# MACROINVERTEBRATE RELATIONS WITH ECOREGIONS AND AQUIFERS AS INFLUENCED BY THE HYDROLOGY AND WATER QUALITY OF WESTERN TEXAS SPRING ECOSYSTEMS

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## INTRODUCTION

The most accessible sources of groundwater issue from springs and seeps (Kalff 2007). Discharges from springs maintain base flows in streams and rivers, particularly during periods of low rainfall, and remote spring-wetland systems provide water and habitat for a variety of plants and animals, particularly in arid to semi-arid ecosystems (Levick et al. 2007). Demand for fresh water is likely to increase (World Resources 1992) and potential changes in water availability associated with global climate change are poorly understood. Because of increasing demands of groundwater for human uses (Edwards et al. 2004) and the desire to maintain diverse ecological resources in these ecosystems, improved understanding of spring hydrology, potential sources of contamination, and the environmental factors that support aquatic organisms in these springs is required (Williams and Williams 1998, Kalff 2002).

Discharges from spring-fed ecosystems are diminishing due to decreases of groundwater levels (Edwards et al. 2004). Decreased groundwater levels are a consequence of increased human groundwater uses and technological advances in the extraction of groundwater resources (Edwards et al. 2004). Human impacts to desert spring ecosystems include over-pumping of aquifers for agricultural and residential development, water-quality contamination of aquifers, reservoir creation, and the introduction of non-native and invasive species (Edwards et al. 1989, Bowles and Arsuffi 1993, Edwards et al. 2004, Kennedy et al. 2005). Additionally, land-use practices such as

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deforestation and overgrazing adversely affect the stability of the landscape, exposing organic soils to erosion processes (Brune 1975, 1981). These processes result in immediate runoff of precipitation instead of natural slow infiltration, which may increase turbidity, export nutrients, and ultimately reduce recharge to local aquifers (Brune 1981).

Springs are a resource for aquatic flora and fauna; however, they also provide important habitats for terrestrial organisms (Shepard 1993). As a result, desert spring ecosystems are areas of heightened biodiversity because water is a crucial resource for many desert organisms (Shepard 1993). If not via stream or other aquatic connections, immigration of aquatic insects and other invertebrate organisms could presumably occur as a result of flying from nearby permanent water bodies, being transported by winds, or being carried in by animals (e.g. via the feet or feathers of birds, livestock, wildlife, or other insects); however, geographic and climatic constraints exert a large influence on the distribution and abundance of macroinvertebrates (Sweeney and Vannote 1978, Thorp and Covich 2001, Lencioni 2004). Topographic barriers (i.e. canyons, volcanic rifts), may affect macroinvertebrate dispersal. As a result, springs are presumably islands in geographic isolation which provide niches for endemic organisms to genetically evolve in the absence of predators in an environment with reduced durations in geological time (Gooch and Glazier 1991).

The occurrence and distribution of macroinvertebrates in and among springs can be influenced by many factors. The emphasis on studying these spring systems is borne out of a desire to gain a better understanding of ecological systems, to comprehend the potential links between biodiversity and ecosystem function (Schwartz et al. 2000, Loreau et al. 2001, Bond and Chase 2002), and to increase the efficacy of biodiversity conservation efforts. Traditionally community ecologists focused primarily on processes that structure diversity at *local* scales thereby implicitly considering the study environments as "closed" systems (Ricklefs 2004, Leibold et al. 2004). Ecologists have realized, however, that understanding *regional* scale processes are important to the understanding of local diversity patterns (Huston 1999, Shurin et al. 2000, Leibold et al. 2004, Ricklefs 2004, Freestone and Inouye 2006). In addition, patterns of regional taxonomic composition are influenced by local biotic and abiotic processes and the dispersal of individuals between local habitats (Ricklefs and Schluter 1993, Binks et al. 2005, Freestone and Inouye 2006). Historically, consideration of spatial dynamics of a regional-scaled community has not been considered in conjunction with local-scaled community spatial dynamics (Leibold et al. 2004, Holyoak et al. 2005). The metacommunity theory, which is designed to examine ecological processes across multiple spatial scales, examines both local and regional scales. Metacommunity theory is gaining wider acceptance in the effort to understand relationships across spatial scales in ecology (Caley and Schluter 1997, Shurin et al. 2000, Cottenie et al. 2003).

A metacommunity is a set of local communities that are associated by migration of various potentially intermingling species (Gilpin and Anski 1991, Wilson 1992). Mechanisms that influence biological diversity patterns at various spatial scales have been a substantial concentration in ecology (Cody and Diamond 1975, Connell 1978, Huston 1994, Ricklefs 2004, Freestone and Inouye 2006). Comprehension of possible relationships between biodiversity and ecosystem function is needed to enhance understanding of ecological systems in order to benefit conservation strategies. Currently studies considering multi-spatial scale processes across larger geographic regions are scarce and need to be explored. The consideration of metacommunities may aid in the avoidance of restricting

analysis to a particular scale (Leibold et al. 2004, Holyoak et al. 2005), which may result in a more unbiased and consistent comprehension of the ecosystem (Chase and Leibold 2002).

It has been documented that endemic and rare spring species, those which do not occur in larger water bodies (e.g., streams, rivers), contribute to aquatic diversity in semi-arid areas (Bowman 1981, Anderson and Anderson 1995, Cao et al. 1998). Endemic organisms have been found in warm isolated spring systems (Erman and Erman 1995). Gooch and Glacier (1991) contended that springs in cooler, eastern areas in North America lack the geologic permanence required for the evolution of endemic taxa, hence, signifying that documentation of macroinvertebrates found in warm-climate, Chihuahuan Desert spring systems could enhance understanding of spring systems by providing evidence of the particular organisms that live in the springs. Baseline data regarding aquatic fauna inhabiting these springs are sparse; however, published studies have indicated loss of endemic fish and freshwater mussel species in perennial streams and rivers of the region (e.g., Bestgen and Platania 1988, Hubbs 1990, Howells and Garrett 1995, Garrett and Edwards 2001).

According to Glazier (1991), considering non-emergent life, macroinvertebrate behavior patterns are less important than factors associated with their maintenance in stable environments. Water temperature is one of the major local factors determining the distribution of aquatic insects along regional gradients of latitude and elevation (Vannote and Sweeney 1980, Ward 1992), which can be influential when considering differences or changes in climate. Geographically-secluded spring systems often exhibit minimal chemical and temperature fluctuations over time (Forester 1991), indicating relatively buffered habitats (Danks 1971b). These stable physicochemical properties support endemic species (MacArthur 1965, Holsinger 1993, Anderson and Anderson 1995). Because a variety of water chemistry and habitat characteristics determine their occurrence and distribution, macroinvertebrates are frequently used as indicators of the health of aquatic ecosystems (Metcalfe 1989, Plafkin et al. 1989, Rosenberg and Resh 1993, EPA 1999).

The presence or absence of macroinvertebrate taxa can be used to derive indices of water quality, and therefore, serve as an indicator of environmental condition or degradation (Schindler 1987). An example of an environmental monitoring program for the National Park Service (NPS) in the study region is the Chihuahuan Desert Network's (CHDN) vital signs program (Reiser et al. 2009). The objectives of this program include an examination of the current status of water quantity, water chemistry, and aquatic invertebrate communities in springs, streams, and rivers, followed by monitoring changes in these environmental indicators over time. Permanent springs (and associated invertebrate communities) can better indicate long-term climate changes than streams or rivers because of historically documented contamination of many streams and rivers that have been influenced by point and/or nonpoint-source contaminants. Results from this study will benefit the NPS vital signs program by providing baseline macroinvertebrate and water-quality information for many springs within the National Park system and adjacent areas of west Texas.

Springs have been characterized as investigational laboratories (Odum 1971) from which we can learn about the interactions between biotic communities and abiotic factors (Huston 1979, Wood et al. 2005). Macroinvertebrate populations in western Texas springs are poorly understood (Bane and Lind 1978, Davis 1980a, b, and c, Khan and Richerson 1982, Meyerhoff and Lind 1987a and b, Mora and Wainwright 1997, Green 1998, Garrett and Edwards 2001, Ordonez 2005, Baumgardner and Bowles 2008, Fordham 2008). The current study provides findings of aquatic macroinvertebrate communities and water chemistry analyses from 41 springs located in six west Texas counties within an area of several thousand square kilometers. The site selection process was based primarily on the ability to access the springs. Results were analyzed in relation to several hypotheses that were presumed to be important for explaining local and regional differences in macroinvertebrate communities:

- (1) Taxonomic composition in springs is influenced by ecoregion (regional factor): Ecoregions frequently are used by State and Federal agencies to stratify results of environmental studies (Omernick 1987, Plafkin et al. 1989, Griffith et al. 2004). Ecoregions are presumed to be relatively homogeneous units with similar climate, physiographic, geochemical, vegetation, soils, and land-use characteristics (Griffith et al. 2004); however, the boundaries of ecoregions and major groundwater aquifers are not necessarily coincident. This hypothesis would be rejected if indicators of macroinvertebrate community structure did not differ significantly among Texas Level 4 Ecoregion categories.
- (2) Taxonomic composition in springs is influenced by differences in aquifer properties (regional factors): Differences in aquifer geochemistry and spring discharge are likely to influence macroinvertebrate populations because of taxon-specific requirements for calcium and/or other major ions, or sensitivities to sub-optimal water-quality conditions (e.g. naturally-occurring metals in certain aquifers). The quantity and persistence of spring discharges may differ in relation to the specific aquifer that maintains spring flows. This hypothesis would be rejected if the distribution of macroinvertebrate taxa did not differ significantly among springs draining different aquifer systems.

- (3) Taxonomic composition in springs is influenced by differences in elevation (regional factor): Macroinvertebrate populations are known to differ in relation to elevation because of differences in climate, notably cooler temperature regimes and shorter warm-weather seasons, as well as differences in bedrock geochemistry that could influence water quality and differences in the occurrences of macroinvertebrate populations in permanent water bodies that could serve as an immigration source to springs. This hypothesis would be rejected if indicators of macroinvertebrate community structure did not vary significantly with increases in elevation.
- (4) Taxonomic composition in springs is influenced by the distance of the spring from a permanent water source such as perennial stream, river, or wetland (regional factor): The taxonomic richness of spring macroinvertebrate communities is hypothesized to decrease with increasing distance from a permanent water body because of reduced potential for species immigration to the spring pool. Springs near permanent water bodies also would be expected to contain a larger percentage of aquatic insects in comparison with non-insect invertebrates that are incapable of flight. The percentage of endemic taxa is hypothesized to increase with distance from a permanent water body. This hypothesis would be rejected if these indicators of macroinvertebrate community structure did not vary significantly with the distance of the spring from a permanent water body.

The objective of this study was to test these 4 hypotheses to facilitate greater understanding of the factors that determine the distribution of aquatic invertebrate species in western Texas and to provide documentation of organisms collected, hopefully, to inform effective management and conservation practices. Results from this research

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provide new (baseline) macroinvertebrate data for 41 springs that should benefit ecological knowledge in several National and State Park systems and, with any luck, stimulate additional interest in measuring the status and trends of macroinvertebrate metacommunities within and between springs throughout the southwestern U.S.

## CHAPTER 1

#### METHODS

### Study Area

Western Texas topography is as diverse as the geological processes that have influenced the landscape: faulting and uplifting, tertiary volcanism, paleo-climatic events, and salt dissolution (Uliana and Sharp 2001). Notably, extended periods of geologic activity created bolsons (typically salt-pan depressions) that formed vast aquifers. The aquifers are a significant source of water in this very dry region (typically less than 300 mm of rainfall per year; NCDC 2004) where surface waters (e.g., Pecos River and Rio Grande) are, in some cases, too saline to be of agricultural or domestic value (Sharp 2001). Freshwater springs are widely dispersed. Wells drilled into aquifers are the primary source of drinking water for humans (Sharp 2001). This area is part of the Chihuahuan Desert and includes some of the most biologically-diverse regions in Texas (Blair 1950).

