

**ECOLOGICAL CHARACTERIZATION OF THE RIO GRANDE FISH ASSEMBLAGES
IN BIG BEND AND LOWER CANYON AREAS**

by

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SUMMARY

Proposed objectives of this study were to quantify the current fish assemblage, to assess historical changes in the fish assemblage, to determine spatial and seasonal trends in the fish assemblage, to assess patterns in spatial and temporal habitat associations of the fish assemblage, and to quantify reproduction and food habits for obligate riverine fishes within the Big Bend reach of the Rio Grande.

Section I of this report satisfies the proposed objectives of the study. Spatial and temporal trends in fish occurrence, abundance, and habitat associations are provided for fishes in the Big Bend reach of the Rio Grande. Reproduction and food habits are described for only one obligate riverine fish (Tamaulipas shiner *Notropis braytoni*), which is sufficiently abundant in the Big Bend reach to allow a thorough assessment. Notes on the diet and population structure are provided for another obligate river fish (blue sucker *Cycleptus elongatus*).

Section II provides additional information on the distribution and diets of larval fishes in the Big Bend reach of the Rio Grande. Maintenance of viable riverine fish populations usually depends on the amount and availability of nursery areas for fish larvae. In addition, success of the repatriation efforts of Rio Grande silvery minnows depends on adequate nursery habitats. This study quantifies occurrences and abundances of larval and juvenile fishes within known Rio Grande nursery habitats and documents food items consumed by the larval and juvenile fishes.

Section III describes the spatial and temporal distributions and habitat associations of macroinvertebrates in the Big Bend reach of the Rio Grande. Macroinvertebrate communities generally are more susceptible to certain anthropogenic modifications (water pollution) than fishes. Collectively, assessment of fish and macroinvertebrates provide a much broader perspective on how anthropogenic modifications (water pollution for macroinvertebrates; reduced instream flow for fishes) impact the biotic integrity of arid systems.

Appendix I contains a published article that was generated during this project. During early stages of field collections, an exotic tapeworm (*Bothriocephalus acheilognathi*; Cestoda: Pseudophyllidea) was observed in larval fishes. Morphological and genetic analyses confirmed the first record of the exotic tapeworm in the Rio Grande drainage. Occurrence of *Bothriocephalus* is problematic for fishes in the Rio Grande, especially those of conservation concern.

SECTION I

Spatial and temporal patterns in the Rio Grande fish assemblage with comments on reproduction and life history of the Tamaulipas shiner (Cyprinidae)

Abstract— The study of relative abundance, habitat associations and life histories of obligate riverine species in the Rio Grande has important implications for future conservation, management and reintroduction programs within this system. Anthropogenic activities along the Rio Grande have significantly impacted biotic and abiotic conditions within this system. The Big Bend reach of the Rio Grande, between the confluence of the Rio Conchos and the Pecos River along the border between West Texas and Mexico, maintains a relatively healthy fish assemblage when compared to other reaches of this river. Species of concern, which have been extirpated from much of their historical range, still occur within the Big Bend reach. Habitat associations were analyzed from data collected monthly from January 2006 to December 2006. Seven study sites were established over 193 river kilometers between Contrabando Creek, near Lajitas, Texas and Maravillas Canyon in Black Gap Wildlife Management Area. Abiotic habitat measurements and species sampling were conducted simultaneously along transects spaced 20 meters apart in areas that contained a diverse collection of geomorphic units. The life histories of *Notropis braytoni* and *Cycleptus elongatus* were also assessed. *Cyprinella lutrensis* and *Notropis braytoni* were the most abundant species comprising 46% and 35% of the assemblage respectively. Canonical Correspondence Analysis of habitat associations explained 12% ($P = <0.01$) of the total variation. *Notropis braytoni* exhibited reproductive activity between February and August, a 2.5 year life span, and the diet of a generalist invertivore. Age-0 *Cycleptus elongatus* exhibited habitat and diet shifts as total lengths progressed beyond 45 mm.

Introduction

Obligate riverine fishes, species which are dependant on a lotic system for at least one part of their life history, have declined in response to in-stream habitat alterations due to their dependence on natural flow regimes (Holden 1979). Dam construction and water diversions alter physical, chemical, and biological components of main stem rivers. Mean discharge and magnitude and frequency of scouring flood events are reduced along with changes in channel morphology, sediment transport, substrates, and habitat types. Aspects of water quality such as turbidity, temperature, and conductivity also are altered by dam and diversion construction on main stem rivers (Bain et. al. 1988; Ligon et. al. 1995; Poff et. al. 1997; Richter et. al. 1997; Richter et. al. 2003; Bunn and Athington 2002). Stream habitat alterations, while negatively affecting some native fish species, can also benefit other native species through the gradual homogenization of available habitat, as well as unique ichthyofaunal assemblages. This process of native invasion is observed in other impacted streams as well (Scott and Helfman 2001).

Over 50% of the inland fish species of concern in the state of Texas occur in the Rio Grande drainage (Hubbs et al. 1991). Historically, common and widespread species

now exhibit reduced abundance and distribution (Trevino-Robinson 1959; Hubbs et. al. 1977; Edwards and Contreras-Balderas 1991; Edwards et. al. 2002; Calamusso et. al. 2005). Populations of obligate riverine cyprinid species, in particular, are highly impacted in the Rio Grande (Anderson et. al. 1995), with Rio Grande silvery minnow *Hybognathus amarus*, Rio Grande shiner *Notropis jemezianus*, blue sucker *Cycleptus elongatus* and Rio Grande speckled chub *Macrhybopsis aestivalis* currently extirpated from large portions of their ranges, and the phantom shiner *Notropis orca* and bluntnose shiner *Notropis simus* currently extinct (Bestgen and Platania 1990; Hubbs et. al. 1991; Platania and Altenbach 1998).

Losses to the ichthyofaunal community in the Rio Grande are associated with anthropogenic activities that have greatly altered the river from its natural state. The creation of reservoirs, channelization, stream diversions for agricultural purposes, reduced water quality, ground water depletion, overgrazing, and the introduction of non-native species have altered the river (Miller 1961). The Rio Grande now has reduced channel widths with more stable banks and is less subject to progressive seasonal changes (Schmidt et. al. 2003). However, a relatively intact fish assemblage is located between the confluence of the Rio Conchos and the confluence of the Pecos River in the Big Bend area of Texas and Mexico. Information is needed on habitat requirements and life history requirements of the Rio Grande fishes to better conserve, manage, and possibly restore this river and its unique fish assemblage.

Objectives of this study were to document seasonal Rio Grande fish assemblage structure, to quantify habitat associations, to describe the life history of an endemic minnow, the Tamaulipas shiner *Notropis braytoni*, in the Big Bend reach of the Rio Grande, and to describe habitat associations and the diet of young of the year blue suckers *Cycleptus elongatus*. To date, only historical trends in fish abundance are available for the Big Bend reach of the Rio Grande and limited information exists on taxon-specific habitat associations. Likewise, general life history information is not available for *Notropis braytoni*, or the *Cycleptus* species found in the Rio Grande drainage. These species are two of several imperiled and endemic fishes in the Rio Grande (TPWD 2005).

Methods

Between the urban centers of El Paso and Presidio, Texas, the Rio Grande is a narrow, heavily impacted intermittent stream until its confluence with the Rio Conchos near Presidio where the flow increases (Hubbs et. al. 1977; Bestgen and Platania 1988). The section of river between Presidio and the confluence of the Pecos River primarily borders Big Bend Ranch State Park, Big Bend National Park (NP), Black Gap Wildlife Management Area (WMA), or is managed by the National Parks Service as a Wild and Scenic River. The Big Bend reach of the Rio Grande has sustained minimal impact by human activities when compared to other reaches of the Rio Grande, although this reach should be regarded as a highly impacted stream in general because of upstream modifications to the drainage in Mexico and USA (Hubbs et. al. 1977; Moring, 2002; Edwards 2005). For example, the magnitude of the 2-year recurrence flood was reduced by 49% downstream of the Rio Concho's confluence with the Rio Grande since 1915

(Schmidt et. al. 2003). This reach is also unique in its dependence on the Rio Conchos basin as its main source of water and nutrients and its more varied geomorphology, including areas with meandering channels with multiple geomorphic units (riffle, run, pool, etc.) over diverse substrates (silt, sand, gravel, cobble) (Hubbs et. al. 1977; Armantrout 1998; Moring 2002; Goldstein and Meador 2004).

Site selection was based on accessibility, longitudinal distribution, and availability of various types of geomorphic units. We chose seven study sites spanning a distance of 193 river km (Fig 1). At each site, we recorded geographic coordinates with a Garmin GPSMAP 60CSx GPS unit in the UTM coordinate system, Zone 13 R, NAD 27. Main stem sampling sites were located near the confluence of Contrabando Creek (Site 1; E 612395, N 3239287), downstream from Santa Elena Canyon (Site 2; E 635271, N 3226912), near the USGS Gauging Station within Big Bend National Park (Site 3; E 656882, N 3212601), near Johnson Ranch campground of Big Bend NP (Site 4; E 658721, N 3211332), near the confluence of Tornillo Creek (Site 5; E 695091, N 3229250), upstream from Boquillas Canyon in Big Bend NP (Site 6; E 702576, N 3231651), and near the confluence of Maravillas Creek in Black Gap WMA (Site 7; E 715509, N 3272178). Study sites contain a mixture of geomorphic units at seinable depths and are bordered upstream and downstream by deep slow runs. Sites are bounded by thick bank vegetation consisting mostly of giant reed *Arundo donax* and salt cedar *Tamarix sp.* (Edwards 2005).

We collected fish monthly from January - December 2006. We established multiple transects perpendicular to the stream bank (Simonson et al. 1994). Along the length of each transect we conducted a minimum of twenty samples of discrete geomorphic units (Dudley and Platania 1997). We used seining and substrate kicking with a block seine in place because of the effectiveness for capturing small cyprinids and because of the reduced efficiency of electrofishing in rivers with high specific conductivity, such as the Rio Grande (Matthews 1986; Onorato et al. 1998). We sampled geomorphic units with one seine (3 m X 1.8 m; mesh size = 3.1 mm) haul up to 5-meters in length in a downstream direction or one 5-meter kick into a blocking seine. All fishes were identified to species (except larval *Lepomis*), measured to the nearest millimeter (total length) and released, except for 10 *Notropis braytoni* from each site. Retained *Notropis braytoni* were lethally anesthetized with MS-222 and preserved in 10% formalin. For each seine hauls, we recorded current velocity (Marsh-McBirney, Inc. Flowmate Model 2000) and depth from two evenly spaced points positioned across the width of each seine haul area. We distinguished substrate type by size at ten randomly selected points through the haul area with reference to the modified Wentworth classification (Cummins 1962).

We used canonical correspondence analysis (CCA; CANOCO V. 4.5) to analyze multivariate aspects of habitat associations of the fish assemblage. Habitat variables analyzed in CCA included current velocity (m/s), depth (m) substrate and geomorphic unit. We also calculated the weighted mean of current velocity (m/s) and depth (m) associations for two size classes (<25 mm & >25 mm) of *Notropis braytoni*, *Macrhybopsis aestivalis* and *Cyprinella lutrensis*.

In the laboratory, we measured total length (mm) and wet mass (g) determined for at least 10 adult *Notropis braytoni* collected from sites 2, 5 and 6. Gonads from both sexes were removed and weighed in order to create a gonadosomatic index (GSI). We

calculated GSIs by [(gonad weight/fish weight)*100] to determine reproductive condition (Williams and Bonner 2006). We used FISAT II program to determine age groups of *Notropis braytoni* pooled across sites by month in 2 mm length groupings. We examined the gut content of three *Notropis braytoni* monthly from sites 2, 5 and 6. Contents from the first two thirds of the digestive tract were identified to the lowest possible taxonomic level. We quantified stomach contents by determining the mean percentage of contents by wet mass (mg) (Murphy and Willis 1996; Williams and Bonner 2006).

We opportunistically retained 22 age-0 blue suckers *Cycleptus elongatus* for diet analysis. We separated age-0 *Cycleptus elongatus* into two size classes: 24 – 45 mm TL and 50 – 103 mm TL. Stomach contents of both size classes were analyzed similar to that for *Notropis braytoni*. Habitat associations for the two size classes were compared using depth and current velocity measurements recorded from habitats with *Cycleptus elongatus*.

Results

Seinable geomorphic units primarily consisted of runs (56 – 89% among sites) and riffles (2.7 – 25%). Among geomorphic units and sites, cobble was the dominant substrate (41% - 89%), except at Site 4 which had large amounts of gravel (43%) and silt (33%) substrates. Sand, boulder, and bedrock comprised <15% of the available substrates (Table 1).

A total of 10,565 fishes were collected from 2,393 seine hauls taken monthly in 2006. Sites 3 and 4 were not sampled in July, 2006 due to a large rain event that washed out the primitive road to those sites. Twenty-three species were identified among seven sites, including 2 *Lepomis* (*Lepomis megalotis* and *Lepomis cyanellus*). Site 2 had the largest number of species, whereas Site 5 had the least number of species. Five fluvial specialist taxa (*N. braytoni*, *N. jemezianus*, *M. aestivalis*, *R. cataractae*, *C. elongatus*) and six imperiled taxa (the five fluvial specialists and *N. chihuahua*; Hubbs et. al. 1991; TPWD 2005) were taken from at least one site. Four non-native taxa (*Cyprinus carpio*, *Menidia beryllina*, *Fundulus zebrinus* and *Oreochromis aureus*) were taken or observed (*O. aureus* was observed during a fish kill at Site 2 in December, 2006) and represented <1% of the total assemblage. Among fishes taken, *Cyprinella lutrensis* was the most abundant overall (46% of the total assemblage) and was the most abundant species at sites 1 through 4 (range: 45 – 69% in relative abundance). Endemic *Notropis braytoni* was the second most abundant (35%) and was the most abundant species at sites 5 through 7 (range: 40 – 51%). The six imperiled taxa comprised about 39% of the total assemblage (Table 2).

The CCA analysis explained 12% ($P = <0.01$) of the taxonomic variation in habitat associations. Axis I described a current velocity and geomorphic unit gradient with swifter current velocities and run and riffle geomorphic units having positive loadings on Axis I and backwater habitats, side channels, and shallow depths having negative loadings on Axis I. Axis II described a depth and substrate gradient with shallow water and gravel to cobble substrates having negative loadings on Axis II. Fishes with strong habitat associations included *Rhinichthys cataractae* (riffle habitats and gravel to cobble substrates), *Ictalurus furcatus*, *Notropis jemezianus*, *Cycleptus*

elongatus (run habitats with swift current velocities), *Notropis chihuahua*, *Gambusia affinis*, *Lepomis*, and *Carpoides carpio* (backwater habitats with silt substrates). Fishes without strong habitat associations included *Cyprinella lutrensis*, *Notropis braytoni*, and *Astyanax mexicanus*.

Current velocity and depth plots for the more abundant taxa indicated habitat differences between size classes of fish (Figure 3). Mean current velocity (\pm SD) of smaller fish (<25 mm) was 0.10 m/s (\pm 0.04) for *Notropis braytoni* ($N = 390$), 0.10 m/s (\pm 0.03) for *Cyprinella lutrensis* ($N = 1,168$), and 0.17 m/s (\pm 0.06) for *Macrhybopsis aestivalis* ($N = 14$). Mean depth (\pm SD) of smaller fish was 0.26 m (\pm 0.04) for *Notropis braytoni*, 0.26 m (\pm 0.04) for *Cyprinella lutrensis*, and 0.17 m (\pm 0.04) for *Macrhybopsis aestivalis*. Larger fishes shifted to swifter current velocities. Mean current velocity (\pm SD) of larger fish (>25 mm) was 0.30 m/s (\pm 0.06) for *Notropis braytoni* ($N = 2,003$), 0.19 m/s (\pm 0.04) for *Cyprinella lutrensis* ($N = 2,172$), and 0.48 m/s (\pm 0.06) for *Macrhybopsis aestivalis* ($N = 279$). Shifts to greater depths only occurred with *M. aestivalis* (0.38 m; \pm 0.04).

Life History of N. braytoni

Maximum length of *N. braytoni* was 76 mm TL and maximum age was 2.5 years. Age-0 fish were collected from April through December in the Rio Grande (Figure 4). Age-1 fish were collected throughout the year, and age-2 fish were collected from January 2007 through April 2007. Female GSIs ($N = 180$) were elevated from February through August (Figure 5). Gonadal quiescence extended from September through January.

Digestive tracts of the 108 *N. braytoni* examined consisted of 66% aquatic insects, 21% unknown aquatic insect parts, terrestrial insects 3%, detritus 3%, plant material 2.5%, algae 2.2%, Ostrocooda 1.8%, stone 0.3%, fish scales < 0.1%. Among identified aquatic insects Ephemeroptera was the most abundant by weight 47.2% followed by Simuliidae 25.7%, Trichoptera 8.6%, Odonata 7.4%, Corixidae 4.4%, Chironomids and adult Diptera 3.8%, Coleoptera 2.4%, Megaloptera 0.5%. (Table 3).

Age-0 Cycleptus elongatus Life History

A total of 22 age-0 *C. elongatus* (24 – 103 mm TL) were taken from all sites except sites 3 and 4. Smaller individuals (24 - 45 mm TL) were taken from current velocities between 0.0 to 0.40 m/s and from depths between 10 and 80 cm. Larger individuals (50 - 103 mm TL) were taken from current velocities between 0.48 to 1.4 m/s and from depths between 40 and 90 cm. Stomach contents in smaller individuals consisted of >90% chironomids and stomach contents of individuals between 50 and 103 mm consisted of > 80% trichopterans.

