INVERTEBRATE COMMUNITY STRUCTURE AND HABITAT ASSOCIATIONS IN
THE ARID DAVIS MOUNTAINS REGION OF WEST TEXAS

by

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A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
May 2019

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ACKNOWLEDGEMENTS

Firstly, I would like to thank my advisor, Dr. Weston Nowlin. He was always my biggest advocate and always pushed me to think bigger. Weston saw me through a lot of life changes during my time at Texas State University and I will be forever grateful for his unwavering support. He taught me what a true advisor should be. I am not often sweet, so I hope he does not take any of my kind words as sarcasm. I’d also like to thank Chad Norris for organizing and implementing this project, I always appreciated the way that Chad would ask my opinion and include me in planning, he always treated me as an equal rather than a lowly grad student. I’d like to thank Dr. Benjamin Schwartz for going out of his way to teach a biologist some hydrogeology things and for always treating me as if I were one of his own students. I’d also like to thank Dr. Astrid Schwalb, she has a brilliant mind and was always willing to help me especially with regard to data analysis.

In any study of this size- it takes a village. I’d like to thank the state and federal employees who helped in the field and the lab including: Randy Gibson, Morgan Brizendine, Marty Kelly, Karim Aziz, and especially Pete Diaz who spent countless hours at the scope helping me to ID bugs. From Texas State University, I’d like to thank: Matthew Stehle, Christina Vance, Kayla Robicheaux, and Gaby Timmins for their help in the field and in the lab. A scientist is only as good as her mind- and I appreciate those in my life who have supported me mentally and emotionally throughout this process, including but not limited to: my family, my mellons, with special thanks to Tori Rivers, Megan Phillips, Forrest Cortes, Taylor Gold Quiros, Parvathi Nair, and Ryan de Mello for their loyal companionship and support.
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<td>ES</td>
<td>East Sandia</td>
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<td>ESA</td>
<td>Endangered Species Act</td>
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<td>HC</td>
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ABSTRACT

In arid regions, springs often represent ecologically important aquatic habitats which are patchily distributed across the landscape. This separation can lead to localized endemic populations of organisms that exhibit small species distributions and are adapted to local environmental conditions. Desert spring aquatic organisms are also frequently of high conservation priority and are listed as imperiled and in need of protection. This study examined invertebrate community structure and habitat associations at seven sites in and around the Davis Mountains in the Trans Pecos region of west Texas. The overall purpose of this study was to determine mesohabitat associations and estimate population sizes of three endangered aquatic invertebrates found in the region: the Phantom springsnail (*Pyrgulopsis texana*), the Phantom tryonia (*Tryonia cheatumi*), and the diminutive amphipod (*Gammarus hyalleloides*). I conducted stratified random sampling at all sites quarterly for a year starting in March of 2017. Results indicate that the abundance of most of the endangered species was most strongly influenced by site (the particular location that was sampled) and that mesohabitat conditions were substantially less important in influencing the density of species. In addition, I found that two species of non-native and invasive snails (*Melanoides tuberculata* and *Tarebia granifera*) were found at most of the study sites sometimes at densities higher than populations of native invertebrates. These results suggest that regionally distributed invertebrates with low dispersal potential (such as snails and amphipods with no desiccation-resistant life stages) exhibit high site-specific occurrence. In addition, these results indicate that conservation of these populations in the wild should focus on site-specific objectives to preserve water
quality and habitat conditions. However, this management strategy is complicated by the fact that these spring systems are interconnected by a larger regional groundwater pool. With agricultural demands and oil and gas development increasing in the Trans-Pecos region, the risk for groundwater over-pumping and contamination place individual and collective regional populations at risk.
1. INVERTEBRATE COMMUNITY STRUCTURE AND HABITAT ASSOCIATIONS IN THE ARID DAVIS MOUNTAINS REGION OF WEST TEXAS

Introduction

On a global scale, freshwater ecosystems support a disproportionate amount of biodiversity when compared to the rest of the earth, (Sada et al., 2005; Dudgeon et al., 2006). However, biodiversity in these systems face a variety of threats, including: over-pumping, pollution, flow modification, habitat destruction, and introduction of non-native invasive species (Dudgeon et al., 2006). In arid and semi-arid regions, perennial aquatic habitats are often dependent upon groundwater discharges (Shepard, 1993). The value of spring-fed systems in a desert landscape cannot be overstated as they typically provide the only reliable source of surface water found in the region (Bogan et al., 2014; Stanislawczyk et al., 2018). The presence of water in an arid region not only provides habitat for aquatic organisms (increasing regional biodiversity) but also provides opportunities for more complex interactions between aquatic and terrestrial environments (Shepard, 1993).

