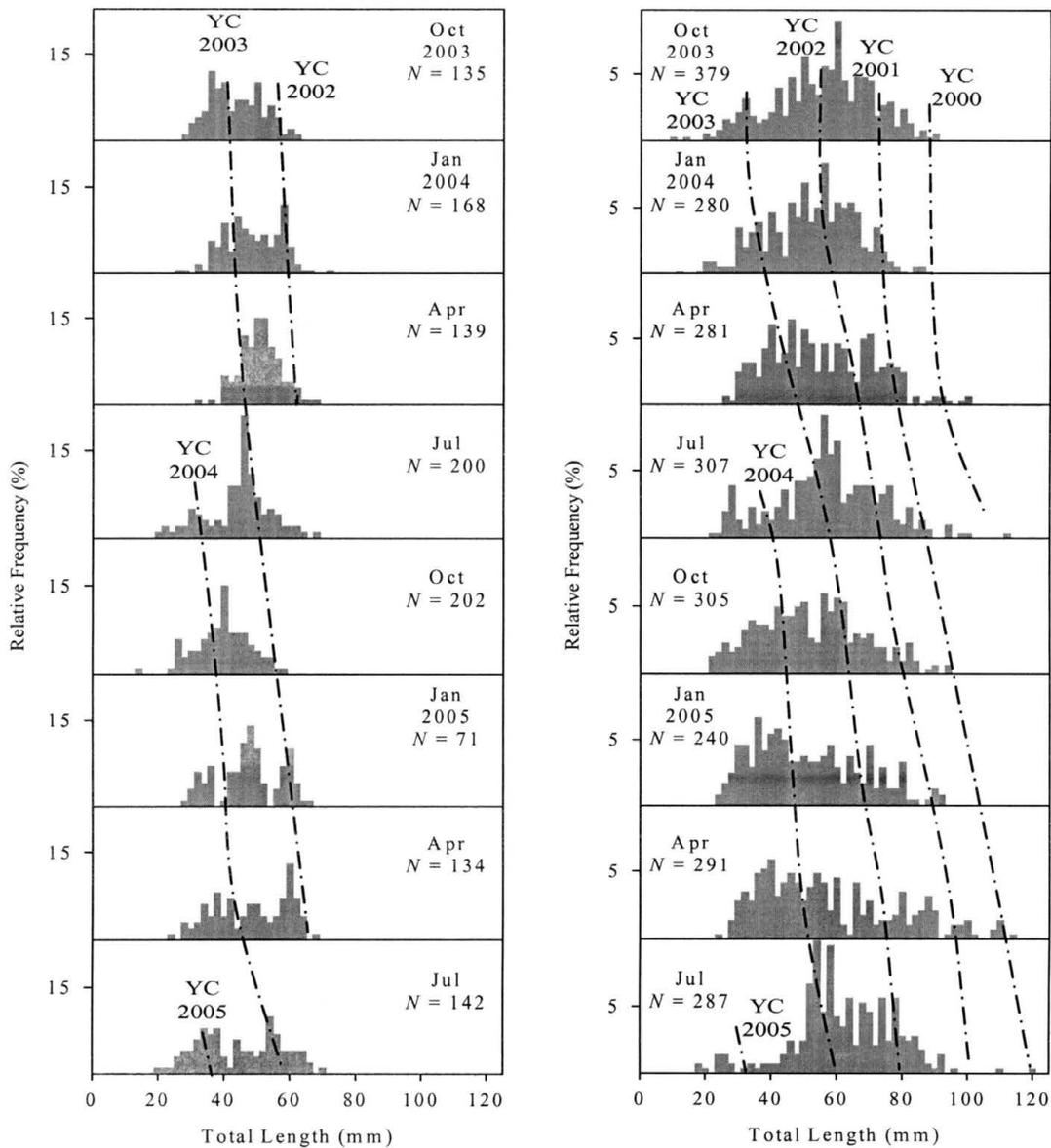


Figure 1-5. Seasonal length frequency histograms for *N. amabilis* and *C. venusta* collected from the Blanco River, Texas from October 2003 through July 2005. Dashed lines represent mean length for age groups determined from modal class progression analysis.