Discussion

Occurrence and abundance of fishes found in this study area are similar to those reported in historical accounts taken from the Big Bend Reach of the Rio Grande. However, the relative abundance of *Notropis braytoni* in the Big Bend reach of the Rio Grande appears to be on the increase when compared to historical assemblage data (Table 4) (Hubbs 1958; Hubbs et al. 1977; Platania 1990; Edwards et al. 2002b; Garrett 2002;

Moring 2002; Edwards 2005). The relative abundance of *Notropis braytoni* also increased in our downstream sites (Table 2). *Notropis jemezianus* represented a very low relative abundance in the Big Bend reach throughout our study, which is a concern in that this species has been extirpated from much of its range (Sublette et. al 1990; Hubbs et. al. 1991).

Though some changes have occurred in the Big Bend reach fish assemblage, this assemblage has changed less than other reaches of the Rio Grande. Obligate riverine species such as *Notropis braytoni*, *Macrhybopsis aestivalis*, *Notropis jemezianus*, *Rhinichthys cataractae* and *Cycleptus elongatus* are absent or found in very low abundance in other reaches of the Rio Grande drainage where they were once present in large abundances. However, these species are still present in the Big Bend Reach. *Cycleptus elongatus*, *Macrhybopsis aestivalis* and *Notropis jemezianus* have been extirpated from the Rio Grande in New Mexico (Sublette et. al 1990). The fish assemblages in the lower Pecos River and the lower Rio Grande have also lost species. In the lower Pecos endemic species including *Notropis braytoni*, *Macrhybopsis aestivalis* and *Cycleptus elongatus* are found in reduced abundances while *Notropis jemezianus* and *Rhinichthys cataractae* have been extirpated (Hoagstrom 2003). Introduced species such as *Fundulus grandis*, *Menidia beryllina* and *Cyprinodon variegatus* have increased in abundance in the lower Pecos (Hoagstrom 2003). In the lower Rio Grande obligate riverine endemics have also declined. *Notropis braytoni*, *Macrhybopsis aestivalis* and *Notropis jemezianus* have declined or have been extirpated from areas of the Rio Grande's lower reach while tributary fish such as *Cyprinella venusta* and marine species have increased in abundance (Edwards and Contreras-Balderas 1991; Contreras-Balderas et. al. 2002).

Similar to other reaches of the Rio Grande, the Big Bend reach is impacted by modified flows, chemical pollution, nutrient enrichment, changes in channel morphology, exotic taxa, periodic and fish kills (Davis 1980c; Miyamoto et. al. 1995; IBWC 2003; Schmidt et. al. 2003; Edwards 2005; Marfurt 2007). We observed the affects of a fish kill event at site 2 in December, 2006. However, biologically sufficient flows originating from the Rio Conchos in Mexico, the lack of channel maintenance activities or major dams and weirs throughout the reach have allowed the fish assemblage to remain relatively complete (Edwards 2005).

The Big Bend reach also maintains a sufficient heterogeneity of habitats necessary to the life histories of the members of the fish assemblage. Our analysis of fish habitat associations and a concurrent study of larval fish in the same reach have demonstrated the necessity for varied habitats to support the life histories of the endemic members of the fish assemblage (Runyan 2007).

Potential threats to the Big Bend reach include declines in water quality and quantity from the upstream portion of the reach or the Rio Conchos. Reductions of flow would homogenize habitats to the benefit introduced and lentic species while negatively impacting obligate riverine species that depend on a heterogeneous habitat structure. Any future restoration of flow regimes would serve to destabilize the river banks and remove introduced riparian vegetation. This would increase areas of braided stream channels and create a more natural heterogeneous habitat structure which should benefit endemic obligate riverine species.

Our CCA habitat analysis showed *Notropis braytoni* exhibiting a habitat generalist position in the assemblage similar to *Cyprinella lutrensis*. Our analysis of the weighted mean of juvenile *Notropis braytoni* (< 25 mm) showed current velocity and depth associations almost identical to juvenile *Cyprinella lutrensis*. *Cyprinella lutrensis* has been documented as a successful species in impacted systems (Bonner and Wilde 2000). The observed similarities between *Cyprinella lutrensis* and *Notropis braytoni* may benefit *Notropis braytoni* as well. Typical of other *Notropis* species, *Notropis braytoni* has short life cycle (~2.5 years) and rapid growth rates. *Notropis braytoni* also is a generalist invertivore, which along with its opportunistic habitat associations could be factors in this species success in the Big Bend reach of the Rio Grande.

Twenty two *Cycleptus elongatus* in both metalarval and juvenile stages were identified from five of our study sites the metalarval individuals were utilizing low current velocity areas over fine substrate while the juveniles were found utilizing higher current velocities (Yeager and Semmens 1987). There was also a change in diet as these individuals increased in total length. The *Cycleptus* species endemic to the Rio Grande drainage has recently been found to be genetically distinct from the two *Cycleptus* species in the Central and Southeastern United States (Bessert 2006).

The presence of this species in all life stages within the Big Bend reach further bolsters the importance of the reach as a refuge for Rio Grande drainage endemics that have been extirpated from much of their historic range such as *Macrhybopsis aestivalis* and *Cycleptus elongatus*. The relative quality of the reach also makes it the likely area of reintroduction for federally listed *Hybognathus amarus* (Edwards 2005).

A relatively intact fish assemblage makes the Big Bend reach a high conservation priority. Other studies have shown that even impacted systems can maintain much of their natural assemblage if the riverine system is intact (Fausch et. al. 2002; Fegan et. al. 2002; Williams and Bonner 2006; Runyan 2007). The Big Bend reach continues to maintain connectivity throughout the reach which allows endemic species to maintain relatively stable populations. Much of the reach is bounded by protected areas such as Big Bend National Park. This and the lack of major urban areas along the Rio Grande between the confluence of the Rio Conchos and Amistad reservoir have served to protect the intact condition of the reach. The relative quality of the Big Bend reach can be preserved as long as the quantity on quality of water from the Rio Conchos is maintained or increased and the reach remains intact.

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TABLE 1. Overall and per site relative abundance of geomorphic units and substrate types encountered from January 2006 through December 2006 in the Big Bend reach of the Rio Grande.

Site #	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
N of seine hauls	349	467	256	338	389	303	291
Percent geomorphic unit							
Run	55.6	74.9	89.1	88.8	74.7	79.2	82.5
Riffle	24.8	15	5.5	2.7	22.2	11.6	6.2
Side channel	3.5	5.8	3.1	2.1	1.3	0.3	5.2
Backwater	6.1	1.1	1.2	3.6	—	3	2.4
Pool	8.6	1.5	1.2	1.8	0.5	0.3	1.4
Eddy	1.4	1.7	—	1.2	1.3	5.6	2.4
Percent substrate							
Cobble	48	40.7	88.5	5.5	65	53.3	59.7
Gravel	8.2	38.7	7.1	43.1	27.9	30.7	11.1
Silt	13.9	12.1	3.7	33.2	3.2	10	15.6
Boulder	15.4	1.5	0.4	3.9	1.5	0.3	13.4
Sand	0.9	7	—	14.3	2.2	5.7	0.1
Bedrock	10.9	—	0.3	—	0.2	—	0.1
Percent Vegetation	2.7	—	—	—	—	—	—

TABLE 2. Overall and per site relative abundance of ichthyofauna encountered from January 2006 through December 2006 in the Big Bend reach of the Rio Grande.

Species	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Total
<i>C. lutrensis</i>	68.7	44.9	51.4	60.2	31.9	43.3	40.1	46.33
<i>N. braytoni</i>	20.3	26	19.1	19.9	50.6	46.3	40.4	34.97
<i>Ca. carpio</i>	1.8	9.9	1.5	6.4	3.8	0.7	1.1	4.1
<i>M. aestivalis</i>	1.9	6.2	7.4	4.5	1.6	1.9	0.4	3.15
<i>G. affinis</i>	2.1	1.4	11.3	4.8	2.8	1.8	2	2.98
<i>I. punctatus</i>	1.7	1.5	2.9	1.2	4.3	1.7	2.4	2.36
<i>I. furcatus</i>	2.4	0.6	1.1	1.7	1.5	1.4	4.2	1.62
<i>A. mexicanus</i>	0.6	0.2	1.1	0.1	2.1	1.2	5.7	1.33
<i>Cy. carpio</i>	0.3	1.9	1	0.3	0.4	0.2	0.5	0.65
<i>M. beryllina</i>	—	2	0.2	0.1	0.5	0.3	0.7	0.61
<i>F. zebrinus</i>	—	2	0.5	0.1	0.4	—	0.1	0.51
<i>R. cataractae</i>	—	2	—	—	—	—	—	0.36
<i>P. olivaris</i>	0.1	0.5	1.3	—	—	0.3	0.6	0.29
<i>C. elongatus</i>	0.1	0.1	—	—	—	0.7	0.5	0.21
<i>Lepomis sp.</i>	0.1	0.2	0.8	0.4	—	—	0.5	0.19
<i>L. osseus</i>	—	0.1	0.3	0.2	—	0.1	0.7	0.14
<i>N. chihuahua</i>	—	0.6	—	0.1	—	—	—	0.12
<i>N. jemezianus</i>	—	0.2	—	0.1	—	—	—	0.05
<i>I. bubalus</i>	—	—	—	0.1	—	—	—	0.02
Total <i>N</i>	1,225	1,938	611	1,441	2,511	2,033	806	10,565

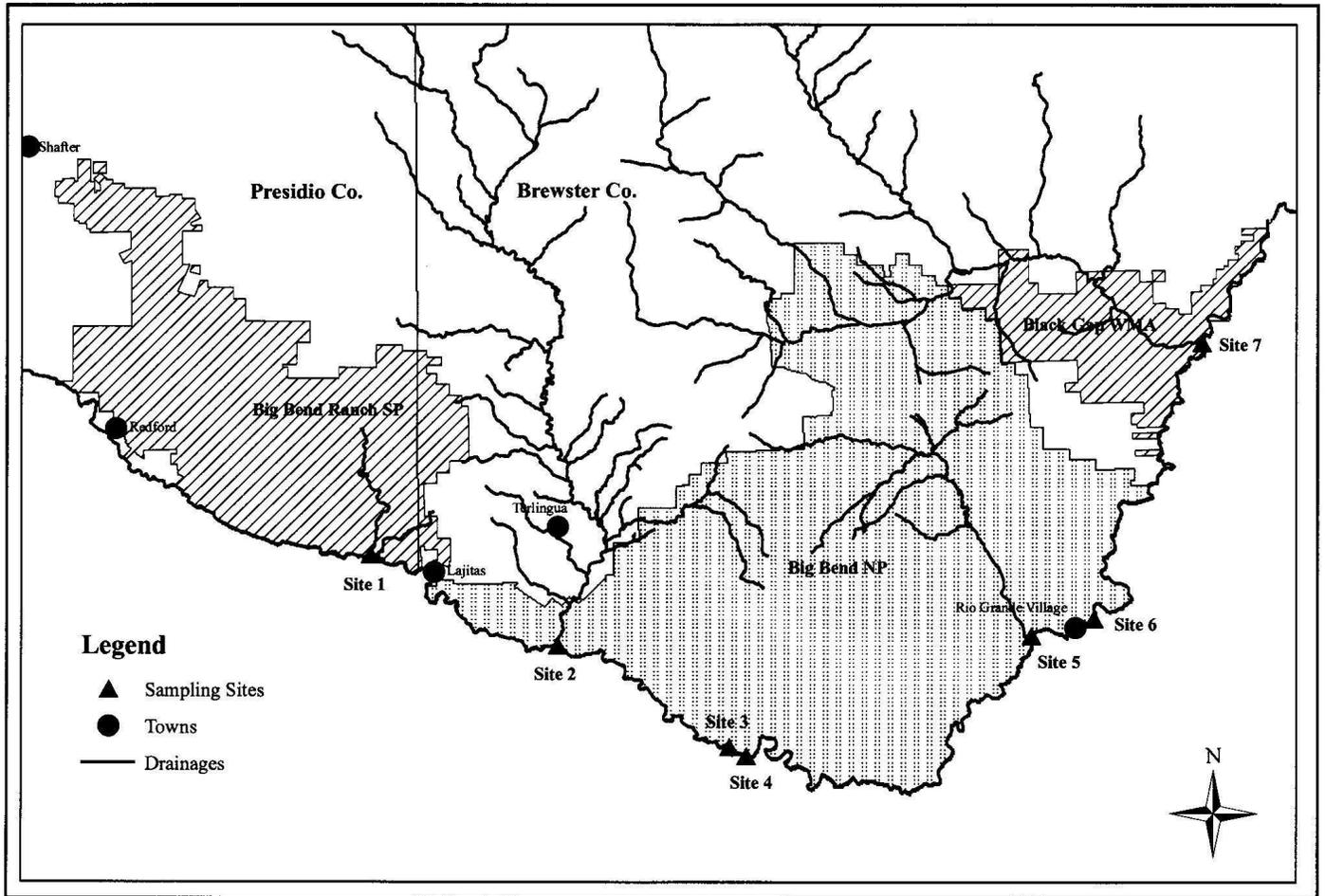
TABLE 3. Mean percentage of identified aquatic insects by wet mass (mg) in stomach contents of adult *Notropis braytoni* from sites 2, 3, and 6 from January 2006 through December 2006.

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	%
Ephemeroptera	11.9	1.8	44.2	76.6	37.4	66.5	20.2	0.6	—	2.5	21.1	65.6	47.2
Simuliidae	27	1.8	2.6	5.6	5.5	0	0	0	0.2	87.5	58.9	0.2	25.7
Trichoptera	—	8.2	0.1	4.2	10.6	28.5	—	—	—	—	0.6	11	8.6
Odonata	2.4	—	15.8	—	29.5	—	—	6.6	—	—	—	—	7.4
Corixidae	—	1.4	10.5	0.3	1.4	—	12.8	6.3	—	—	—	—	4.4
Coleoptera	—	10.8	7.2	—	0.1	—	—	—	—	—	—	—	2.4
Chironomidae	7.9	0.6	4.8	0.2	—	—	—	0.9	0.5	—	—	0.2	2.1
Adult Dipteran	7.1	1.5	—	—	—	—	—	—	—	1.9	2	—	1.7
Megaloptera	—	3.9	—	—	—	—	—	—	—	—	—	—	0.5

TABLE 4. Historical fish assemblage with relative abundances from the Big Bend reach of the Rio Grande. X indicates species was present.

Species	1954	1977	1990	1992	1993	1999	2004	2006
<i>Cyprinella lutrensis</i>	0.7	19.4	76.6	61.9	87.00	27.22	16.20	46.33
<i>Notropis braytoni</i>	16.4	2.0	2.3	0.2	2.00	25.11	59.00	34.97
<i>Carpionodes carpio</i>	20.4	0.5	3.9	10.7	—	5.49	9.80	4.10
<i>Macrhybopsis aestivalis</i>	1.3	3.3	0.3	—	0.60	10.34	0.03	3.15
<i>Gambusia affinis</i>	0.7	2.7	2.0	—	7.10	0.21	2.90	2.98
<i>Ictalurus punctatus</i>	1.3	0.6	0.1	0.1	0.04	3.16	—	2.36
<i>Ictalurus furcatus</i>	45.4	1.3	—	0.9	0.20	5.06	2.10	1.62
<i>Astyanax mexicanus</i>	0.7	0.8	2.5	1.3	0.01	—	6.60	1.33
<i>Cyprinus carpio</i>	0.7	0.2	0.1	0.5	—	1.90	0.20	0.65
<i>Menidia beryllina</i>	—	0.6	1.7	6.2	0.30	—	—	0.61
<i>Fundulus zebrinus</i>	—	—	—	0.2	—	1.27	0.10	0.51
<i>Rhinichthys cataractae</i>	—	57.7	8.8	—	0.40	1.48	0.20	0.36
<i>Pylodictis olivaris</i>	3.9	0.3	0.1	0.8	0.03	4.85	0.40	0.29
<i>Cycleptus elongatus</i>	—	3.8	—	—	—	2.95	—	0.21
<i>Lepomis species</i>	—	0.5	0.2	0.1	0.17	1.05	0.26	0.19
<i>Lepisosteus osseus</i>	0.7	0.1	0.1	—	—	0.84	1.00	0.14
<i>Notropis chihuahua</i>	—	0.1	—	2.0	0.02	—	—	0.12
<i>Notropis jemezianus</i>	7.2	3.4	1.2	—	0.30	7.38	—	0.05
<i>Ictiobus bubalus</i>	0.7	—	—	—	1.70	0.21	0.40	0.02
<i>Dorosoma cepedianum</i>	—	2.1	—	—	—	1.27	0.20	—
<i>Campostoma ornatum</i>	—	—	—	—	0.01	—	—	—
<i>Dionda episcopa</i>	—	—	—	—	—	—	0.03	—
<i>Notropis stramineus</i>	—	—	—	—	0.20	—	—	—
<i>Pimephales promelas</i>	—	0.1	—	4.5	0.20	—	—	—
<i>Moxostoma austrinum</i>	—	—	—	1.3	0.02	—	—	—
<i>Ictalurus lupus</i>	—	—	—	0.1	0.02	—	0.40	—
<i>Cyprinodon eximius</i>	—	0.3	—	—	—	—	—	—
<i>Morone chrysops</i>	—	—	0.1	0.3	—	—	—	—
<i>Micropterus salmoides</i>	—	0.1	—	1.0	—	—	—	—
<i>Aplodinotus grunniens</i>	—	—	—	—	—	0.21	0.03	—
<i>Oreochromis aureus</i>	—	—	—	8.2	—	—	—	X
Total	152	2077	1376	992	8964	474	3044	10565

References: Hubbs 1958; Hubbs et al. 1977; Platania 1990; Edwards et al. 2002b; Moring 2002; Edwards 2005; Heard, this study.



Tom C. Heard
 GIS Data Acquired from the Texas General Land Office

01/24/06

FIGURE 1. Map of study area and sampling sites, Big Bend reach, Rio Grande, January 2007 through December 2007.