Spring (crenic) habitats are biologically important aquatic habitats which are often patchily distributed across landscapes, and this spatial separation leads to local adaptation of populations and speciation with narrow endemic ranges (Murphy, et al. 2012). Desert springs are particularly important because they often provide habitat for “remnant” populations of organisms “trapped” in remaining surface water in arid and water-limited landscapes (Unmack and Minckley, 2008). Obligate aquatic invertebrates in particular, are more likely to have narrow ranges of endemism due to their inability to disperse
relatively long distances (Murphy et al., 2012). These species are often highly adapted to very specific water quality parameters due to the stability in flow, water chemistry, and temperature of these groundwater-fed systems (Unmack and Minckley, 2008). Aquatic organisms restricted to desert springs often are of high conservation priority and many are listed as imperiled and in need of protection through legislation, such as the Endangered Species Act of 1973 (the ESA).

Despite the importance of desert crenic habitats in maintaining endemic populations, they are often highly imperiled ecosystems and are disappearing globally. In particular, the extraction of groundwater has been identified as the “killer of springs” and the negative effects of groundwater pumping on springs in arid regions has been extensively documented worldwide (Unmack and Minckley, 2008). The preservation of these crenic habitats is vital to the conservation of spring-obligate species which have narrow ranges, making these species even more vulnerable to extinction (Box et al., 2008; Fensham et al., 2011). Fensham et al. (2011) believes that efforts to conserve entire crenic systems should be implemented because they are “biodiversity hotspots” and preserving the system allows for the conservation of many endangered and threatened species at once. Additionally, the conservation of crenic habitats and springflows also protect downstream surface water uses and helps keep common regional species common.

The Trans-Pecos region encompasses nine counties in west Texas (Brewster, Culberson, El Paso, Hudspeth, Jeff Davis, Pecos, Presidio, Reeves, and Terrell) lying west of the Pecos River. The Trans-Pecos is dominated by groundwater with minimal surface water (Uliana and Sharp, 2001) and is a unique ecoregion that includes desert and the highest mountains in the state. Approximately 300 mya, a Permian Sea covered this
region and resulting sedimentary geologic formations (including salt and anhydrite) now contribute to the relatively high specific conductivity of the region’s ground and surface water, rendering much of the water non-potable (Jensen, et al., 2006). Exposed rocks in and around Balmorhea, Texas are marine sediments of the Lower and Upper Cretaceous, volcanic deposits, and alluvial deposits (Chowdhury, 2004). The modern Trans-Pecos region is arid, ranging from 20 to 50 cm precipitation per year (Uliana and Sharp, 2001). The close association between ground and surface waters in the region results in their high sensitivity to changes in both water quantity and quality. If these desert spring ecosystems are to be protected, and the region’s water supplies maintained, then quantity and quality of both surface and groundwaters must be preserved, including maintenance of flows required to ensure the persistence of federally threatened and endangered species found in the region’s springs.

Texas Parks and Wildlife Department (TPWD) has identified 16 threatened, endangered or imperiled species whose habitats could be affected by reductions in local water quality and quantity (Norris, 2016). Indeed, most of these species, including fish, crustaceans, and gastropods are spring associated taxa and all have limited geographic distributions making them highly susceptible to extinction. Springs in this region have been impacted by groundwater withdrawals for agricultural irrigation are now facing increased pressure from fossil fuel exploration; both activities can reduce flows at springs and also create possible contamination hazards (Jensen, et al., 2006). Some examples of impacted springs in the region include San Solomon Springs (Balmorhea State Park, Balmorhea, TX) where median discharge from 1920-1936 was 972.68 l/s and since 2001 is 722.08 l/s; a 25.7% decrease in discharge from 1920 to present (TPWD, 2005). Discharge at Comanche Springs (Ft. Stockton, TX, 90 km east of Balmorhea State Park)
began to decline in 1947 and ceased in 1961 due to groundwater pumping for agricultural purposes and discharge of other springs in the area, such as Toyah, Saragosa, and Leon springs, have either ceased to flow or have had their flows greatly diminished in volume and persistence (Brune, 2002).