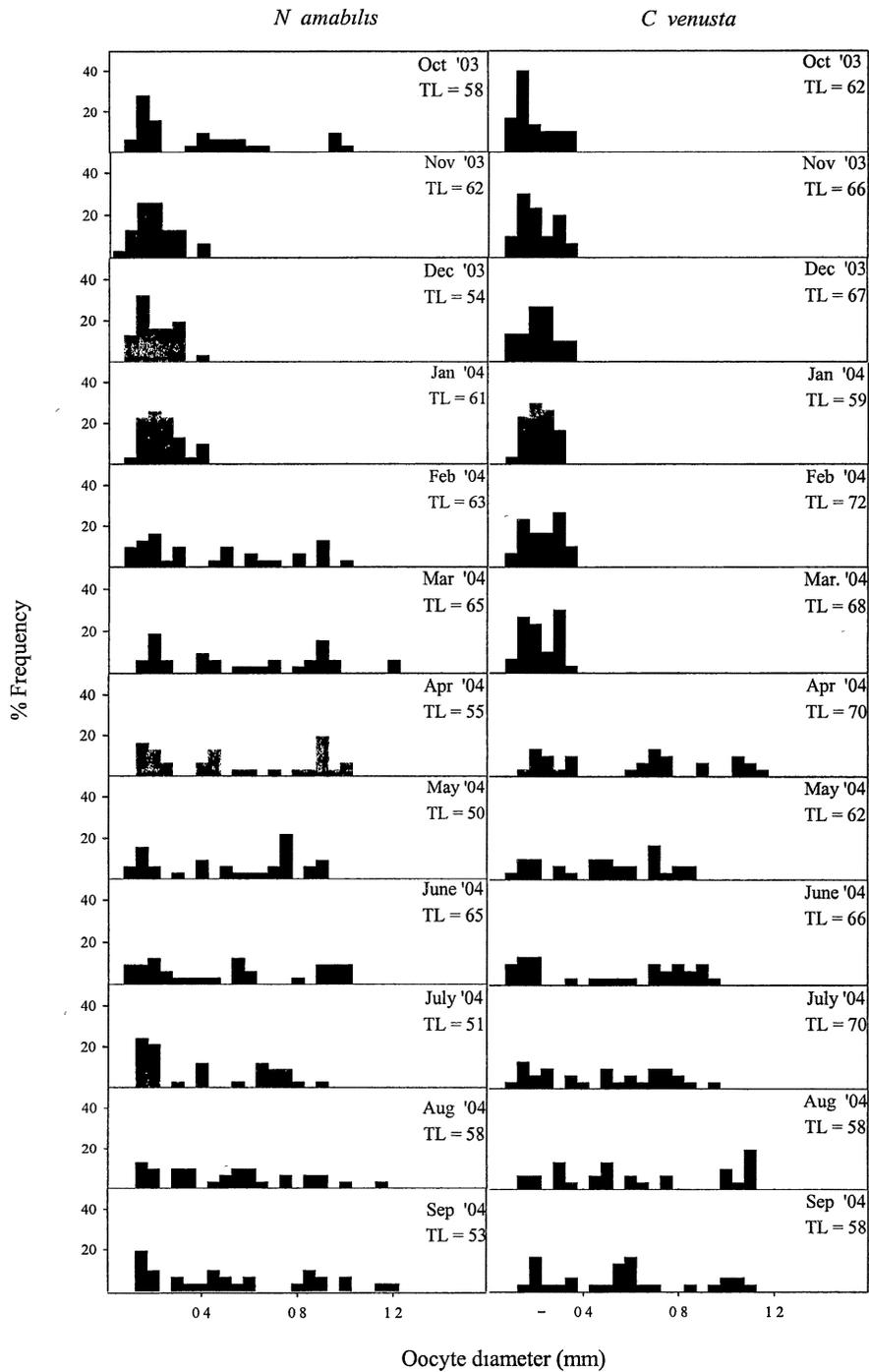


Figure 1-6. Oocyte diameter distributions ($n = 30$) from individual *N. amabilis* and *C. venusta* females collected each month from October 2003 through July 2004 from the Blanco River, Texas.

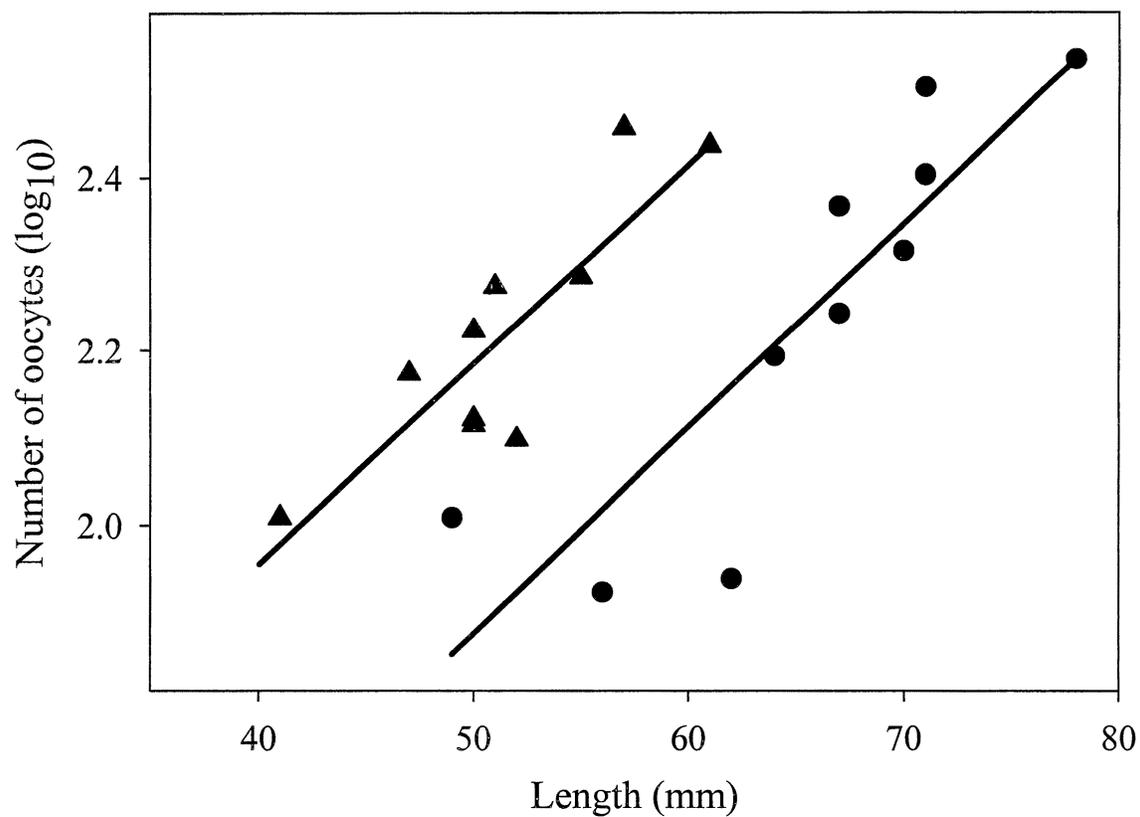


Figure 1-7. Number of mature oocytes versus total length for 10 *N. amabilis* (triangles) and 10 *C. venusta* (circles) collected from the Blanco River, Texas.