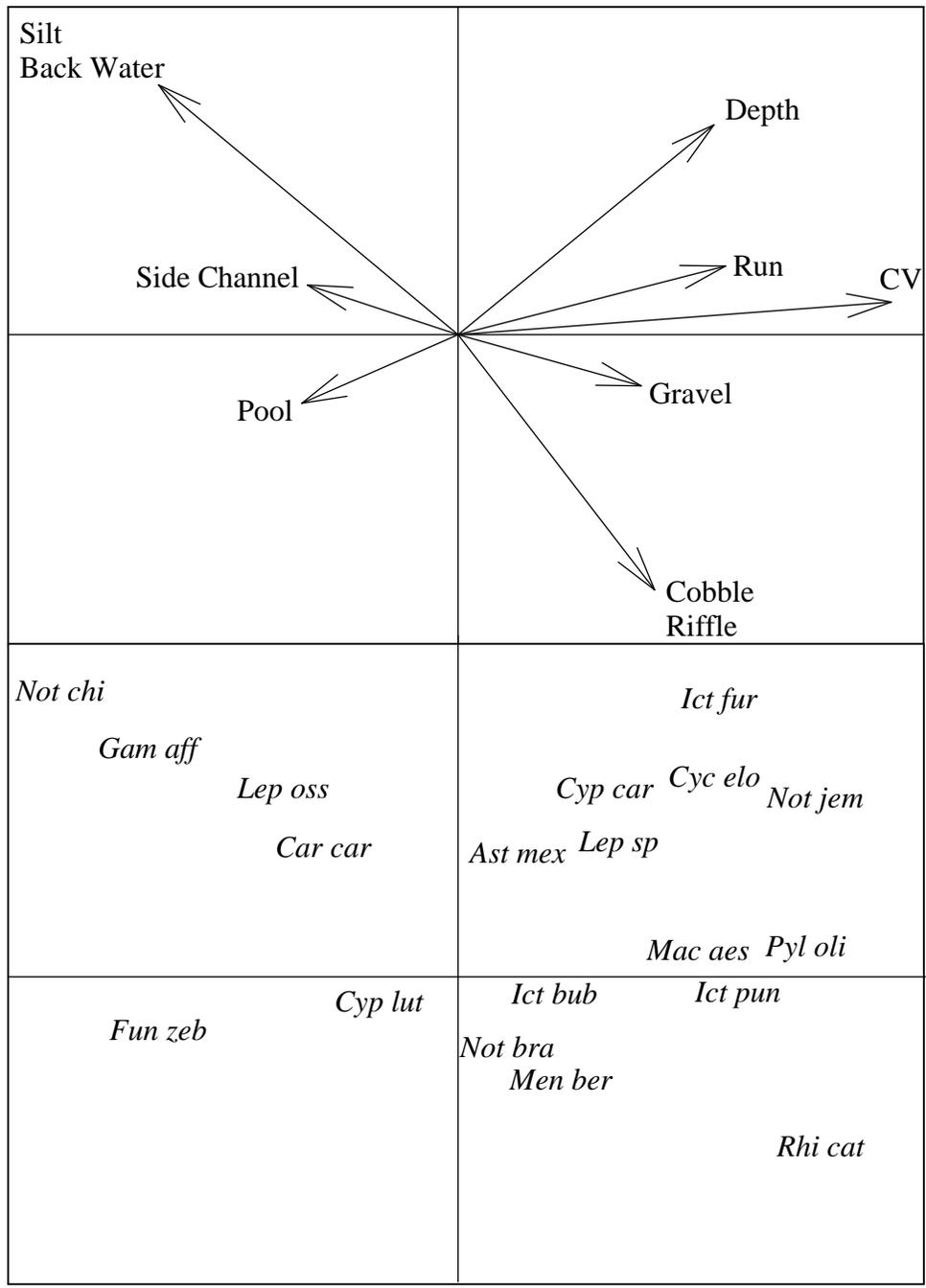


FIGURE 2. Simplified Canonical Correspondence Analysis (CCA) ordination plot for habitat associations of the fish assemblage of the Big Bend Reach, Rio Grande, January through December 2007.

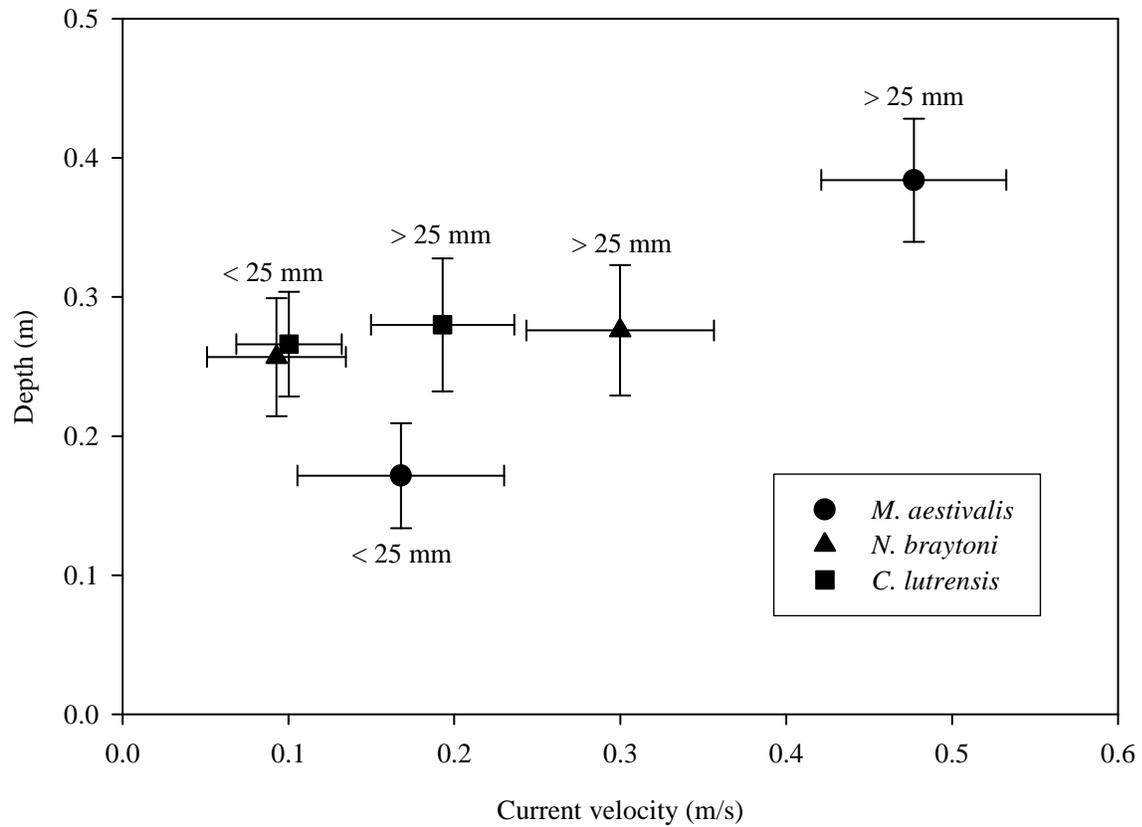


FIGURE 3. Weighted mean (\pm SE) of current velocity (m/s) and depth (m) associations for two size classes (< 25 mm & >25 mm) of *Notropis braytoni*, *Macrhybopsis aestivalis* and *Cyprinella lutrensis* in the Big Bend Reach, Rio Grande, January 2007 through December 2007.

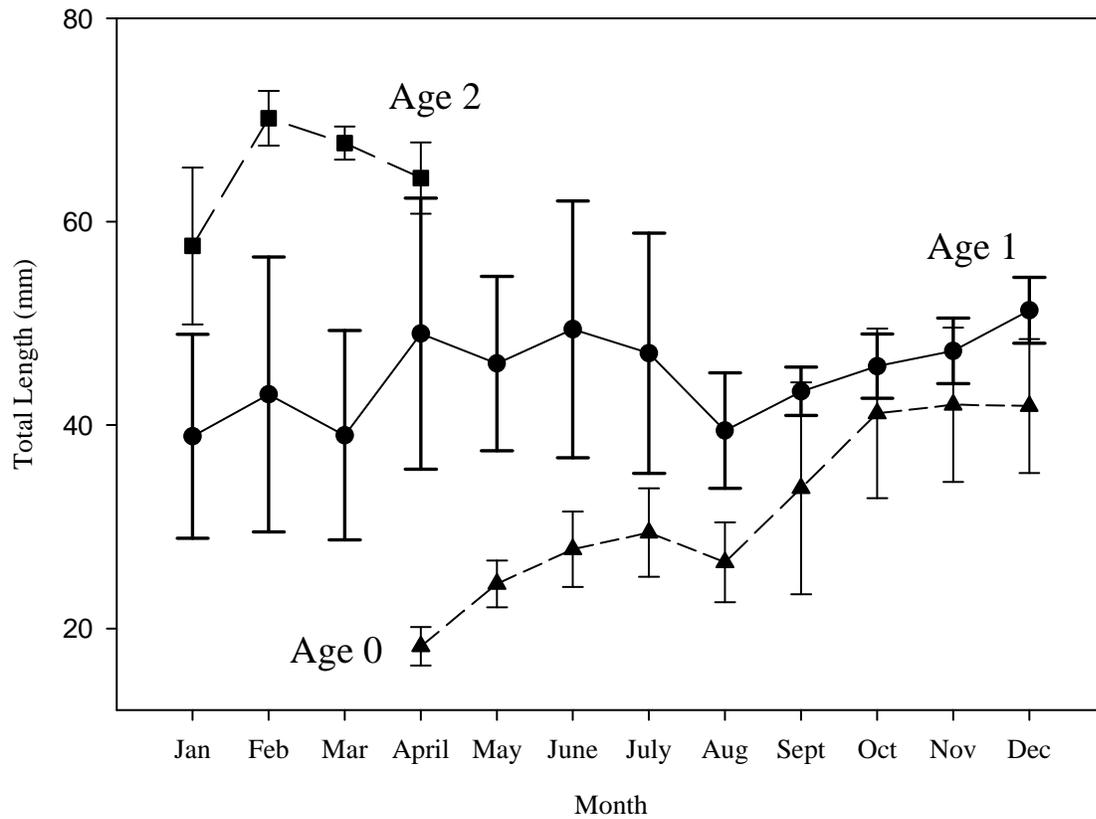


FIGURE 4. Monthly total length (\pm SD) of *Notropis braytoni* in the Big Bend Reach, Rio Grande, January 2007 through December 2007.

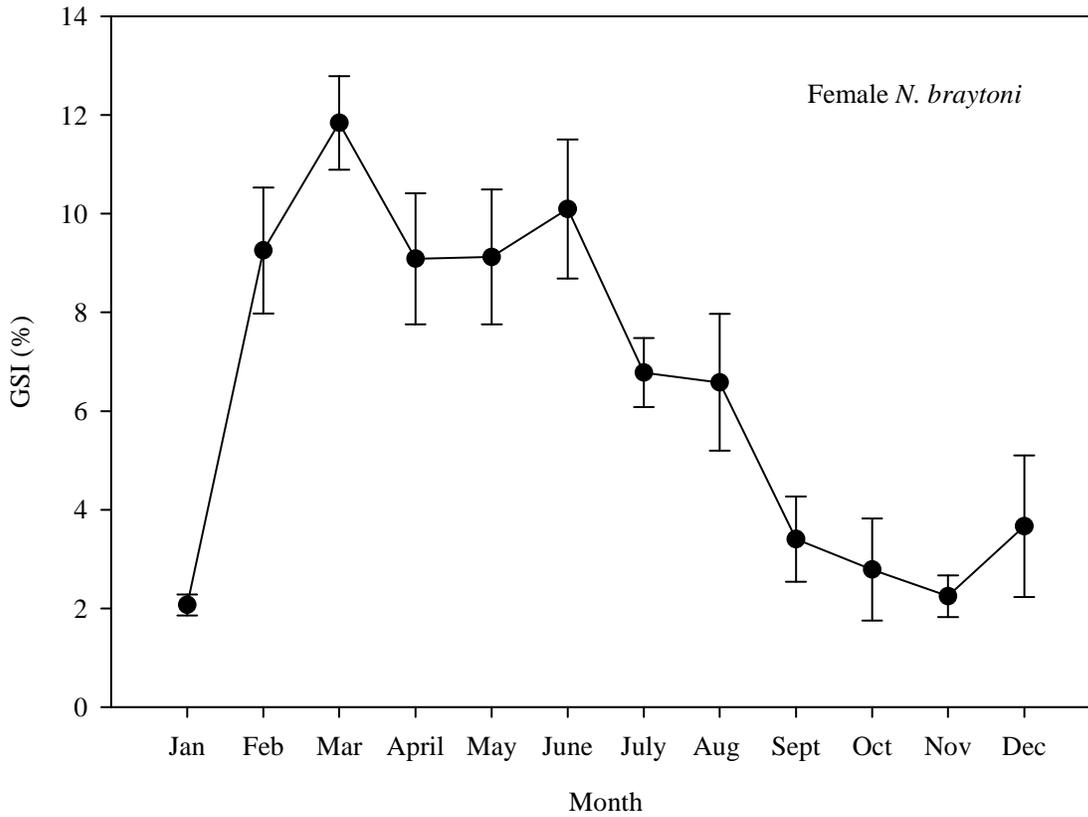


FIGURE 5. Mean (\pm SE) monthly gonadosomatic index (GSI) for female *Notropis braytoni* from sites 2, 5 and 6, Big Bend Reach, Rio Grande, January 2007 through December 2007.

SECTION II

Distribution and diet of larval and juvenile fishes in the Rio Grande, Texas

Abstract--Fishes of the Rio Grande in the Big Bend region of Texas represent an imperiled fauna because of direct anthropogenic alterations and continued growth of municipalities upstream. Fish species once populous are now extirpated and several are now extinct as a product of habitat modification. The larval and juvenile fish assemblage was assessed by sampling discrete geomorphic units at three mainstem Rio Grande sites and two tributary sites in Big Bend National Park. *Cyprinella lutrensis* comprised 75% of the overall assemblage followed by *Notropis braytoni* (14%), *Carpionodes carpio* (5%), and *Gambusia affinis* (3%). Specific habitat associations were determined that suggest at least 12 fish species found therein utilized slackwater habitats (i.e., backwaters) predominantly. Fishes were not evenly distributed among sites, geomorphic units, or chronologically and Canonical Correspondence Analysis explained 21% ($P = 0.044$) of the total variation. Variance partitioning to assess pure effects of first order interactions explained all but 2% of the total variation. Stomach content analysis of fishes indicated opportunistic feeding of Diptera and other aquatic insects. No chronological difference in diet was found among time periods using Analysis of Similarity ($R: 0.096$, $P = 0.13$).

Introduction

The study of age-0 fishes (larval and juvenile) is an essential component in understanding species-specific information regarding reproductive success, year-class strength, and habitat associations (Snyder et al. 2005). Furthermore, fish assemblages are a good indicator for instream flow as they comprehensively incorporate aspects of an aquatic community (Osting et al. 2004). In the southwestern USA and Chihuahuan desert region of northern Mexico, many aquatic habitats are endangered as a result of modern development of municipalities and increased demand upon limited water resources (Edwards et al. 2002). A high degree of endemism among aquatic fauna exists in this region as a result of physiographic isolation and its arid nature (Smith and Miller 1986). Fishes found in headwater springs, streams, and large rivers of this region are under great threat from anthropogenic alteration (Minckley and Deacon 1968; Karges 2003).

Native fishes of the Rio Grande drainage represent imperiled fauna (Edwards et al. 2002); at least 50% are of conservation concern (Hubbs et al. 1991). Many species once prevalent in the Chihuahuan desert and Trans Pecos regions are now extinct or extirpated, and several more are endangered or threatened (Hubbs et al. 1991; Dudley and Platania 1997). Alteration of fish assemblages here are largely attributed to habitat degradation and loss (Karges 2003) as flow has been reduced substantially in the Rio Grande and Río Conchos (confluence upstream of study area). Studies of faunal composition and abundance change in major rivers of this region suggest a shift from dominance of obligate riverine fishes, to an assemblage dominated by habitat generalists (Hoagstrom 2001; Edwards et al. 2003).

Preservation of habitat via maintenance of river flow is vital for conservation of many species in the Rio Grande (e.g., *Hybognathus amarus* and *Macrhybopsis aestivalis*) as their

spawning behavior requires adequate flow for longitudinal distribution and development of larvae (Richter et al. 1996; Dudley and Platania 1997; Platania and Altenbach 1998). Obligate riverine fishes such as *H. amarus* often have specific habitat requirements for larval development (Dudley and Platania 1997). Natural river flows (variable intra-annual discharge, pulse floods) are required to create, maintain, and alter these habitats (Collier et al. 1996; Richter et al. 1996). Protection of larval and juvenile fish habitats as well river flow is necessary in order to maintain faunal integrity (Scheidegger and Bain 1995). The objectives of this study were to determine larval and juvenile fish distribution among discrete geomorphic units and to investigate diets across the larval and juvenile fish assemblage.

Study Area

Five sites, three main-stem and two tributary locales, were selected in the Big Bend region of the Rio Grande. The region's arid climate, sandy soil, and mountainous physiography resulted in a river system subject to rapid hydrologic changes and geomorphic alteration; its course through the study area alternates between deep canyons and lowland floodplains. Santa Elena and its tributary influence, Terlingua Creek, Johnson Ranch, and Hot Springs and its tributary, Tornillo Creek, were sites utilized in this study. The most upstream site, Santa Elena, and Hot Springs, furthest downstream, are separated by approximately 115 river km. A series of alternating narrow, deep canyons and meandering river stretches separate these sites.

Santa Elena and Hot Springs provided ample opportunity for sampling myriad habitats due to tributary influence and the cobble, gravel, sand, and silt substrates in constant flux; however, Johnson Ranch exhibited the least geomorphic change throughout the sampling period maintaining a long continuous run with shifting gravel and sand bars. Both tributary sites are characterized by intermittent flow from springs and flash flooding from intense rainfall, and the oft temperate waters flow over gravel and sand to their main-stem confluence.