The purpose of this study was to 1) gather baseline quantitative data on macroinvertebrate community at four spring-fed systems, 2) define mesohabitat associations of several endangered and exotic invertebrates in the region and 3) create site specific population and density estimates for *Tryonia cheatumi*, *Pyrgulopsis texana*, and *Gammarus hyalleloides*, as well as other species deemed as significant. This study is part of a larger and intensive monitoring program initiated by the TPWD at four spring-fed systems (at 7 sites) in and around Balmorhea, TX in Reeves and Jeff Davis counties (Figure 1b), which are home to many threatened and endangered species including the three invertebrate species of interest addressed in this study: the Phantom Springsnail (*Pyrgulopsis texana*), the Phantom Tryonia (*Tryonia cheatumi*), and the Diminutive Amphipod (*Gammarus hyalleloides*). These species have narrow ranges and are only known to occur in one or more of the seven sites (Bradstreet et al., 2010). In addition, several of these sites have been invaded by two species of non-native invasive snails (*Melanoides tuberculata* and *Tarebia granifera*), making conservation efforts of the endangered species more critical and complicated. Data gathered as part of this project will help inform natural resource managers make informed decisions on matters that may impact springs in the Balmorhea area.

I predicted that the species of interest in this study (*T. cheatumi*, *P. texana*, and *G. hyalleloides*) will exhibit specific mesohabitat associations across the spring sites. I also predicted that, based on the site-specific population estimates, the species of interest will
exhibit patterns of high site-specific associations and will not be distributed uniformly across sites.

Materials and Methods

Site description

The study sites were seven spring-fed aquatic systems in Texas’s Trans-Pecos region: East Sandia (ES), West Sandia (WS), San Solomon Springs, including San Solomon Pool (SSP), San Solomon Canal (CAN), Hubbs Cienega (HC), and San Solomon Cienega (SSC), and Phantom Lake Spring (PHA) (Table 1). Balmorhea State Park contains San Solomon Springs; an artesian spring system that fills a man-made pool (San Solomon Pool), which is a regionally-important recreation, diving, and swimming destination (Norris 2016). Discharge from the springs flows out of the headwater pool into a canal (CAN), which feeds two cienegas (San Solomon Cienega and Hubbs Cienega) before leaving the State Park. The canal transports water 5.6 km east to Balmorhea Lake Reservoir, where it is stored for recreational use and distribution for irrigation.

Approximately 2.4 km southwest of Balmorhea State Park, Phantom Lake Spring discharges artesian groundwater from a large cavern system (Brune 2002). The spring once supplied a lake near the cavern entrance, but that is now reduced to a small pool that feeds into an irrigation canal. The spring ceased flowing in 1998 and flow has since been artificially maintained with a pump; there has been no significant decrease in precipitation in the region so over-pumping is likely the cause (White, et al. 1941; Uliana and Sharp, 2001). Regionally, Phantom Lake Spring and San Solomon Springs are connected along a groundwater flow path that originates in the Salt Basin and flows
through the Apache Mountains (Uliana and Sharp 2001; Veni 2013). Previous studies have shown that East and West Sandia (Figure 1b) have Na\(^+\):Cl\(^-\) and SO\(_4^{2-}\):Cl\(^-\) ratios similar to that of San Solomon Springs and Phantom Lake Spring, indicating that the water from these two springs systems are from a similar groundwater source (Chowdhury et al. 2004). East and West Sandia are gravity-fed springs on property owned by the Nature Conservancy and are approximately 4.8 km northeast of Balmorhea State Park (White, et al. 1940). In 2000, West Sandia was described as being “reduced to a moist soil area” wherein no aquatic snails were found (Landye). In May 2017 during a trip to Balmorhea (unrelated to spring sampling), a few upwellings and a long, narrow flowing canal of water was found with an approximate average depth of 15 to 20 cm and a maximum depth of 90 cm amongst tall, dense stands of *Phragmites*.