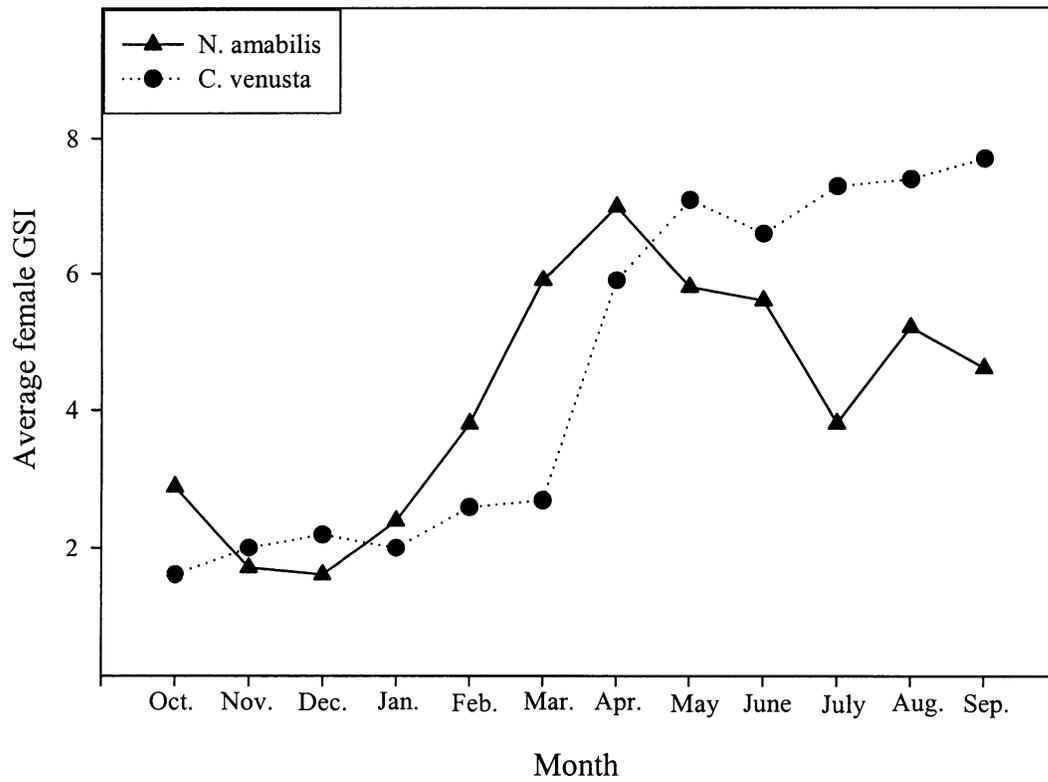


Figure 1-8. Average female GSI values for *N. amabilis* and *C. venusta* collected from the Blanco River, Texas from October 2003 to September 2004.

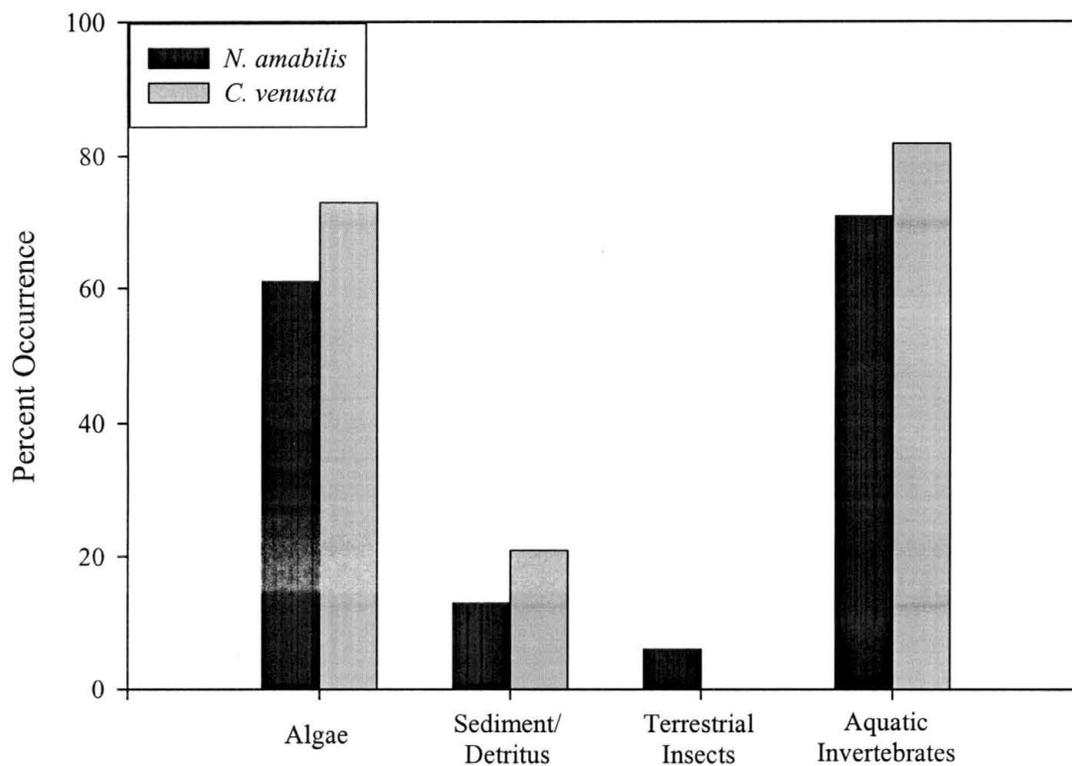


Figure 1-9. Percent occurrence of various food items in the alimentary tracts of 36 *N. amabilis* and 36 *C. venusta* collected from the Blanco River, Texas from October 2003 through September 2004.