Methods

Monthly collections started with the first occurrence of age-0 fishes in April 2006 and extended through December 2006. At each main stem site, I selected geomorphic units (e.g., backwater, eddy, channel margin) where larval and juvenile Rio Grande fishes are known to occur (Dudley and Platania 1997). Geomorphic units were not proportionally represented by sampling. Instead, an effort was made to sample all and replicates of the available geomorphic units. At each tributary site, all geomorphic units (i.e., pool, runs, and riffles) were sampled up to 100 m upstream from the confluence. Fish collections consisted of multiple passes with a larval fish seine (1.2 x 1.8 m; 800 μ m mesh size) or a small aquarium dipnet (on one occasion amidst boulder substrate) until a large number of fishes were captured. Consequently, sampling effort was not consistent among geomorphic units. Likewise, numbers of seine hauls in geomorphic units without fish were not recorded. Captured fishes were anesthetized with a lethal dose of MS-222 (80 mg/L) to prevent regurgitation of stomach contents (Mendelson 1975) and fixed in 10% buffered formalin. For each collection, geomorphic unit type was recorded along with substrate type, and current velocity was determined using an ordinal scale (no flow, low flow, and moderate flow).

In the laboratory, fishes were identified, measured (total length; mm), and enumerated. Only fishes less than specific size limits were considered for further analyses to maintain a strictly larval and juvenile assemblage based on estimated total length at sexual maturation. *Cyprinella lutrensis* (Laser and Carlander 1971; Farringer et al. 1979) and *Notropis braytoni* and *N. chihuahua* (length inferred from *C. lutrensis*) were retained if less than 30 mm, *Macrhybopsis aestivalis* if less than 45 mm (inferred from *M. hyostoma*; C. S. Williams pers. comm. 2007), *Astyanax mexicanus* if less than 50 mm (Estrada 1999), *Fundulus zebrinus* if less than 25 mm (Bohnam 1962), *Gambusia affinis* if less than 10 mm (Haynes and Cashney 1995), and *Lepomis megalotis* if less than 45 mm (Jennings and Philipp 1992). *Lepisosteus osseus* (Haase 1969), *Cyprinus carpio* (Farabee 1979; Ross 2001), *Carpionodes carpio* (Bass and Riggs 1959), and *Ictalurus sp.* (Appelgate and Smith 1950; Jenkins 1956) mature at lengths exceeding those I collected thus all individuals were retained for analyses.

Habitat analyses consisted of determining relative abundance of species that occurred in geomorphic units by time period, by site, and modeling species and habitat parameters throughout the sampling period using Canonical Correspondence Analysis (CCA; ter Brack 1986). Variance partitioning was used to determine the pure effects of environmental variables, sites, and time period and Monte Carlo simulation tests (999 permutations) were run to test significance (Williams et al. 2005). Species richness (*S*) was determined by site and geomorphic unit.

Samples were separated for diet analysis into two time periods, Period I (April–August) and Period II (September–December), combined together by geomorphic unit maintaining species integrity within each time period, and up to 5 vouchers were retained for each sample when possible. Five individuals of each species were randomly drawn from combined samples for diet analysis. Fish were eviscerated and gut contents from the most anterior end of the stomach to the first turn of the large intestine were examined (Heins and Clemmer 1975; Bowen 1996). *Carpionodes carpio* was an exception to this rule because of its long coiled intestine; the anterior 25% of the gut tract was examined.

Stomach contents were identified to the lowest practical taxonomic level and recorded as occurrence by food item for each species (Hyslop 1980; Bowen 1996). Similarity matrices (Bray and Curtis 1957) were determined from relative abundance of food items and tested with analysis of similarity (ANOSIM; $\alpha = 0.05$; 9,999 permutations) across time periods using PRIMER 6.1.6 (Clarke 1993; Clarke and Warwick 2001). Permutation analysis indicated average rank dissimilarity within and between samples (Clarke and Gorley 2006). Species that occurred only during one period, *Lepisosteus oculatus*, *N. chihuahua*, *Ictalurus sp.* and *Lepomis megalotis*, were excluded from this analysis because their inclusion generated specious significance. Additionally, these species composed only a minor portion of the overall relative abundance across periods.

Results

A total of 8,364 individuals was collected between April and December 2006. Among these, 6,928 fishes were determined to be larval or juvenile fishes and were retained for analyses. Eight families consisting of 12 species were represented by the larval and juvenile assemblage from 75 geomorphic units. Among all larval and juvenile fishes taken, *Cyprinella lutrensis* was

the most abundant (75% in relative abundance) followed by *Notropis braytoni* (14%), *Carpionodes carpio* (5%), and *G. affinis* (3%) (Table 3.1).

Larval and juvenile fishes ($N = 5,325$) were taken from 38 geomorphic units during Period I (Table 3.2). Channel margins was the most speciose with 11 species of fishes, followed by backwaters ($S = 7$), tributaries ($S = 5$), and eddies ($S = 3$). Among the 11 taxa, two (*C. lutrensis* and *N. braytoni*) were taken from all geomorphic unit types, three were taken from three geomorphic unit types, three were taken from two geomorphic units, and three were taken from only one geomorphic unit (channel margins).

Larval and juvenile fishes were taken from all geomorphic unit types during Period II ($N = 1,603$). Channel margins were again the most speciose with 8 species, followed by eddies and tributaries ($S = 6$) and backwaters ($S = 4$). Among the 9 fishes collected, two species (*C. lutrensis* and *A. mexicanus*) were taken from all geomorphic units. Three species were taken from three geomorphic units, two species were taken from two geomorphic units, and two species taken from only one geomorphic unit.

Multivariate ordination of site, habitat parameters, and time period explained 21% ($P = 0.04$) of fish assemblage variation (Figure 3.1). Using variance partitioning, pure effect of site explained 11% ($P = 0.017$) of assemblage variation, pure effect of habitat variables explained 7% ($P = 0.266$), and time period explained 1% ($P = 0.543$). Two percent of the total variation was not explained by first order interactions. Significant site effects were attributed to the occurrence or high abundance of a few species at only one site location (i.e., *L. megalotis* and *Ictalurus* at Johnson Ranch site; *F. zebrinus* at Tornillo Creek site). Lack of significance among environmental variables was attributed to ubiquitous habitat associations of few, dominate taxa (e.g., *C. lutrensis* and *N. braytoni*). Nevertheless, some taxa (i.e., *L. megalotis*, *G. affinis*, *L. osseus*, and *A. mexicanus*) were strongly associated with habitat parameters.

Environmental factors with the greatest positive biplot scaling scores on Axis I were gravel substrate (0.52) and current velocity (0.50). The greatest negative biplot scores among environmental variables were silt substrate (-0.45) and vegetation (-0.13). Fish species with high positive biplot scaling scores were *F. zebrinus* (1.9), *N. chihuahua* (1.5), and *A. mexicanus* (1.0). Fishes with the greatest negative biplot scores were *G. affinis* (-1.2), *Lepomis megalotis* (-1.1), *Lepisosteus osseus* (-0.96), *Carpionodes carpio* (-0.76), and *Ictalurus sp.* (-0.70). Sites with positive biplot scaling scores were Tornillo Creek (0.66), Hot Springs (0.17), and Terlingua Creek (0.14). Sites with negative biplot scores were Johnson Ranch (-0.63) and Santa Elena (-0.04).

Differences in species diets between time periods were not different (ANOSIM; Global R: 0.096, $P = 0.13$); species-specific diets of most fishes were similar between periods (Figure 3.2). Consequently, diet information was combined for both periods. Among nine food categories (Copepoda, insect parts, Ephemeroptera, Coleoptera, Hemiptera, Diptera, fish, organic detritus, and silt), Diptera were the most common food item consumed by all taxa ranging from 13% ($N = 8$) to 100% ($N = 6$) (Table 3.3). Unidentifiable insect parts, due to mastication or digestion or both, was also common and occurred in all but 2 species. Other notable diet contents included silt consumed by *Carpionodes carpio* (88%; $N = 26$), *Cyprinus carpio* (25%; $N = 16$), and *N. braytoni* (22%; $N = 45$), Coleoptera were consumed by *Lepomis megalotis* (25%; $N = 4$), *N. braytoni* (16%; $N = 7$), and *A. mexicanus* (13%; $N = 8$). Fishes were only consumed by *Lepisosteus osseus* whereas Hemiptera were only consumed by *Lepomis megalotis*. Across taxa and grouped by geomorphic unit, fishes from margin and tributary geomorphic units consumed the most diverse food items (Table 3.4). Diptera and insect parts were consumed among all

geomorphic units whereas copepods and ephemeropterans were consumed only in the margins and tributaries, and hemipterans were consumed only in the margins.

Discussion

Larval and juvenile fishes captured in this study represented the more common adult taxa found in the Rio Grande (concurrent study; T. Heard, unpublished data). The use of slackwater habitats as nursery areas is common among riverine fishes (Floyd et al. 1984; Grift et al. 2003) and verified for Rio Grande-Big Bend area fishes by this study. One unexpected result was lower taxonomic diversity in tributary streams compared to mainstem sites. Tributaries provide stable refugia for larvae and juveniles of many main stem taxa (Clark 1973; Sheldon 1988) but did not necessarily support more age-0 Rio Grande fishes than main stem slackwater areas. This might be attributed to few perennially flowing tributaries in arid reaches of the Rio Grande, or that the Rio Grande, especially in the Big Bend area and downstream from the confluence with the Devils River, historically was much wider and shallower (Contreras-Balderas et al. 2002; Calamusso et al. 2005); consequently, sufficient areas of slackwater occurred within the main channel and refugia in tributaries were not necessary. Conversely, many tributaries have been altered by anthropogenic modifications and no longer suitable as nursery habitats (Edwards et al. 2002). The latter explanation is somewhat supported by fish assemblage changes in Terlingua Creek, which historically supported a unique fish assemblage (Hubbs and Wauer 1973) but currently supports an assemblage similar to the Rio Grande main stem (Edwards et al. 2002).

Fishes were not equally distributed among main stem geomorphic units or sites, and abundance differed among time periods. Target-sampling, as done in this study, prevented predictions of species-habitat association; however, CCA was useful in conveying larval and juvenile distributions among sites and environmental parameters. The pure effects of site and environment each explained about one half of the total variation in the complete CCA model. These results were further supported by observed differences in fish species richness and individual species abundance among sites. Taxa richness was greatest at Johnson Ranch. *Lepisosteus osseus*, *M. aestivalis*, *Carpiodes carpio*, *Ictalurus sp.*, *G. affinis*, and *Lepomis megalotis* were associated with Johnson Ranch and its predominately silt substrate. Species associated with more intermediate environmental variables, those with centroids near the origin, cobble, sand, and vegetation, and sites, Santa Elena, Terlingua Creek, and Hot Springs, were primarily the most abundant taxa in this study, *C. lutrensis* and *N. braytoni*, but also included *Cyprinus carpio*. *Fundulus zebrinus* was largely associated with Tornillo Creek, gravel substrate, and moderate current velocity.

Little variation in occurrence of food items examined was observed between periods for each species. Using ANOSIM to wholly compare data suggested significant similarity of prey items among the fish assemblage and commonality of individual food items by species. All fishes examined appeared to feed opportunistically; prey selectivity could not be determined and was not an objective of this study though Diptera and other insects were the most commonly ingested and likely the most available food items. Diet of all species aligned with published analyses of larval and juvenile fishes. Some species ontogenetic shifts in diet preference (e.g., *Lepisosteus osseus* shift from invertivory to piscivory; Echelle and Riggs 1972); however, the immature fishes examined herein were mostly classified as invertivores. *Cyprinella lutrensis* (Simon 1999), *M. aestivalis* (inferred from *M. hyostoma*; Starrett 1950), *A. mexicanus* (Edwards

1977), *Ictalurus sp.* (inferred from *I. punctatus*; Bailey and Harrison 1945), *F. zebrinus* (Bonham 1962), *G. affinis* (Simon 1999), and *Lepomis megalotis* (Applegate 1966) were all classified as invertivores. *Cyprinus carpio* (Simon 1999) and *Carpiodes carpio* (Ross 2001) were classified as omnivorous, which was evidenced herein by a relatively high occurrence of silt.

Numerous large-bodied and small-bodied fishes have suffered from declines in abundance, and were extirpated from or are now extinct in the Rio Grande. *Atractosteus spatula* is extirpated above Falcon Reservoir (Garrett 2002), *Scaphirhynchus platyrhynchus* once ranged as far upstream as Albuquerque, New Mexico, but is now extirpated from the Rio Grande and much of Texas (Hubbs et al. 1991), *Cycleptus elongatus* has been reported as rare to abundant and is likely unique in the drainage (summarized by Garrett 2002), and *Ictalurus furcatus*, which may also be endemic (Hubbs et al. 1991), has recently suffered from a fish kill of unknown cause (pers. observ. at Santa Elena Canyon, December 2006). Additionally, *Hybognathus amarus*, *N. orca*, and *N. simus simus* are extirpated or extinct, and abundance of *N. jemezianus* and *Etheostoma grahami* has substantially declined (Hubbs et al. 1991). Fishes in the Rio Grande and other large rivers rely on specific habitats maintained by both pulse and base flows for reproduction and foraging (Platania and Altenbach 1998; Grift et al. 2003). To what extent that population declines in the Rio Grande are associated with the lack nursery habitats is unknown, but dewatering, main stem impoundments, channelization, and invasive riparian vegetation collectively have decreased slackwater habitats and floodplain connectivity throughout Rio Grande, especially in areas where Rio Grande endemics are extirpated, extinct, or in rare abundance (Contreras-Balderas et al. 2002; Calamusso et al. 2005). To maintain current assemblage and endemic taxa (i.e., *N. braytoni*, *M. aestivalis*), main stem slackwater habitats should be maintained by flow regime or by mechanical alterations (Porter and Massong 2004a, 2004b). In addition, maintenance of these slackwater habitats likely would benefit repatriation efforts of *H. amarus* in the Big Bend reach of the Rio Grande (USFWS 2006), which seems to be a limiting factor for their successful reproduction in the upper reaches of the Rio Grande in New Mexico (Porter and Massong 2004a, 2004b).

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TABLE 1. Relative abundance of Rio Grande drainage larval and juvenile fishes in Big Bend National Park collected throughout the sampling period.

Species	Relative Abundance
<i>Cyprinella lutrensis</i>	75
<i>Notropis braytoni</i>	14
<i>Carpionodes carpio</i>	5
<i>Gambusia affinis</i>	3
<i>Fundulus zebrinus</i>	2
<i>Cyprinus carpio</i>	0.36
<i>Ictalurus sp.</i>	0.26
<i>Lepisosteus osseus</i>	0.22
<i>Astyanax mexicanus</i>	0.16
<i>Macrhybopsis aestivalis</i>	0.12
<i>Lepomis megalotis</i>	0.07
<i>Notropis chihuahua</i>	0.01
<i>N</i>	6,928

TABLE 2. Relative abundance of larval and juvenile fishes by time period and geomorphic unit.

Species	Period I				
	Backwater	Eddy	Margin	Riffle	Tributary
<i>Lepisosteus osseus</i>	0.08		1		
<i>Cyprinella lutrensis</i>	81	64	67	1	75
<i>Cyprinus carpio</i>	0.06		0.87		
<i>Macrhybopsis aestivalis</i>			0.09		
<i>Notropis braytoni</i>	8	35	11	99	16
<i>Notropis chihuahua</i>					
<i>Carpiodes carpio</i>	6		12		2
<i>Astyanax mexicanus</i>		2	0.09		
<i>Ictalurus sp.</i>			2		
<i>Fundulus zebrinus</i>	0.61		2		5
<i>Gambusia affinis</i>	4		4		2
<i>Lepomis megalotis</i>			0.44		
Total N:	3,586	55	1,149	139	396
Geomorphic Unit N:	10	1	20	1	7
Species	Period II				
	Backwater	Eddy	Margin	Riffle	Tributary
<i>Lepisosteus osseus</i>					
<i>Cyprinella lutrensis</i>	78	97	65	100	76
<i>Cyprinus carpio</i>			0.71		1
<i>Macrhybopsis aestivalis</i>		0.85	0.85		
<i>Notropis braytoni</i>	16		31		15
<i>Notropis chihuahua</i>		0.85			
<i>Carpiodes carpio</i>		0.85	0.57		0.56
<i>Astyanax mexicanus</i>	1	0.85	0.57		0.42
<i>Ictalurus sp.</i>					
<i>Fundulus zebrinus</i>	4		2		7
<i>Gambusia affinis</i>			0.14		
<i>Lepomis megalotis</i>					
Total N:	67	118	707	2	709
Geomorphic Unit N:	3	3	18	1	11

TABLE 3. Percent occurrence of food items by species combined by time period. Total length (TL) range for dissected fishes is given in millimeters.

Food Item	Species											
	<i>L. osseus</i>	<i>C. lutrensis</i>	<i>Cy. carpio</i>	<i>M. aestivalis</i>	<i>N. braytoni</i>	<i>N. chihuahua</i>	<i>Ca. carpio</i>	<i>A. mexicanus</i>	<i>Ictalurus sp.</i>	<i>F. zebrinus</i>	<i>G. affinis</i>	<i>L. megalotis</i>
Copepoda		7					4	13			20	
Insect parts		68	44	33	33	100	35	75	60	25	40	
Ephemeroptera	13	2								3		100
Coleoptera					16			13		3	7	25
Hemiptera												25
Diptera	13	34	81	100	49	100	69	50	80	83	73	75
Fish	88											
Detritus		11			18		8			3	20	
Silt		18	25		22		88		20	3		
TL Range	27–74	9–29	13–81	9–29	12–58	29	12–58	9–49	24–51	9–24	7–10	11–27
<i>N</i>	8	44	16	6	45	1	26	8	5	38	15	4

TABLE 4. Percent occurrence of food items by geomorphic unit combined by time period.