The forty-one springs included in this study extend across 6 counties in west Texas and span an area of over twenty-one thousand miles<sup>2</sup> (Figure 1, Table 1). Because many springs are on private land or protected public land, site choice was governed primarily by access. We sampled springs where access was granted. To assist with orientation and discussion, most of the sampled sites are grouped according to familiar landmarks in Table 1. Those not included are two isolated sites: Vanderbeek Springs, which is on a private ranch

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close to Independence Creek Preserve, and Post Springs, located near the town of Marathon, Texas in Brewster County.



Figure 1. Locations of Springs in Study Area

Shortened Designations in text	Familiar Grouping (County)	Sampled Springs
BBNP	Big Bend National Park (Brewster)	Burro Springs, Buttrill Springs, Oak Springs, Glenn Springs, McKinney Springs, Mule Ear Springs, Peña Springs
BBSP	Big Bend Ranch State Park (Presidio)	Cinco Tinajas, Las Cuevas, Ojito Adentro, Smith House (AKA: Madrid Springs)
Devil's	Devil's River State Natural Area	
	(Val Verde)	Blue Springs (AKA: Finnegan)
Devil's	The Nature Conservancy's Dolan Fall's Nature Preserve (Val Verde)	Dolan Springs
Independence	The Nature Conservancy's Independence Creek Preserve (Terrell)	Caroline Springs (AKA:T5)
Davis	The Nature Conservancy's Davis Mountains Preserve (Jeff Davis)	Bridge Springs, Toab Springs
Davis	Davis Mountains (Jeff Davis)	Limpia Springs, Lower Tuly, Upper Tuly
Cibolo	The Cibolo Creek Ranch (Presidio)	Cave Springs, Cienaga Springs, High Elevation Springs, Indian Cave Springs, La Morita Springs, North Springs, Ojo Carrizal Springs
02	The O2 Ranch (Brewster)	Cottonwood Springs, Davenport Springs, Duff Springs, Terlingua Springs, Whirlwind Springs
GUMO	Guadalupe Mountains National Park (Culberson)	Choza Springs, Guadalupe Springs, Smith Springs, Upper Pine Springs
Lower Canyons	Wild and Scenic River: lower Canyons of the Rio Grande (Brewster, Terrell)	Asa Jones Springs, Kyle Springs (AKA Big Canyon), Mystery Springs, Son of Hot Springs

 

 Table 1. Locations of Sample Sites in Relation to Familiar Parks, Counties and Natural Areas

For the purpose of effective land management, the U.S. Environmental Protection Agency (EPA) has established ecoregions to designate areas of general similarity in ecosystems and environmental resources (Griffith et al. 2004). These designations are intended to provide a spatial framework for the evaluation of ecosystems and their mechanisms. The compilation is based on the principle that ecological regions can be determined by examination of spatial patterns of biotic and abiotic phenomena, including hydrology, geology, land use, climate, soils, physiography, and vegetation (Griffith et al.

2004). Sites in this study fall within the "Arizona/New Mexico Mountains,"

"Chihuahuan Desert," and "Edwards Plateau" - Level 3 EPA Ecoregions. Each spring

location is categorized according to the Level 4 EPA Ecoregion specification (Figure 1,

Table 2).

Acronym	Level 4 Ecoregion	Description
AMW	Montane Woodlands	Areas of Guadalupe Mountains above 1,676.4 m with oaks, juniper, pinyon pine, Texas madrone, and big tooth maple. Limited areas with high elevation firs and pines. Scarce surface water. Rainfall seeps through limestone and emerges from lower sandstone as springs.
CDS	Chihuahuan Desert Slopes	Slopes (below 1,676.4 m) of the Guadalupe Mountains composed of limestone, shale, and sandstone. Yucca, stool, lechuguilla, ocotillo, and cacti compose the landscape.
CDG	Chihuahuan Desert Grasslands	Fine soils such as silt and clay provide for higher water retention than coarse soils. Higher annual rainfall (25.4-45.7 cm) than more elevated landscapes. Grasses include black, blue, and side oats grama, bush muhly, tabosa, beargrass and galleta with interspersed creosote bush and cholla cactus.
CMW	Chihuahuan Montane Woodlands	Above 1,524 m where oaks, juniper, and pinyon pines are prevalent. Geologically similar to AMW.
CLMB	Low Mountains and Bajadas	Mixed geology with shallow soil, exposed bedrock, coarse, rocky substrate. Alluvium contains rubble, sand, and gravel which, at times, form bajadas at the base of mountains. Soils and vegetation similar to CDS.
STK	Stockton Plateau	Geologically a continuation of Edwards Plateau Cretaceous- age limestone but differs in its ecology in that it occurs in a drier area with low precipitation and thus produces more jagged hills and to W of the Pecos transitioning into desert.
SEP	Semi-Arid Edwards Plateau	Low precipitation, dry climate, Edward's Cretaceous limestone, lies E of the Pecos. Geologically similar to STK.

 Table 2. Categorical Descriptions of Level 4 Ecoregions of West Texas

Griffith et al. 2004.

The method for determining the location of springs within ecoregions was a twostep process. The first step was an overlay of the Ecoregions of Texas Map (Griffith et al. 2004) with a map of point data representing each sampled spring location. Since the spring data coordinates and ecoregion maps use a different geographic coordinate system (springs used World Geographical Survey (WGS) 84; Ecoregions of Texas are drawn on Clarke 1883), not all points fell within the correct ecoregion. The course-scale of ecoregion groups required ground truthing. Upper Tuly, Lower Tuly, Bridge, and Toab springs in were incorrectly projected onto Ecoregion CLMB and corrected as Ecoregion CMW post hoc.

## Aquifers

Multiple aquifers are known to influence the study area. Because of fracturing and the fact that aquifers do not necessarily line up below springs they feed, researchers are not positive; this is still speculative as no one has clear tangible proof. The Capitan Aquifer of the Capitan Reef Complex, an ancient reef which existed during the Permian age, consists of limestone, talus, and dolomite (Rees 1987), and produces outcrops with high water quality in the Guadalupe Mountains National Park (GUMO) in Culberson County. The springs in the GUMO are known to be fed by the Capitan aquifer (Table 1, APPENDIX 6). The Igneous aquifer is thought to be predominant in Jeff Davis, Brewster, and Presidio Counties (Ashworth and Hopkins 1995, Chastain-Howley 2001) which includes the Davis Mountains, O2 Ranch, Cibolo Creek Ranch, the Big Bend Ranch State Park (BBSP), and the Big Bend National Park (BBNP; APPENDIX 6). Chastain-Howley (2001) indicates that the Igneous aquifer consists of areas where volcanic rocks protrude into the landscape; therefore, these geographically spread out Igneous strata probably do not comprise one cohesive aquifer. The Edwards-Trinity aquifer (hard, alkaline, high silica, fresh waters, but including areas of Permian evaporite sediments that can produce saline water) has been documented as influencing the eastern area of Big Bend National Park, the lower canyons of the Rio Grande, the Devil's River

area, and the Independence Creek area (Anaya 2001; APPENDIX 6). According to Ashworth and Hopkins (1995), the Edward-Trinity Aquifer produces water with various levels of dissolved solids consisting of mostly calcium and bicarbonates which means it is very hard. The Marathon aquifer (hard, limestone) feeds the city of Marathon (Smith 2001) where the single site, Post spring occurs.

#### Study Design

Springs were sampled from October 2004 through January 2007 for water chemistry and macroinvertebrates (Table 3). To deal with unequal sampling effort, the macroinvertebrate samples were matched up with the most relevant water sample. If available, macroinvertebrate samples were matched with the water sample that corresponded with the same sampling date. If corresponding sampling dates were not available, the water sample with similar season to the macroinvertebrate sample was used. If a similar season water sample was not available, the mean water parameter and chemical measurements were used. Topographic location (latitude (degrees minutes), longitude (degrees minutes) and elevation (m)) was recorded using a Trek Garmin<sup>®</sup>GPS. Distance from permanent water source was measured in ArcGIS (2005) using shortest straight line from spring site to river.

Site	Sampling Dates
Asa Jones	1.4.06
Blue	8.22.06
Bridge	1.14.05, 5.19.05, 3.16.06, 8.30.06
Burro	10.9.04, 3.11.06, 8.27.06
Buttrill	3.11.05, 5.14.05, 8.16.05, 12.15.05, 3.10.06, 8.26.06
Caroline	8.12.04, 1.13.05, 5.13.05, 8.15.05, 12.16.05, 3.9.06, 1.14.07
Cave	1.12.07
Choza	10.15.04, 4.16.05, 5.18.05, 8.20.05, 12.10.05, 3.18.06
Cienaga	5.16.05, 8.17.05
Cinco Tinajas	8.28.06
Cottonwood	9.1.06

|--|

Site	Sampling Dates
Davenport	9.1.06
Dolan	8.25.06
Duff	8.19.05, 12.13.05, 3.14.06, 9.1.06
Glenn	10.08.04, 3.12.05, 3.9.06
Guadalupe	10.16.04, 4.16.05, 8.20.05, 12.10.05, 3.17.06
High Elevation	1.12.07
Indian Cave	3.13.06, 8.29.06
Kyle	1.3.06
La Morita	8.11.04, 3.13.05, 3.13.06, 8.29.06
Las Cuevas	8.11.04, 3.13.05, 5.15.05, 1.11.07
Limpia	1.14.05, 5.17.05, 12.12.05, 3.15.06, 8.30.06
Lower Tuly	8.18.05, 12.5.05, 3.15.06, 8.31.06
McKinney	10.8.04, 3.11.05, 3.10.06
Mule Ear	12.14.05, 3.11.06, 8.28.06
Mystery	1.7.06
North	8.29.06
Oak	10.9.04, 3.11.05, 5.15.05, 12.15.05, 3.11.06, 8.27.06
Ojito Adentro	1.11.07
Ojo Carrizal	8.29.06
Pena	3.12.06, 8.27.06
Post	10.10.04, 3.11.05, 5.13.05, 8.16.05, 12.15.05, 3.9.06
Smith	10.15.04, 4.16.05, 5.18.05, 8.20.05, 12.10.05, 3.17.06
Smith House	5.15.05, 8.17.05, 12.14.05, 3.12.06
Son of Hot Springs	1.5.06
Terlingua	8.19.05, 12.13.05, 3.14.06
Toab	1.14.05, 3.16.06, 8.30.06
Upper Pine	10.15.04, 4.16.05, 5.18.05, 8.20.05, 12.10.05, 3.17.06
Upper Tuly	8.18.05
Vanderbeek	8.15.05
Whirlwind	8.19.05, 12.13.05, 3.14.06

# Table 3-Continued. Sites and Sample Dates

Note that bold sites were sampled only once and sample types and data are in APPENDIX 2.

Macroinvertebrate samples were collected within the first 100 m of spring head (eucrenal zone; Wood et al. 2005). Qualitative macroinvertebrate collection took place for no longer than 2 hrs (or until no new genera were found) using D-frame nets (150  $\mu$ m mesh). Macroinvertebrates were placed in 150 mL sample bottles containing 80% ethanol. Basic water quality parameters [pH, dissolved oxygen (DO), specific conductance (SpC), and water temperature (°C)] were measured with a YSI 556 multiple

parameter sampler (MPS). Where applicable, a Marsh-McBirney Model 201 portable water current meter was used to measure velocity at the spring head. The discharge formula (Q=A\*V) was used for total discharge calculation. Discharge measurements were taken sporadically, so general designations (low, med, high) were used as relative Q (RELQ) for statistical analyses.