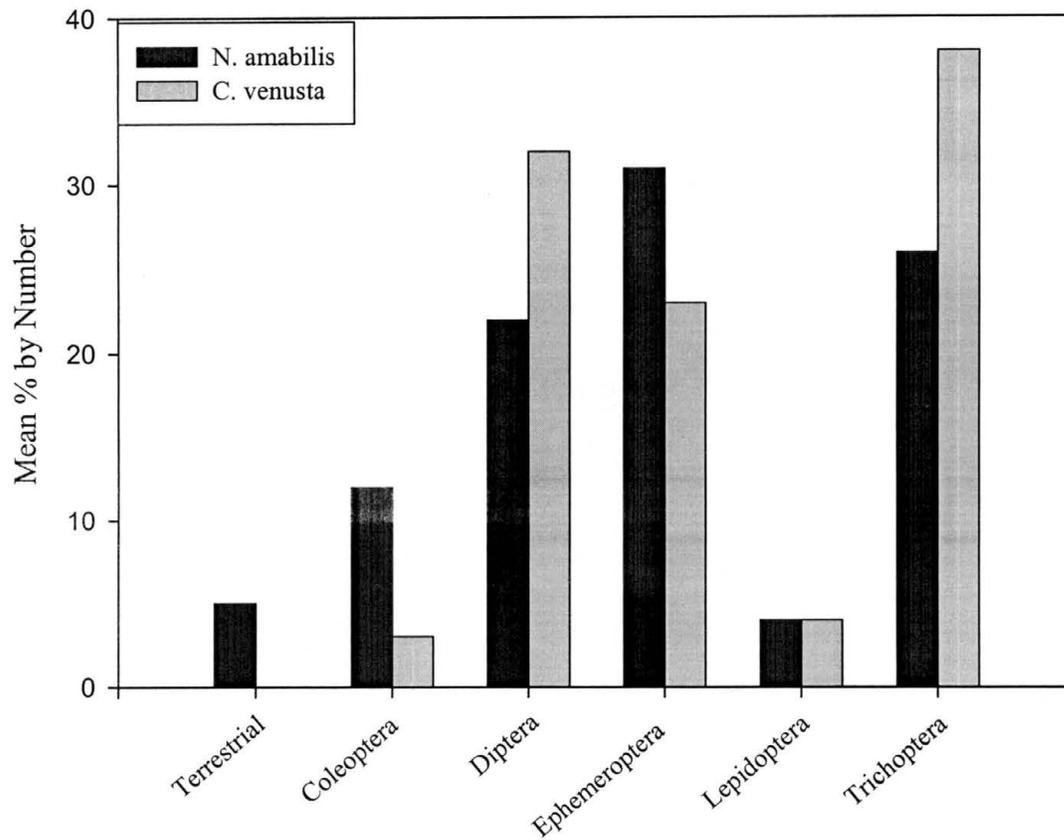


Figure 1-10. Mean percent by number of various aquatic invertebrates identified from alimentary tracts of 36 *N. amabilis* and 36 *C. venusta* collected from the Blanco River, Texas from October 2003 through September 2004.

TABLES

Table 1-1. Loadings and percent variation explained for PC axes one and two.

Parameter	PC	
	1	2
Run	0.030	0.132
Riffle	-0.184	0.006
Pool	0.092	-0.080
Backwater	0.015	-0.062
Plunge Pool	0.001	0.004
Side Channel	0.000	-0.017
Mainstem	-0.022	0.064
Tributary	0.022	-0.064
Reservoir	0.046	0.017
Mean Velocity (m/s)	-0.451	0.219
Maximum Velocity (m/s)	-0.458	0.241
Mean Depth (m)	0.384	0.061
Maximum Depth (m)	0.394	0.116
Stream width	0.233	0.378
Silt substrate (%)	0.244	-0.257
Sand substrate (%)	0.090	0.106
Gravel substrate (%)	-0.161	-0.222
Cobble substrate (%)	-0.203	-0.253
Bedrock substrate (%)	0.102	0.552
Boulder (%)	0.143	0.099
Detritus (%)	0.097	-0.215
Cover (%)	0.076	-0.077
Vegetation (%)	0.048	-0.374
<i>Percent Variance Explained</i>	<i>21%</i>	<i>12%</i>

Table 1-2. Correlations between seasonal abundance of *N. amabilis* and *C. venusta* over a two-year period and PC axes 1 and 2. Values in bold represent statistically significant correlations.

	<i>N</i> observations	<i>N</i> fish	PC I		PC II	
			<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
<i>Cyprinella venusta</i>						
Fall	50	2,786	0.20	0.16	0.34	0.01
Winter	49	2,747	-0.17	0.24	-0.07	0.64
Spring	54	3,227	0.24	0.08	0.44	<0.01
Summer	45	3,158	0.12	0.44	0.41	0.01
<i>Notropis amabilis</i>						
Fall	50	726	0.36	0.01	0.24	0.09
Winter	49	507	0.07	0.65	-0.33	0.02
Spring	54	1,481	0.26	0.06	0.18	0.18
Summer	45	594	0.26	0.09	0.12	0.42

Table 1-3. Mean total length (mm) \pm 1 standard deviation by age for *N. amabilis* and *C. venusta* collected from the Blanco River in January 2004 and 2005, representing annual growth for each age group.

Species	Date	Total length (mm) at age							
		<u>0</u>		<u>1</u>		<u>2</u>		<u>3</u>	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
<i>Notropis amabilis</i>	January 2004	43.6	4.6	56.8	2.8				
	January 2005	44.4	10.8	60.5	2.2				
<i>Cyprinella venusta</i>	January 2004	34.5	8.1	56.9	7.2	73.6	3.5	85.1	2.4
	January 2005	45.0	10.4	66.0	7.0	90.5	1.6	-	-

Table 1-4. Ovarian classification of female *N. amabilis* and *C. venusta* collected from the Blanco River from October 2003 to September 2004.

