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# Evaluation and Refinement of Guadalupe Bass Conservation Strategies to Support Adaptive Management

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**Abstract:**

Guadalupe Bass has experienced declines in parts of its range due to habitat degradation and introgressive hybridization with Smallmouth Bass. Efforts to restore Guadalupe Bass have been ongoing in the Guadalupe River drainage for over twenty years, but have recently been expanded to the Llano River drainage. Conservation and restoration efforts in the South Llano River have included supplemental stocking of non-hybrid fingerlings as well as landscape-level restoration projects in riparian and upland areas to benefit the river system. To further this effort, we undertook a multifaceted approach to evaluate Guadalupe Bass population characteristics as well as habitat use patterns in order to refine conservation and restoration strategies. Catch per unit effort was highest in sites containing swift moving water and large woody debris while abundances were lower in pool habitats with bedrock substrates. Additionally, population estimates resulted in densities of Guadalupe Bass that were highest in the sub-reaches possessing greater amounts of riffle and run habitats. Evaluation of current introgression rates indicates that introgression has declined from 3.9% to 0.9% as stocking has occurred. Our results suggest that maintaining flows is critical to providing highly utilized habitat types and that supplemental stocking is an effective management tool for reducing introgression rates in Guadalupe Bass.

## Introduction

Guadalupe Bass *Micropterus treculii* is an important species in the Edwards Plateau ecoregion in the Texas Hill Country, both economically as an important sport fish and ecologically as one of the most abundant aquatic predators. Forty-two percent of anglers in the Texas Hill Country indicated Guadalupe Bass as a primary angling target and anglers in the Texas Hill Country generate an estimated \$76,000,000 in economic activity annually (Thomas et al. in press). Though it is an ecologically and economically important species, Guadalupe Bass faces a wide array of threats that have contributed to declines in abundance in parts of its range.

Declines in Guadalupe Bass abundance have been attributed to habitat degradation (Edwards 1980) as well as to hybridization with Smallmouth Bass *Micropterus dolomieu* (Garrett 1991, Bean et al. 2013). Smallmouth Bass were intensively stocked within the range of Guadalupe Bass in the 1970's and led to introgression with Smallmouth Bass. Hybridization between the two species was first documented by Edwards (1979) and later determined to be introgressed (Whitmore 1983). A series of studies (Whitmore and Butler 1982, Whitmore 1983, Garrett 1991, Morizot et al. 1991) found that hybridization was widespread across many populations and the first attempts at restoration of Guadalupe Bass populations then began in the early 1990's.

The Guadalupe and Blanco rivers were first targeted for restoration efforts. The Guadalupe River was stocked with over 950,000 fingerlings between 1992 and 2010 and hybridization rates significantly decreased (Fleming et al. in press). In contrast, the Blanco River was stocked with Guadalupe Bass fingerlings for only two years (1994-1995). A follow-up study, Littrell et al. (2007), found that non-introgressed Guadalupe Bass had become extirpated from the Blanco River and that only Smallmouth Bass and hybrids remained. Additionally, Bean et al. (2013) detected introgression in three populations where it had not been known to previously occur, including the South Llano River.

In an assessment of changes in introgression since the Garrett 1991 study, Bean et al. (2013) detected introgression in three populations where it had not been known to previously occur, including the Llano River. New restoration efforts have begun in the Llano River basin as part of the Guadalupe Bass Restoration Initiative (GBRI) under the National Fish and Wildlife Foundation's National Black Bass Initiative. These efforts include stocking of non-introgressed fingerlings as well as watershed-scale land restoration efforts that have resulted in the conservation and restoration of riparian and upland habitats with the goal of improving instream habitats for Guadalupe Bass. Restoration began in the Llano River and, to date, >567,000 Guadalupe Bass fingerlings have been stocked in South Llano River and over 2630 acres of active restoration projects have been undertaken in the Llano River basin.

With these restoration efforts underway, evaluations of current restoration strategies as well as filling knowledge gaps for Guadalupe Bass are needed to enable refinement of management strategies. We conducted multiple studies to generate population estimates, evaluate relationships

between habitat and abundance, and assessed the integration of hatchery reared fish into the population for Guadalupe Bass in the South Llano River. Because of its co-occurrence with Guadalupe Bass, we also generated population estimates, evaluated relationships between habitat and abundance, and evaluated age structure and growth of Gray Redhorse *Moxostoma congestum* in the South Llano River. Gray Redhorse was selected due to its contrasting life history strategy. Gray Redhorse is a migratory, benthic insectivore/detritivore belonging to the lithophilic spawning guild. It is also numerically abundant and gregarious.

## Methods

### *Study Area:*

The South Llano River arises in eastern Edwards County, Texas, and flows for 53 km through Kimble County before its confluence with the North Llano River in the city of Junction, Texas. The substrate in the South Llano River is comprised primarily of limestone and flows are derived largely from spring flows from the Edwards Aquifer. The fish assemblage consists largely of Blacktail Shiner *Cyprinella venusta* and Texas Shiner *Notropis amabilis* (Cheek et al. *accepted*) and is similar to other fish assemblages of the Edwards Plateau (Bean et al. 2007, Shattuck 2010). We sampled two reaches of the South Llano River for this project and consisted of four sub-reaches (Fig. 1). Sampling sites were comprised of multiple mesohabitats consisting of run-riffle-pool complexes. We mapped instream habitats using side-scan sonar following Kaeser and Litts (2010) as part of previous projects on the South Llano River (Groeschel 2013, Cheek et al. *accepted*) and resulted in GIS layers of instream habitat at mesohabitat (e.g., run, riffle, or pool) and microhabitat (e.g., substrate type) scales. We acquired riparian habitat data from the Texas Ecological Systems Classifications Project provided by Texas Parks and Wildlife Department. We sampled four sub-reaches between four and seven times between November 2012 and December 2013. The most upstream sub-reach occurred between the two crossings of US 377 (377 Site) on the South Llano River, the next downstream sub-reach occurred between the South Llano River State Park and the upstream end of the Texas Tech Llano River Field Station property (State Park Site), the third sub-reach was along the length of the Texas Tech Llano River Field Station property (TTU Site), and the fourth sub-reach occurred between the Flatrock Lane and Texas Loop 481 crossings (Flatrock Site) in Junction, TX. Public access to the 377 Site is limited to two road crossings at the upper and lower ends of the site, whereas the State Park, TTU, and Flatrock sites have greater access via state, county, and city parks as well as the South Llano River Paddling Trail that encompasses the State Park and TTU sites.

### *Fish Sampling:*

We conducted fish sampling using boat electrofishing with pulsed DC at 120 Hz with output adjusted to maintain approximately 4-5 amps. We made a single electrofishing pass in each sampling site and all Guadalupe Bass and Gray Redhorse encountered were collected. Fish were

placed in an aerated holding tank and water changes were performed between sampling sites. Guadalupe Bass and Gray Redhorse were measured to the nearest mm total length, 3-5 scales were removed for aging, and a small portion of pectoral fin was collected from Guadalupe Bass for genetic analyses. Individuals over 170 mm (fish under 170 mm in initial tagging did not recover well from tagging event) were then tagged with a uniquely numbered T-bar anchor tag (Floy Tag and Manufacturing, Seattle, Washington) adjacent to the anterior portion of the dorsal fin (Fig. 2). After the first sampling event, all captured fish were checked for the presence of a tag and untagged fish were then tagged, except during the final sampling event where fish were only checked for the presence of a tag. For all recaptured fish, the tag number and total length at the time of recapture were recorded.

#### *Relationship of Fish Abundance to Habitat:*

For each sampling site, the meso- and microhabitats occurring between the beginning and endpoint for the site and a 50 m buffer of riparian habitat around the sampling site were included for analysis (Fig. 2). Polygons of meso- and microhabitats and riparian habitat for each sampling site were exported as raster files and analyzed in FRAGSTATS v.4 (McGarigal et al. 2012) to determine the proportion of each habitat type occurring within the sampling site. A principal component analysis (PCA) of the habitat data was performed to reduce the number of variables to a lesser number of axes representing multivariate habitat gradients (McGarigal et al. 2000). A scree plot (Cattell 1966) was used to determine the number of axes to retain for further analysis. Number of Guadalupe Bass or Gray Redhorse per linear meter (i.e. site length) at each sampling site was used as a measure of catch per unit effort (CPUE). The relationship between CPUE and habitat gradients identified in the PCA axes was then evaluated by examining correlations between CPUE and samples scores on each axis retained from the PCA.

#### *Population Estimation:*

Estimates of population size as well as 95% confidence intervals for Guadalupe Bass and Gray Redhorse in each sub-reach were generated using the Schnabel method (Schnabel 1938) for multiple mark and recapture events in Package *fishmethods* v. 1.7 (Nelson 2014) for R v. 3.1.2 (R Core Team 2014). Estimates were generated at the sub-reach level instead of the reach level because not all sub-reaches in a reach were sampled on the same date.