	Geomorphic Unit				
	Backwater	Eddy	Margin	Riffle	Tributary
Copepoda			7		4
Insect parts	43	63	38	33	31
Ephemeroptera			6		4
Coleoptera		10	1	33	8
Hemiptera			1		
Diptera	57	50	65	22	63
Fish	7		5		
Detritus	16	10	4		12
Silt	30	23	17	44	18
<i>N</i>	44	30	84	9	49

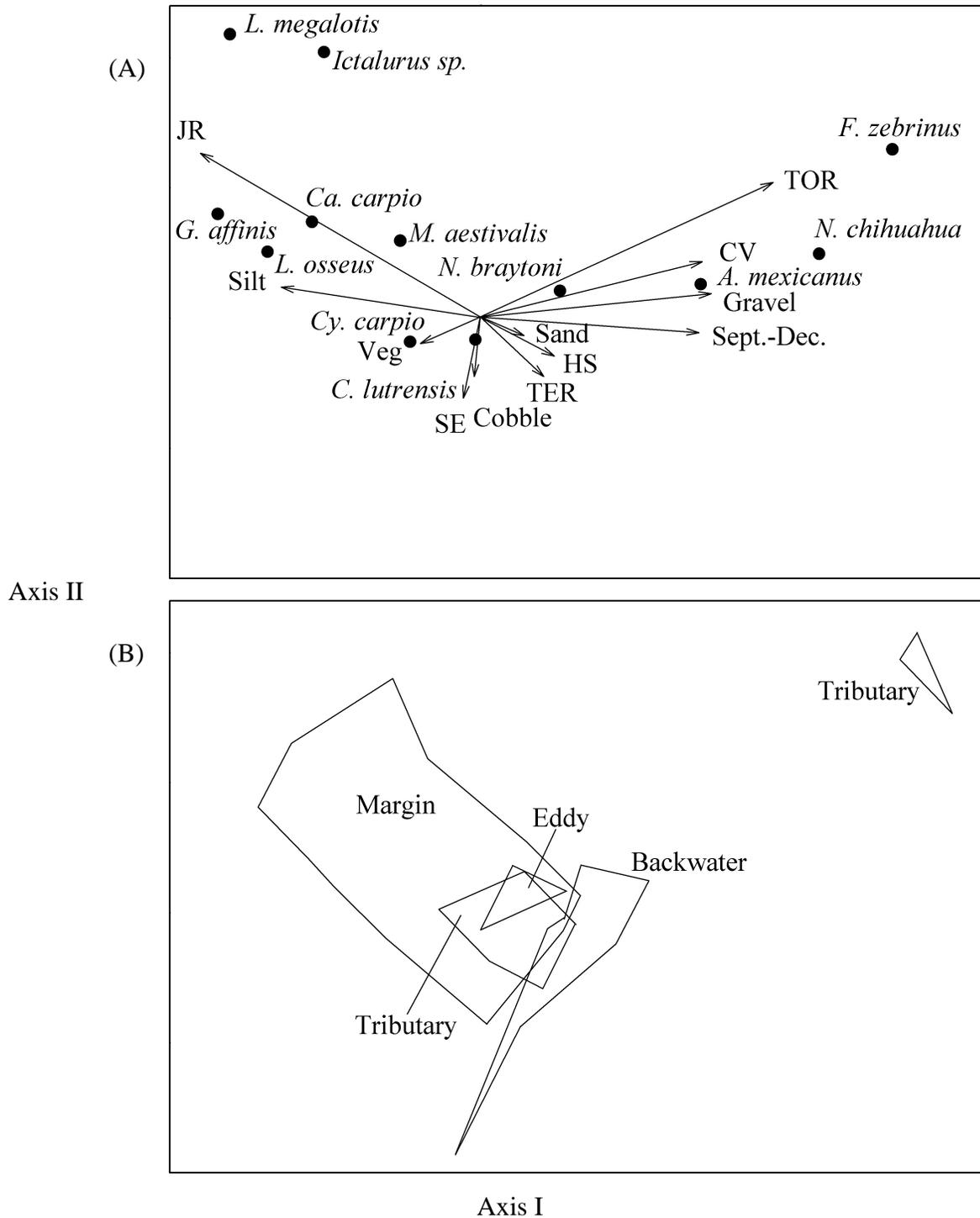


FIGURE 1. Canonical Correspondence Analysis of species and environmental variables (A) and habitat polygons (B) inferred from biplot scaling scores. Sites, Santa Elena (SE), Terlingua Creek (TER), Johnson Ranch (JR), Hot Springs (HS), and Tornillo Creek (TOR), and current velocity (CV) were abbreviated for clarity.

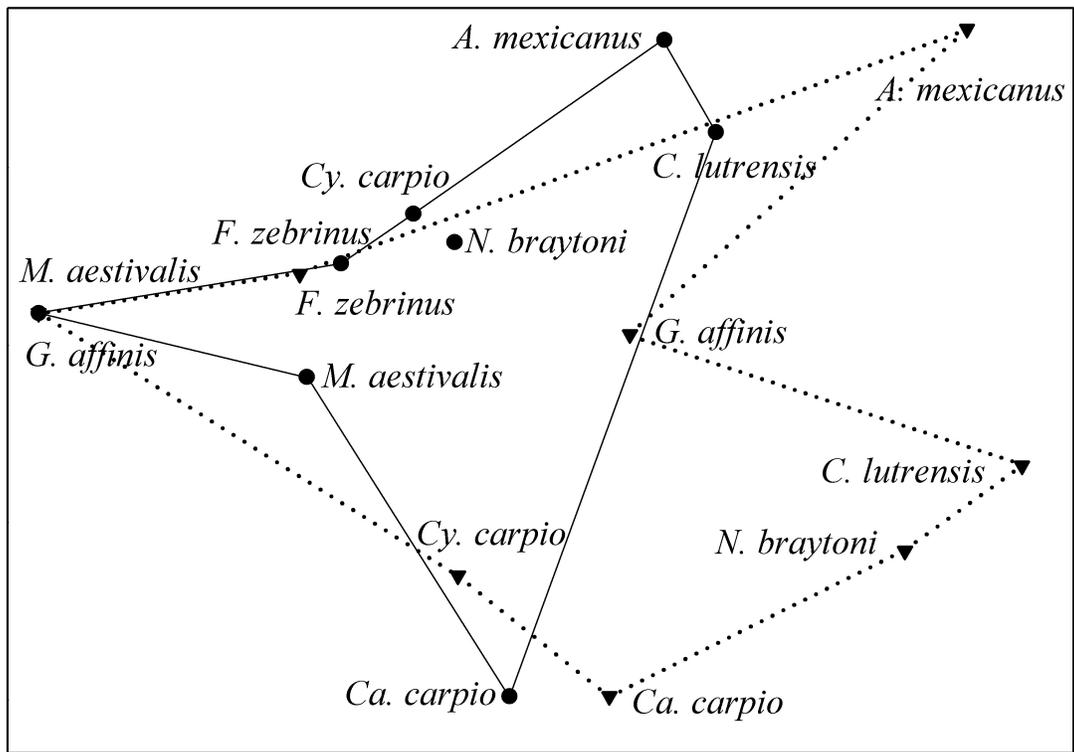


FIGURE 2. Multi-dimensional scaling plot of species and relative abundance of stomach contents. Period I species are represented by inverted triangles and are outlined by the dotted line. Period II species are represented by solid circles and are outlined by the solid line.

SECTION III

Macroinvertebrate abundance and habitat associations in the Big Bend Region of the Rio Grande

Abstract--Arid aquatic environments represent unique and ecologically distinct systems, ranging from highly stable to highly fluctuating water bodies/streams along expansive discharge, water temperature, and salinity gradients. These environments typically support a diverse macroinvertebrate assemblage with a number of endemic taxa. The primary objective of this study included quantifying monthly occurrence and abundance, longitudinal distribution, and habitat associations of the macroinvertebrate community in the Rio Grande, located in northern Chihuahuan Desert. Leptophlebiidae (Order: Ephemeroptera) comprised the most abundant family (21% in relative abundance), followed by Cheumatopsyche (Order: Trichoptera; 14%) and Simuliidae (Order: Diptera; 7%). Macroinvertebrate assemblage changed along a downstream gradient, suggesting that upstream pollution inputs favor dipteran taxa at sites 1 and 2, although generalist species occurred at all sites. As water quality improved longitudinally, downstream assemblages shifted to favor ephemeropteran and trichopteran taxa.

Introduction

Arid aquatic environments represent unique and ecologically distinct systems ranging from highly stable ones (i.e., endorheic springs) to highly fluctuating (i.e., streams and rivers) along expansive discharge, water temperature, and salinity gradients (Fisher and Gray 1983; Herbst and Bromley 1984; Castleberry and Cech 1986; Stanley et al. 1994; Watson 2006). Wide ranging and fluctuating environmental conditions along with relatively few interconnections within and among drainages collectively influence the speciation of arid aquatic organisms, many of which are endemic to only small geographic regions (Stanley 1994; Poff et al. 1997; Richter et al. 2003; Fritz and Dodds 2005). Unfortunately, these habitats exhibit high susceptibility to anthropogenic perturbation. Dams, excessive surface and groundwater withdrawals, and point and non-point source pollution alter a suite of habitat characteristics, including flow regime, channel morphology, sediment transport, substrate components, nutrient availability, and riparian vegetation (Brown and Ford 2002; Bunn and Arthington 2002; Schmidt et al. 2003; Richter et al. 2003; Strayer 2006). Likewise, dams and dewatered sections of streams limit longitudinal connectivity of aquatic taxa (Bunn and Arthington 2002), thus impacting natural source-sink dynamics of metapopulations (Ligon et al. 2006).

The Rio Grande originates in the southern Rocky Mountains of western North America and meanders 2,800 km to the Gulf of Mexico. The majority of the basin lies within the Chihuahuan Desert ecoregion. Although a small number of studies exist on the macroinvertebrate assemblage in the Big Bend of the Rio Grande, little is known about the spatial and temporal associations of macroinvertebrates along the mainstem of the river. Davis (1980a) reported 83 taxonomic groups, including 14 ephemeropteran genera, 7 odonate genera, and 8 trichopteran genera, taken from eight sites in the Rio Grande between El Paso (TX) to Del Rio (TX).

The Rio Grande historically and currently is threatened with alterations to its biotic integrity, or pristine state, because of anthropogenic modifications. Geomorphology of the river changed dramatically during the past century through flow alteration, reduced sediment transport, invasive taxa, and water pollution (Schmidt et al. 2003). The intensity of flood events is reduced by 76% with the construction of dams for irrigation and recreation since 1915 (Schmidt et al. 2003). Consequently, dams converted the once shallow, braided river system to a single channel with steep banks throughout most of lower reaches of the Rio Grande (Dahm et al. 2005). Establishment of invasive plants, such as salt cedar (*Tamarix* sp.) and giant reed (*Arundo donax*), further exacerbate channel incision by stabilizing river banks and helping to prevent the river from changing course (Schmidt et al. 2003). Furthermore, these plants aid in dewatering portions of the river, especially during periods of low flow because of their high rates of evapo-transpiration (Shafroth et al. 2005). Slower decomposition rates of the invasive plants additionally alters nutrient processing rates and subsequently macroinvertebrate communities in the river (Bailey et al. 2001, Andersen et al. 2003; Kennedy and Hobbie 2004;). Decreased flow combined with a high concentration of people (> 1,000,000) and their wastewater discharge along the Rio Grande degrades water quality. Often, the Texas Commission on Environmental Quality (TCEQ) issues warnings because of the high concentration of coliform bacteria in the Rio Grande in Texas (www.ibwc.state.gov/wad/flowdata.htm) even in remote areas of the Rio Grande such as Big Bend National Park (BBNP). Historical flows have gradually decreased within the Rio Grande during the 1900s (Figure 1). The Big Bend reach of the Rio Grande probably represents the least impacted stretch of the Rio Grande because of its distance from heavily populated areas and because of less obvious alteration of stream flow through the area as compared to those in the New Mexico and lower Rio Grande near the Gulf of Mexico. Therefore, ecological integrity should be the greatest in Big Bend reach (Schmidt et al. 2003).

Rio Grande mainstem macroinvertebrates serve as critical components to the riverine and terrestrial communities. Macroinvertebrates play a vital role in the complex aquatic food webs, but also process nutrients and bacteria in the water, rendering the water more suitable for human use (Wallace and Merritt 1980). Additionally, because emergent macroinvertebrates typically live briefly as adults and die on land (Meffe and Minckley 1987; Gray 1981), these insects provide an important energy and nutrient flux to the surrounding arid terrestrial environment (Grimm 1988). Declines in macroinvertebrate abundance and diversity therefore can affect a multitude of aquatic and terrestrial organisms, by reducing nutrient and bacteria processing and cause losses of species diversity within the macroinvertebrate community (Goodnight 1973).

To develop a current understanding of macroinvertebrate populations within the Big Bend region of the Rio Grande, the objectives of this study included describing the current status of the macroinvertebrate community and to assess the influence of environment on macroinvertebrate distribution. Specifically, we assessed spatial and temporal patterns in physical and chemical habitat parameters among four sites within the Big Bend reach of the Rio Grande, described spatial and temporal patterns macroinvertebrate occurrence and abundance, and associated macroinvertebrate abundance with spatial and temporal patterns in physical and chemical habitat parameters. Understanding of macroinvertebrate distribution and habitat associations in the Big Bend reach will provide a baseline index for macroinvertebrate diversity within the drainage and to better predict how current and future anthropogenic modifications or restoration efforts will influence changes in the macroinvertebrate assemblage.

Study Area

The Big Bend region is located in the central stretch of the Rio Grande downstream from the confluence of Rio Conchos in Mexico and upstream from Lake Amistad along the United States/Mexico border (Figure 2). Four sites were selected along the mainstem of the Rio Grande in the Big Bend region. Site 1 occurred within Big Bend Ranch State Park near the dry bed of Contrabando Creek. Site 2 can be found at Santa Elena Canyon within Big Bend NP at the confluence of Terlingua Creek. Site 3 was located at Hot Springs within Big Bend NP at the confluence of Tornillo Creek; and Site 4 occurred farthest downstream at Texas Parks and Wildlife Department, Black Gap Wildlife Management Area (Black Gap WMA). All four sites have large riffle areas suitable for macroinvertebrate sampling with the use of a Hess sampler to provide comparable and quantitative efforts among sites and through time.

Methods

Macroinvertebrates were collected monthly from the four sites in 2006 following sampling protocols described by Barbour et al. (1999). At each site, two samples were collected using a 0.086 m² Hess sampler with 363 µm mesh. All samples were collected from shallow riffle areas with substrates <50 cm in diameter. For each Hess sample, two individuals cleaned rocks for 120 seconds, ensuring that all insects were removed from substrata before discarding. We used an invertebrate kick net (1.0 m x 1.0 m, 0.5 mm mesh) in swifter and deeper habitats. One collector disturbed substrate in a one meter area by continuously kicking for 60 seconds, allowing for invertebrates and debris to catch in the net downstream. After collection, specimens were picked from the debris for 15 minutes, or until it took several minutes to find the next invertebrate (Growth et al. 1997). Hess and kicknet samples were stored in separate containers with 70% ethanol. Samples were then sorted in the laboratory and macroinvertebrates were identified to the lowest practical taxonomic level, usually Genus (Merritt and Cummings 2005). Macroinvertebrate taxa were also classified into functional feeding groups.

After macroinvertebrate collection, physical and chemical habitat parameters were estimated for each site. We established 16 to 30 quadrats in riffle areas within transects spaced 20 meters apart. At each transect, the team took two current velocity measurements (m/s), two depth estimations (cm), and ten substrate points from three to six quadrats (about 5 x 8 m) spaced equi-distance apart and spanning the width of the river, except during high flow conditions. Current velocities were measured with a Marsh-McBirney, Inc. Flo-Mate Model 2000. Substrate identification included the proportion of silt, sand, gravel (mean diameter < 11.5 mm), cobble (<33 mm), and boulder (> 70 mm; Parker 1989). Averages of current velocity, depth, and substrate type per quadrat provided monthly estimates of current velocity, depth, and percent substrate estimation per site and by month. A YSI Model 600 multiprobe water quality meter was used to measure temperature (°C), dissolved oxygen (mg/l), specific conductivity (µS/cm), pH, and turbidity (NTU) at each site. However, the average annual mean temperature, dissolved oxygen, specific conductivity, and pH measurements were obtained from two Texas Commission on Environmental Quality (TCEQ) monitoring stations located Castilon and Rio Grande Village to accurately estimate chemical habitat parameters of the Big Bend reach (http://www.ibwc.state.gov/Water_Data/rtdata.htm). TCEQ measurements did not include

turbidity, so monthly turbidity measurements were taken by site taken with the multiprobe water quality meter.

Statistical analyses

Spatial and temporal patterns were assessed in physical habitat parameters with Principal Components Analysis (PCA). Quadrat estimations of mean current velocity, mean depth, and percent substrate type (i.e., silt, sand, gravel, cobble, boulder, and bedrock) by site and month required z-scored transformation (Krebs 1999) and analysis with SAS (Proc Princomp; version 9.1; Cary, NC). Quadrat scores along PC axes 1 through 3 were enveloped to infer site differences along habitat gradients. Chemical habitat parameters were not included in PCA to avoid the influence different sampling times (e.g., diel fluctuations) among sites.

We assessed spatial and temporal patterns in macroinvertebrate abundance with semi-quantitative kicknet samples and Hess samples. The data was analyzed with an Analysis of Similarity (ANOSIM $\alpha = 0.05$; 9,999 permutations) using PRIMER 6.1.6 software package (Clarke 1993; Clarke and Warwick 2001). ANOSIM specifies average rank of matrices of similarity and dissimilarity (Bray and Curtis 1957) using binary data within and between samples (Clarke and Gorley 2006; Grown et al. 1997). Similarity percentage breakdowns (SIMPER) determined the most common taxa between sites as well as the least common taxa between sites. Diversity indices were determined among the four sample sites using the Shannon-Wiener Index. This function examines both species richness and evenness to determine the likelihood of an individual selected from a population at random (Margalef 1957; MacArthur and MacArthur 1961).