Water samples were collected (a minimum of one sample from each spring) from the spring head (where possible) and dispensed into 250 mL plastic sample bottles containing preservatives specific to the chemical analyses. Samples were fixed with 1 mL HNO<sub>3</sub> for metal analyses and 1 mL H<sub>2</sub>SO<sub>4</sub> for nutrient analyses. Sample bottles were placed on ice until they were returned to the lab where they were stored at ~4°C. As samples were brought into the lab, pH and temperature were recorded. If the pH measured above 2, the sample was adjusted to a pH of 2 for nutrients and <2 for metals. The Edwards Aquifer Research and Data Center (EARDC) laboratory analyzed water samples for ions and nutrient levels (Table 4). For samples from oligotrophic systems, phosphorus analyses were performed with a Quick Chemistry 8500 FIA Automated Ion Analyzer.

Tuble in Water Quality Multiplical Methods	
Water Quality Parameter	Method Used
Total Alkalinity (HCO <sub>3</sub> )	SM 2310B <sup>(2)</sup>
Silica (SiO <sub>2</sub> )	EPA370.1 <sup>(1)</sup>
Lead (Pb)	SM 3113 <sup>(2)</sup>
Chloride (Cl)	EPA 325.3 <sup>(1)</sup>
Sulfate $(SO_4)^{2-}$	EPA 375.4 <sup>(1)</sup>
Nitrate (NO <sub>3</sub> ), Nitrite (NO <sub>2</sub> )	EPA 353.2 <sup>(1)</sup>
Fluoride (F)	EPA 340.2 <sup>(1)</sup>
Total Hardness (CaCO <sub>3</sub> )	EPA 130.2 <sup>(1)</sup>
Total Phosphate (P)	EPA 365.2 <sup>(1)</sup>

**Table 4. Water Quality Analytical Methods** 

Water Quality Parameter	Method Used
Arsenic (As), Calcium (Ca <sup>+</sup> ), Chromium (Cr), Magnesium	
(Mg <sup>+</sup> ), Manganese (Mn), Potassium (K), Selenium (Se),	
Silver (Ag), Sodium (Na)	SM 3113B <sup>(2)</sup>
Mercury (Hg)	EPA 245.1 <sup>(1)</sup>
Aluminum (Al), Boron (B), Bromide (Br), Strontium (Sr),	
Thallium (Ti)	EPA 200.7 <sup>(1)</sup>
(1)EPA, 1999	

Table 4-Continued. Water Quality Analytical Methods

(2)APHA, 1998

In the laboratory, macroinvertebrates were sorted under magnification, with aid of a SMZ800 Nikon microscope, and identified to lowest possible taxonomic unit.

Taxonomic references include, but are not confined to, the following: Pennak (1989),

Merritt and Cummins (1996), Lugo-Ortiz and McCafferty (1998), Wiggins (1998), and

Thorp and Covich (2001). Insects were preserved in 80% ethanol. The complete list of

taxa is presented in APPENDIX 1.

## Analyses

All organisms were identified to the lowest possible taxonomic category.

Because it was not possible to identify all organisms to genus, statistical analyses

required an aggregation to a common taxonomic level. The richness count at the family

level was used for maintenance of taxonomic integrity because not all organisms were

identified to genus level. The aggregation scheme is outlined below.

- Insecta, Mollusca, and Platyhelminthes were identified down to genus and aggregated to family
- Remaining non-insect arthropods were identified down to order and counted to order
- Annelida were identified down to class and aggregated to phylum
- Rotifera, Nematomorpha, Nematoda were identified to phylum and counted to phylum

Taxonomic richness (S; hereafter referred to as 'richness') was calculated as the presence

of individuals at the taxonomic levels summarized above. For example, where two

genera of Coleoptera were identified within the family Elmidae in Blue Spring, Elmidae was counted as a value of 1 for *S*.

In reference to whether taxonomic composition is influenced by differences in aquifer properties a Piper Trilinear Diagram (Aquachem© 2003) categorized all 41 spring sites into groups as functions of their dominant ion concentrations. A Pearson product-moment correlation matrix (Systat© 2007) tested for any correlations between the ions considered in the Piper diagram and remaining chemical parameters (not considered in the Piper diagram) from the water chemistry. Although a total of 41 spring sites were sampled, only 30 spring sites had analogous collection dates of macroinvertebrate and chemical samples, and so, a 30 spring subsample was used to test the hypotheses. In order to approximate a normal distribution required for some of the statistical analyses, water chemistry data were transformed by means of square root or a log<sub>10</sub> as needed. Because of limited samples, if there was a missing value for a site, when data was available for the site from a similar season, that datum was used, if similar seasonal data was not available, the mean value from the entire data set was used.

To test whether taxonomic composition is influenced by differences in ecoregions, elevations, aquifer properties, and/or permanent water source distances and to ordinate sites in relation to these community-environmental relationships, canonical correspondence analyses (CCA; ter Braak 1986) were conducted using Canoco for Windows® Version 4.5 (Canoco© 2002). The statistical significance of the relation between the occurrence of macroinvertebrate taxa and environmental data was evaluated using Monte Carlo permutation tests and the forward selection procedure in CANOCO software (ter Braak and Smilauer 1998). Environmental variables with p-value estimates less than 0.05 were retained for the final CCA analysis for environmental ordination. Hierarchical cluster analysis (CA), using Ward's method for clustering run in Systat for Windows® Version 12.01.01 (Systat© 2007), was applied to CCA site scores for the first two CCA axes to assist with defining groups of CCA sites in relation to community composition and environmental variables. To analyze for differences in macroinvertebrate composition and sites constrained by environmental variables in Table 5, an ANOVA (Systat© 2007) was run.

Variables Analyzed by ANOVA					
Dissolved oxygen (DO)	Total Phosphorus (TP) (µg/L)	$NO_3^-, N$	K	Al	
Alkalinity (HCO <sub>3</sub> )	Cl	F	Mg		
Hardness (CaCO <sub>3</sub> )	SiO <sub>2</sub>	Ca	Fe		
Specific conductance (SpC) (µs/cm)	SO <sub>4</sub>	Na	Mn		

Table 5. Environmental variables (mg/L)

Environmental variables with p-value estimates greater than 0.05 were compared among CCA groups derived from the analysis of variance (ANOVA; Systat© 2007). Post hoc Tukey tests (Systat© 2007) were used to determine significant differences between groups. Significant environmental data site values were correlated (Pearson product-moment correlation analysis; Systat© 2007) with CCA site scores to assist with defining environmental gradients on the first two CCA axes. To specifically address the hypothesis concerning taxonomic composition being influenced by the distance of the spring from its permanent water source (PWS), a Pearson correlation matrix was run to test for significance of *S* in macroinvertebrate samples, exclusive from CCA composition results.

## **CHAPTER 2**

#### RESULTS

## Macroinvertebrates

A total of 17,174 macroinvertebrates were collected and identified from the 41 sites. Eighty five taxonomic groups were identified representing 7 phyla. Within these phyla 12 classes, 22 orders, and 73 families were identified (APPENDIX 1). Presence/absence counts were recorded. The most common and taxonomically rich organisms across all springs were midges (Chironomidae), which occurred at 36 of the 41 sites (88%) and mayflies (Baetidae), which occurred at 34 of the 41 sites (83%). Less common taxa included Dytiscidae, Veliidae, and Coenagrionidae (80%) followed by Podocopida (73%) and Libellulidae (71%). The more rare taxa included: Staphylinidae, Scizomyidae, Ephemeridae, Crambidae, Corydalidae, Corduliidae, Leptoceridae, Cambaridae, Ancylidae, Pomatiopsidae, Valvatidae, and Rotifera (2%).

Some interesting taxa, discovered in the current study, include a few rare individuals either previously undocumented or found within an extension of the present range. A single amphipod, Crangonyctidae: *Stygobromus (near russelli)*, was collected from Caroline Springs in Terrell County. This specimen was verified by John Holsinger (Old Dominion University, Sept. 8, 2006). The occurrence of *Stygobromus russelli* in Caroline Springs represents a range extension of this organism to the west (from Kerr

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County) by approximately 209 km (Holsinger 2006). However, it is possible, that this is an undescribed species. Another single *Stygobromus (near limbus)* was collected from Oak Springs in Brewster County. This specimen was verified by John Holsinger via photograph. This collection is similar to the Terrell County specimen in that, even if it is *Stygobromus limbus*, this occurrence represents an approximate 322 km SE (from Culberson County) range extension for the taxon (Holsinger 2006). Another interesting amphipod was collected from Caroline Springs: Gammaridae: *Gammarus (near pecos)*. One hundred ninety-two specimens were identified from Caroline Springs. Some of these specimens were verified by Randy Gibson (USFWS-San Marcos, TX, August 2006). This may be yet another case of a range extension or possible undescribed species. Holsinger (1972) has revealed that these groundwater inhabiting crustaceans, "stygobionts" are restricted and regionally scarce.

A few rare isopods were collected from the Lower Canyons of the Rio Grande. Most were collected from thermal springs and all were members of Sphaeromatidae: *Thermosphaeroma*. Several specimens were collected from thermal springs, Son of Hot Springs and Asa Jones. These specialized inhabitants are restricted to hot springs (Bowman 1981). A single, morphologically distinct specimen (possibly from the family Cymothoidae; Van Name 1936), was collected from Mystery Spring. Mystery Spring is not thermal and contained fish; this isopod may or may not be parasitic. If the identifications of these specimens are correct, their presence in these locations would constitute a range extension (Bowman 1981) or they may represent an undescribed species. These findings support the contention that geographic endemism is occurring. The fact that these subterranean taxa exist in few locations cause these populations to be highly vulnerable to habitat destruction or reductions in spring flows; very few have federal protection as designated threatened or endangered species (Thorp and Covich 2001).

A single Dytiscid beetle: Crinodessus amyae was collected from Cottonwood Springs in Brewster County. This species, verified via photograph by Gil Challet (Bohart Museum University of California-Davis, Mar 2008), is rare. The holotype for this taxon was designated only recently (1985) in Presidio County, and was described as a new genus (Miller 1997). It was the only specimen of this taxon collected in this study. Another relatively unusual taxon included several specimens of an undescribed species of Trichoptera: Odontoceridae: Marilia. These were collected from Guadalupe, Indian Cave, Ojo Carrizal, Buttrill, and Peña Springs. A few specimens were verified by David Bowles (Missouri State University, Oct 2007), who is in the process of describing the species. David Bowles also verified a "never before seen" Lepidostomatidae: Lepistoma and Lepidopteran. Several of the Lepistomatidae specimens were collected from Upper Pine in Culberson County and two were collected from Indian Cave and North Spring in Presidio County. Lastly, Thiaridae: *Thiara granifera*, verified by Robert Howells (Texas Parks and Wildlife Department, June 2008), were collected from Caroline Springs (Terrell County) and Glenn Springs (Brewster County). Occurrence of these organisms in the T5 spring represents range extensions for these taxa (Robert Howells, Texas Parks and Wildlife Department, June 2008).

## Macroinvertebrates in Hot springs

The thermal springs, Asa Jones, Kyle, and Son of Hot Springs were relatively depauperate when considering macroinvertebrate taxa richness. What appears to be a thermal endemic in 2 of these springs is the isopoda *Thermosphaeroma*. When considering endemics in thermal springs, Bowman (1981) states that thermal isopod occurrence may be attributed to their lack of competitive ability and their restricted reproductive timing in relation to other benthic invertebrates (e.g. amphipods). Besides the restrictive thermal property, these springs tend to contain elevated sulfate and chloride levels. A theory for marine invertebrates suggests that the thermal limits of many species are largely determined by physiological shifts from aerobic to anaerobic pathways (Merritt and Cummins 1996). Although not demonstrated for freshwater invertebrates, it is possible that respiration and osmoregulation are directly related to thermal tolerance, thus limiting diversity in thermal environments. At extremely warm temperatures metabolic demands outpace the ability to collect and circulate oxygen to the tissues (Pörtner 2002); thus, rendering thermal springs as less populated by predators and/or competitors who would, in more viable temperatures, pose conflict for organisms that can live in thermal extremes.