#### *Age and Size Structure:*

In order to assess size structure, length-frequency histograms using 20-mm bins were created for both Guadalupe Bass and Gray Redhorse. The age-length key for Guadalupe Bass in the South Llano River developed by Groeschel (2013) and lengths of bass collected in this study were used to generate age distributions of Guadalupe Bass in the South Llano River. Length-frequency histograms and age distributions were created separately for the upstream reach (i.e., 377 Site) and the downstream reach (i.e., State Park, TTU, and Flatrock sites) to allow for comparisons of the size and age structure between the two reaches as angling pressure is expected to be greater

in the downstream reach where greater public access (i.e., state, city, and county parks and an established paddling trail) exists.

#### *Gray Redhorse Age and Growth:*

Gray Redhorse from the South Llano River were aged from scales collected during electrofishing surveys. Scales were used in lieu of otoliths as fish collected were subsequently tagged and released for the population estimation as described above. Scales were prepared by soaking in water and removing any soft tissues still attached to the scale. Scales were photographed under a dissecting microscope fitted with a digital camera. Three scales per fish were photographed as regenerated scales are common in Gray Redhorse. From the digital images, the distance from the focus to the margin as well as the distance from the focus to each annulus was measured in the program ImageJ (Abramoff et al. 2004). Length at age for Gray Redhorse was back-calculated using the direct proportion method (Devries and Frie 1996) and assuming a hatch date of January 1 for all fish. An age-length key was then developed for Gray Redhorse using ages determined from scales and length at capture.

A von Bertalanffy growth curve ( $L_t = L_{\infty} [1 - e^{-K(t-t_0)}]$ ) (von Bertalanffy 1938) was fit to back-calculated length at age data for Gray Redhorse in the South Llano River. Residuals from the von Bertalanffy growth curve were then calculated to be used for correlations with hydrologic data.

No streamflow monitoring station was in operation during this study. Thus, to determine flows in the South Llano River, flows from the North Llano River (USGS Station 08148500) were subtracted from flows in the Llano River at Junction, TX (USGS Station 08150000). Resulting daily streamflow data for the South Llano River were analyzed in IHA (Richter et al. 1996) to produce summary statistics characterizing frequencies of varying levels of flow from extreme low flows to large floods as well as the durations of these events for each year. Principal component analysis (PCA) was used to reduce these summary statistics to a lesser number of axes that represent gradients in the patterns of streamflow and a scree plot was evaluated to determine the number of axes to retain for further analyses. Correlations between residuals from the von Bertalanffy growth curve and PCA sample scores of the corresponding year were examined using a Pearson product moment correlation coefficient to evaluate the potential effects of streamflow on Gray Redhorse growth.

#### *Current Introgression Levels and Integration of Stocked Guadalupe Bass:*

A subset of 224 Guadalupe Bass were submitted to Texas Parks and Wildlife Department for genotyping at 34 microsatellite loci that make up two panels of loci with differing purposes. The first panel consists of 10 loci that are informative for identifying hybridization between Guadalupe Bass and Smallmouth Bass. The second panel consists of 24 loci that exhibit great allelic diversity and are informative for parentage analysis for the purposes of identifying whether individual bass were derived from hatchery stock.

To identify hybrids, the microsatellite data from the hybridization panel were analyzed in STRUCTURE (Pritchard et al. 2000). The Bayesian clustering algorithm in STRUCTURE assigns probabilities of belonging to a particular group with the criteria of minimizing departures from Hardy-Weinberg equilibrium and minimizing linkage disequilibrium. In the case that groups represent two separate species, the assignment probabilities for individuals can be interpreted as the proportion of a particular individual's genome derived from each parental species. A cutoff proportion of 0.05 was used to classify individuals as hybrids or non-hybrids. Individuals with assignment probabilities  $>0.05$  for both Guadalupe and Smallmouth Bass ancestry were classified as hybrids whereas individuals with an assignment probability  $>0.95$  to a single species were classified as non-hybrids.

Genotyping of Guadalupe Bass to distinguish between hatchery-reared fish and wild fish is currently underway by the Texas Parks and Wildlife Department Fish Health and Genetics Lab. Additionally, 485 hatchery-reared fingerlings were tagged with PIT tags and released at three sites on the South Llano River in 2011 as part of another project (Groeschel 2013). Fish captured in this study were scanned for PIT tags and capture locations and the unique PIT tag number were recorded for recapture PIT tagged fish.

#### *Guadalupe Bass Spawning Habitat Use:*

During the timeframe of this study, the South Llano River experienced abnormally high turbidity levels of unknown cause. As this study was dependent upon high visibility in order to observe Guadalupe Bass in their spawning habitats, extremely elevated turbidity levels precluded the completion of this objective using visual survey approaches. Attempts were also made to locate Guadalupe Bass on nest sites using low-cost side scan sonar (Humminbird, Eufala, Alabama), following the standard survey methods described by Kaeser and Litts (2010), Groeschel (2013), and Cheek et al. (*in press*). However, no putative nest sites were observed using this approach likely due to the propensity of Guadalupe Bass to spawn immediately adjacent to the riverbank (Enriquez 2013) where the side-scan sonar was unable to resolve them.

#### *Hybrid Performance:*

Several attempts were made over a two year period to make  $F_1$  crosses of Guadalupe Bass and Smallmouth Bass for hybrid performance laboratory experiments. Guadalupe and Smallmouth bass were collected from the South Llano and Devils Rivers, respectively, and held in 500 gallon fiberglass tanks at the Texas Parks and Wildlife Heart of the Hills Fisheries Science Center in Mountain Home, Texas. Several procedures were attempted to produce hybrid fry and included: allowing Guadalupe Bass x Smallmouth Bass pairs collected during spawning season to spawn on their own, injection with carp pituitary or Ovaprim (Western Chemical, Inc., Ferndale, Washington) and strip spawning fish. Ripe ova and milt were produced with Ovaprim injections. However, the spawns did not result in the production of fry.

## Results

### *Relationship of Fish Abundance to Habitat:*

Evaluation of the scree plot of the proportion of variance explained by each principal component resulted in the retention of the first three principal components for further analyses. The first three principal components cumulatively accounted for 49% of the variance. The first principal component of the PCA of instream and landscape habitat data represented a gradient primarily from run and riffle habitats with cobble/gravel substrates to pool habitats with gravel/sand substrates (Fig. 3). The variables with the strongest loadings (Table 1) were run mesohabitats (-0.512), cobble/gravel substrate (-0.410), riffle mesohabitats (-0.356), pool mesohabitats (0.299), forested riparian areas (0.303), and gravel/sand substrate (0.401). The second principal component represented a gradient from sites with high concentrations of boulder substrate instream and barren riparian areas to sites with urban development in the riparian areas and instream submerged aquatic vegetation. The variables with the strongest loadings for the second principal component were barren riparian areas (-0.380), boulder substrate (-0.350), urban riparian areas (0.333), and submerged aquatic vegetation (0.512). The third principal component represented a gradient from sites with pool mesohabitats with bedrock substrate and shrubland riparian areas to sites with riffle habitats with gravel/cobble substrates and large woody debris (Fig. 4). The variables with the strongest loadings for the third principal component were pool mesohabitats (-0.415), shrubland riparian areas (-0.385), bedrock substrate (-0.329), large woody debris (0.288), riffle mesohabitats (0.340), herbaceous riparian vegetation (0.416), and gravel/cobble substrate (0.435). CPUE of Guadalupe Bass ( $r = 0.20$ ,  $P = 0.003$ ) and Gray Redhorse ( $r = 0.16$ ,  $P = 0.021$ ) were positively correlated with site scores of the third principal component which indicated lower abundance of both species in habitats with lower current velocities and bedrock substrates and greater abundance in habitats with greater finer gravel/cobble substrates and herbaceous riparian vegetation. Gray redhorse CPUE was not significantly correlated with site scores of the first ( $r = -0.06$ ,  $P = 0.367$ ) or second ( $r = 0.095$ ,  $P = 0.171$ ) principal components from the instream and landscape habitat PCA. Gray redhorse CPUE was positively correlated with site score of the third principal component ( $r = 0.16$ ,  $P = 0.021$ ).

### *Population Estimation:*

A total of 352 Guadalupe Bass  $\geq 170$  mm in total length were tagged in the study area between November 2012 and December 2013. Forty-eight recaptures occurred and no individuals were recaptured more than one time. Sub-reach population estimates ranged from 65 (95% CI 35-228) in the Flatrock Site to 1,149 (95% CI 825-1,788) in the State Park Site (Table 3). These corresponded to density estimates of 25 individuals/km in the Flatrock Site to 261 individuals/km in the TTU Site. With the exception of the Flatrock Site that includes Lake Junction as a large proportion of the site, densities of Guadalupe Bass increased from the upstream 377 (128 individuals/km) site to the TTU Site downstream.