**Species of interest**

The Phantom Tryonia (*T. cheatumi*) and the Phantom Springsnail (*P. texana*) are aquatic snails in the family Hydrobiidae. Hydrobiids make up the largest and most diverse group of freshwater snails in North America, consisting of 285 species in 35 genera (Hershler, et al. 1998; Brown, et al. 2008), and ~74% of hydrobiids are considered at risk of extinction due to their narrow endemic ranges and recent reductions in habitat due to groundwater pumping (Thorpe and Rogers, 2011). The Diminutive Amphipod (*G. hyalleloides*) is one of at least seven distinct species of amphipod in the *Gammarus pecos* species complex, which are endemic to springs in the Trans-Pecos region (Cole, 1985; Gervasio et al., 2004; Seidel 2009). The diminutive amphipod (*G. hyalleloides*) is the smallest known *Gammarus* species in North America (Cole 1976; Gervasio, et al. 2004). All three species of interest are considered federally endangered by the Unites States Fish and Wildlife Service.
Field sampling

Each of the seven sites were *a priori* subdivided into distinct mesohabitat types that were mapped/defined prior to sampling. For the purposes of this study, mesohabitat was defined as “visually distinct units of habitat within the stream, recognizable from the bank and with an apparent physical uniformity” (Pardo and Armitage, 1997). Unique combinations of sediment and vegetation types in each spring were used to define each mesohabitat assigned in each site. Mesohabitat types at each location were determined by identifying substrate types and areas with vegetation cover. Thus, mesohabitats were designated for each site as a combination of substrates and vegetation types, projected onto a GIS-generated map for each spring location. For example, the area occupied by both silt and the macrophyte *Chara* in an individual site would be designated as a specific mesohabitat type and the sampling points for that mesohabitat type would then be distributed throughout it.

Spring systems and their biota were sampled four times throughout 2017, beginning in March. Sampling followed a stratified random design in each site, with 10 to 25 randomly generated points (dependent upon size and the diversity of mesohabitats at each site) distributed among the various mesohabitat types in each spring system. To collect benthic invertebrates, a welded stainless steel-framed sampler (Lang benthic basket sampler; 10cm x 10cm x 3 cm, 500 µm mesh) was used at each sampling point. The sampler was developed by USFWS staff to gather quantitative data in spring fed ciénegas in New Mexico and was deemed appropriate for use in this study. Benthic samples were preserved in the field with 95% ethanol, and organism sorting, counting, and identifications were performed in the Aquatic Ecology Lab in the Freeman Aquatic Station at Texas State University.
In addition to invertebrate collection, the following parameters were recorded to establish mesohabitat identification: substrate size and mixture, organic matter type (vegetation type, algae, detritus, etc.), and water quality using a pre- and post-calibrated Eureka multiprobe (pH, temperature, specific conductivity, and dissolved oxygen). The substrate at each sampling point was defined by its primary sediment and secondary sediment type according to the Wentworth Scale (Wentworth, 1922). All sampling events began at ES and WS because there had been no previous record of the invasive aquatic snails *M. tuberculata* or *T. granifera* at either of these sites.

**Data analysis**

For all analyses, the only species analyzed were *T. cheatumi*, *P. texana*, *G. hyalleloides*, *M. tuberculata*, and *T. granifera* as they comprised 97.5% of all individuals collected (Wang et al., 2003). Principal Component Analysis (PCA) was utilized to determine the mesohabitat and water quality parameter ranges that are distributed across the sampled sites using RStudio Version 1.1.456 (Diaz, et al. 2015). All sediment and organic matter types were assigned their recorded percentage cover (0 to 100) prior to analysis. All data were z-score transformed prior to analysis. PCA scores for each sampling point were identified by site to assess the range of mesohabitats and water chemistry parameters across sites.

Redundancy Analysis (RDA) was utilized to determine the mesohabitat and water physicochemistry associations of each of the species. Significance ($\alpha = 0.05$) was determined by completing 999 permutations with the `envfit` function found in the *vegan* package installed into RStudio Version 1.1.456. Prior to analysis, species count data were Hellinger transformed and fourth-root normalized to minimize the impact of rare species at each sampling point (Legendre and Gallagher, 2001). An initial RDA was used to
determine site associations of each species followed by a second RDA to determine the mesohabitat and water physicochemistry associations of each species to determine which factors had significant influence in predicting the density of species present. A global RDA encompassing site, mesohabitat, and water physicochemistry data was run to determine which were significant in predicting the density of species present. Finally, I ran two partial RDAs (the first to determine the pure effects of site location while controlling for the influence of mesohabitat and water physicochemistry data and the second to determine the pure effects of mesohabitat and water physicochemistry data while controlling for the influence of site location) and variance partitioning to evaluate combined and pure effects of the two predictor sets (King et al. 2005; Becker et al., 2014). To determine the mesohabitat associations of each species within each site, I ran an additional RDAs in each site.