#### **Richness**

Taxa richness varied from 1 (Son of Hot Springs) to 46 (Caroline Springs), with the mean ( $\pm$  1 SD) site *S* = 22.6  $\pm$  10.8.. The highest taxa richness was found in Caroline Springs (46), Duff Springs (42), Indian Cave Spring (38), North Spring (37), Choza and Lower Tuly (35), Oak Springs (33), Buttrill, Limpia, and Post (32) (Table 6). Note that springs were not sampled with equal frequency; some springs were sampled several times whereas others were sampled only once.

Site	N	S = Richness	Total Macroinvertebrates
Caroline	3	46	1375
Duff	5	40	676
Indian Cave	3	38	1027
North	1	37	1027
Choza	4	37	53/
Lower Tuly	7	35	798
Oak	5	33	425
Buttrill	7	33	840
Limpia	4	32	881
Post	2	32	468
Smith House	3	29	550
Guadalupe	4	27	403
Oiito Adentro	1	27	297
Oio Carrizal	1	27	312
Smith	4	27	656
Upper Pine	5	26	1944
Burro	2	25	355
Cottonwood	1	25	371
Mystery	1	25	272
Dolan	1	24	318
High Elevation	1	24	267
Bridge	3	23	460
Mule Ear	3	23	454
Glenn	1	20	205
Vanderbeek	1	20	141
Peña	3	19	393
Blue	1	18	294
Cienaga	1	18	131
Davenport	1	18	159
Whirlwind	4	18	378
La Morita	3	17	227
Terlingua	3	16	239
Las Cuevas	1	14	139
Toab	2	13	229
Upper Tuly	1	13	308
McKinney	1	12	120
Cinco Tinajas	1	6	20
Asa Jones	1	3	31
Cave	1	3	19
Kyle	1	3	7

 Table 6. Total Macroinvertebrates/Taxonomic Richness for West

 Texas Springs(N=number of samples; Richness for springs

 sampled multiple times are cumulative)

Table 6-Continued.	Total Macroinvertebrates/Taxonomic Richness
for West Texas S	Springs(N=number of samples; Richness for
springs sa	mpled multiple times are cumulative)

Site	N	S = <b>Richness</b>	Total Macroinvertebrates
Son of Hot Springs	1	1	7

#### Physical measurements

Ranges of physical measurements of water temperature, specific conductance, dissolved oxygen, and pH of the systems are reported in Table 7. Discharge measurements were taken sporadically, so general designations (low, med, high) were used for comparison (designations in APPENDIX 3; actual data in APPENDIX 2).

Parameter	Mean	Std Dev	Median	Min	Max
Temperature (°C)	20.4	5.67	21.4	8.4	32.8
SpC (ms/cm)	553	206	534	107	944
DO (mg/L)	5.5	1.9	5.6	1.4	10.2
рН	7.5	0.4	7.4	6.6	8.8

 Table 7. Water parameter measurements for all collected samples

#### Water Chemistry

Mean values and standard deviation of measured chemical concentration are in

Table 8. Nitrate levels were largest (2.4 mg/L) in Buttrill Springs; however,

concentrations were less than the analytical detection limit (0.10 mg/L) in other springs (data in APPENDIX 3).

Table 6. Water chemistry measurements for an conceled samples					
Parameter	Mean	Std Dev	Median	Min	Max
TP (µg/L)	94.8	117	49	10	640
NO <sub>3</sub>	0.87	0.76	0.80	0.09	2.4
SiO <sub>2</sub>	33.0	36.3	25.0	3.95	237
CaCO <sub>3</sub>	180	93.0	189	9.40	423

 Table 8. Water chemistry measurements for all collected samples

\*units are in mg/L unless otherwise indicated
Mean values and standard deviations of the anion and cation concentrations considered in the Piper Trilinear diagram (Figure 2) are in Table 9.

Site N HCO<sub>3</sub> Cl **SO₄** Ca Na K Mg Asa Jones 1 196 130.00 50.00 62.50 58.80 4.60 19.00 Blue 1 226 16.00 9.00 65.80 14.50 1.20 15.20 Bridge 3 57 5.67 13.33 12.97 7.00 1.13 4.97 2 Burro 137 31.50 60.50 34.75 58.00 7.66 1.60 Buttrill 2 209 37.50 188.00 74.85 44.70 3.28 15.10 Caroline 2 222 71.50 107.50 79.77 36.50 2.07 17.60 Cave 5.00 43.00 32.70 20.70 0.90 14.60 1 166 25.25 Choza 2 274 9.00 9.50 59.75 3.90 3.13 Cienaga 1 208 10.00 23.00 30.00 3.10 3.90 18.50 92 1 4.00 12.00 19.90 13.80 7.00 2.10 Cinco Tinajas Cottonwood 252 5.00 14.00 46.90 36.70 2.60 10.00 1 Davenport 1 212 6.00 11.00 33.50 80.30 2.60 6.90 Dolan 1 216 16.00 8.00 69.70 6.50 1.00 13.10 Duff 2 363 17.00 136.00 40.10 17.10 2.50 10.90 Glenn 1 332 9.00 94.00 88.70 82.20 4.20 8.40 2 27.75 Guadalupe 282 9.00 14.50 61.30 6.25 3.14 70.80 2.00 High Elevation 1 238 5.00 62.00 20.30 18.40 1 Indian Cave 232 11.00 21.00 34.30 47.30 3.10 3.50 Kyle 1 210 59.00 175.00 69.20 69.60 4.90 21.50 2 3.35 20.17 343 21.00 29.00 53.15 47.40 La Morita Las Cuevas 2 259 11.00 24.0080.80 30.05 3.05 4.71 Limpia 2 115 0.75 12.50 22.45 8.55 1.40 7.60 Lower Tuly 3 95 5.67 13.67 18.77 11.27 2.93 5.07 1 278 33.00 197.00 151.80 62.20 3.24 10.60 McKinney 2 26.50 Mule Ear 271 10.00 79.15 39.50 0.70 7.05 1 172 117.00 10.00 49.00 5.20 3.20 14.00 Mystery North 14.00 31.90 1 176 6.00 14.70 1.10 5.30 Oak 2 173 9.50 63.50 44.35 22.95 1.40 2.40 Ojito Adentro 1 204 8.00 21.00 70.30 1.60 3.30 19.00 Ojo Carrizal 1 174 7.00 22.00 31.90 14.70 1.50 5.90 2 94.95 9.20 Peña 285 29.50 134.30 74.15 0.56 9.00 124.00 51.50 54.20 4.13 38.30 Post 1 366 2 Smith 277 9.00 9.50 58.85 3.10 3.06 23.05 Smith House 1 252 1.00 22.00 53.00 2.80 2.50 5.00 Son of Hot Springs 1 196 149.00 45.00 60.00 49.20 4.60 18.00 Terlingua 1 176 11.00 24.00 13.80 8.10 2.20 2.40 Toab 1 30 0.75 10.00 8.85 4.10 0.50 3.40 2 Upper Pine 271 9.00 10.50 60.35 4.05 3.02 25.25

 Table 9. Anion and Cation Averages (mg/L; N=number of samples)

				9	0, 1			====)
Site	N	HCO <sub>3</sub>	Cl	$SO_4$	Ca	Na	K	Mg
Upper Tuly	1	142	9.00	17.00	0.05	19.60	2.50	4.20
Vanderbeek	1	236	94.00	100.00	90.50	7.90	4.20	25.60
Whirlwind	1	250	26.00	47.00	20.20	8.30	0.80	3.90
	Mean	216	25.2	49.5	52.0	27.8	2.74	12.2
	SD	76.0	36.1	53.5	29.1	24.2	1.61	8.81
	Median	216	9.50	23.0	53.0	19.0	2.60	10.0
	Min	29.5	0.750	8.00	0.050	2.80	0.50	1.60
	Max	366	149	197	152	82.2	7.66	38.3

Table 9-Continued. Anion and Cation Averages (mg/L; *N*=number of samples)

#### Differences in geochemistry among sites

As water travels through an aquifer, it contacts rock formations with a variety of minerals, thereby influencing the ionic composition of the water. The Piper diagram (Figure 2) groups springs as a function of their dominant anion and cation concentrations (APPENDIX 6). Measurements are plotted in percentages of occurrence. Each outer triangle shows percentages of three ions, whereas, the center diamond is an integration of the outer triangles, showing the percentages of six ions. The demonstration of 6 ions was possible because Na<sup>+</sup> and K<sup>+</sup> were grouped together,  $Ca^{2+}$  and  $Mg^{2+}$ , and  $HCO_3^-$  and  $CO_3^{2^2}$ . Most springs clustered in the bicarbonate and calcium groups (Figure 2; APPENDIX 5). As such, the hydrochemical signatures among spring systems are largely similar as seen within the dotted line, designated by Fetter (1988; APPENDIX 5), on the diamond of the Piper diagram (Figure 2). The dominant ions within the dotted line are bicarbonate, carbonate, calcium and magnesium. A Pearson correlation matrix indicated that concentrations of Mg are negatively correlated with Fe (r=-0.67; p=.003), As (r=-0.65; p=.006, Mn (r= -0.68; p=.002) concentrations, and Na concentrations are positively correlated with Si (r=0.64; p=.01).

The geology of BBNP (Table 1) is complex and is reflected in the Piper diagram (Figure 2, APPENDIX 6) with springs occurring in and out of the dotted line. The

eastern area of BBNP, towards the lower canyons of the Rio Grande, largely receive drainage from the Edwards-Trinity aquifer (Chastain-Howley 2001; APPENDIX 6) whereas, volcanic outcrops feed Pena, Mule Ear, Oak, Burro, McKinney, and Glenn Springs (Gary et al. 2007). According to the Piper Diagram the springs in GUMO all have similar water quality with dominant Ca-Mg-HCO<sub>3</sub> characteristics. Regarding the O2 springs (Table 1), Brune (1981) classified Whirlwind Spring as receiving discharge from conglomerate in the Eocene Pruett formation and, according to the Piper table (APPENDIX 6), Terlingua and Duff Springs also issue from a similar source. Brune (1981) classified Cottonwood Spring as receiving discharge from the Igneous aquifer; the Piper table (APPENDIX 6) indicates a similar chemical signature for Davenport Spring. The Piper diagram shows that most of the springs have a chemical signature of either Igneous or Edwards-Trinity sources.



Spring Chemical Distribution

Figure 2. Piper Trilinear Graph of Major Ion Concentrations

#### Site ordination in relation to organismal and environmental relationships

Canonical Correspondence Analysis (CCA; ter Braak 1986) was used to relate macroinvertebrate community composition to known variation in the environment, ordinating sites in relation to species-environmental relationships (Figure 3). Environmental data (APPENDIX 9) consisted of physical, chemical, and landscape data associated with the springs, including a set of latitude and longitude coordinates (APPENDIX 8) as indicators of the location of the springs in relation to Level 4 ecoregions (Table 2) and known aquifer systems (APPENDIX 6) in the study area. Significant environmental variables included relative spring discharge (RELQ), spring distance from a permanent water body (PWS), latitude (LAT), longitude (LONG), spring elevation (ELEV) and water temperature (WTEMP).