A total of 916 Gray Redhorse  $\geq 170$  mm in total length were tagged in the study area. Eighty-three recaptures occurred with 61 individuals being recaptured once and 11 individuals being recaptured twice. Sub-reach population estimates ranged from 573 (95% CI 309-2,007) in the Flatrock Site to 8,548 (6,411-12,309) in the State Park Site (Table 4). These corresponded to density estimates of 226 individuals/km in the Flatrock Site to 1,563 individuals/km in the State Park Site.

#### *Age and Size Structure:*

The length-frequency histograms for Guadalupe Bass exhibited three modes in the lower half of the length distribution (Fig. 5 and Fig. 6). These corresponded to Age-0, Age-1, and Age-2 Guadalupe Bass. The upper half of the length distribution that corresponded to Age-3 through Age-7 Guadalupe Bass did not exhibit distinct modes. Size and age structures in the two reaches (Fig. 7 and Fig. 8) were similar between reaches and is also reflected in a lack of difference ( $F_{1,933} < 0.001$ ,  $P > 0.99$ ) in mean total length between the upstream (Mean TL = 180.86 mm) and downstream (Mean TL = 180.85 mm) reaches.

The length-frequency histogram for Gray Redhorse exhibited four distinct modes (Fig. 9). The mode centered around 120 mm individuals represented both Age-0 and Age-1 individuals based on the Gray Redhorse age-length key (Table 5). Age-0 individuals in this mode were captured in the later sampling events once they became susceptible to the electrofishing gear. Evaluation of the age distribution of (Fig. 10) indicates that Gray Redhorse are fully recruited to the electrofishing gear by Age-2. The mode centered around 420 mm individuals represented Age-4 through Age-8 individuals. The estimated von Bertalanffy growth curve parameters were  $L_{\infty} = 456.61$ ,  $K = 0.4629$ , and  $t_0 = 0.3366$ . Predicted lengths (Fig. 11) at Age-1 through Age-8 were 121 mm, 245 mm, 324 mm, 373 mm, 404 mm, 423 mm, 435 mm, and 443 mm.

Based on evaluation of the scree plot for the PCA of flow metrics, three principal components were retained for further analyses. The first principal component represented a gradient in hydrologic characteristics of years with greater numbers of extreme low flow days to those with greater minimum flow levels. The second principal component was characterized by years with positive scores having higher 3-day maximum flows and greater frequencies of floods of all magnitudes. The third principal component represented a gradient from years with greater frequencies of large magnitude floods to years with greater frequencies of extreme low flow days. Residuals from the von Bertalanffy growth curve were negatively correlated with the first ( $r = -0.119$ ,  $P = 0.001$ ) and second ( $r = -0.077$ ,  $P = 0.038$ ) principal components and positively correlated with the third principal component ( $r = 0.104$ ,  $P = 0.006$ ). Residuals from the growth curve were also positively correlated with mean air temperature between May and September ( $r = 0.095$ ,  $P = 0.011$ ).

### *Current Introgression Levels and Integration of Stocked Guadalupe Bass:*

A total of 224 bass from the South Llano River were genotyped at 10 microsatellite loci. Of the 224 bass, 222 were non-introgressed Guadalupe Bass and 2 displayed introgression between Guadalupe and Smallmouth Bass. In the two introgressed individuals, admixture proportions estimated in STRUCTURE indicated a contribution of 5% of their genome from Smallmouth Bass.

Among Guadalupe Bass PIT tagged by Groeschel (2013), three fish were recaptured in this study. Two individuals were captured within the area of release and one individual was recaptured on 9 November 2012, 2.56 km upstream of the nearest, and most upstream, release site. PIT tagged bass ranged from 76 - 102 mm at the time of release on 31 May 2012 and reached lengths of 165 mm by 9 November 2012, 175 mm by 13 July 2013, and 232 mm by 15 November 2013.

## **Discussion**

### *Relationship of Fish Abundance to Habitat:*

While Guadalupe Bass were found in a wide variety of habitat types, their abundance was greatest in habitats with moderate to swift current velocities, gravel/cobble substrates, and large woody debris present as indicated by the correlation with the third principal component in the PCA of habitat data. Within these habitats, Guadalupe Bass were often collected within the immediate vicinity of large woody debris or other types of instream cover such as boulders, root wads, and undercut banks. Because of their location within the instream cover, the current velocities experienced by the bass are often much less than immediately outside of the cover. Perkin et al. (2010) similarly found that Guadalupe Bass were associated with instream cover in habitats with moderate current velocities and other stream-adapted species of the genus *Micropterus* show similar patterns of habitats use (Stormer and Maceina 2008) where instream cover in areas of moderate current velocity is greatly utilized.

Although there were correlations between Guadalupe Bass abundance and the third principal component of the PCA of habitat data, there was no correlation between abundance and or the first or second principal components. The gradient along the first principal component was primarily one of mesohabitat types and substrates and variables that provide instream structure had relatively lower loadings along this axis. The second principal component represented a gradient of urbanization and instream aquatic vegetation. While urbanization is known to affect fish assemblages and lead to homogenization (Walters et al. 2003), Guadalupe Bass abundances were not related to levels of urbanization within the South Llano River watershed. The Flatrock Site did, however, have the lowest estimate of adult bass per river kilometer and were nearer to the city of Junction, TX. The site also had greater amounts of aquatic vegetation due to the

presence of a dam just upstream of the confluence with the North Llano River. Development within the riparian area was not restricted to the Flatrock Site, but was present throughout much of the watershed. The South Llano River, though, is a minimally disturbed stream within the region and the scale at which urbanization is occurring might not be to the extent that effects on Guadalupe Bass are realized.

Gray Redhorse also showed similar patterns in that abundance was correlated only with the third principal component of the habitat data PCA. Diets of Gray Redhorse indicate that much of their feeding occurs in habitats with low current velocities (Bean and Bonner 2008). However, abundances of Gray Redhorse were associated with greater proportions of habitats with faster current velocities. Anecdotally, captures of large numbers of juveniles occurred in vegetated riffles and runs and likely account for the observed association with higher current velocity habitats.

#### *Population Estimation:*

Estimates of the number of adult Guadalupe Bass per river kilometer were greatest in the TTU and State Park sites and lowest in the 377 and Flatrock sites. The TTU and State Park sites had greater amounts of riffle habitats and instream structures such as large woody debris and root wads. The 377 reach consisted of long pools separated by short riffles and lesser amounts of woody debris compared to the TTU and State Park sites. Additionally, much of the Flatrock site consists of lentic habitats due to the presence of Lake Junction. As such, the patterns in density estimates from the mark-recapture study are consistent with observed patterns in relationships between CPUE and habitat variables. On the other hand, estimates of Gray Redhorse of fish per river kilometer were similar among the sub-reaches that did not include Lake Junction. Estimates of Gray Redhorse populations were very high (e.g., over 8,500 in the State Park site). However, due to the larger size at maturity (Bean and Bonner 2008) and the size range of fish in this study (i.e., >170 mm), the estimate includes not only adult fish, but sub-adult fish as well.

#### *Age and Size Structure:*

Guadalupe Bass size and age distributions were created for both the upstream reach and downstream reach to facilitate comparisons between an area expected to have greater fishing pressure (i.e., paddling trail and state park in the lower reach) and an area expected to have lower fishing pressure (i.e., between two road crossings, but with no additional access points available). Size and age distributions were similar between the two reaches and indicate recruitment to the sampling gear by Age-2 and then a steady decline through Age-5 with only a few fish making it to Age-6 and Age-7. If angling pressure is, in fact, greater in the lower reach, the highly similar size structures would suggest that harvest of Guadalupe Bass by anglers is very low. Thomas et al. (2014) reported that issues related to harvest were less important to anglers in the Texas Hill Country than issues related to river access. Additionally, angler attitudes towards catching fish to eat as a reason to fish was ranked as 'neutral' to 'very unimportant' by 77% of respondents, with

40% of anglers viewing it as very unimportant. This suggests that harvest of fish, even in the face of increasing angling pressure, is unlikely to affect Guadalupe Bass size structure

Gray Redhorse were fully recruited to the sampling gear by Age-2 and a substantial drop in abundance occurred between Age-2 and Age-3 followed by steady declines thereafter. The minimum length of individuals with developing oocytes detected by Bean and Bonner (2008) correspond primarily to Age-3 and Age-4 as determined in this study and suggests that mortality is higher in sub-adult Gray Redhorse than in adults.