Canonical correspondence analysis (CCA; ter Braak 1986) was utilized to assess associations between stream habitat (e.g., substrate, current velocity, and depth), and macroinvertebrate community collected from quantitative Hess samples. Canonical correspondence analysis is a direct gradient analysis where an ordination of one multivariate matrix is constrained by a multiple linear regression on variables in a second matrix (McCune and Grace 2002).

Results

The first three axes of PCA explained 66% of the total variation in physical habitat parameters among the four sampling sites (Figure 3). The first PCA axis explained 28% of the total variation and contrasted relatively deeper and swifter habitats with cobble substrate from habitats with slower current velocities, shallower depths, and gravel and sand substrates. Generally, PCA axis I described habitat differences along a longitudinal gradient with upstream habitats (Sites 1 and 2) having shallower depths and more sand and gravel substrates and downstream habitats (Sites 3 and 4) having greater depths, primarily cobble substrates, and swifter current velocities. The second PCA axis explained 21% of the total variation and described a substrate gradient. Site 1 consisted primarily of bedrock and large boulders, whereas substrates at downstream sites consisted primarily of cobble. The third PCA axis accounted for 16% of total variation, describing a gradient of swift current velocity and sand substrates to habitats with slower current velocities dominated by cobble and bedrock substrate. Sites 1 and 4 overlapped in multivariate space, as both sites experienced swift currents and large substrate

size. Sites 2 and 3 also overlapped considerably in multivariate space, as both of these sites experience a strong stream influence.

Macroinvertebrates were collected from sites with shallow depths (range of means: 17.4 – 24.9 cm) and swift current velocities (0.39 – 0.64 m/s; Table 1). Cobble was the most abundant substrate among all sites (47.0 - 73.2%), followed by gravel (4.2 – 33.6%) and bedrock (0 – 14.3%). Sand and silt collectively were <6% of the mean substrate composition by site. Annual mean turbidity ranged from 234.8 to 379.1 NTU by site. Mean (± 1 SD) or median chemical parameters, which were obtained from two TCEQ monitoring stations, ranged between 23°C (± 6.0) and 26 (± 5.2) °C for water temperature, 6.0 (± 2.3) and 6.0 (± 3.4) mg/l for dissolved oxygen, 1,699 (± 555.3) to 1,858 ($\pm 1,092.1$) μ S/cm for specific conductivity, and 7.0 (range: 6.0 – 8.0) and 7.5 (range: 7.0 – 8.0) for pH.

Overall, 9,505 macroinvertebrates were collected from Site 1 ($n = 1,716$), Site 2 ($n = 1,777$), Site 3 ($n = 1,829$), and Site 4 ($n = 4,183$). The Big Bend region of the Rio Grande macroinvertebrate community differed among sites (ANOSIM; Global $R = 0.104$, $P = 0.01$) with the macroinvertebrate community at Site 1 differing significantly ($P < 0.01$) from those at sites 3 and 4. The Tricopteran genus *Cheumatopsyche*, which comprised 24% of the total invertebrate assemblage, dominated Site 1, followed by two dipteran families, Chironomidae (27.3%) and Simuliidae (21.4%; Table 2). Members of genus *Thaurodes* dominated Site 2, comprising 23.2% of the invertebrate assemblage of the site, followed by family Simuliidae (14.6%), and genera *Traverella* (12.9%) and *Helichus* (12.5%). Site 3 was dominated by genus *Traverella* (50.4%) followed by *Cheumatopsyche* (14.1%), *Erpetogomphus* (4.8%), and *Helichus* (4.3%). Site 4 was dominated by family Leptophlebiidae, specifically the genera *Traverella* (66.91%), *Thaurodes* (4.81%), *Helichus* (10.2%), and *Cheumatopsyche* (4.8%). Total assemblage of the four study sites consisted primarily of filterer and collector-filterer taxa (68.9%), followed by collectors-gatherers (24.1%), predators (6.5%), collectors-detritivores (0.7%), scrapers (0.4%), and shredders (0.2%; Merritt and Cummings 2005). Shannon-Wiener diversity indices suggested low diversity, the highest at Site 2 (1.78), followed by Site 3 (1.77), Site 1 (1.74), and Site 4 (1.31).

Habitat, season, and site explained 43% of the variability within the macroinvertebrate assemblage (Figure 4). Pure effects for season explained 8% of the assemblage variation ($P < 0.01$), whereas pure site effects explained 11% ($P = 0.17$) and pure habitat effects explained 14% ($P = 0.20$). Significance difference among seasons was attributed primarily to the high abundance of some taxa (i.e., Simuliidae and Chironomidae) in late fall and winter before spring emergence, and the high abundance of other taxa (i.e., *Traverella* and *Thaurodes*) during the summer before their Fall emergence (Figure 5). Although macroinvertebrate community at Site 1 differed from the remaining three (ANOSIM), site did not explain significant variation in the macroinvertebrate community with habitat and season held as co-variates. Likewise, habitat did not explain significant variation in the macroinvertebrate community. However, site and environmental gradients expressed by CCA axes still provide predictors of community structure because of pure and partial (2- and 3-way) interactions among season, site, and habitat.

Environmental factors with the highest positive centroids on CCA axis I were July-Sept (0.96), Black Gap (0.93), and Apr-June (0.76). Habitat factors with the highest negative centroids were Contrabando (-0.76), silt substrate (-0.71), and Jan-Mar (-0.71). Macroinvertebrate species were highly correlated ($r^2 = 0.89$) to the CCA axis I. Biplot scaling score included *Neptopsyche* (1.38), *Neochoroterpes* (1.36), *Hygrotus* (1.13), *Haplotaxida* (1.08), *Trichocorixa* (0.98), *Gyretes* (0.88), *Corbicula* (0.72), *Stenophysa* (0.52), *Traverella* (0.50),

Helichus (0.46), *Microcylloepus* (0.21), *Argia* (0.17), *Hetaerina* (0.16), *Erpetogomphus* (0.11), and *Fallceon* (0.09). Taxa negatively associated with axis I included *Protoptila* (-1.00), *Ambryus* (-0.71), *Corydalis* (-0.69), *Callibaetis* (-0.61), *Sphaerium* (-0.55), Chironomidae (-0.50), *Cryphocricus* (-0.45), Simuliidae (-0.44), Tabanidae (-0.43), *Tricorythodes* (-0.33), *Cheumatopsyche* (-0.10), and *Thaurodes* (-0.02).

Discussion

Dominance of ephemeropterans, dipterans, and trichopterans (84% across all sites) in the study measured less than the overall abundance of these three taxonomic groups (93%) in 1975 - 1977 (Davis 1980a). Overall relative abundances increased from 39.6% in 1975 – 1977 to 54.8% in 2006 for ephemeropterans, slightly decreased from 21.5% to 17.7% for dipterans, and decreased from 31.6% to 11.4% for trichopterans. Average diversity indices changed from 2.14 in 1975 to 1.65 in 2006. Shifts in diversity indices are probably a result of taxonomic resolution within Diptera, as the 1975-1977 study classified dipteran taxa to Genus or Species level. Observed differences in macroinvertebrates abundance and diversity through time likely did not relate to differences in water years between time periods because mean daily discharge (\pm SD) in 1976 ($28.8 \pm 33.4 \text{ m}^3/\text{s}$) and 1977 ($19.6 \pm 33.4 \text{ m}^3/\text{s}$) fell within the range of mean daily discharge in 2006 ($19.1 \pm 31.8 \text{ m}^3/\text{s}$) and all three measured lower than the mean daily discharge (34.4 ± 75.7) on record (1936 – 2007; International Boundary Water Commission gauging station at Castolon; 08-3750). Instead, we expect observed differences to be a result of improvements in water quality through time. This is illustrated by the increased abundance of ephemeropterans, which are often associated with higher water quality (Baumgardner and Bowles 2005) and decreased abundance of dipterans and *Cheumatopsyche*, which generally are associated with areas of lower water quality such as water with high levels of nutrients and bacteria (Fuller et al. 1988; Edwards 1987; Wallace and Merritt 1980).

Despite moderate improvements of water quality, the Rio Grande macroinvertebrate community is still impacted by poor water quality. For example, we found higher abundances of dipteran taxa at Site 1, which decreased along a downstream gradient. This was contrasted by lower abundances of ephemeropteran taxa at Site 1, which increased at the downstream sites. Although I found habitat similarities between sites 1 and 4 as well as sites 2 and 3, macroinvertebrate assemblages at these sites instead changed longitudinally. Davis (1980a) found similar assemblage trends in his study, although 87% of the most upstream site consisted of *Corbicula manilensis*, *Homoeoneuria*, *Hydrobaenus*, *Oligochaeta*, *Palpomyia tibialis*, and *Paraclodopelma*. Farther downstream at Lower Presidio, the 71% of the dominant taxa consisted of *Cheumatopsyche*, *Simulium*, *Thaulodes*, *Traverella*, and *Orthocladius*. The five dominant taxa at each site continued to drop in percentages after reaching a small increase in percent dominant taxa at Santa Elena (Site 2 of this study). Both Davis (1980a) and this study suggest that there is an upstream bacterial pollution input that occurs above the Big Bend region of the Rio Grande that affects macroinvertebrate assemblages. This study also illustrates water quality improvement along a downstream gradient as reflected by the dominant taxa.

Furthermore, a 2003 water quality survey by the Texas Clean Waters Program at the International Boundary and Water Commission reported that water from the Rio Grande at Lajitas (in close proximity to Site 1) was unfit for human consumption due to elevated levels of chloride, sulfate, total dissolved solids, and fecal coliform bacteria. The survey concludes that

ingestion of the bacteria present in the river by humans could lead to gastrointestinal disease. The source of the pollution at this site has not been determined, although there are confirmed pollution inputs upstream at Presidio from wastewater (IBWC 2003). As a result of the large populations of filter feeding macroinvertebrates in the upstream reaches of the study region (Sites 1 and 2), the macroinvertebrate assemblages downstream (Sites 3 and 4) have reduced densities of filter feeding taxa. In this sense, the filter feeding taxa upstream are reducing their own downstream densities by consuming detritus in drift and allowing for a greater diversity of macroinvertebrates at Sites 3 and 4 (Wallace and Merritt 1980).

Although the likely pollution influences are likely a result of nutrient inputs from agricultural or municipal waste, other types of pollution, such as DDT and toxaphene, may pose an additional threat to the ecological integrity of the Rio Grande. Elevated levels of DDE and toxaphene were reported in fish and avians in the early 1980s in the lower Rio Grande Valley (White et al. 1983). Additionally, Davis (1980c) suggested elevated levels of the pesticide residues DDT and DDE in sediments from the Rio Conchos may reduce macroinvertebrate diversity in the areas directly downstream, thus favoring more tolerant taxa. Leptophlebiids, such as the *Traverella*, were also reported to have deformities of the eyes and appendages as a possible result of these pesticide residues (Davis 1980a).

Though impacted, the macroinvertebrate community in the Big Bend reach is similar to those in other rivers within the Rio Grande drainage and in other rivers in more humid areas of the southwest. The Devils River, a relatively non-impacted and spring-flow influenced tributary located downstream from this study area, has at least 26 taxa in common with the Big Bend region (Davis 1980a). Likewise, the Pecos River, a highly impacted tributary also located downstream of this study area, consists primarily of ephemeropterans (Leptophlebiidae; 30.7%), trichopterans Hydropsychidae (7.3%), and Hydroptilidae (5.4%) and dipterans Chironomidae (6.8%) and Simuliidae (1.0%; Davis 1980b), 26 of which were found at my study sites within the Big Bend Region of the Rio Grande. Because these similar taxa are found in both disturbed systems, such as the Rio Grande and Pecos River, as well as less impacted systems such as the Devils River, it can be inferred that these generalist taxa are present as a result of their tolerance to natural stressors from an arid environment. Although human alterations to the environment have increased the number of filter-feeding taxa within the Big Bend region of the Rio Grande in the past thirty years (Davis 1980a) in response to increase nutrient levels (Wallace and Merritt 1980), the diversity of west Texas arid rivers is inherently low. The Rio Grande, although similar to other arid drainages, varies substantially from more temperate regions, such as areas of central Texas. In the Blanco River of central Texas, dipterans Chironomidae and Simuliidae are the most abundant macroinvertebrates within the assemblage, the two families comprising approximately 20% of relative abundances (Pendergrass 2006). The Rio Ayuquila of west-central Mexico consists primarily of 79 major taxa, including Corydalidae, Elmidae, Baetidae, Leptophlebiidae, Tricorythidae, and Hydropsychidae (Weigel et al. 2002). Both the Blanco River and the Rio Ayuquila experience fewer flash floods, thus housing fewer generalist taxa than rivers in arid regions, such as the Rio Grande.

Physical water parameters between upstream (Sites 1 and 2) and downstream (Sites 3 and 4) reaches of the Rio Grande did not differ indicating that the differences in macroinvertebrate assemblages between study sites can not be attributed to differences in pH, dissolved oxygen, temperature, and conductivity. Habitat characteristics (as seen in PCA) are similar as well, differing only in comparison between Sites 1 and 4. Sites 2 and 3 shared very similar habitat characteristics in the PCA, likely because both are influenced by small desert streams and have

very similar substrate. Further alterations in habitat as a result of dams or irrigation may favor more generalist species and dramatically reduce overall species diversity within the system.

Overall, the macroinvertebrate assemblage from the benthic regions of these study sites provided an accurate picture of the aquatic ecosystem of the Rio Grande. Limitations of this study include a lack of macroinvertebrate sampling from deep pools, eddies, and side channels. Additionally, further information could be acquired from stream inputs, such as Terlingua Creek and Tornillo Creek; greater macroinvertebrate diversity has been found in small tributaries and ephemeral pools within Big Bend (Bane and Lind 1978). These results could be used to continue monitoring water quality in the Rio Grande. Decreased flows alone may produce a more uniform macroinvertebrate assemblage, consisting primarily of Simuliidae and Chironomidae.

Filter feeding macroinvertebrate taxa play an important role in the overall structure and function of the aquatic ecosystem of the Rio Grande – primarily through the reduction in nutrient load and bacterial content in water and sediments. This is of particular importance for human recreational purposes, specifically at Lake Amistad in the middle Rio Grande downstream from Big Bend National Park. Because this lake supports a large bass population, it attracts numerous recreational anglers from both the United States and Mexico. The ability of the river to repair itself through nutrient processing by macroinvertebrates has allowed for a not only a successful fishery, but also cleaner water availability for downstream municipalities.

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TABLE 1. Mean water quality and substrate values of Big Bend sample sites, January through December, 1996.

Variable	Site 1	Site 2	Site 3	Site 4
Current Velocity (m/s)	0.39 ± 0.24	0.64 ± 0.10	0.53 ± 0.12	0.56 ± 0.16
Depth (cm)	17.4 ± 7.5	17.8 ± 4.2	17.5 ± 4.0	24.9 ± 4.9
Substrate (%)				
Silt	0.3	0.9	1.7	0.0
Sand	0.0	0.7	4.1	0.0
Gravel	14.4	33.6	19.8	4.2
Cobble	47.0	64.6	73.2	71.8
Boulder	24.0	0.3	1.7	24.0
Bedrock	14.3	0.0	0.0	0.0
Temperature (°C)	18.6 ± 7.3	18.02 ± 7.4	22.54 ± 6.2	20.03 ± 6.3
pH	8.18	8.26	7.98	8.09
Turbidity *	370.66 ± 511.5	234.77 ± 458.6	379.09 ± 563.8	326.01 ± 531.6

* Nephelometric turbidity units

TABLE 2. Taxonomic classification and relative abundance of macroinvertebrates in the Rio Grande, Big Bend region, Texas, January through December 2006.