Species	Month	N	Resting or Immature	Developing	Mature	Spent
<i>N. amabilis</i>						
	October-03	15	53%		20%	20%
	November-03	16	100%			
	December-03	12	100%			
	January-04	17	76%	24%		
	February-04	12	50%	33%	17%	
	March-04	11	27%	36%	36%	
	April-04	20	10%	15%	75%	
	May-04	16	0%	69%	31%	
	June-04	15	0%	73%	27%	
	July-04	12	17%	58%	25%	
	August-04	14	0%	0%	86%	14%
	September-04	17	6%	0%	71%	24%
<i>C. venusta</i>						
	October-03	13	100%			
	November-03	14	100%			
	December-03	13	100%			
	January-04	16	100%			
	February-04	8	100%			
	March-04	16	100%			
	April-04	13	31%	46%	23%	
	May-04	13		62%	38%	
	June-04	18		44%	56%	
	July-04	11		18%	82%	
	August-04	14			100%	
	September-04	11			100%	

CHAPTER 2

STATUS OF AN INTROGRESSED GUADALUPE BASS POPULATION IN A CENTRAL TEXAS STREAM

ABSTRACT

Introductions of non-native smallmouth bass *Micropterus dolomieu* into central Texas streams resulted in introgressive hybridization with an endemic allopatric congener, the Guadalupe bass *M. treculii*. In an attempt to restore a dominant Guadalupe bass population and genetically swamp the smallmouth bass genome, 80,000 hatchery-reared Guadalupe bass fingerlings were stocked in the Blanco River (Guadalupe River drainage, Texas) in 1994 and 1995. Objectives of this study were to examine the genetic influence of Guadalupe bass, smallmouth bass, and their hybrids 10 years following supplemental stockings of Guadalupe bass in the Blanco River by analyzing allele frequencies at fourteen unlinked microsatellite loci. Genetic analysis identified 40% of individuals as smallmouth bass, 51% as smallmouth bass x Guadalupe bass hybrids, and 9% as other *Micropterus* hybrids. Pure Guadalupe bass were not collected. Despite supplemental stockings, introgression continues in the Blanco River and has likely continued or spread in other areas documenting the need for a current review of the genetic integrity of the Guadalupe bass throughout its range.

INTRODUCTION

Intentional stocking of non-native sport fishes accounts for the majority of introduced fishes in North America (Rahel 2000, Rahel 2002). Although non-native sportfish stockings are accepted and viable management options used by many federal and state agencies to enhance fishing opportunities (Heidinger 1993, Li and Moyle 1993), some stockings are detrimental to resident fishes. This is especially true when the introduced species is an allopatric congener of a resident fish, and weakly-developed reproductive isolating mechanisms increase the likelihood of hybridization and introgression (Hubbs 1955). Consequently, genetic contamination has occurred in several native fishes as a result of intentional sportfish stockings, including Paiute cutthroat trout *Salmo clarki seleniris* (Busack and Gall 1981), Atlantic salmon *S. salar* (Verspoor 1988), largemouth bass *Micropterus salmoides* (Whitmore and Hellier 1988), spotted bass *M. punctulatus* (Koppelman 1994, Pierce and Van Den Avyle 1997), smallmouth bass *M. dolomieu* (Turner et al. 1991), redeye bass *M. coosae* (Pipas and Bulow 1998), and Guadalupe bass *M. treculii* (Edwards 1979, Whitmore and Butler 1982, Whitmore 1983, Garrett 1988).

Smallmouth bass were stocked intensively in central Texas waterways beginning in 1974 (Garrett 1991). However, several streams in the Brazos River, Colorado River, Guadalupe River, and San Antonio River drainages of central Texas supported an endemic allopatric congener, the Guadalupe bass (Hubbs et al. 1991). In the mid-1970s, morphometric evidence of hybridization was found between smallmouth bass and Guadalupe bass in the Guadalupe River drainage (Edwards 1979) with hybridization and introgression later verified by electrophoretic techniques (Whitmore and Butler 1982,

Whitmore 1983). By 1990, smallmouth bass x Guadalupe bass hybrids were common (e.g., incidence of hybridization >45% in some areas) in central Texas streams (Garrett 1991). Genetic contamination and habitat modifications to lotic environments resulted in a decline in abundance and distribution of Guadalupe bass (Edwards 1978, Edwards 1980, Morizot et al. 1991). Consequently, the Guadalupe bass is listed by several authors and agencies as a species of conservation concern (Johnson 1987, USDI 1989, Hubbs et al. 1991, Warren et al. 2000). In an effort to protect and restore Guadalupe bass populations, smallmouth bass stockings were eliminated within the native range of Guadalupe bass by 1990. In addition, hatchery-produced Guadalupe bass were stocked in two central Texas streams to restore a dominant Guadalupe bass population and to genetically swamp the smallmouth bass genome (Garrett 1991).

In the Blanco River, a tributary of the San Marcos River (Guadalupe River drainage), 130,000 smallmouth bass were stocked from 1977 through 1980. In 1991, 30% of black basses ($N = 88$) collected from the Blanco and San Marcos rivers were genetically identified as interspecific hybrids between smallmouth bass and Guadalupe bass (Morizot et al. 1991). As part of the restoration effort, 80,000 hatchery-produced Guadalupe bass were stocked in the Blanco River in 1994 and 1995. One objective of this study was to determine the proportion and genetic influence of Guadalupe bass, smallmouth bass, and their hybrids in the Blanco River 10 years following the supplemental stocking of hatchery-produced Guadalupe bass, and thus assess the effectiveness of supplemental stocking as a management option to restore a native fish population genetically contaminated by an introduced allopatric congener. A second

objective was to examine gene flow among our collection sites in order to identify superior stocking locations for future restoration efforts.

MATERIALS AND METHODS

The Blanco River is a 571 km² limestone-dominated drainage along the eastern edge of the Edwards Plateau in Kendall, Blanco, and Hays counties of central Texas. Bass were collected from three sites on the Blanco River (Figure 2-1). Site 1 consisted of an 11-km segment of river immediately downstream from Wimberley, Texas. Site 2 was a 2-km stretch between Site 1 and the city of Kyle, Texas. Site 3 comprised a 10-km segment downstream from Five-Mile dam to the confluence with the San Marcos River. Habitats at sites 1 and 2 were dominated by wide shallow runs with bedrock substrate whereas habitat at Site 3 was more heterogeneous with fast riffle and deep pool habitats interspersed over cobble and gravel substrates. Although smallmouth bass are present in the upper reaches of the Blanco River in low abundance (T. Bonner, unpublished data), the area was not sampled because previous studies (Morizot et al. 1991) collected only largemouth bass in this section of the river.