#### *Current Introgression Levels and Integration of Stocked Guadalupe Bass:*

Over 567,000 Guadalupe Bass have been stocked in the South Llano River since 2011 for the purposes of reducing rates of introgression in the system. Prior to stocking, the rate of introgression was 3.9% (Bean et al. 2013) and has since been reduced to 0.9%. Similarly, Fleming et al. (in press) found substantial reductions in introgression rates associated with restorative stocking efforts in the Guadalupe River. Starting introgression rates were much higher in the Guadalupe River (20-100%) and stocking occurred over a longer time. However, both Fleming et al. (in press) and the present study demonstrate that stocking of hatchery reared fingerlings can be an effective management strategy for reducing introgression rates. While stocking can be effective, it should also be noted that introgression rates are highest where both Guadalupe Bass and Smallmouth bass still co-occur (Bean et al. 2013) and introgression is ongoing. Therefore, prioritizing the protection of non-introgressed populations and using stocking to reduce introgression rates in systems where Smallmouth Bass are no longer present may be the most effective strategy for future management of Guadalupe Bass.

#### *Gray Redhorse Age and Growth:*

Predicted length at age from the von Bertalanffy growth curve corresponded well with modal groups of length frequency histograms. Size at maturation of Gray Redhorse (350-400 mm; Bean and Bonner 2008) was typically reached by Age-3, similar to some other redhorse species such as Golden Redhorse *Moxostoma erythrurum* (Jenkins 1980). Growth rates were greatest in younger fish and decreased greatly after maturation. This pattern of slowed growth after maturation was evident from both the von Bertalanffy growth curve as well as in the grouping of reproductive-aged fish in a single mode in the length frequency histogram. Similar growth patterns have been documented in other sucker species (Bacula et al. 2009), and likely result from changes in energy allocation upon reaching maturation.

Redhorse growth variation across years was not strongly linked to flows. Correlations were extremely weak ( $\approx 0.10$ ), and what correlation does exist indicates higher growth in years with lower flows (negative correlation of first principal component of South Llano River flow data with residuals from von Bertalanffy growth curve). In contrast, annual variation in growth in Guadalupe Bass was related to flow characteristics in this system (Groeschel 2013), with greater growth occurring in years with fewer extreme low flow days. A possible explanation for the

observed differences is that Gray Redhorse and Guadalupe Bass utilize different habitats. Guadalupe Bass typically occur in habitats with greater velocities that are likely affected to a greater degree by changes in streamflow, whereas the effects of lower streamflows on pool habitats where Gray Redhorse typically occur are likely much less. Additionally, Gray Redhorse growth was positively correlated with air temperatures during the period from May through September.

*Summary:*

Management actions to restore Guadalupe Bass by reducing hybridization rates have been successful in the South Llano River. Determining the long-term success of this effort will require continued monitoring. However, the absence of non-introgressed Smallmouth Bass in the system suggests that hybridization rates will likely remain low as the potential for new  $F_1$  hybrids and subsequent introgression is absent. As such, preventing the introduction of Smallmouth Bass or their alleles through hybrids is vital to the maintenance of the long term conservation goals for this population.

Abundance varied with availability of suitable habitat (e.g., current velocity, substrate, and instream habitat structure) among sites. Woody debris was an important instream habitat structure affecting the abundance of Guadalupe Bass. However, it is a less-permanent structure than boulder substrates and requires replenishment in the long-term from fallen trees from the surrounding riparian area. Jones (2008) found that there is a lack of tree recruitment in Pecan Bottomland communities that form much of the riparian area of the South Llano River and suggested that grazing by native and non-native ungulates is likely a major cause. Given the importance of woody debris to Guadalupe Bass, riparian protection and restoration to ensure replenishment of woody debris as instream structure has clear benefits to bass conservation.

Guadalupe Bass size structure was similar among reaches despite variation in abundance and greater angler access in the downstream reach. Two potential explanations for this observation include similar rates of angling pressure among the reaches or low harvest rates and low incidental mortality associated with anglers practicing catch-and-release. Examination of rates of exploitation of Guadalupe Bass in the South Llano River as well as among other populations would allow for greater understanding of factors affecting variation in abundance and size structure. It would also facilitate further refining of management strategies to meet conservation goals and also continue to provide high-quality recreational fisheries in rivers of the Edwards Plateau.