To calculate the estimated population size of each species in each site, I calculated the mean of each species per sample per mesohabitat and extrapolated that mean to the total area of each site. The min to max range was calculated in the same manner using the minimum observed number of each species per mesohabitat in each site to maximum observed number of each species per mesohabitat in each site.

**Results**

Of all the individuals collected, five species dominated and comprised 90,186 of the 92,495 individuals collected: *T. cheatumi* (11,565 individuals), *P. texana* (21,733 individuals), *G. hyalleloides* (14,633 individuals), *M. tuberculata* (11,719 individuals), and *T. granifera* (30,505 individuals). *P. texana* were found at all sites except for the San Solomon Cienega (WS being a new local for this species), and *T. cheatumi* were found at
all sites except for West Sandia. *G. hyalleloides* were found at all sites, including West Sandia which is a new locale for this species (Table 3). *M. tuberculata* and *T. granifera* were found at all sites except for East and West Sandia.

*Water physicochemistry and mesohabitat availability gradients*

To examine variation in mesohabitat conditions, the water physicochemistry (Table 2) and mesohabitat PCA accounted for 28.2% of the variation among sites on the first two axes (Figure 2a), the third axis accounted for an additional 9.4% of the variation among sites (Figure 2b). Principal component axis I (PC I) explained 16.6% of the variation among sites. In general, the positive loadings on PC I were the presence of sand, gravel, and spike rush while the negative loadings on PC I were the presence of silt and increased pH and DO. Sites were arranged across this axis from SSC having the most negative loading values and CAN having the most positive indicating the substantial range of conditions available found in Balmorhea State Park. Principal component axis II (PC II) explained 11.6% of the variation among sites. In general, the positive loadings on PC II were the presence of concrete and increased values of specific conductivity while the negative loadings on PC II were increased temperature, pH, and DO values. All sites in Balmorhea State Park and PHA had substantial overlap across PC II but ES and WS, while having substantial overlap with each other, had minimal overlap with any other sites due to the higher specific conductivity values at these sites. The PCA showed that East and West Sandia had generally lower temperatures and higher specific conductivity values when compared to the other sites (Figure 2a). The sites in Balmorhea State Park were quite variable. SSC and HC had generally higher pH, dissolved oxygen (DO), and temperature (Figure 2a). Principal component axis III (PC III) explained an additional 9.5% of the variation among sites. In general, the positive loadings on PC III were the
presence of *Chara*, spike rush, and silt while the negative loadings were increased pH and the presence of concrete and periphyton. All sites had a great area of overlap across PC III but there were a few outlying points belonging to SSP, which had very negative values. These outlying points can be explained due to the substantial concrete and periphyton cover found in SSP since most of the shallow swimming area has a concrete substrate. SSP and CAN had generally overlapping data points with no clear water quality parameter association, but CAN was more associated with spike rush than any of the other sites. PHA was the most centralized of all sites sampled; all points hovered around the origin of both axes indicating intermediate conditions when compared to ES, WS, and the sites in Balmorhea State Park.

*Community mesohabitat associations*

Variance partitioning indicated that the pure effects of environmental factors accounted for 3% of the variation and the pure effects of site location accounted for 17%, explaining ~5.6x more variation than environmental factors. Variance partitioning indicates significant collinearity between environmental factors and site location (31%), because many substrate and vegetation types were site-specific. For example, spike rush was only found in CAN, the majority of concrete was found in SSP, and detritus was only found in ES and WS.

The site location RDA was able to separate the seven studied sites into three major groups, the first including ES and WS, the second including PHA and SSP, and the third including CAN, HC, and SSC (Figure 3a). *G. hyalleloides* dominated the community structure in ES and WS, the endangered hydrobiids dominated the community structure in PHA and SSP, and the invasive aquatic snails dominated the community structure in CAN, HC, and SSC (Figure 3a). The environmental factor RDA
further supported the pattern of separation across sites (Figure 3b, 3c).