The CCA explained a total of 63.5 percent of the total species-environmental relation. Springs with positive scores on the first CCA axis (APPENDIX 11). Values in AX1 and AX2 columns are percentages of the total variance explained in the first row. The bold values are where the majority of the variance lies. Springs were relatively larger (higher relative discharge) and located at relatively lower elevations in the eastern portion of the study area (Figure 4). By contrast, springs with negative scores on the first axis were relatively small (lower relative discharge) and located at higher elevations in the western portion of the study area. Springs with positive scores on the second CCA axis were located at relatively greater distances from permanent water bodies, at relatively higher elevations in the northwestern portion of the study area where water temperature was relatively cooler. By contrast, springs with negative scores on the second axis were relatively lower elevations in the southeastern portion of the study area.







Figure 4. CCA of sites in environmental space. Nested within CCA tri-plot.

CA of site scores from the first two CCA axes revealed 3 groups of sites with similar macroinvertebrate and environmental conditions (Figure 3, Figure 4). Group 1 contains springs in the Guadalupe Mountains, Cibolo Creek Ranch, and Big Bend National Park. These are springs with relatively higher water temperature and moderate discharge, located relatively close to permanent water bodies at moderate elevations in the southwestern portion of the study area. Group 2 sites contain springs in the Davis Mountains, O2 Ranch, and Big Bend Ranch State Park. These are springs with relatively low discharge, located at relatively high elevations that are distant from permanent water bodies. Group 3 sites contain low-elevation springs in the eastern portion of the study area with relatively higher discharge and warm water temperature.

These include springs in the Lower Canyons of the Rio Grande and those near Independence Creek, suggesting influence from the Edwards-Trinity aquifer.



Figure 5. Ward's hierarchical cluster analysis of 3 site groupings

#### Relation of site groups to CCA site scores

Analysis of variance (Figure 6) followed by Tukey significance tests (Table 10) indicated that mean CCA axis 1 scores differed significantly among CCA groups derived from Cluster Analysis (Figure 5, Figure 6). Mean scores for Group 3 were highest, followed by Group 1, and Group 2. Mean CCA axis 2 for Group 1 were significantly lower than Groups 2 or 3, which did not differ significantly on the second CCA axis.

### Water chemistry relations among CCA site groups

Mean specific conductance values differed significantly among CCA groups (Figure 6, Table 10). Mean specific conductance at sites within CCA Group 3 (~ 700 μS/cm) was over

twice the average value observed at sites within CCA Group 2 (~  $300 \mu$ S/cm), and was significantly larger than the average value observed at sites within CCA Group 1 (~  $525 \mu$ S/cm) (COND). This result appears to reflect environmental-species relations with aquifer geochemistry. Sites within CCA Group 2 contained significantly lower mean concentrations of chloride, hardness, and alkalinity than the other two CCA groups (Figure 6). By contrast, mean concentrations of nitrate, calcium, and magnesium were significantly higher at sites within CCA Group 3 than Group 2. Although these constituents were not used as environmental variables in the CCA, it appears that the first CCA axis is positively correlated with increases in geochemical variables associated with salinity (specific conductance and chloride concentrations), water hardness (including indicators of alkalinity), and nutrient enrichment (nitrate concentrations).



Figure 6. ANOVA results illustrate relations between CCA Site Groups and Conductance



Figure 7. ANOVA results illustrate relations between CCA Site Groups and Chemical parameters

Post Hoc Tukey Significant Difference Test for previous ANOVAs							
CCA	axis1	1		CCA	axis 2		
Site g	group	Difference	p-value	Site g	group	Difference	p-value
1	2	1.373	0.000	1	2	-2.125	0.000
1	3	-2.768	0.000	1	3	-1.674	0.001
2	3	-4.141	0.000				
Ca	CO <sub>3</sub>	Difference	p-value	N	Ig	Difference	p-value
1	3	-4.465	0.043	1	3	-0.533	0.020
2	3	-6.283	0.010				
C	Ca	Difference	p-value	SI	рС	Difference	p-value
2	3	-3.283	0.044	1	2	196.562	0.013
N	03	Difference	p-value	1	3	-170.563	0.057
2	3	-0.871	0.006	2	3	-367.125	0.000
Α	lk	Difference	p-value	(	Cl	Difference	p-value
1	2	3.581	0.006	1	3	-0.517	0.032
2	3	-3.858	0.018	2	3	-0.829	0.002

Table 10. CCA chemical parameters test of significance

## Relation of ecoregions to CCA site scores

Analysis of variance (Figure 8) followed by Tukey significance tests (Table 11) indicated significantly higher CCA axis 1 site scores for SEP/STK than the other 3 ecoregion groups; however, no significant differences were observed among CDG, CLMB/CDS, and CMW/AMW. For CCA axis 2 scores, mean values for CMW/AMW were significantly larger than those for CDG and CLMB/CDS; however, mean values for SEP/STK did not differ significantly from other ecoregions.



Figure 8. ANOVA results considering relations between ecoregions and CCA site scores

	8							
Post Hoc Tukey Significant Difference Test								
CC axis1				CC axis 2				
Eco (A)	Eco (B)	Difference	p-value	Eco (a)	Eco (b)	Difference	p-value	
CDG	SEP/STK	-3.337	0.019	CDG	CMW/AMW	-1.997	0.018	
CLMB/CDS	SEP/STK	-2.584	0.031	CLMB/CDS	CMW/AMW	-1.796	0.002	
CMW/AMW	SEP/STK	-2.775	0.030					

 Table 11. Differences in ecoregions

Average CCA axis 1 site scores for springs located in the Stockton Plateau/Semi-Arid Edwards Plateau ecoregions (STK/SEP; Table 2, Figure 8) were significantly larger than for other ecoregions (Table 11). These ecoregions overlie the Edwards-Trinity aquifer. Average CCA axis 2 scores for the Montane Woodlands ecoregions (Chihuahuan Desert, CMW; Arizona/New Mexico, AMW) were significantly higher than those for the Chihuahuan Desert Slopes (CDS), Chihuahuan Desert Grasslands (CDG), and Chihuahuan Low Mountains and Bajadas (CLMB) ecoregions; however, no significant difference was observed between the Montane Woodlands (CMW/AMW) and the Plateau (SEP/STK) ecoregions. Group 2 sites primarily are located at higher elevations in the Montane Woodlands ecoregions where rainfall and surface waters are scarce (Table 2), whereas Group 1 sites are located at lower elevations in desert grassland ecoregions where annual rainfall is relatively higher (e.g. CDG) or in drier portions of the GUMO and BBNP/BBSP regions.

#### Taxa scores in relation to Environmental Variables

A CA (Figure 10) of CCA taxa scores (APPENDIX 12) revealed 3 groups of taxa (Figure 9) that relatively matched up with the 3 site groups. Taxa found commonly at sites in Group 1 include *Simulium, Marilia, Probezzia, Libellula, Peltodytes,* and *Orthemis*. Of the 6 taxa, one is a Trichopteran. Group 1 total Ephemeropteran/Trichopteran (ET) taxa is seventeen. These taxa are influenced primarily by CCA axis 2 and are characteristic of relatively lower-elevation, warm springs in the southern part of the study area that are relatively close to permanent water bodies. The predatory taxa number of the Odonata/Coleoptera/Hemiptera genera totals 67. There is a possibility that the paucity of species is somehow related to the fact that the group contains a higher occurrence of top aquatic predators. This group has the highest Elmidae and Dryopidae taxa number at 7.



Figure 9. CCA bi-plot of taxa in environmental space.



Figure 10. Ward's hierarchical cluster analysis of organisms

Taxa found commonly at sites in Group 2 include Physidae, *Enallagma, Anopheles, Archilestes, Limnoporus, Notonectus, Laccophilus, Microvelia*, Podocopida, *Stictotarsus, Callibaetis*, Cyclopoida, and *Agabus*. Of thirteen taxa there is one Ephemeropteran in this group; the total number of ET taxa is lowest in Group 2 totaling 10. The predatory taxa number is second highest at 48 genera. These taxa are characteristic of relatively moderate-higher elevation, warmer to cooler springs in the northwestern part of the study area, springs with some of the lowest discharges (RELQ) of the three groups, and relatively distant from permanent water bodies. The fact that lentic habitats are where Cyclopoida prefer and that there is evidence that Cyclopoida distributions may be influenced by temperature (Thorp and Covich 2001) may explain why they occur in Group 2, in cooler systems, as opposed to Group 1 or 3 which tend to be warm. The Ephemeropteran, *Callibaetis*, and the Zygoptera, *Enallagma* and *Archilestes*, have external gills and the Dipteran, *Anopheles*, has an external siphon, all which aid in their physiological tolerance of lower levels of dissolved oxygen. The Hemiptera in this group, *Limnoporus, Notonectus*, and *Microvelia* are neustic, mostly inhabiting the surface of the water, being able to occur regardless of DO content. Also consistent with a low discharge habitat is the observation that of this group contained the lowest number of Elmidae and Dryopidae genera (2).

Found commonly at sites in Group 3 include *Caloparyphus, Hyalella, Euparyphus, Tricorythodes, Tabanus, Helicopsyche* (can tolerate warm waters), Oligochaeta, *Phylloicus, Ambrysus, Macrelmis*, and *Berosus*. Of eleven taxa there is one Ephemeropteran and two Trichoptera in this group; the total number of ET taxa is highest in Group 3 totaling 23. The predatory taxa number is lowest for this group at 38. These taxa are primarily influenced by CCA axis one and characteristic of relatively lower-elevation, warmer springs in the eastern part of the study area with relatively higher (and perhaps more persistent) discharge (RELQ). Water quality in Group 3 springs is characterized by hard, alkaline, waters with higher specific conductance and nutrient concentrations. Persistence of spring flows may be supported by the occurrence of *Hyalella* which are very established taxa in that they have no way of colonization besides through water or animal transport. Higher levels of calcium which may be supported by occurrence of taxa with needs for calcium carbonate (i.e. *Caloparyphus* and *Euparyphus* who have these crystals on their larval bodies). This group has the second highest Elmidae and Dryopidae number at 5.

#### Water-chemistry relations among CCA taxonomic groups

Analysis of variance of water-quality constituents based on species groups revealed two additional results (Figure 11). Concentrations of aluminum were significantly higher in CCA Group 1 than in other CCA Groups, whereas silica concentrations were significantly lower in CCA Group 2 than in other groups (Table 12).



Figure 11. ANOVA results show relations of CCA Taxa Groups with silica and aluminum.

Al		Difference	p-value	
1	2	0.871	0.008	
1	3	0.947	0.005	
SiO2				
SiO	2	Difference	p-value	
<b>SiO</b> 1	<b>2</b>	Difference 2.199	<b>p-value</b> 0.056	

 Table 12. Taxa Post hoc Tukey significant difference test

#### **CHAPTER 3**

#### DISCUSSION

Results from this investigation provide baseline macroinvertebrate and waterquality data for a large number of springs in the Trans-Pecos region of Texas with which subsequent studies may be compared. These results will be important to the National Park Service's Vital Signs program to determine the current status and future trends in the condition of springs and macroinvertebrate communities over time that could become altered as a result of changes in climate and/or human land- or water-use practices. Macroinvertebrate and water-quality results differ significantly among major groupings of springs, indicating that a null hypothesis that all springs contain the similar water quality and macroinvertebrate-community structure can be rejected. Differences in community structure among springs are related to differences in environmental condition relative to landscape variables (e.g. elevation and the physical location of the springs), relative discharge (or presumed persistence) of spring flows, water temperature, and water chemistry, for example, concentrations of major ions, alkalinity, nutrients, and metals.