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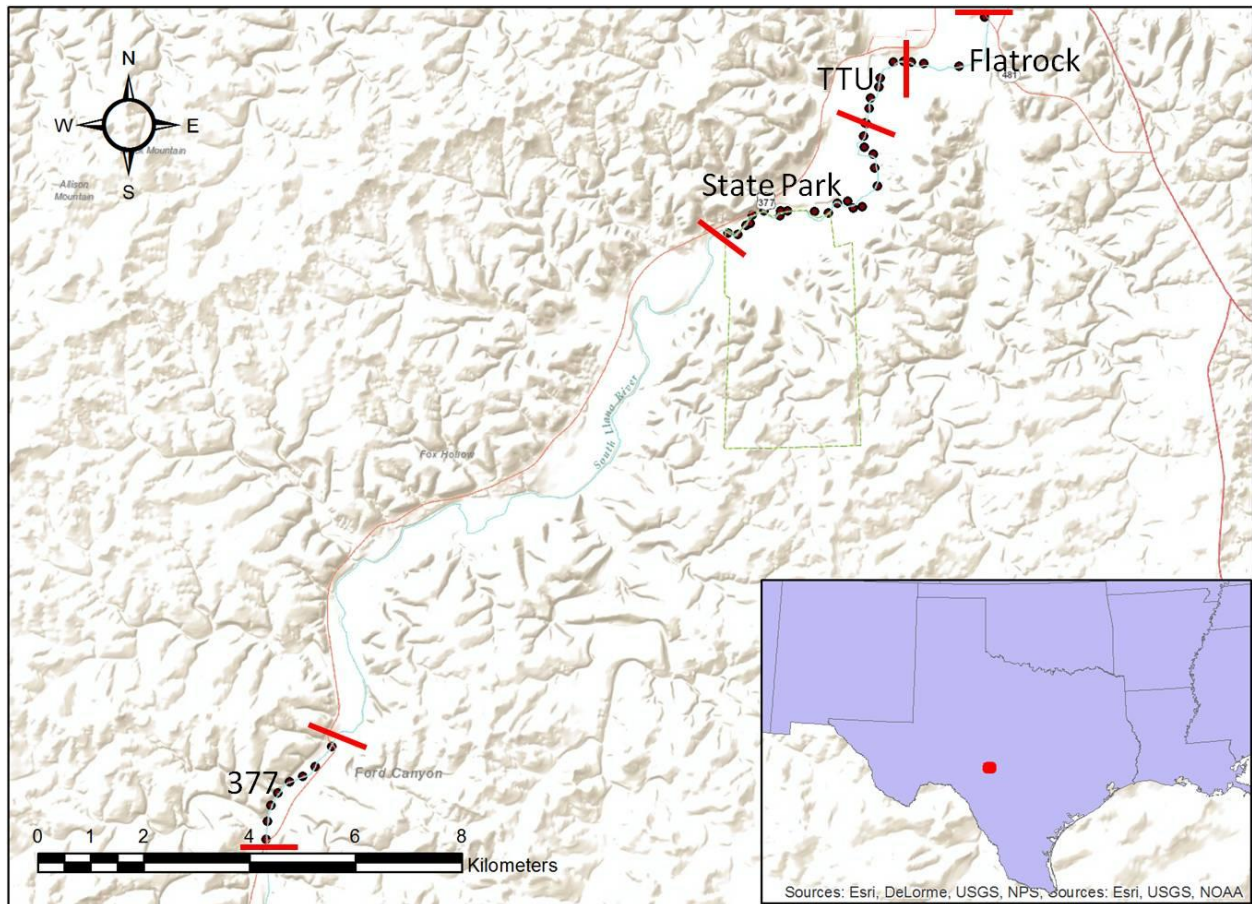
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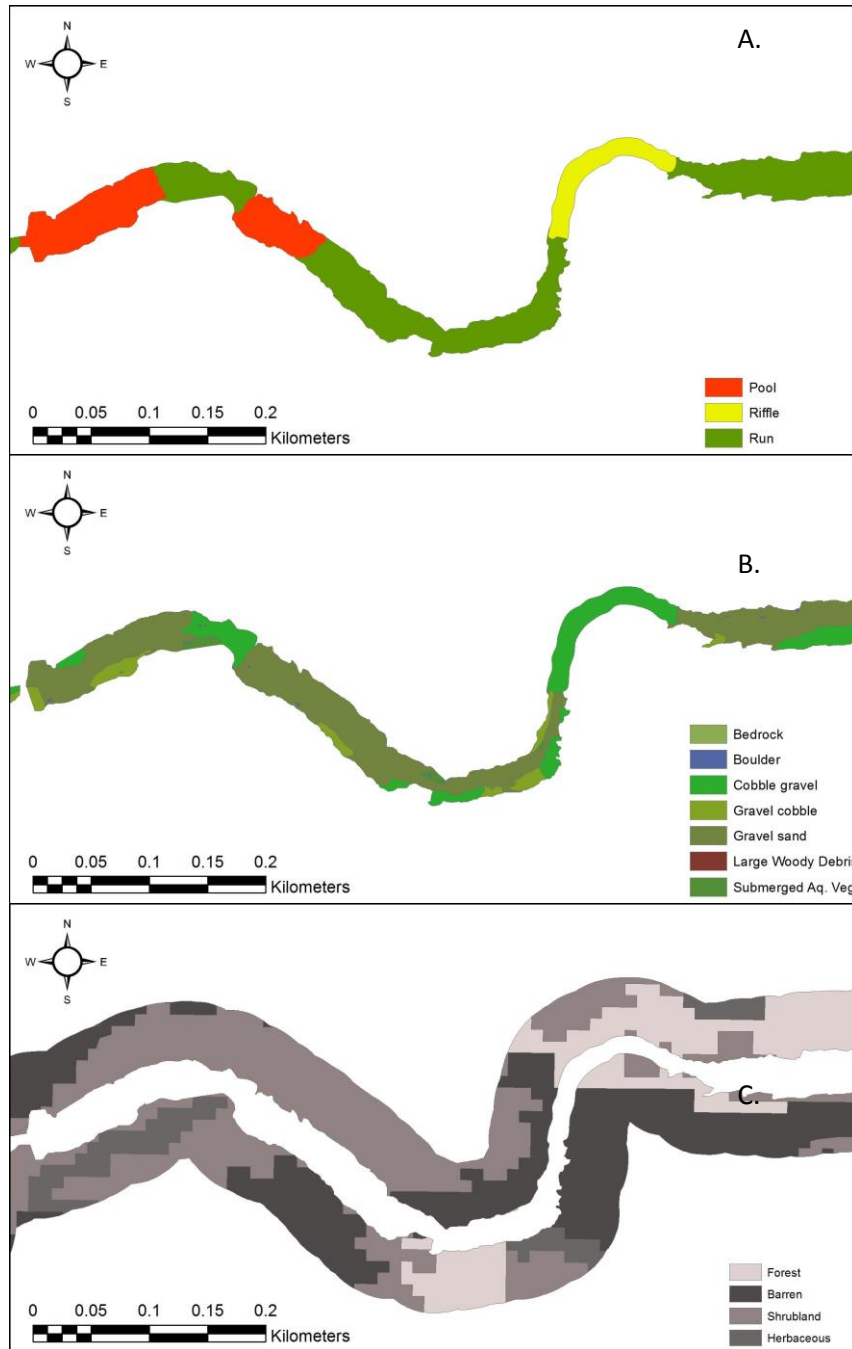
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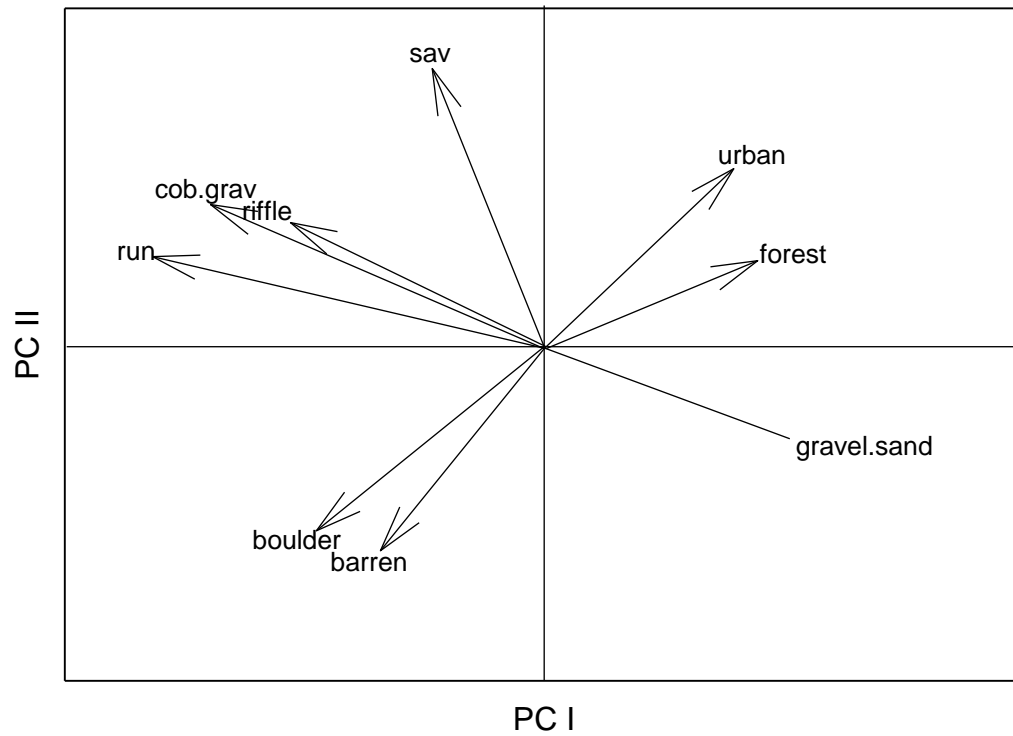
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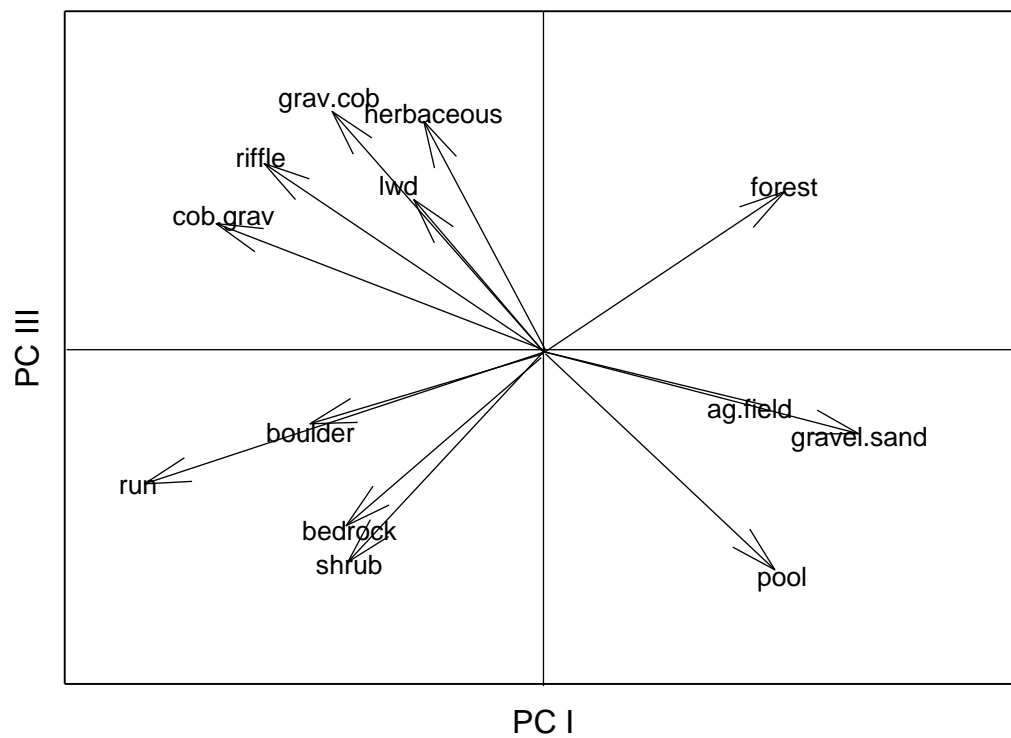
**Figure 1:** Map indicating the distribution of study sites in the South Llano River. Sites on the southwestern portion of the map comprised the upstream reach whereas the sites on the northeastern portion of the map comprised the downstream reach and included the State Park, TTU, and Flatrock sites.