Class	Order	Family	Genus or Scientific Name	Trophic Guild	Percent Abundance			
					Site 1	Site 2	Site 3	Site 4
				Collectors-gatherers			0.11	
Bivalvia	Pelecypoda	Sphaeriidae	<i>Corbicula</i>	Filterers	0.12		0.05	0.38
			<i>Sphaerium</i>	Filterers		0.11		
Gastropoda	Limnophila	Physidae	<i>Stenophysa</i>	Scrapers	0.06	0.06	0.38	
Oligochaeta	Haplotaxida			Collectors-gatherers	0.87		0.16	0.65
Arachnida	Acarina	Acari		Predators			0.11	
Insecta	Ephemeroptera	Baetidae	<i>Calibaetis</i>	Collectors-gatherers	1.77	0.51		1.05
			<i>Fallceon</i>		1.28	3.83	1.75	0.31
		Heptageniidae	<i>Neochoroterpes</i>		0.12		0.27	
		Leptophlebiidae	<i>Thaurodes</i>		8.16	23.19	3.94	4.81
			<i>Traverella</i>	Collectors-filterers	1.81	12.90	50.41	66.91
		Tricorythidae	<i>Tricorythodes</i>	Collectors-gatherers	3.73	1.97	3.66	0.24
	Odonata	Coengrionidae	<i>Argia</i>	Predators	0.70	1.18	4.43	1.98
		Calopterygidae	<i>Hetaerina americana</i>			0.11	0.44	0.22
		Gomphidae	<i>Brachemorhoga</i>			0.11		0.07
			<i>Erpetogomphus</i>		0.93	0.96	4.81	0.79
		Macromiidae	<i>Macromia</i>		0.06			
	Hemiptera	Corixidae	<i>Trichocorixia</i>		0.06		0.16	0.10
		Naucoridae	<i>Ambryus</i>		0.41	0.17		0.05
			<i>Cryphocricos</i>		0.12	0.39	0.22	
		Veliidae	<i>Helocharus</i>			0.06		
	Megaloptera	Corydalidae	<i>Corydalis cornutus</i>		1.17	0.96	0.22	0.84
	Trichoptera	Glossomatidae	<i>Protoptila</i>	Scrapers	0.12	0.39	0.11	
		Leptoceridae	<i>Neptopsyche</i>	Shredders			0.05	0.43
		Hydropsychidae	<i>Cheumatopsyche</i>	Collectors-filterers	23.78	11.42	14.05	4.81
	Lepidoptera	Pyalidae	<i>Petrophila</i>	Shredders	0.12			
	Coeloptera	Dytiscidae	<i>Hygrotus</i>	Predators			0.05	0.02
		Elimidae	<i>Microcylloepus</i>	Collectors-detritivores	0.93	0.51	0.77	0.41
		Dryopidae	<i>Helichus</i>	Scrapers, Collectors-gatherers	4.78	12.49	4.26	10.23
		Gyrinidae	<i>Gyretes</i>	Predators	0.17	0.73	0.60	0.36
	Diptera	Simuliidae		Collectors-filterers	21.40	14.63	3.32	4.26
		Tabanidae		Predators	0.06	0.28	0.16	0.17
		Chironomidae		Collectors-gatherers, filterers	27.27	11.09	5.47	0.93
			N		1,716	1,777	1,829	4,183

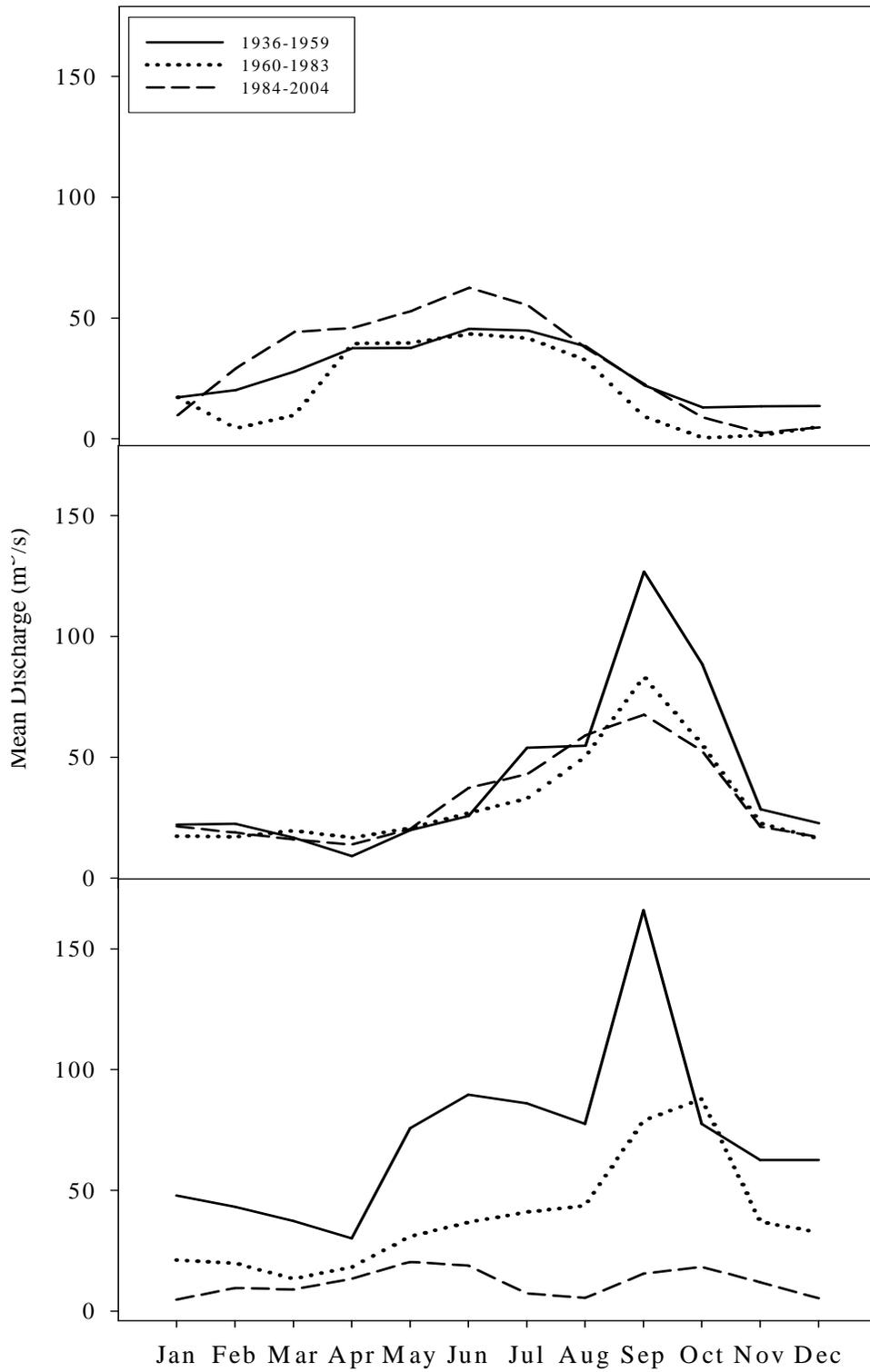


FIGURE 1. Mean annual discharge of the Rio Grande at a) Elephant Butte, New Mexico, b) Rio Grande Village, Big Bend National Park, Texas, and c) Brownsville, Texas.

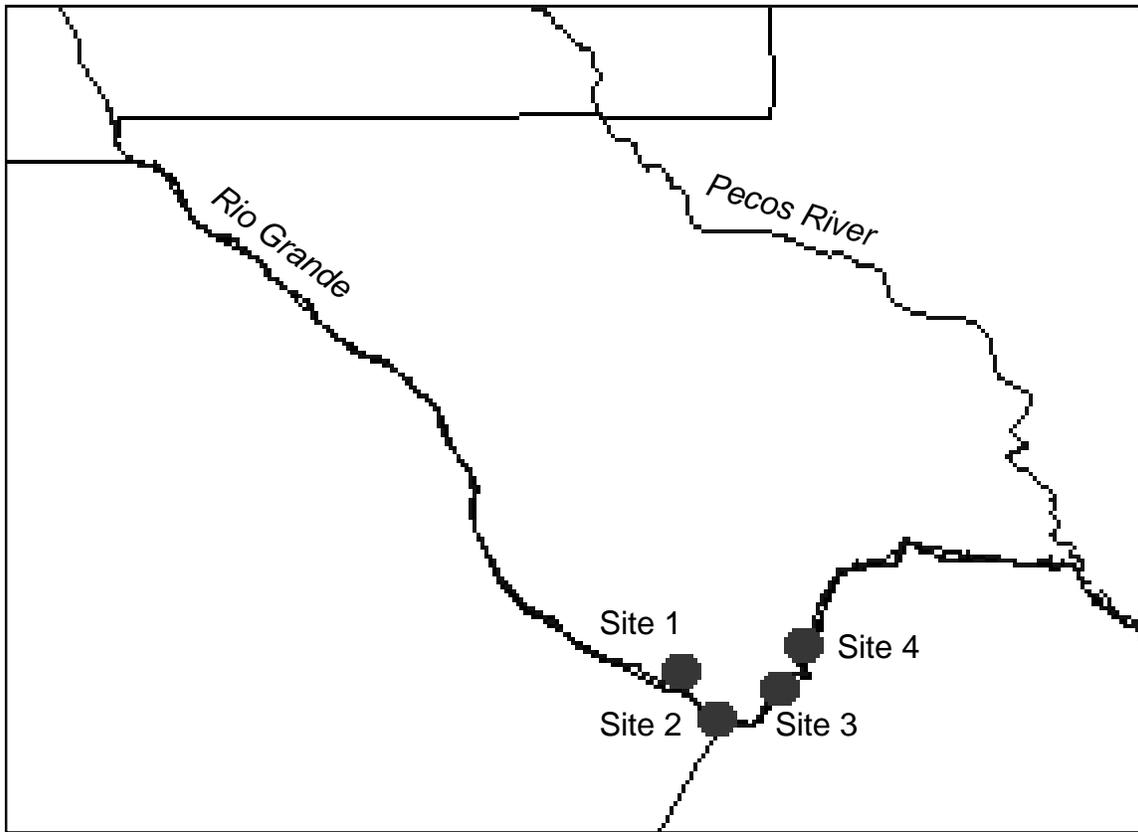


FIGURE 2. Locations of sampling sites in the Big Bend region of Texas. We categorized Contrabando as Site 1, Santa Elena as Site 2, Hot Springs as Site 3, and Black Gap as Site 4.

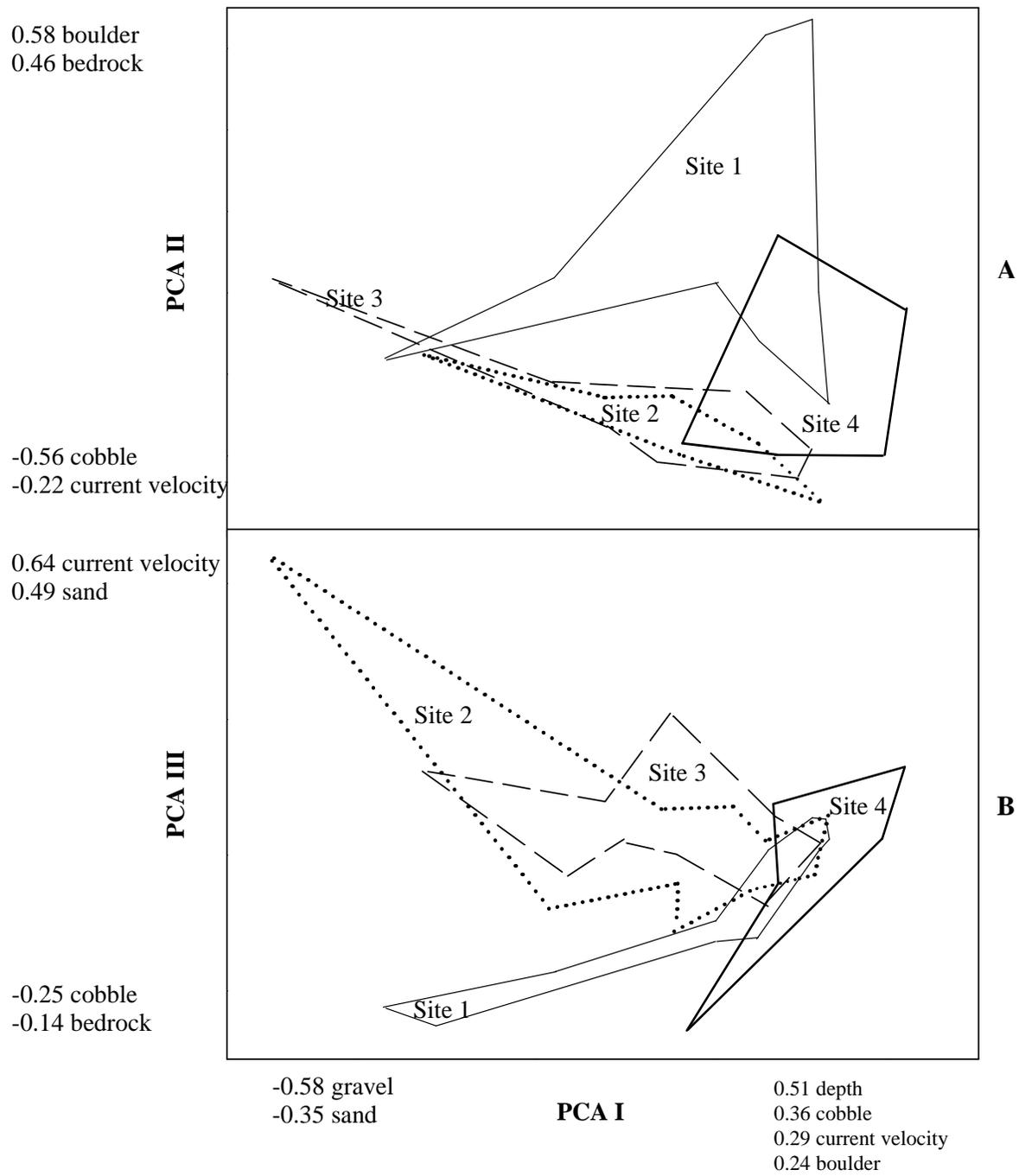


FIGURE 3. Principal components analysis (PCA) of habitat data collected from the Big Bend region. Individual sites are contained in ordination space, and habitat loadings of a) PCA I and PCA II and b) PCA I and PCA III are located in the margins.

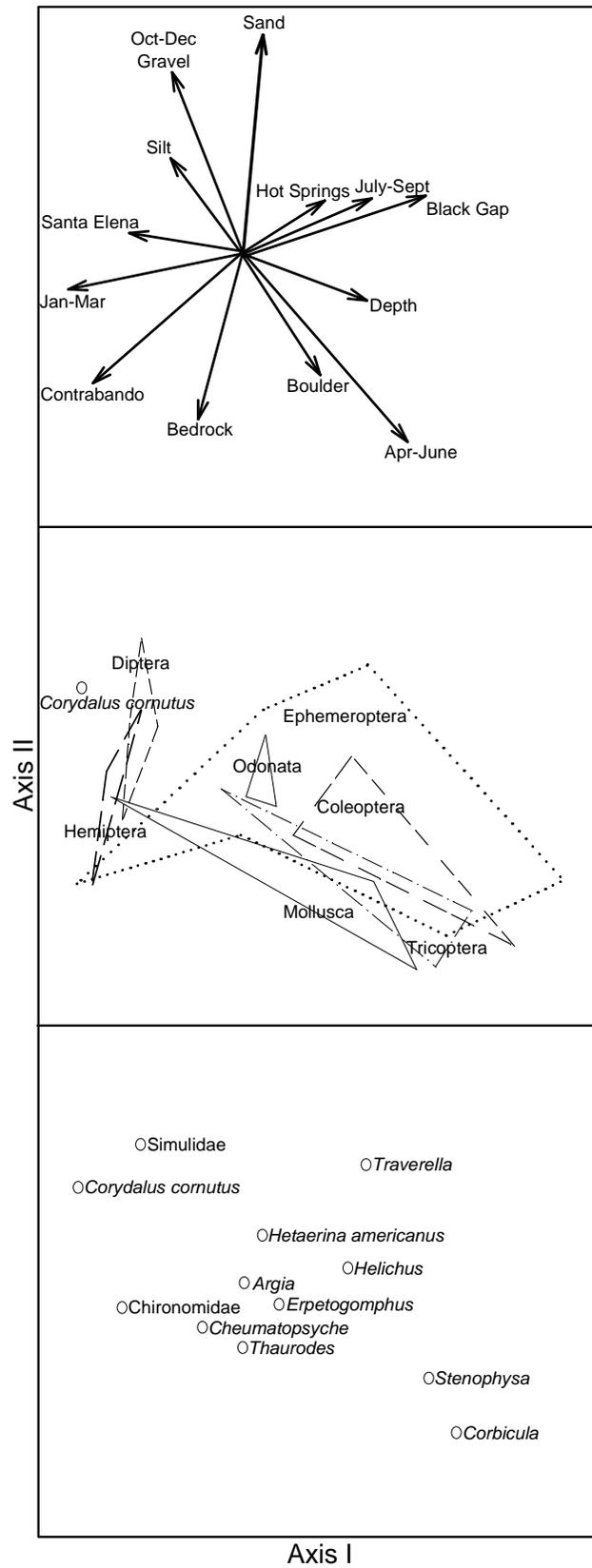


FIGURE 4. Canonical components analysis (CCA) of species and habitat data collected from the Big Bend region, Texas.

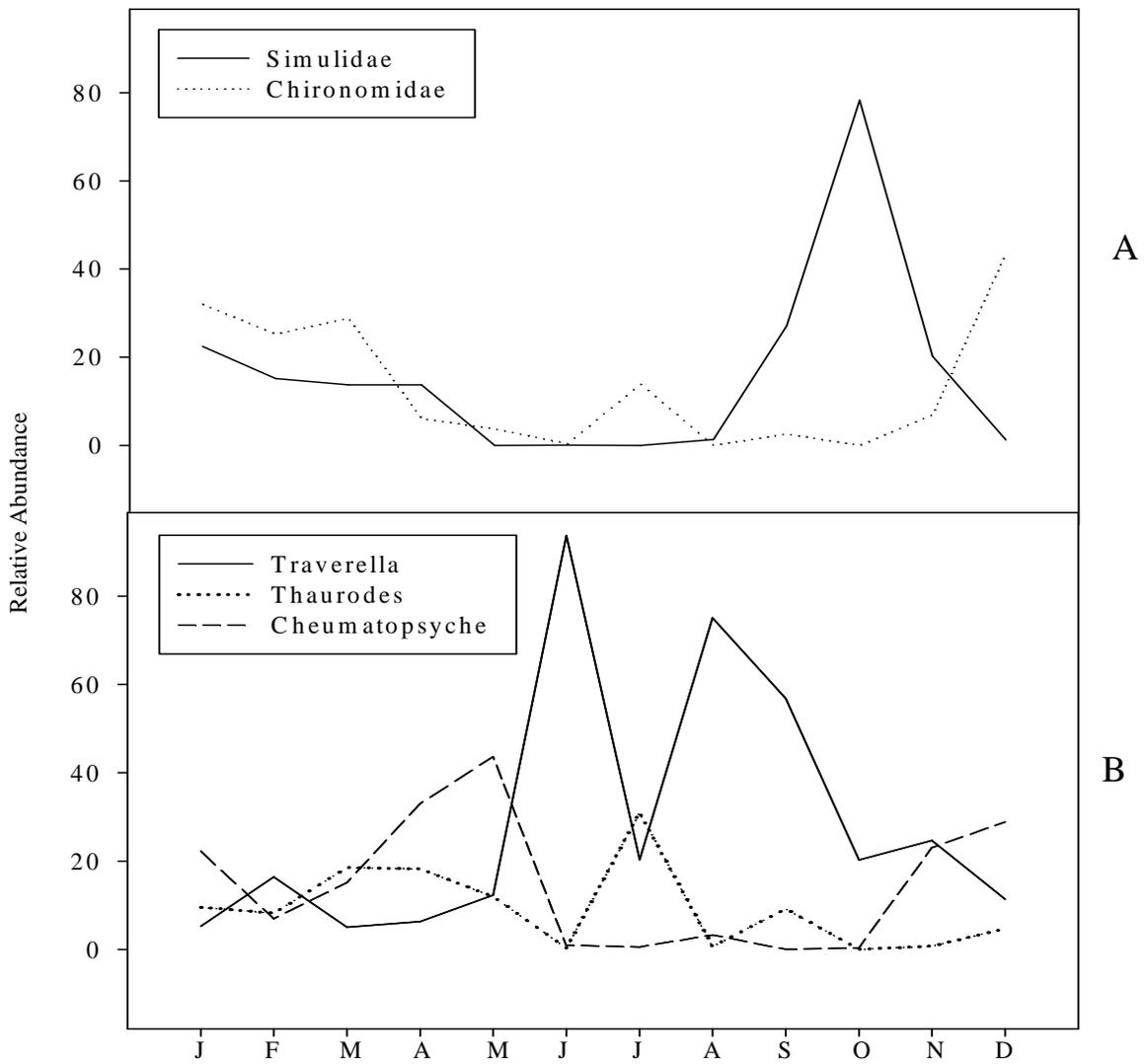


FIGURE 5. Monthly relative abundance of a) summer emergent macroinvertebrate taxa versus b) spring emergent macroinvertebrate taxa. Specimens collected from the Big Bend region,, Texas, January through December 2006.