Many of these factors appear to be related, spatially, to the aquifers from which spring flow originates. Ecoregions recently have been used to stratify environmentalstudy designs and to interpret results from surface waters such as streams and rivers, theboundaries of ecoregions were determined solely on the basis of land-surface features. Although there is some overlap between the spatial distribution of major aquifers and ecoregions in west Texas, notably a separation between the Edwards-Trinity carbonate

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aquifer system from those derived from volcanic and metamorphic processes in western parts of the study area, consideration of Texas Level 4 ecoregions as an explanatory factor for macroinvertebrate and water-quality conditions in springs resulted in poor discrimination among major groups of springs detected in this study, thus the null hypothesis of similar macroinvertebrate and water-quality conditions among ecoregions cannot be rejected. Spring relations with the distribution of major aquifers are confounded by a relatively poor understanding of the boundaries of specific aquifers relative to land-surface features.

#### Major ion chemistry

Differences in major ion concentrations among springs, as revealed on the Piper Trilinear Diagram (Figure 2) were relatively small. Most springs could be classified, geochemically, as calcium bicarbonate types; however, the major-ion "signature" of certain springs in the Big Bend National Park and Post-Independence regions departed from springs in other topographic regions because of increases in relative concentrations of chloride, sulfate, calcium, and magnesium. This is likely a function of differences in the water chemistry among different aquifers, for example, springs influenced by discharges from the Edwards-Trinity aquifer in eastern portions of the study area. The major-ion chemistry of several springs in the Davis Mountains, Cibolo Creek Ranch, and Lower Rio Grande Canyons regions differed from the majority of other sites in those regions, possibly because they drain different aquifers that are small (and relatively unknown) but important locally.

#### Macroinvertebrate-Environmental Relations

Multivariate ordination revealed that spatial position (latitude and longitude), elevation and relative discharge from the spring, as well as water temperature and the distance to a permanent stream or river explained a large percentage of the variance relative to the distribution of macroinvertebrate taxa (CCA). Several of these variables are not totally independent, for example, elevation tends to increase with longitude (i.e. elevation increases from east to west), and the distance from a spring to the nearest permanent water source tends to increase from the south-eastern to the northwestern part of the study area. Although water temperature does tend to decrease with increases in elevation, temperature was not related to spatial position or other environmental components selected for analysis.

Cluster Analysis, followed by ANOVA and post-hoc analyses indicated 3 statistically distinct groups of sites based on differences in multivariate component factors and the presence or absence of macroinvertebrate taxa. Although the site groups are influenced by differences of very basic indicators of physical location, spring discharge, and temperature, a comparison of independent water-chemistry variables (those not used in the CCA ordination) among these groups reveals significant differences in the quality of spring waters among groups. The three groups represent a gradient of dissolved-ion concentrations, with mean specific conductance values varying from about 300  $\mu$ S/cm (Group 2) to about 525  $\mu$ S/cm (Group 1) to 700  $\mu$ S/cm (Group 3), suggesting fundamental differences in geochemistry among groups. Significant differences in average concentrations of chloride, nitrate, aluminum, silica, and indicators of alkalinity and hardness (calcium and magnesium) were found among groups. These

variations are reflective of differences in the regional condition of aquifers that provide flow to springs within the three groups. For example, increases in alkalinity, hardness, chloride, and nitrate most likely are associated with the Edwards-Trinity aquifer, whereas differences in mean concentrations of aluminum and silica separate the southern and northern groups of springs in west Texas. Regional scale differences in chemical composition and relative discharge among springs account for some of the local differences among macroinvertebrate distributions. For example, larger nitrate concentrations in the discharge from Edwards-Trinity springs could stimulate the growth of algae and other aquatic plants in springs, resulting in increased food-web and habitat diversity, thereby providing increases in the number of specific niches, and thus taxa, in those springs.

Group 1 sites were characterized by waters with moderate specific conductance, alkalinity, hardness, and nutrient concentrations but elevated concentrations of aluminum, and perhaps other metals. Springs in Group 1 were located in the southern part of the study area, at relatively lower elevations, which partially explains why water temperature was warmer at Group 1 sites than those in the other two groups. Macroinvertebrate communities were characterized primarily by taxa in the Group 1 species ordination but shared some affinity with Group 2 taxa (Physidae; APPENDIX 12), possibly because of similarity in alkalinity levels between the two groups (Table 10). Macroinvertebrate communities at Group 1 sites contained less common taxa than did Group 2 or 3. Heightened predatory composition of taxa, in comparison to the other two groups, may have rendered Group 1 scarce of common taxa. Although Group 1 springs were close to permanent sources of water (e.g. Rio Grande, Pecos River), and springs appeared to

contain the most diverse assemblage of taxa, a Pearson correlation matrix showed no significant difference in taxonomic richness (APPENDIX 7) when considering distance of PWS (r=0.15; p=.42). Therefore, the null hypothesis concerning distance to a permanent water source cannot be rejected.

Group 2 sites were characterized by waters with low specific conductance, with relatively lower alkalinity, hardness, silica, and nutrient concentrations than other groups. Springs in Group 2 typically were located in the northwestern part of the study area, at relatively higher elevations located distant from permanent water sources. Water temperature was warm to cool, and relative discharge from these springs was low. Macroinvertebrate communities at Group 2 sites were characterized primarily by lentic taxa including Cyclopoida, *Callibaetis, Anopheles, Stictotarsus,* and *Laccophilus,* in addition to three neustic genera *Limnoporus, Notonectus, and Microvelia.* Low discharges are also consistent with the occurrences of *Enallagma* and *Archilestes* genera which, because of lamella, have higher tolerances to low DO (Thorp and Covich 2001).

Group 3 sites were characterized by warm waters with relatively high specific conductance, alkalinity, hardness, chloride, and nutrient concentrations. Relative discharge from Group 3 springs was large, and the persistence of springflow from these springs may be longer than those in Group 2. Other common taxa in this group included *Helicopsyche*, which tolerates warm temperatures, and *Tricorythodes*, which is tolerant to low dissolved-oxygen. Group 3 ET taxa was highest and the top predatory taxa was the lowest of the groups. The primary distinguishing features of Group 3 springs are their relatively high discharge patterns, limestone-based aquifer geochemistry, and eastern distribution in the study area. Calcium is an important water-quality factor for many

macroinvertebrate taxa (i.e. *Caloparyphus* and *Euparyphus*) and the presumed permanence of these high-flow springs probably enhances the ability of taxa with longer aquatic life histories (e.g. *Helicopsyche, Stygobromus*) to maintain populations in springs. Most of the springs in Group 3 receive drainage from the Edwards-Trinity aquifer. Exceptions include Smith Spring which is influenced by the Bell Canyon limestone of the Capitan Aquifer (Brune 1981).

Hypotheses supported by this research include that local taxonomic composition in springs is influenced by regional differences in 1) aquifer properties and 2) elevation. These null hypotheses can be rejected because of the evidence that each factor explained some variance in species-environmental relations. This study may have benefited from considerations of habitat diversity (e.g., substrate composition) and larger sample size. Further analyses and sampling of more systems will benefit the understanding of these relationships.

The discharge and relative permanence of springs currently is threatened by increased pumping of aquifers in locations proximate to or up gradient from the springs in west Texas (Porter et al. 2009). Because of the intrinsic value of springs in arid to semi-arid climates, a political solution needs to be achieved to balance human needs (i.e. water for livestock and irrigation) and ecological values. Potential threats to springs are concerns because of their roles in water quality, indicators of pollution, and ultimately, geographic isolation. Not only are these oases required for the existence of living things in this arid ecosystem, but these springs, in their geographic isolation contain endemic organisms that augment the regional diversity. When the springs are no longer extant there will be evolutionary consequence.

## **APPENDIX 1.**

## TAXONOMIC LIST BY SPRING

50 page Excel document included

## **APPENDIX 2.**

## INDIVIDUAL SAMPLE DATA

20 page Excel document included

Values are averaged according to all measurements recorded in this study (including records from 2004). Discharge measurements were sporadic, therefore, grouped as Low: 0-0.5 Med: 0.5-3 High: >/= 4 (with consideration of field measurements, observation, and historical data).

	Water Temp	SpC	DO		Discharge
Site	(°C)	(ms/cm)	(mg/L)	pН	(cms)
Asa Jones	31.90	791.00	5.83	7.10	Low
Blue	22.58	500.00	6.05	7.34	High
Bridge	9.20	237.50	6.45	7.83	Low
Burro	23.27	516.33	3.92	7.92	Low
Buttrill	21.48	477.78	4.65	7.40	Low
Caroline	21.85	842.38	6.27	6.79	High
Cave	16.12	407.00	4.40	8.41	Low
Choza	16.93	527.75	7.09	7.29	Low
Cienaga	24.78	489.00	4.96	7.03	Low
Cinco Tinajas	32.81	235.00	5.43	8.77	Low
Cottonwood	22.80	534.00	3.47	7.07	Low
Davenport	23.85	441.00	5.85	7.61	Low
Dolan	22.53	487.00	7.72	7.45	High
Duff	24.45	821.75	4.38	7.29	Med
Glenn	21.39	771.33	3.96	7.33	Low
Guadalupe	13.19	541.86	6.86	7.53	Low
High Elevation	15.21	538.00	6.56	8.17	Low
Indian Cave	22.24	510.67	3.46	7.38	Med
Kyle	31.00	872.00	5.60	7.14	Med
La Morita	18.98	663.20	4.79	7.11	Low
Las Cuevas	22.76	589.25	1.44	6.61	Low
Limpia	12.76	287.40	4.21	6.75	Low
Lower Tuly	16.41	301.40	4.06	7.26	Low
McKinney	18.45	873.25	3.94	6.97	Low
Mule Ear	24.12	574.50	6.51	7.64	Low

Table 13. Chemical and physical analyses

Site	Water Temp	SpC (ms/cm)	DO (mg/L)	nH	Discharge
Mystery	12.79	386.00	10.20	7.85	Low
North	23.02	370.00	5.14	7.39	Low
Oak	18.72	404.63	5.80	7.65	Med
Ojito Adentro	18.45	473.00	-	8.07	Low
Ojo Carrizal	23.95	379.00	3.83	7.11	Low
Peña	20.78	885.00	3.54	7.69	Low
Post	14.60	944.00	7.64	7.51	Med
Smith	14.86	534.25	8.10	7.40	Low
Smith House	21.49	541.67	2.71	7.59	Low
Son of Hot Springs	31.88	746.00	5.82	7.10	High
Terlingua	18.98	605.67	9.17	8.62	Low
Toab	8.41	106.67	9.43	6.97	Low
Upper Pine	15.34	540.43	5.99	7.09	Low
Upper Tuly	18.00	322.00	7.66	-	Low
Vanderbeek	24.15	933.00	2.81	-	Med
Whirlwind	20.48	659.50	5.61	7.87	Low

Table 13-Continued. Chemical and physical analyses

Note that bold sites were sampled only once.