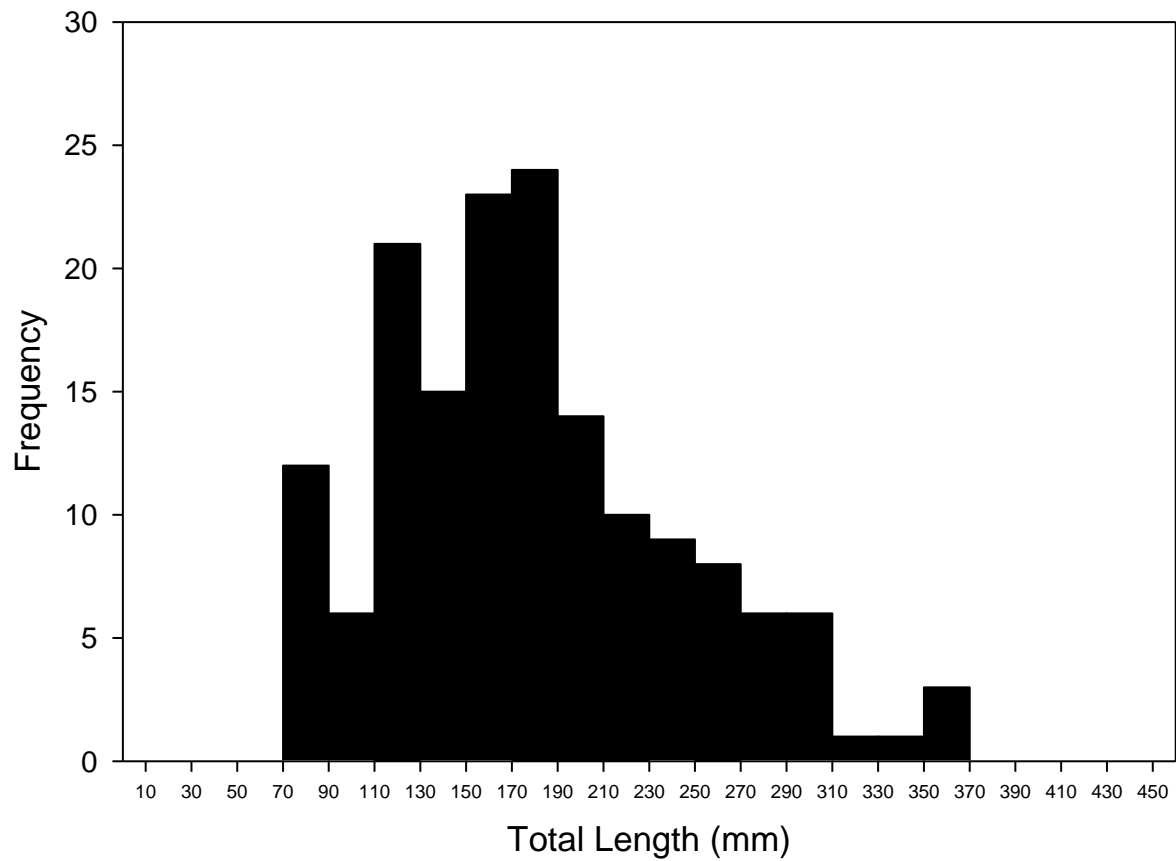
**Figure 2:** Maps illustrating the scale and resolution of habitat data from side-scan sonar mapping and the Texas Ecological Systems Classification project for: (A) mesohabitats, (B) microhabitats, and (C) riparian land cover.



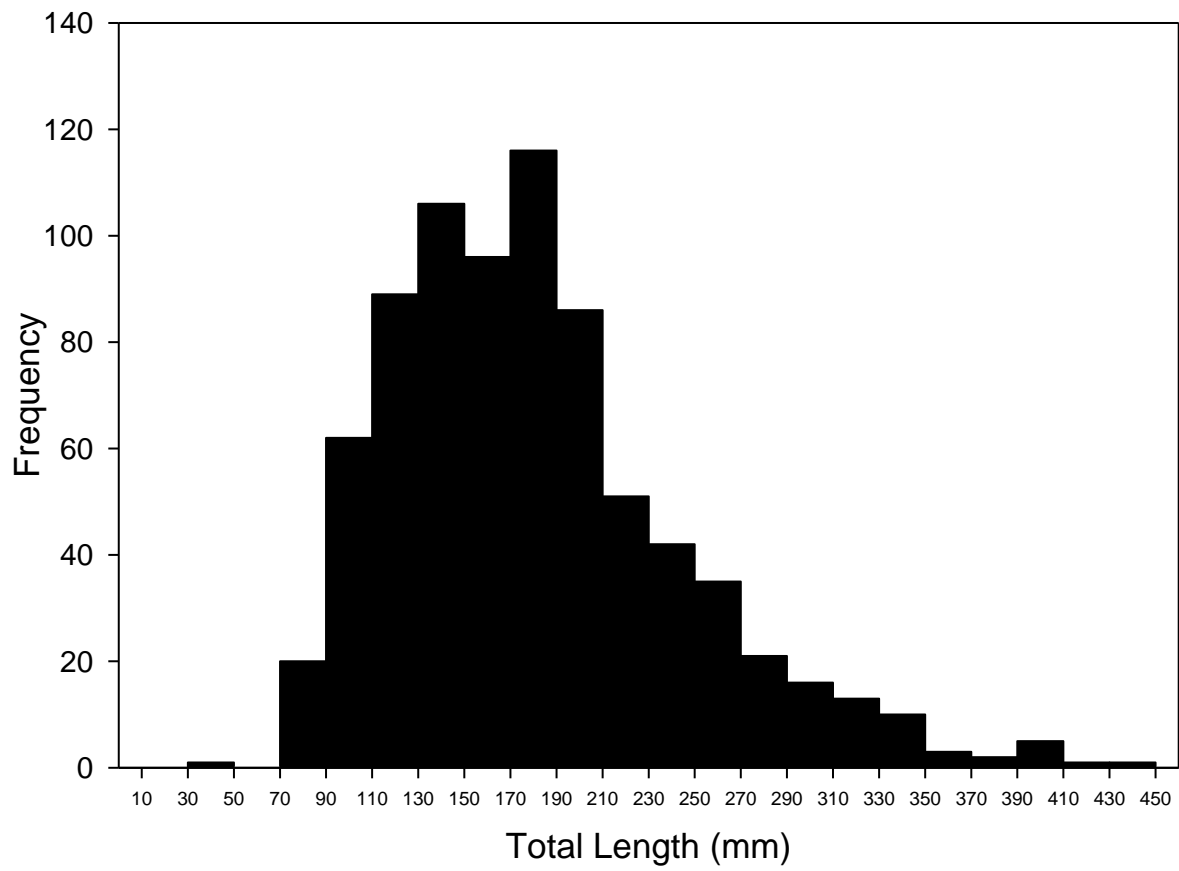
**Figure 3:** Graph of loadings for the first and second principal components from a principal components analysis of mesohabitat, microhabitat, and riparian habitat data from the study sites in the South Llano River, Texas.



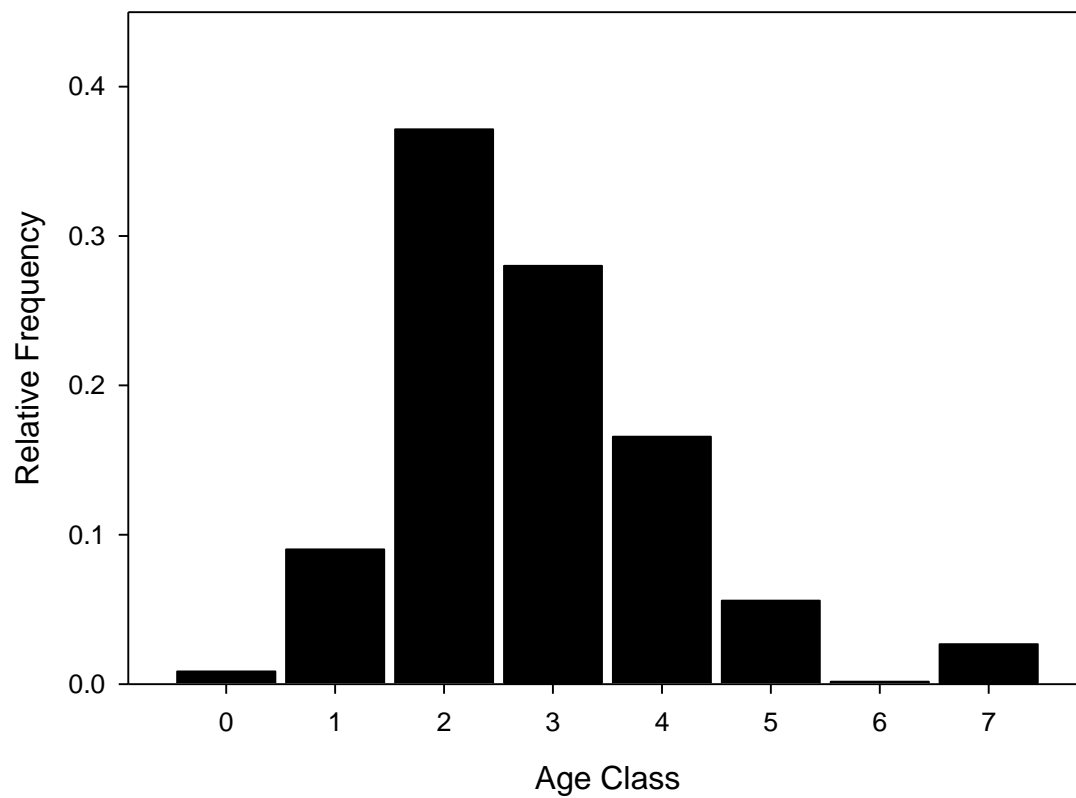
**Figure 4:** Graph of loadings for the first and third principal components from a principal components analysis of mesohabitat, microhabitat, and riparian habitat data from the study sites in the South Llano River, Texas.