APPENDIX I

First Record of *Bothriocephalus acheilognathi* in the Rio Grande with Comparative Analysis of ITS2 and V4-18S rRNA Gene Sequences

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Abstract.—*Bothriocephalus acheilognathi* is an introduced tapeworm in North America often reported as a serious ecological threat to native fishes. In this paper, we report the first record of *B. acheilognathi* in the Big Bend region of the Rio Grande in Texas (known as the Río Bravo del Norte in Mexico). Identification of *B. acheilognathi* was confirmed by morphologic and genetic techniques (sequences of ITS2 and V4-18S rRNA genes). Its prevalence was 27% and its intensity ranged from 1 to 5 individuals in a January 2006 collection of 115 red shiners *Cyprinella lutrensis*. In addition, it was found in the Tamaulipas shiner *Notropis braytoni*, a Rio Grande endemic and a new host record. The occurrence of *B. acheilognathi* might have negative ecological impacts on endemic fishes in the Rio Grande. Several of the fishes that could serve as definitive hosts are of conservation concern. Its occurrence also might affect the success of reintroducing the Rio Grande silvery minnow *Hybognathus amarus*, which is federally listed as endangered, in this portion of the Rio Grande.

The tapeworm *Bothriocephalus acheilognathi* infects over 100 species of fish in Africa, America, Asia, Australia, and Europe and is considered a threat to populations of endemic, commercial, and hatchery fishes (Körting 1975; Hoffman 1980; Hoffman and Schubert 1984; Salgado-Maldonado and Pineda-López

2003). It is recognized as a causative agent of detrimental infection in aquaculture operations in Asia and Europe, where it has been reported to cause 100% mortality in some hatchery ponds (Liao and Shih 1956; Körting 1975). *Bothriocephalus acheilognathi* requires as little as 2 weeks to complete its life cycle in the intermediate host and has low definitive and intermediate host specificity (Körting 1975). Eggs are passed with the feces of the fish and mobile coracidia emerge from the eggs after embryonation. The coracidia are consumed by the intermediate host, cyclopoid copepods (e.g., those of the genera *Acantocyclops*, *Macrocylops*, *Mesocyclops*, *Tropocyclops*, and *Diacyclops*; Körting 1975; Marcogliese and Esch 1989; Díaz-Castaneda et al. 1995). The life cycle is completed when fish ingest infected copepods.

Low host specificity enables *B. acheilognathi* to rapidly colonize new drainages (Marcogliese and Esch 1989; Dove and Fletcher 2000). The natural geographic range of *B. acheilognathi* is Japan (where it was originally described by Yamaguti in 1934), China, and the Amur River basin in the Russian Far East (Bauer and Hoffman 1976; Pool and Chubb 1985; Pool 1987; Scholz 1997). One of the tapeworm's native hosts is the grass carp *Ctenopharyngodon idella* (Choudhury et al. 2006). *Bothriocephalus acheilognathi* was introduced into nonnative areas around the world, including North America, when infected grass carp were

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imported for macrophyte control (Hoffman 1980; Andrews et al. 1981). It occurs in six drainages in Mexico and in Lake Winnipeg in Canada (Salgado-Maldonado and Pineda-López 2003; Choudhury et al. 2006). In the USA, *B. acheilognathi* occurs in the Colorado River drainage in Arizona, the Virgin River in Nevada, Arizona, and Utah, Belevs Lake in North Carolina, the Yampa River in Colorado, Peter Lake in Wisconsin, and the South Platte River in Nebraska (Granath and Esch 1983b; Heckmann and Deacon 1987; Brouder and Hoffnagle 1997; Ward 2005; Choudhury et al. 2006). The tapeworm also has been reported in Kentucky, Arkansas, and New Mexico (Choudhury et al. 2006). Transfer into new drainages within the USA is attributed to baitfish introductions (Heckmann et al. 1993).

Methods

In January 2006, 115 red shiners *Cyprinella lutrensis* (total length, 19–39 mm) were collected from the Rio Grande (known as the Río Bravo del Norte in Mexico) at Santa Elena Canyon near the confluence with Terlingua Creek in Big Bend National Park (Figure 1). Fish were taken with a 3-m × 1.8-m seine (mesh size, 1.8 mm) and preserved in 10% solutions of formalin. In the laboratory, the gastrointestinal tracts of the fish were removed. Tapeworms were teased from the intestinal lining and initially identified by their heart-shaped scolex with a pair of deep bothria (Scholz 1997). Tapeworms were enumerated in each fish to determine the prevalence and intensity of infection (see Margolis et al. 1982 for terminology).

Additional seine hauls made in January 2006 captured red shiners and Tamaulipas shiners *Notropis braytoni*. Five red shiners (samples 06/31–35) were preserved in 70% ethanol for genetic analysis (ITS2 and V4-18S rRNA genes) of *B. acheilognathi*. The remaining fish were kept alive in aerated containers and transported to the laboratory. Gastrointestinal tracts were removed from freshly killed red and Tamaulipas shiners and tapeworms removed from the intestinal lining. These specimens were stained with Mayer's hydrochloric carmine solution and mounted in Canada balsam as permanent preparations deposited in the U.S. National Parasite Collection, Beltsville, Maryland (collection number USNPC 98874) and the helminthological collection of the Institute of Parasitology of the Academy of Sciences of the Czech Republic (collection number C-15).

Total DNA was extracted from 0.5 cm of strobila using the DNeasy Tissue Kit (Qiagen, Sigma, St. Louis, Missouri). To amplify the sequences of ITS2 and the V4 region of the 18S rRNA gene, the primer sets Proteo1 (5'-CGG TGG ATC ACT CGG CTC-3'),

Proteo2 (5'-TCC TCC GCT TAT TGA TAT GC-3'), Ces1 (5'-CCA GCA GCC GCG GTA ACT CCA-3'), and Ces2 (5'-CCC CCG CCT GTC TCT TTT GAT-3') were used (Škeříková et al. 2001; Scholz et al. 2003). The polymerase chain reaction (PCR) program was as follows: 15 min at 95°C (Hotstar *Taq* DNA polymerase, Qiagen, Sigma); 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, and 2 min extension at 72°C; and final extension for 10 min at 68°C.

The PCR products were cloned into pGEM-T Easy system 1 (Promega, Madison, Wisconsin) and sequenced in both directions using T7 and SP6 primers. DNA sequencing was performed on an ABI PRISM Model 310 automated sequencer (PE-Biosystems, Foster City, California) using the GenomeLab DTCS-Quick Start Kit (Beckman Coulter, Fullerton, California). The sequences were deposited in GenBank under the accession numbers DQ866988–DQ866997.

The sequences were sent to the Basic Local Alignment and Search Tool (BLAST) program in GenBank for comparison with other sequences in public databases. *Bothriocephalus acheilognathi* from a kawar *Leuciscus lepidus* collected by Shamall Abdullah in Iraq were used as a reference sample (GenBank accession numbers AY 340121 [ITS2 sequence], AY 340106 [V4-18S rRNA sequence]; see Škeříková et al. 2004). To assess the similarity among the sequences obtained, the Martinez-Needleman-Wunsch method, as implemented in the program MEGALIGN (DNASTar, Nevada City, California), was used.

Results

The lengths of the ITS2 and V4-18S rRNA gene sequences obtained from the five Rio Grande samples were 783–795 and 460 base pairs, respectively (Table 1). The tapeworms were identified as *B. acheilognathi* on the basis of their similarity to the reference sequences of this cestode species available in GenBank.

The ITS2 sequences of the five samples showed a similarity of 93.8–99.1%, and comparison with the sequence of the Iraqi reference sample from GenBank revealed 95.1–96.8% similarity (Table 1). Comparison with the other 27 sequences of the ITS2 gene of *B. acheilognathi* from different localities accessible in GenBank showed similarities between 94.2% and 99.9% (data not shown). The greater similarity of the ITS2 sequences between the Texas samples and previously sequenced samples than within the Texas samples themselves might indicate multiple, independent colonization of *B. acheilognathi* into the Rio Grande. However, previous studies have demonstrated

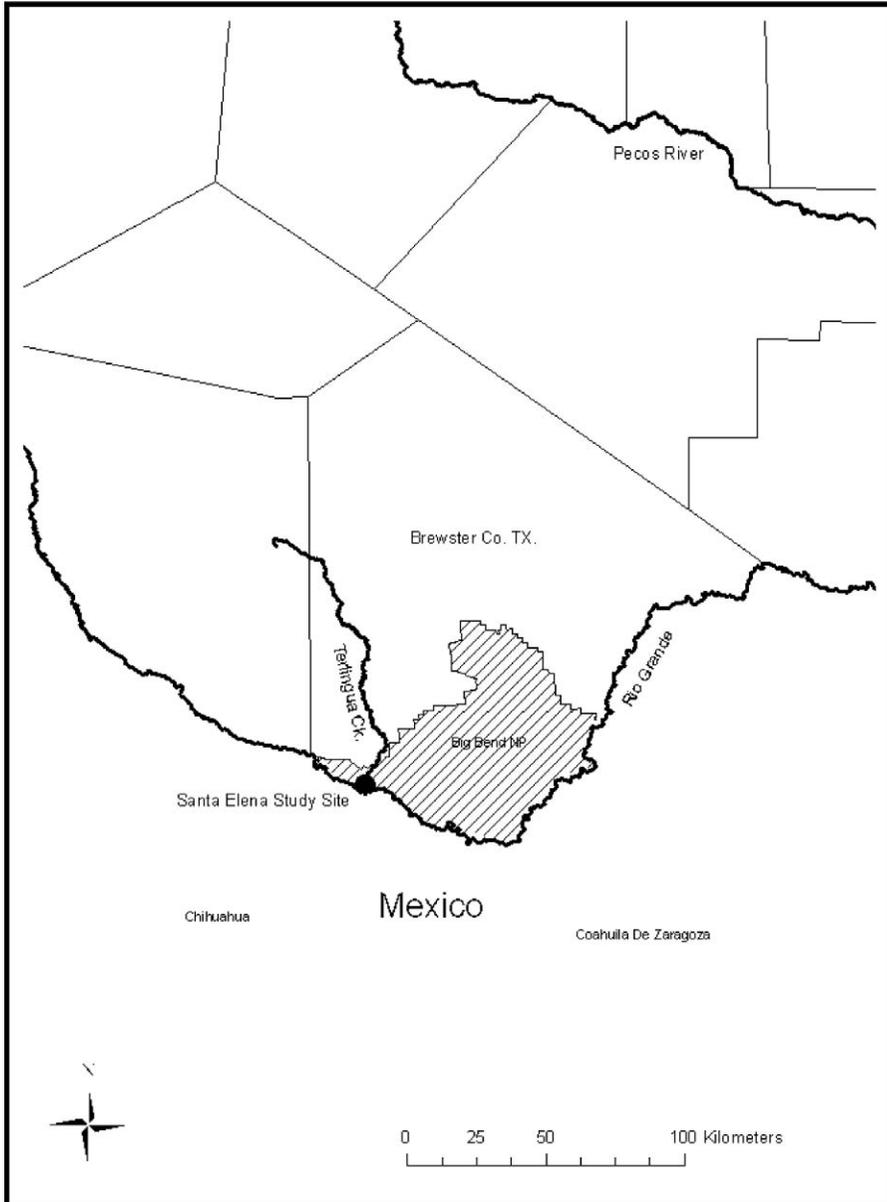


FIGURE 1.—Map of the Big Bend region, Texas, showing the location of the study site at Santa Elena Canyon.

high intraspecific variation in ITS sequences in several species of *Bothriocephalus* (Luo et al. 2002; Scholz et al. 2004; Škeříková et al. 2004).

Comparison of the V4-18S rRNA sequences demonstrated only negligible differences among the samples from the Rio Grande (similarity, 99.8–100%), indicating that the specimens are conspecific. The similarity of the Rio Grande sequences to those of the Iraqi samples in GenBank varied from 99.3% to 99.6% (Table 1).

In view of this sequence similarity and data on other species of *Bothriocephalus* (Škeříková et al. 2004), it is possible to consider all of the North American samples examined thus far to be conspecific with *B. acheilognathi*. This genetic analysis, combined with morphological comparisons, suggests that all of the tapeworms from the Rio Grande were indistinguishable from those from the wide spectrum of fish hosts and different geographical regions deposited in the helminthological collection of the Institute of Parasitology of the

TABLE 1.—Percent similarity and length of individual sequences of the tapeworm *Bothriocephalus acheilognathi* from red shiners from the Rio Grande, Texas (06/31–35) and kawar from Iraq (GenBank accession numbers AY 340106 and AY 340121). The ITS2 sequences are above the diagonal, the V4-18S rRNA gene sequences below the diagonal.

Sample ^a	06/31 (795 bp)	06/32 (790 bp)	06/33 (788 bp)	06/34 (783 bp)	06/35 (794 bp)	AY 340121 (805 bp)
06/31		94.8	94.1	93.8	94.1	96.8
06/32	100		99.0	96.4	99.1	95.7
06/33	99.8	99.8		96.2	98.6	95.1
06/34	100	100	99.8		96.9	95.2
06/35	100	100	99.8	100		96.3
AY 340106	99.6	99.6	99.3	99.6	99.6	

^a All samples 460 base pairs (bp) except AY340106, which is 459 bp.

Academy of Sciences of the Czech Republic (Scholz and Di Cave 1993; Scholz 1997).

Having confirmed the identification of *B. acheilognathi*, we found that its prevalence in red shiners was 27% and that its intensity ranged from one to five tapeworms per fish. We report the Tamaulipas shiner as a new host record for *B. acheilognathi* and expand the range of this invasive tapeworm in North America to include the Rio Grande.

Discussion

Bothriocephalus acheilognathi is considered to be a serious threat to endemic fishes in Mexico (Salgado-Maldonado and Pineda-López 2003). Pathogenic effects can include intestinal blockage and perforation, distended abdomen, necrosis, inflammation, hemorrhaging, loss of intestinal microvilli, loss of enterocytes, reduced growth, significantly decreased survivorship, and mortality (Scott and Grizzle 1979; Hoffman 1980; Granath and Esch 1983a; Hoole and Nisan 1994; Hansen et al. 2006). Consequently, the occurrence of *B. acheilognathi* might have negative ecological impacts on native fishes in the Rio Grande. The Rio Grande drainage fish assemblage includes several endemic cyprinids that are listed as species of conservation concern because of anthropogenic modifications (Hubbs et al. 1991) that are potential hosts for the tapeworm. These include the Rio Grande silvery minnow *Hybognathus amarus*, which is listed as endangered by the Texas Parks and Wildlife Department (TPWD), the U.S. Fish and Wildlife Service (USFWS), and Mexico (CONABIO 2002); the Devils River minnow *Dionda diaboli*, listed as threatened by TPWD and USFWS and as endangered by Mexico (CONABIO 2002); the Chihuahua shiner *Notropis chihuahua*, listed as threatened by TPWD and Mexico (CONABIO 2002) and classified as threatened by Hubbs et al. (1991); the Mexican stoneroller *Campostoma ornatum*, listed as threatened by TPWD and classified as threatened by Hubbs et al. (1991); the Rio Grande shiner *Notropis jemezianus*, listed as threatened by Mexico (CONABIO 2002) and classified as

threatened by Hubbs et al. (1991); and the Tamaulipas shiner, listed as threatened by Mexico (CONABIO 2002).

One fish of importance in the Big Bend region is the Rio Grande silvery minnow. This fish, the distribution of which once spanned 4,825 km of the Rio Grande from Colorado to Texas (Ikenson 2002), is now extirpated from Texas and only found in scattered locations in the Rio Grande in New Mexico (Hubbs et al. 1991; Bestgen and Platania 1991). The recovery plan for the Rio Grande silvery minnow (USDI 1999) lists the reach from the town of Presidio to Amistad Reservoir, which includes Big Bend National Park, as one of six reaches having the best reestablishment potential. With this discovery of *B. acheilognathi* in the Rio Grande, the success of reintroductions might be seriously jeopardized.

Acknowledgments

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