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

Total genomic DNA was isolated from 3 to 5 mm³ of anal fin tissue using a modified version of the Purgene protocol for fish tissue (Gentra Systems, Inc., Minneapolis, MN, USA). Genomic DNA was quantified by fluorometry (Hofer DyNA

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

Species classification and hybridization were assessed by comparing allele frequencies of unknown Blanco River individuals to pure individuals from a pre-existing database which included collections of largemouth bass from Lake Kickapoo, TX ($N = 28$) and the Devils River, TX ($N = 36$), smallmouth bass from the Devils River, TX ($N = 26$) and the Possum Kingdom State Fish Hatchery, TX ($N = 15$), and Guadalupe bass from the Guadalupe River ($N = 53$). The Bayesian inference algorithm implemented in STRUCTURE (v. 2.0, Pritchard et al. 2000) was used to infer the genetic contribution of each species to each individual. This program defines the probability of each individual belonging to a group or the joint probabilities that it belongs to two or more groups by clustering individuals based on their multilocus genotypes and the principles of Hardy-

Weinberg and linkage equilibrium; these clustering probabilities approximate genomic proportions. Using a clustering value of three, the *a priori* samples were clustered into three groups corresponding to their species status. Clustering probabilities were arcsin transformed for normality and confidence intervals of ± 1.96 SD were used to create classification threshold values. Unknowns were assigned to each group and designated as hybrids if transformed clustering probabilities did not meet threshold cutoff values.

Genetic differentiation among sites was analyzed by calculating pairwise F_{ST} values; significance levels were evaluated by permutating individuals among samples using the program ARLEQUIN (v. 2.000, Schneider et al. 2000). Numbers of effective migrants among sites per generation was estimated using the method of Slatkin (1995). A Bayesian method, implemented in BAYESASS (v. 1.2, Wilson and Rannala 2003), was used to estimate recent migration rates in each direction between sites with data pooled for sites 1 and 2. Default settings were selected after consistent results were obtained for multiple parameter settings.

RESULTS

A total of 83 micropterids ranging in size from 195 – 376 mm TL was collected from the Blanco River. Sixty-six were collected by angling from Site 1 ($N = 48$) and Site 3 ($N = 18$). Seventeen were collected by electrofishing from Site 2. Proportions of genotypes did not differ between samples collected by electrofishing from Site 2 and samples collected by angling from nearby Site 1 (Fisher's exact test, $P = 0.451$) and the assumption that genotypes were equally susceptible to angling was not rejected.

Collectively, 40% of individuals were classified as smallmouth bass and 60% as hybrids (Table 2-1). Among the hybrid genotypes, smallmouth bass x Guadalupe bass hybrids were the most abundant (84%), followed by smallmouth bass x largemouth bass hybrids (10%), and multi-species hybrids (6%), which exhibited introgression from all three species. Pure Guadalupe bass were not collected. Estimated genomic proportions pooled over all sites were predominantly smallmouth bass (0.83), followed by Guadalupe bass (0.16), and largemouth bass (0.01). The mean genetic influence from Guadalupe bass in the smallmouth x Guadalupe bass hybrids was 0.28 (SE 0.028; range 0.051 – 0.798).

Genetic influence varied among sites with mean (\pm SE) smallmouth bass influence ranging from 0.89 (\pm 0.13) at Site 1 to 0.66 (\pm 0.22) at Site 3, and Guadalupe bass influence ranging from 0.09 (\pm 0.13) at Site 1 to 0.33 (\pm 0.22) at Site 3. Largemouth bass influence was consistently low at all sites ranging from 0.01 (\pm 0.01) to 0.03 (\pm 0.05) (Figure 2-2). Genotypes also varied among sites with the percentage of smallmouth bass x Guadalupe bass hybrids ranging from 40% and 35% at sites 1 and 2, respectively, to 94% at Site 3.

Estimates of genetic differentiation among sites indicated that sites 1 and 2 were not significantly different ($F_{ST} = 0.01$, $P = 0.126$), but both were significantly different than Site 3 ($F_{ST} = 0.07$ and 0.06 , $P < 0.001$ and 0.001). Slatkin's (1995) estimate of gene flow indicated that the number of effective migrants per generation between sites 1 and 2 ($M' = 22.3$) was substantially greater than the number of migrants exchanged between sites 1 and 3 ($M' = 3.3$) or sites 2 and 3 ($M' = 3.9$). Due to the close proximity and genetic similarity of sites 1 and 2, these sites were pooled for analysis of migration rates. Bayesian estimates of migration rates were significantly different from their priors ($P < 0.001$), indicating enough genetic variation was present among sites to detect migration rates between them, and results indicated that migration was asymmetric with a predominant downstream direction (Figure 2-3).

DISCUSSION

Lack of Guadalupe bass genotypes detected in this study suggests possible genetic extirpation of the species in the Blanco River. We attribute this apparent extirpation to introgression with smallmouth bass despite supplemental stocking of Guadalupe bass. Before the supplemental stocking, a total of 218 black basses, excluding largemouth bass, were collected from the Blanco River from 1991 through 1993 (Morizot et al. 1991, Farquhar 1995). In those studies, hybrids comprised 75% of the fish captured, followed by smallmouth bass (17%), and Guadalupe bass (8%). In 2005, ten years after supplemental stocking, Guadalupe bass were not found, smallmouth bass increased to 40%, and hybrids comprised 60% of the of the black bass assemblage excluding largemouth bass. If Guadalupe bass were still present at a rate of 8%, the probability of obtaining at least one in a random sample of 83 fish is 0.999, thus our results suggest that if this fish is not extirpated in the Blanco River it is severely reduced in number. Consequently, the three supplemental stockings totaling 80,000 Guadalupe bass (two in May 1994 and one in July 1995) were not successful in shifting genetic influence from smallmouth bass toward Guadalupe bass.