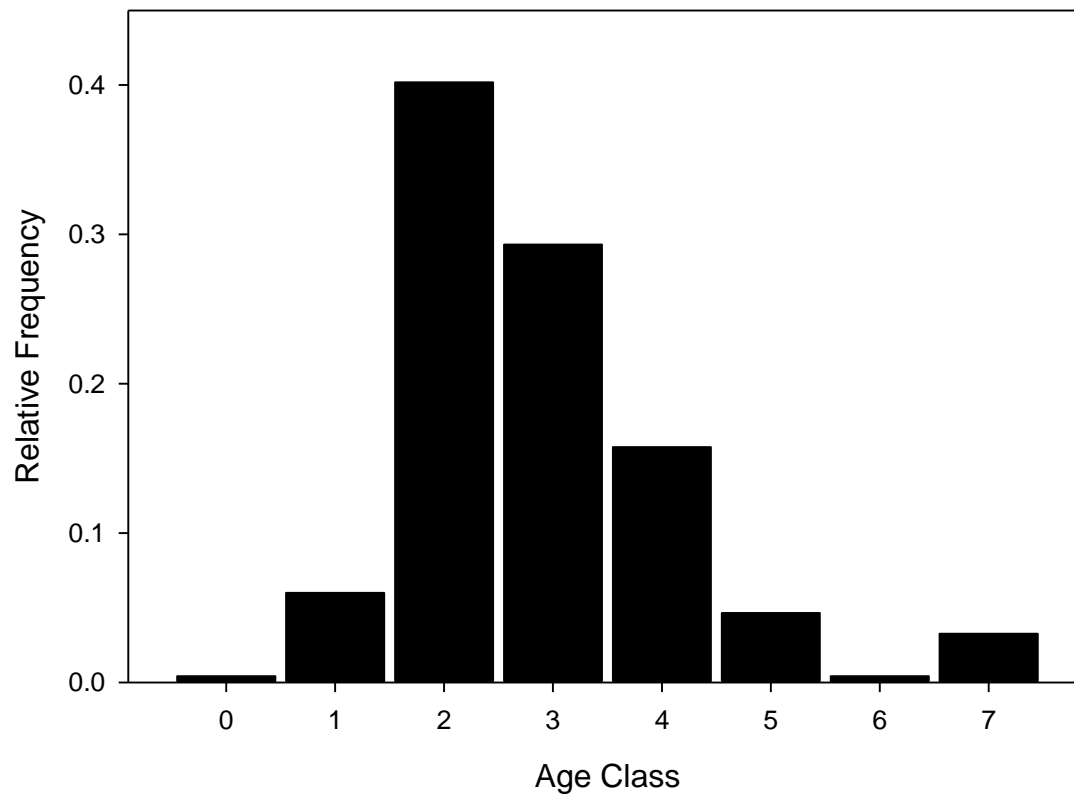
**Figure 5:** Length-frequency histogram for Guadalupe Bass collected between November 2012 and December 2013 in the 377 site of the South Llano River, Texas.



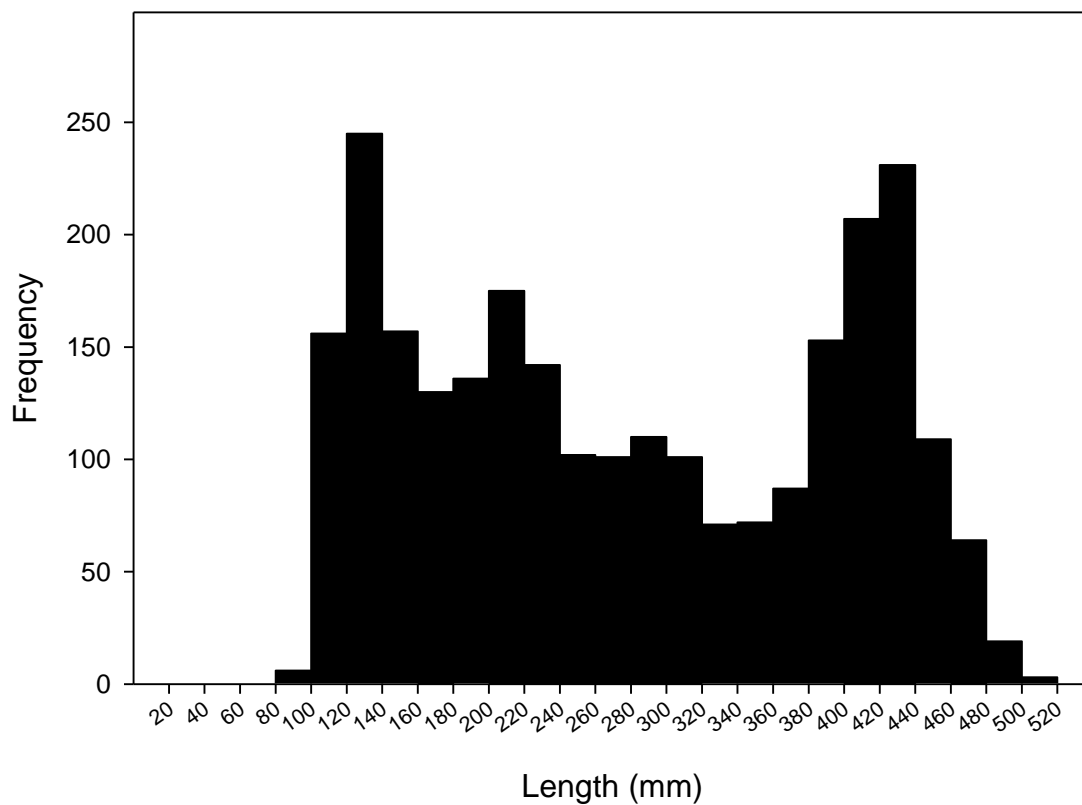
**Figure 6:** Length-frequency histogram for Guadalupe Bass collected between November 2012 and December 2013 in the State Park, TTU, and Flatrock sites of the South Llano River, Texas.



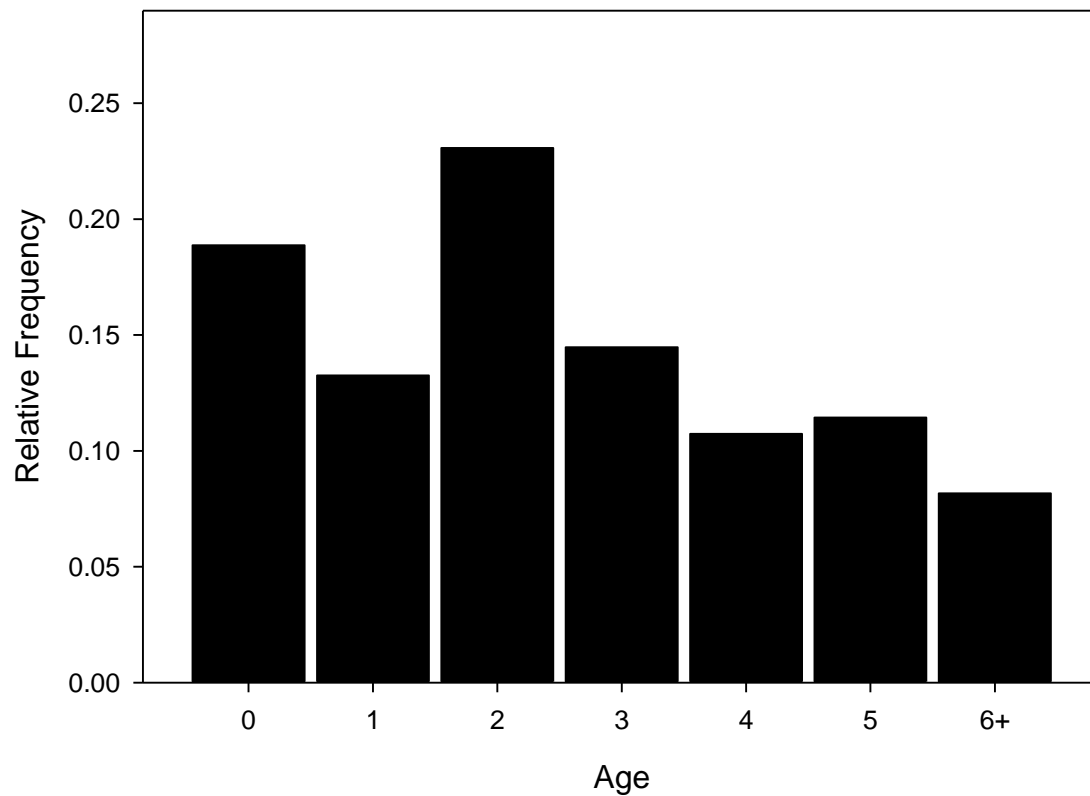
**Figure 7:** Age structure of Guadalupe Bass from the 377 site of the South Llano River, Texas. Ages were assigned from lengths using an age-length key developed by Groeschel (2013).