Table	14. Hydr	ochemio	cal averag	ges (mg/L)	)
		d'O	NO3-	$NO^{2+}$	ſ

Site	P (µg/L)	SiO <sub>2</sub>	NO <sup>3-</sup>	NO <sup>2+</sup>	F	Br
Asa Jones	11	20	1.50	0.09	1.60	0.19
Blue	10	9	1.90	0.09	0.30	0.04
Bridge	238	32	0.09	0.09	0.33	0.04
Burro	127	23	2.40	0.11	3.55	0.24
Buttrill	15	21	0.25	0.09	1.45	0.16
Caroline	29	13	2.05	0.09	1.00	0.12
Cave	49	50	0.20	0.09	1.20	-
Choza	28	6	0.85	0.09	0.15	0.04
Cienaga	210	55	1.80	-	1.70	0.11
Cinco Tinajas	50	21	1.90	0.09	1.00	0.04
Cottonwood	50	37	0.80	0.09	0.60	0.04
Davenport	90	47	0.50	0.09	0.70	0.04
Dolan	10	7	1.80	0.09	0.30	0.04
Duff	80	57	0.09	0.09	1.80	0.25
Glenn	245	19	0.09	0.09	4.40	0.15
Guadalupe	36	8	0.65	0.09	0.25	0.05
High Elevation	130	35	0.09	0.09	0.60	-
Indian Cave	27	33	1.10	0.09	1.50	0.12
Kyle	13	20	1.40	0.09	1.80	0.21
La Morita	130	40	1.00	0.09	2.00	0.21
Las Cuevas	65	33	0.41	0.09	2.05	0.13
Limpia	304	17	0.09	0.09	0.45	0.05
Lower Tuly	226	35	0.10	0.09	0.50	0.04
McKinney	45	25	0.20	0.09	3.90	0.34
Mule Ear	38	56	0.91	0.09	2.60	0.14
Mystery	11	13	1.70	0.09	0.50	0.05
North	250	34	0.09	0.09	1.10	0.04
Oak	23	17	0.86	0.09	3.50	0.07
Ojito Adentro	49	53	2.20	0.09	1.70	-
Ojo Carrizal	640	25	0.40	0.09	0.70	0.04
Peña	105	39	0.09	78.51	3.45	0.22
Post	77	8	0.20	0.09	2.50	0.34
Smith	29	4	0.90	0.09	0.10	0.04
Smith House	45	59	0.09	0.09	1.00	0.13

$NO^{2+}$		
110	F	Br
0.09	1.50	0.18
0.09	1.20	0.06
0.09	0.10	0.02
0.09	0.10	0.04
0.09	2.00	0.12
0.09	1.10	0.17
0.09	1.10	0.22
	0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09           0.09	NO         1           0.09         1.50           0.09         1.20           0.09         0.10           0.09         0.10           0.09         2.00           0.09         1.10           0.09         1.10

Table 14-Continued. Hydrochemical averages (mg/L)

Note that bold sites were sampled only once



Fetter 1988.

Figure 12. Anion and cation hydrochemical group chart

Colors are reflective of Piper color designation of springs.

Site	Piper Designation	Aquifer Source	Reference
		Capitan Reef-Bell Canyon Limestone	Brune 1981, Ashworth
<b>Choza</b>	CaMgHCO3		and Hopkins 1995
		Capitan Reef	Brune 1981, Ashworth
Guadalupe	CaMgHCO3		and Hopkins 1995
		Capitan Reef-Bell Canyon	Brune 1981, Ashworth
Smith	CaMgHCO3		and Hopkins 1995
Linnen Dine	C-M-UCO2	Capitan Reef-Cherry Canyon	Brune 1981, Ashworth
Bridge	CaMgNaHC03504	Igneous	
Limpia	CaMgHCO3	Igneous	
Lower Tuly	CaNaMgHCO3	Igneous	
Toab	CaMgNaHCO3SO4	Igneous	
Upper Tuly	NaHCO3	Igneous	
		Igneous: Oligocene Cottonwood	Brune 1981
Cottonwood	CaNaHCO3	Springs Basalt	
Davenpon		Igneous	
Dull Tarlin ave		Igneous	
	CaHCO3SO4	Igneous Econe Pruett formation	Drupa 1091
Whirlwind		Igneous-Eocene Fluett Ioffilation	Diulie 1981
Cave	CaMgNaHC03S04	Igneous	
Cienega	MgCaHCO3	Igneous	
High Elevation	CaMgHCO3SO4	Igneous	
Indian Cave	NaCaHCO3	Igneous	
La Morita	CaNaMgHCO3	Igneous	
North	CaNaHCO3	Igneous	
Ojo Carrizal	CaNaHCO3	Igneous	
Cinco Tinajas	CaNaHCO3	Igneous	
Las Cuevas	CaNaHCO3	Igneous	
Ojito Adentro	CaHCO3	Igneous	
Smith House	CaHCO3	Igneous	
Burro	NaCaHCO3SO4	Igneous	Gary et al. 2007
		Igneous, Upper Cretaceous	Gary et al. 2007, Brune
Buttrill	CaNaSO4HCO3		1981
		Igneous, Aguja Sandstone	Gary et al. 2007, Brune
Glenn	CaNaHCO3SO4		1975
Making	CoNoLICO2SO4	Igneous, Aguja Sandstone	Gary et al. 2007, Brune
McKinney	CanahC03S04		1981

Table 15	Piner table k	ev for understandin	o spring aquifer source
Table 15.	Piper table K	ev for understanding	ig spring aquiter source

Site	Piper Designation	Aquifer Source	Reference
Mule Ear	CaNaHCO3	Igneous	Gary et al. 2007
		Igneous	Gary et al. 2007, Baker
Oak	CaNaHCO3SO4		and Buszka 1993
Pena	CaNaHCO3SO4	Igneous	Gary et al. 2007
Asa Jones	CaNaMgClHCO3	Edward's Trinity-Glen Rose limestone	Brune 1981
Kyle	CaNaMgSO4HCO3	Edward's Trinity	Brune 1981
Mystery	CaMgClHCO3	Edward's Trinity	
Son of Hot		Edward's Trinity-Glen Rose limestone	
Springs	CaNaClHCO3		
		Edward's Trinity-Georgetown	Brune 1975
Blue	CaMgHCO3	Limestone	
		Edward's Trinity-Georgetown	Brune 1975
Dolan	CaMgHCO3	Limestone	
Caroline	CaNaHCO3SO4Cl	Edward's Trinity	Brune 1975
Vanderbeek	CaMgHCO3ClSO4	Edward's Trinity	
Post	MgCaNaHCO3SO4	Marathon	Smith 2001

 Table 15-Continued. Piper table key for understanding spring aquifer source

		Macro Sample in		Total
Regional Grouping	Sites	APPENDIX 1	Richness	Organisms
GUMO	Choza	15		149
GUMO	Guadalupe	2		174
GUMO	Smith	9		196
GUMO	Upper Pine	7		95
Davis	Bridge	29		200
Davis	Limpia	34		168
Davis	Lower Tuly	39		238
Davis	Toab	36		55
Davis	Upper Tuly	102	13	308
O2	Cottonwood	82	25	371
O2	Davenport	81	18	159
O2	Duff	79		227
O2	Terlingua	74		89
O2	Whirlwind	97		162
Cibolo	Cienega	26	18	131
Cibolo	High Elevation	28	24	267
Cibolo	Indian Cave	21		198
Cibolo	La Morita	24		76
Cibolo	North	19	37	444
Cibolo	Ojo Carrizal	27	27	312
BBSP	Cave	101	3	19
BBSP	Cinco Tinajas	50	6	20
BBSP	Las Cuevas	49	14	139
BBSP	Ojito Adentro	48	27	297
BBSP	Smith House	46		168
BBNP	Buttrill	51		346
BBNP	Burro	66		57
BBNP	Glenn	65	20	205
BBNP	Mule Ear	63		207
BBNP	McKinney	71		49
BBNP	Oak	60		61
BBNP	Pena	69		175
Lower Canyons	Asa	93		31
Lower Canyons	Kyle	94		7
Lower Canyons	Mystery	96	25	272
Lower Canyons	Son of Hot	95		7

# Table 16. Macroinvertebrate richness (used to test for PWS correlation and richness regression).

		Macro Sample in		Total				
<b>Regional Grouping</b>	Sites	APPENDIX 1	Richness	Organisms				
Devil's	Blue	18	18	294				
Devil's	Dolan	17	24	318				
Independence	T5	84		215				
Independence	Vanderbeek	87	20	141				
Marathon	Post	88		326				
	Regional Grouping Devil's Devil's Independence Independence Marathon	Regional GroupingSitesDevil'sBlueDevil'sDolanIndependenceT5IndependenceVanderbeekMarathonPost	Regional GroupingSitesMacro Sample in APPENDIX 1Devil'sBlue18Devil'sDolan17IndependenceT584IndependenceVanderbeek87MarathonPost88	Regional GroupingSitesMacro Sample in APPENDIX 1Devil'sBlue1818Devil'sDolan1724IndependenceT58418IndependenceVanderbeek8720MarathonPost8818				

Table 16-Continued. Macroinvertebrate richness (used to test for PWS correlation and richness regression).

Table 17. Physical result table											
					Latitude	Longitude					
<b>a</b> .	<b>.</b> .		Relative	Water	(decimal	(decimal	Elevation				
Sites	Ecoregion	PWS (km)	Q	Temperature (°C)	degrees)	degrees)	(m)				
Choza	1	76.2	1	16.5	32.00	104.82	1611				
Guadalupe	1	82.3	1	14.0	31.92	104.91	1649				
Smith	3	79.0	1	14.8	31.95	104.91	1828				
Upper Pine	3	79.3	1	14.8	31.95	104.84	1830				
Bridge	3	65.5	2	16.1	30.82	104.43	2117				
Limpia	3	65.5	1	6.9	30.67	104.22	1892				
Lower Tuly	3	63.6	2	19.1	30.83	104.31	1557				
Toab	3	61.4	1	14.2	30.73	104.37	2149				
Upper Tuly	3	66.0	1	18.0	30.89	104.26	1870				
Cottonwood	4	64.0	1	22.8	29.98	103.89	1252				
Davenport	1	71.3	1	23.9	30.03	103.84	1259				
Duff	1	92.0	2	24.6	30.18	103.81	1245				
Terlingua	4	75.0	1	25.9	30.00	103.78	1171				
Whirlwind	1	75.0	1	21.4	30.88	104.26	1233				
Cienega	1	32.2	1	26.1	29.86	104.39	1171				
High Elevation	1	21.8	1	15.2	29.99	104.51	1475				
Indian Cave	1	34.7	2	23.8	30.02	104.27	1236				
La Morita	1	25.4	1	20.2	29.85	104.40	1179				
North	1	24.1	1	23.0	29.91	104.46	1244				
Ojo Carrizal	1	24.2	1	24.0	29.94	104.53	1233				
Cave	3	21.3	1	16.1	30.03	104.44	1542				
Cinco Tinajas	1	19.1	1	32.8	29.71	104.04	1236				
Las Cuevas	1	10.9	1	23.4	29.66	104.14	1099				
Oak	1	12.3	1	18.5	29.64	104.20	1158				
Smith House	1	16.5	1	21.6	29.64	104.11	1224				
Buttrill	4	36.3	1	23.5	30.28	103.33	1036				
Burro	4	13.3	1	24.6	29.35	103.57	966				
Glenn	4	10.1	1	25.7	29.31	103.27	790				
Mule Ear	1	8.7	1	22.9	29.36	103.54	924				
McKinney	1	25.9	1	24.3	29.54	103.16	876				
Oak	1	25.4	2	19.5	29.52	103.39	1236				
Pena	4	3.6	1	18.8	29.25	103.58	762				
Asa	1	0.3	2	31.9	29.79	102.77	483				
Kvle	1	0.6	2	31.0	29.91	102.21	452				
Mystery	1	1.7	1	12.8	29.97	102.43	436				
Son of Hot	1	0.6	3	31.9	29.99	102.66	444				
Blue	2	28.0	3	22.6	30.06	101 17	398				
Dolan	2	36.5	3	22.5	30.00	101.03	447				
T5	2	17.0	3	22.0	30.50	101.86	653				
Vanderbeek	$\frac{1}{2}$	19.5	2	24.0	30.50	101.89	622				
Post	4	863	2	15.8	30.28	103.33	673				
1 051	1 +	00.5	2	13.0	50.20	105.55	075				