Proportions of various hybrid genotypes documented here were similar to those of Morizot et al. (1991), and were dominated by smallmouth bass x Guadalupe bass hybrids. Multi-species hybrids (smallmouth bass x Guadalupe bass x largemouth bass) were identified in low proportions in both studies. In addition, our results identified five individuals as smallmouth bass x largemouth bass hybrids. Although hybrids between these two species are rare in sympatric populations, they were documented in the San

Gabriel, Guadalupe, and Medina rivers (Garrett 1991) and in Squaw Creek Reservoir, Texas after introduction of non-native smallmouth bass (Whitmore and Hellier 1988).

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

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

Introgressive hybridization with smallmouth bass is not only a problem in the Blanco River, but also a problem in other localities throughout the range of the Guadalupe bass. However, pure populations are thought to exist in Gorman Creek

(Colorado River drainage), and the San Saba, Llano, Pedernales, and Medina Rivers, as well as an introduced population in the Nueces River drainage (Garrett 1991). Similar widespread introgression has been noted in several species including westslope cutthroat trout *Oncorhynchus clarki lewisi* (Allendorf and Leary 1988), headwater catfish *Ictalurus lupus* (McClure et al. 2005), and Leon Springs pupfish *Cyprinodon bovinus* (Echelle and Echelle 1997). Allendorf et al. (2001) noted that where non-introgressed populations occur hybridized populations are of little conservation value, and conservation efforts should focus on remaining pure populations. In contrast, with uncommon taxa or rare pure populations, conservation of introgressed populations might be vital to preserving the species (McClure et al. 2005). Eradicating introgressed populations and restocking hatchery-raised fish has been proposed for recovery of introgressed cutthroat trout populations (Allendorf and Leary 1988). Unfortunately, total eradication of introgressed populations results in the loss of localized genetic variation, and given that hatchery stock may be more genetically similar than wild populations this can lead to genetic homogenization at the population and species level (Allendorf and Leary 1988, Dowling and Childs 1992, Ryman and Laikre 1991). Selective removal of the introduced species and hybrid individuals that are morphologically similar to the introduced species is one method proposed to conserve localized genetic variation while decreasing proportions of introduced alleles (Dowling and Childs 1992).

Although stocking of hatchery-raised Guadalupe bass fingerlings was unsuccessful in the Blanco River, this strategy appears to have been successful elsewhere. Localized reduction in the number of hybrids has been noted in Johnson Creek, a tributary to the Guadalupe River, after a series of stockings averaging >32,000 fingerlings

per year from 1992 to 2005 (Gary Garrett, TPWD, personal communication). Results from Johnson Creek indicate that persistent stockings can positively influence hybridization levels after several years (Koppelman and Garrett 2002). However, the effects of persistent stockings on a population already dominated by introduced alleles, such as the Blanco River, are unknown.

Removal of smallmouth bass and morphologically similar hybrids may increase the effectiveness of supplemental stockings. Removal of introduced rainbow trout *Salmo gairdneri* by electrofishing has been shown to positively impact native brook trout *Salvelinus fontinalis* in streams of the Great Smoky Mountains National Park (Riley 1986, Russell 1992). Although eradication of smallmouth bass in the Blanco River is unlikely, removal efforts could reduce proportions of smallmouth bass alleles in the population and thereby increase the efficiency of supplemental stockings.

Hybridization with congeners is not exclusive to the Guadalupe bass, but is documented in several species of *Micropterus*. The popularity of black basses as sport fish resulting in widespread introduction outside their native range exacerbates this problem. Among the seven *Micropterus* species, hybridization with congeners is documented in five of them. Given that four of the seven species are considered rare due to their small native ranges (Koppelman and Garrett 2002), interspecific hybridization and introgression severely threaten genetic diversity of this genus. The development of pre-stocking evaluations (Pipas and Bulow 1998) to assess potential impacts to native species before introductions, and detailed management plans to aid in conserving genetic structure in introgressed populations (Echelle 1991) will be vital to conserving species diversity within this genus.