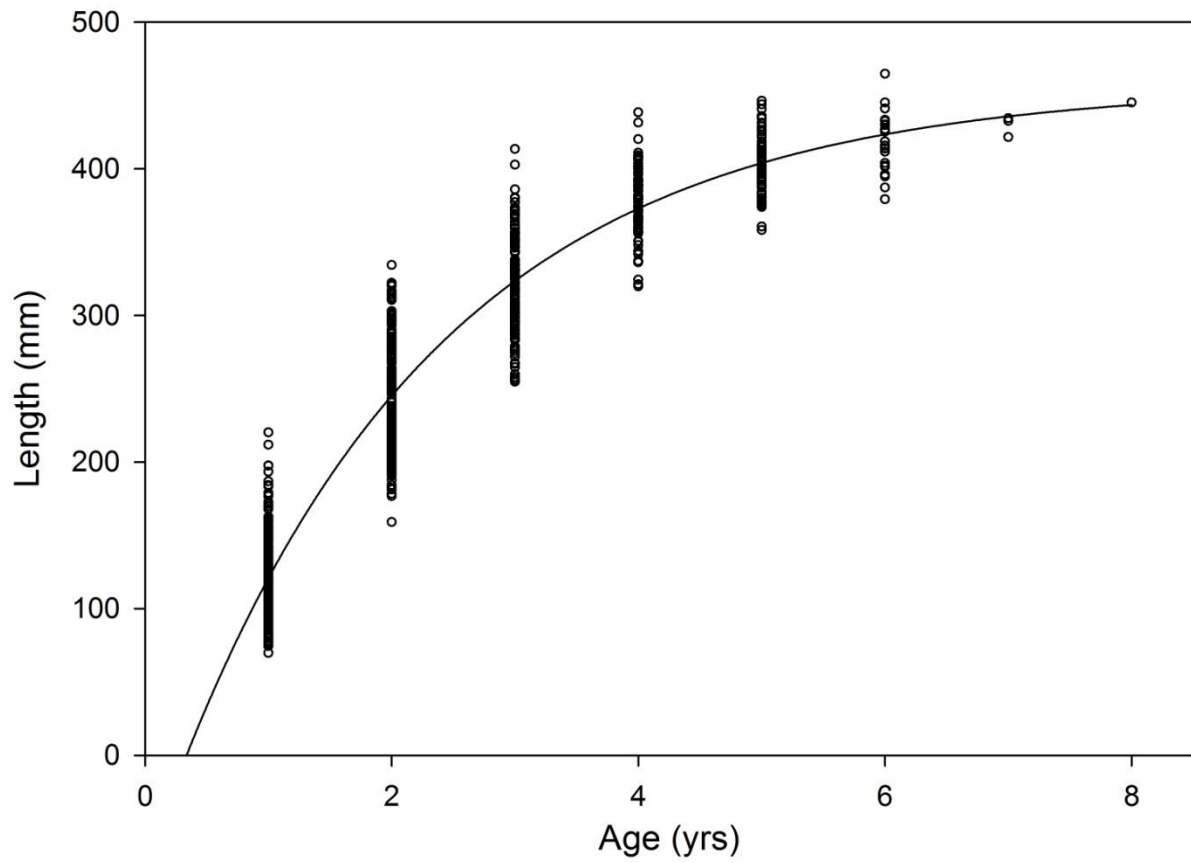
**Figure 8:** Age structure of Guadalupe Bass from the State Park, TTU, and Flatrock sites of the South Llano River, Texas. Ages were assigned from lengths using an age-length key developed by Groeschel (2013).



**Figure 9:** Length-frequency histogram for Gray Redhorse collected between November 2012 and December 2013 in the South Llano River, Texas.



**Figure 10:** Age structure of Gray Redhorse from the South Llano River, Texas. Ages were assigned from lengths using an age-length key developed in this study.



**Figure 11:** The von Bertalanffy growth curve fit to back-calculated length-at age data for Gray Redhorse from the South Llano River, Texas.

**Table 1:** Variable loadings from the principal components analysis of mesohabitat, microhabitat, and riparian habitat data from sampling sites on the South Llano River, Texas.

	<u>Variable Type</u>	<u>PC I</u>	<u>PC II</u>	<u>PC III</u>
Run	Instream	-0.512	0.161	-0.247
Cobble/Gravel	Instream	-0.41	0.261	0.23
Riffle	Instream	-0.356	0.252	0.34
Boulder	Instream	-0.308	-0.35	-0.151
Gravel/Cobble	Instream	-0.268	-0.281	0.435
Bedrock	Instream	-0.249	0.217	-0.329
Shrubland	Landscape	-0.243	-0.172	-0.385
Barren	Landscape	-0.241	-0.38	0.195
Large Woody Debris	Instream	-0.183	0.277	0.288
Herbaceous	Landscape	-0.154	-0.221	0.416
Submerged Aquatic Vegetation	Instream	-0.14	0.512	0.216
Open Water	Landscape	0.119	0.214	-0.22
Urban	Landscape	0.249	0.333	0.119
Agricultural Field	Landscape	0.253	0.177	-0.114
Pool	Instream	0.299	0.209	-0.415
Forest	Landscape	0.303	0.173	0.281
Gravel/Sand	Instream	0.401	-0.18	-0.157

**Table 2:** Correlations between catch per unit effort (CPUE) and site scores from the principal components analysis of habitat data from sampling sites on the South Llano River, Texas.

	<u>PC I</u>	<u>PC II</u>	<u>PC III</u>
Guadalupe Bass CPUE	-0.11	-0.104	0.202*
Gray Redhorse CPUE	-0.063	0.095	0.160*

**Table 3:** Population estimates and 95% confidence intervals for Guadalupe Bass and Gray Redhorse from four sub-reaches of the South Llano River, Texas.

<u>Site</u>	<u>Guadalupe Bass</u>				<u>Gray Redhorse</u>			
	<u>N</u>	<u>Lower CI</u>	<u>Upper CI</u>	<u>Fish/km</u>	<u>N</u>	<u>Lower CI</u>	<u>Upper CI</u>	<u>Fish/km</u>
377	301	181	677	128	3,254	2,015	7,051	1,384
State Park	1,149	825	1,788	210	8,548	6,411	12,309	1,563
TTU	436	218	2,179	261	2,294	1,619	3,670	1,373
Flatrock	65	35	228	25	573	309	2,007	226

**Table 4:** Population estimates and 95% confidence intervals for Gray Redhorse from four sub-reaches of the South Llano River, Texas.

<u>Sub-Reach</u>	<u>N</u>	<u>Lower CI</u>	<u>Upper CI</u>
377 Crossings	3,254	2,015	7,051
State Park	8,548	6,411	12,309
Texas Tech Campus	2,294	1,619	3,670
Flatrock Park	573	309	2,007

**Table 5:** Age-Length key for Gray Redhorse developed from age at capture data from Gray Redhorse captured in the South Llano River, Texas.

Length (mm)	<u>Age-0</u>	<u>Age-1</u>	<u>Age-2</u>	<u>Age-3</u>	<u>Age-4</u>	<u>Age-5</u>	<u>Age-6+</u>
101-120	93	7	-	-	-	-	-
121-140	93	7	-	-	-	-	-
141-160	62	38	-	-	-	-	-
161-180	35	65	-	-	-	-	-
181-200	20	40	40	-	-	-	-
201-220	-	-	100	-	-	-	-
221-240	-	-	100	-	-	-	-
241-260	-	38	63	-	-	-	-
261-280	-	7	71	21	-	-	-
281-300	-	12	47	41	-	-	-
301-320	-	6	28	67	-	-	-
321-340	-	-	29	71	-	-	-
341-360	-	-	-	92	8	-	-
361-380	-	-	12	76	12	-	-
381-400	-	-	-	25	69	6	-
401-420	-	-	-	-	50	36	14
421-440	-	-	-	-	12	62	26
441-460	-	-	-	-	6	44	50
461-480	-	-	-	-	-	50	50