# Table 17. Physical result table

	SpC	_						0110 (11	- <b>B</b> ,)							
Sites	(µS/cm)	HCO <sub>3</sub>	Cl	DO	$(SO_4)^{2-}$	NO <sub>3-</sub>	F	Br	Ca	Na	Κ	Mg	Fe	Al	As	Mn
Choza	570	196	5.0	5.8	43.0	0.2	0.1	0.2	62.5	58.8	0.9	14.6	0.09	0.20	0.09	0.01
Guadalupe	553	226	9.0	6.1	14.5	0.7	0.3	0.0	65.8	14.5	3.1	27.8	0.90	0.45	0.01	0.09
Smith	592	34	9.0	6.5	9.5	0.9	0.1	0.0	8.1	7.3	3.1	23.1	0.05	0.05	0.01	0.01
Upper Pine	570	208	9.0	5.4	10.5	1.3	0.1	0.0	50.8	25.6	3.0	25.3	0.07	0.05	0.01	0.01
Bridge	114	128	5.7	4.0	13.3	0.1	0.3	0.3	26.4	65.2	1.1	5.0	0.09	0.09	0.01	0.04
Limpia	415	166	21.0	4.4	29.0	1.0	0.6	0.0	32.7	20.7	3.4	20.2	0.10	0.02	0.03	0.00
Lower Tuly	219	278	11.0	8.7	24.0	0.4	0.5	0.0	44.5	5.2	3.1	4.7	0.25	2.76	0.05	0.05
Toab	74	208	0.8	0.0	10.0	0.1	0.1	0.0	0.0	0.0	0.5	3.4	0.20	0.09	0.01	0.01
Upper Tuly	322	252	9.0	3.5	17.0	0.1	2.0	0.0	46.9	36.7	2.5	4.2	0.90	0.20	0.01	0.01
Cottonwood	534	92	10.0	5.4	23.0	1.8	0.6	0.0	19.9	13.8	3.9	18.5	0.10	0.09	0.01	0.07
Davenport	441	262	6.0	1.4	11.0	0.5	0.7	0.0	83.7	27.1	2.6	6.9	0.40	0.07	0.01	0.05
Duff	858	212	17.0	5.9	136.0	0.1	1.7	0.0	33.5	80.3	2.5	10.9	1.80	0.20	0.01	0.06
Terlingua	383	216	11.0	7.7	24.0	0.2	1.2	0.0	69.7	6.5	2.2	2.4	1.90	0.20	0.01	0.03
Whirlwind	668	370	26.0	3.5	47.0	2.2	1.1	0.2	43.0	26.7	0.8	3.9	0.09	0.20	0.01	0.01
Cienega	496	332	9.0	4.5	9.5	0.9	0.0	0.2	88.7	82.2	3.1	25.3	0.05	0.05	0.01	0.01
High Elevation	538	264	5.0	7.6	62.0	0.1	0.6	0.1	77.6	6.5	2.0	18.4	0.09	0.90	0.01	0.01
Indian Cave	512	238	11.0	6.6	21.0	1.1	0.0	0.0	70.8	20.3	3.1	3.5				
La Morita	1067	232	33.0	3.5	197.0	0.2	2.5	0.1	34.3	47.3	3.2	10.6	0.03	0.02	0.01	0.08
North	370	210	117.0	5.6	10.0	1.7	1.1	0.2	69.2	69.6	3.2	14.0	0.10	0.07	0.01	0.04
Ojo Carrizal	379	188	9.5	3.6	63.5	0.9	0.7	0.1	37.6	10.7	1.4	2.4	0.13	0.02	0.01	0.01
Cave	407	88	71.5	3.4	107.5	2.1	1.2	0.0	19.1	15.5	2.1	17.6	0.06	4.17	0.06	0.01
Cinco Tinajas	235	262	4.0	6.6	12.0	1.9	1.0	0.1	104.3	35.6	7.0	2.1	0.40	0.47	0.01	0.03
Las Cuevas	585	278	5.0	5.3	14.0	0.8	2.2	0.3	152.0	62.6	2.6	10.0	0.30	0.08	0.01	0.01
Ojito Adentro	473	262	6.0	3.8	14.0	0.1	1.7	0.2	80.8	70.4	1.1	5.3	2.60		0.01	0.06
Smith House	549	512	9.0	4.4	124.0	0.2	1.0	0.3	70.5	65.7	4.1	38.3	0.01	0.02	0.01	0.01
Buttrill	485	172	31.5	10.2	60.5	2.4	0.9	0.1	49.0	5.2	7.7	1.6	0.01	0.02	0.01	0.01
Burro	586	176	37.5	5.1	188.0	0.2	3.0	0.0	31.9	14.7	3.3	15.1	0.01	0.02	0.01	0.01
Glenn	831	204	9.0	0.0	94.0	0.1	4.4	0.0	70.3	19.0	4.2	8.4	0.03	0.02	0.01	0.01
Mule Ear	575	174	0.8	3.8	12.5	0.1	2.8	0.0	31.9	14.7	1.4	7.6	0.09	0.09	0.01	0.01

## Table 18. Results for anions and cations (mg/L)

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Tuble 10 Commutat Repuis for among and carlons (mg/L)																
	SpC									_						
Sites	(µS/cm)	$HCO_3$	Cl	DO	$(SO_4)^{2-}$	NO <sub>3-</sub>	F	Br	Ca	Na	Κ	Mg	Fe	Al	As	Mn
McKinney	1021	148	5.7	5.5	13.7	0.1	3.9	0.1	52.8	23.3	2.9	5.1	4.50	0.20	0.01	0.02
Oak	450	366	8.0	5.7	21.0	2.2	4.0	0.3	51.5	54.2	1.6	3.3	0.09	1.10	0.04	0.01
Pena	779	252	7.0	0.0	22.0	0.4	3.7	13.0	53.0	2.8	1.5	5.9	0.70	8.50	0.01	0.02
Asa	791	278	130.0	8.1	50.0	1.5	1.6	0.0	49.5	4.6	4.6	19.0	0.20	0.07	0.01	0.01
Kyle	872	196	59.0	5.8	175.0	1.4	1.8	0.2	60.0	49.2	4.9	21.5	0.10	0.07	0.01	0.01
Mystery	386	218	10.0	4.4	26.5	0.9	0.5	0.1	49.2	39.9	0.7	7.1	1.70	0.37	0.01	0.03
Son of Hot	746	176	1.0	7.2	22.0	0.1	1.5	0.1	13.8	8.1	2.5	5.0	0.09	0.09	0.01	0.17
Blue	500	25	16.0	4.7	9.0	1.9	0.3	0.0	6.5	4.6	1.2	15.2	0.40	0.07	0.01	0.01
Dolan	487	0	16.0	7.2	8.0	1.8	0.3	0.0	75.7	3.3	1.0	13.1	0.30	0.07	0.01	0.01
T5	781	142	149.0	7.7	45.0	1.5	1.0	0.1	19.6	9.4	4.6	18.0	0.10	0.07	0.01	0.01
Vanderbeek	933	236	94.0	2.8	100.0	1.6	1.1	0.2	90.5	7.9	4.2	25.6	0.09	0.20	0.01	0.01
Post	984	250	29.5	7.2	134.3	0.1	2.5	0.2	20.2	8.3	0.6	9.2	0.20	0.94	0.01	2.69

Table 18-Continued. Results for anions and cations (mg/L)
# **APPENDIX 10**

Sites	CaCO <sub>3</sub>	SiO <sub>2</sub>	TP(µg/L)
Choza	234	19.8	49
Guadalupe	227	8.7	35.8
Smith	33	31.3	29.4
Upper Pine	145	33.9	41.4
Bridge	74	22.9	238.4
Limpia	142	50.1	129.7
Lower Tuly	214	6.9	64.5
Toab	0	0	64.7
Upper Tuly	158	36.6	66
Cottonwood	58	20.5	210
Davenport	227	53.1	90
Duff	112	46.7	80
Terlingua	228	7.2	129
Whirlwind	152	52.3	34.8
Cienega	256	18.6	28.2
High Elevation	336	8.3	130
Indian Cave	253	34.6	27
La Morita	100	32.6	44.5
North	261	19.8	10.5
Ojo Carrizal	147	18.6	23
Cave	70	30.9	29.4
Cinco Tinajas	224	68.6	50
Las Cuevas	423	25.1	50
Ojito Adentro	268	45.5	250
Smith House	297	70.5	76.8
Buttrill	180	13	126.7
Burro	101	33.6	15.2
Glenn	189	52.9	245
Mule Ear	104	24.9	303.5
McKinney	140	12.8	225.8
Oak	265	8	49
Pena	153	59.2	640
Asa	231	5	11.4
Kyle	224	22.1	13.1
Mystery	159	3.9	37.6
Son of Hot	44	41.6	44.9
Blue	28	20	10
Dolan	279	0	10

# Table 19. Water chemistry (mg/L)

Sites	CaCO <sub>3</sub>	$SiO_2$	TP(µg/L)
T5	66	35.6	16.6
Vanderbeek	331	25	49
Post	66	51.8	105

Table 19-Continued. Water chemistry (mg/L)

## **APPENDIX 11**

Values in AX1 and AX2 columns are percentages of the total variance explained in the first row. The bold values are where the majority of the variance lies.

Variance	0.419	0.216	
	AX1: REL Q. LONG.	AX2: PWS, WTEMP,	
Site	ELEV	ELEV, LAT	Regional groups
СН	0.661	-0.446	GUMO
GU	-0.277	0.476	GUMO
SM	2.364	1.782	GUMO
UP	-0.599	0.203	GUMO
BR	-1.075	2.298	Davis
LI	-2.201	1.039	Davis
ТО	-1.613	1.962	Davis
UT	-0.583	3.15	Davis
CO	-1.876	1.546	O2
DA	-1.79	-0.196	O2
WH	-0.76	0.135	O2
CA	-1.727	1.048	Cibolo
CI	0.36	0.43	Cibolo
HE	-1.145	0.733	Cibolo
IN	0.089	-1.149	Cibolo
NO	0.661	-0.446	Cibolo
OC	0.574	-1.607	Cibolo
СТ	-2.201	1.039	BBSP
CU	0.823	-0.58	BBSP
OA	0.341	-0.629	BBSP
BT	0.344	0.383	BBNP
BU	-1.198	-0.536	BBNP
ME	-0.824	-1.592	BBNP
PE	-0.681	-3.012	BBNP
AS	2.603	0.576	Lower Canyons
MY	-0.599	0.203	Lower Canyons
BL	3.109	1.258	Devil's
DO	2.487	0.934	Devil's
T5	2.603	0.576	Independence
VA	2.364	1.782	Independence

Table 20. CCA site scores in environmental space

### **APPENDIX 12**

Values in AX1 and AX2 columns are percentages of the total variance explained in the first row. The bold values are where the majority of the variance lies.

Variation Explained	0.419	0.216
		AX2:PWS, WTEMP,
Org species	AX1 : REL Q, LONG, ELEV	ELEV, LAT
PODO	-0.3245	0.2033
CALL I	-0.4506	0.2563
MCVE L	-0.3700	0.0139
AMBR Y	0.4760	-0.1139
LACCPH	-0.4076	0.0401
HYAL	0.4456	0.3013
NOTONC	-0.4700	0.0264
ARCHIL	-0.3826	-0.1979
TABAN	0.5983	-0.0165
STICTO	-0.4140	0.1911
LIBELL	-0.1619	-0.4952
OLIGO	0.5235	0.0597
HELI CO	0.6659	0.0010
AGA	-0.0344	0.8022
PELTO	-0.2204	-0.5523
PHYSD	-0.2048	-0.2909
TRICOR	0.6094	-0.0775
CALO	0.7465	0.6494
LIMNP	-0.6864	-0.1401
ENALL	-0.2296	-0.0992
BERO	0.6191	-0.2472
ORTH	-0.2226	-0.5681
MACRL	0.4863	-0.2530
CYCLO	-0.4544	0.6691
MAR	0.1116	-0.6138
SIM	0.1052	-1.0044
EUP	0.8188	-0.0397
ANOPH	-0.3307	-0.0852
PHYLL	0.5872	0.1353
PROB	-0.0705	-0.5054

 Table 21. CCA taxa scores

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