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FIGURES

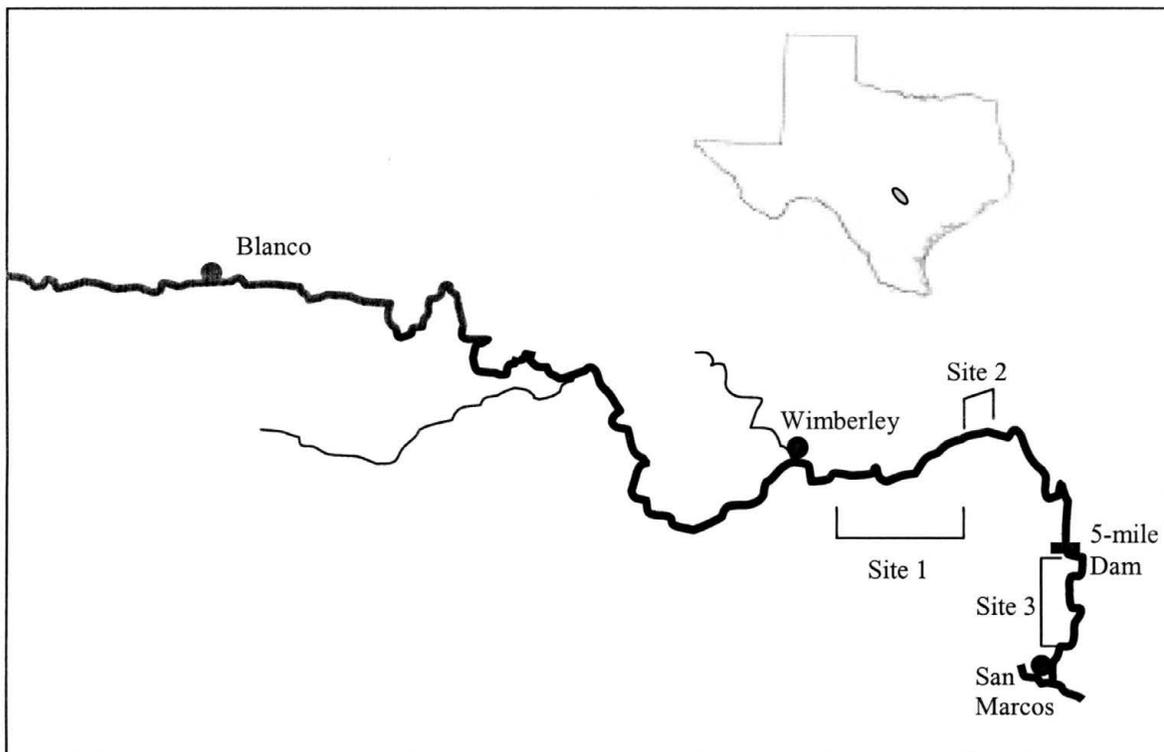


Figure 2-1. Collection sites for 83 micropterids taken from the Blanco River in Hays County, Texas during January and February 2005.

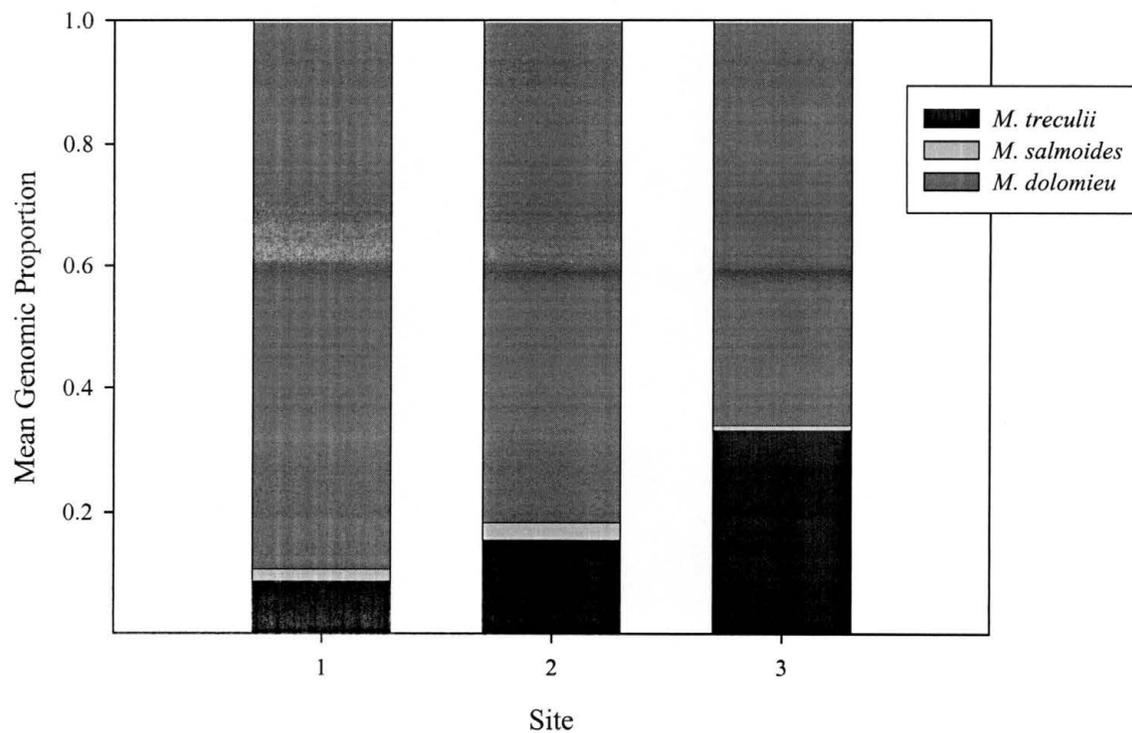


Figure 2-2. Mean genomic proportions of 83 micropterids collected from the Blanco River in Hays County, Texas during January and February 2005 at each of three sampling sites.

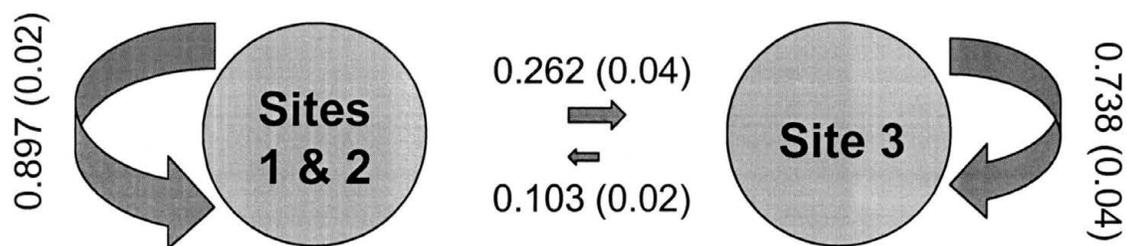


Figure 2-3. Estimated migration rates (\pm SD) between collection sites on the Blanco River using the Bayesian method implemented in BAYESASS (Wilson and Rannala 2003). Large arrows indicate the proportion of non-migrants within each site, smaller arrows indicate migration rates between sites.

TABLES

Table 2-1. Genotype of 83 micropterids collected from the Blanco River in Hays County, Texas during January and February 2005 at each of three sampling sites.

Genotype	Number of individuals		
	Site 1	Site 2	Site 3
Smallmouth bass	24	8	1
Guadalupe bass	-	-	-
Smallmouth bass x Guadalupe bass	19	6	17
Smallmouth bass x largemouth bass	4	1	-
Smallmouth bass x largemouth bass x Guadalupe bass	1	2	-