Two Partial RDAs were run to determine the pure effects of site specification and environmental factors separately. In the first Partial RDA, when controlling for environmental factors, the pure effects of site location RDA axes I and II explained 3.2% and 1.6% of the variation in species distribution ($R^2_{adj} = 0.17$, p<0.05, Figure 4a). In this model CAN, ES, and WS were not significant predictors of species distribution; all species had significant associations with the remaining sites SSP, HC, SSC, and PHA. In the second Partial RDA, when controlling for site location, the pure effects of environmental factors RDA axes I and II explained 1.1% and 0.4% of the variation in the species abundance distribution data, respectively (Figure 4b). In this model, *T. cheatumi*, *G. hyalleloides*, and *M. tuberculata* were the only species with significant associations and the only significant environmental factors displayed were sand, gravel, periphyton, concrete, and silt. Three of the site-specific RDAs indicated significant responses from species densities to the mesohabitats available within the respective sites (ES, SSP, and PHA; Figure 5a-c). *G. hyalleloides* in ES and SSP was associated with the presence of sand and gravel and had no other significant associations with mesohabitats in other sites (Figure 5a, 5b). *T. cheatumi* in SSP was associated with the presence of concrete and had no other identified significant association with mesohabitats in other sites (Figure 5b). *P. texana* was closely associated with *Chara* in PHA and had no other significant associations with mesohabitats in other sites (Figure 5c). Of the population of *P. texana* within PHA, >92% of those individuals were found in the *Chara* mesohabitat. *M. tuberculata* in SSP was associated with *Chara* and silt (Figure 5a). *T. granifera* did not seem to have any identified significant mesohabitat associations within any site.

*Mean densities and population size estimates*
\textit{T. cheatumi} were found in greatest numbers in SSP at Balmorhea State Park, which comprised 88.8\% of the regional population estimate (Table 3) with an average density of 10,006 individuals/m\(^2\) at this site (Table 4). \textit{P. texana} were found in greatest numbers in PHA which comprised 72.3\% of the regional population estimate (Table 3) with an average of 65,696 individuals/m\(^2\) (Table 4). \textit{G. hyalleloides} were found in greatest numbers in CAN at Balmorhea State Park, which contained 41.6\% of the regional population estimate (Table 3), with an average of 7,350 individuals/m\(^2\) at this site (Table 4). \textit{T. granifera} and \textit{M. tuberculata} were both found in greatest numbers in SSC, which encompassed 52.8\% (averaging 19,503 individuals/m\(^2\)) and 59.0\% (averaging 8,495 individuals/m\(^2\)) of the regional population estimates, respectively. In combination, 59.1\% and 79.2\% of the \textit{T. granifera} and \textit{M. tuberculata}, respectively, were collected at SSC and HC (Tables 3, 4).

\textbf{Discussion}

In this study, I found that site associations were the strongest predictor of individual species occurrences and densities, leading to significant differences in community composition among sites. In the RDA, all species had significant relationships with site location when controlling for the influence of environmental factors (Figure 4a), but the three focal endangered species exhibited a gradient of site-specific association. Overall, site-specific associations were strongest for the two hydrobiid species. \textit{T. cheatumi} exhibited a close association with the sites SSP and PHA (Figure 3a, Figure 4a), but \textit{T. cheatumi} was also associated with concrete substrate and periphyton which encompass substantial areas of the man-made portion of SSP (Figure 4b). \textit{P. texana} was strongly associated with the PHA site, encompassing ~72\% of the
estimated regional population across study sites. This finding is particularly interesting because the PHA contains <1% of the total habitat surface area I surveyed across all study sites. Additionally, *P. texana* did not show a significant association with any environmental factor when controlling for site (Figure 4b), reinforcing the strength of this site association. However, within the PHA site, *P. texana* showed a very strong association with one particular mesohabitat type – the macrophyte *Chara*. The strong site association of *P. texana* at PHA suggests that this site serves as an *in situ* refugium for this species in the Davis Mountain region (Figure 5c).

Although the initial RDA examining only the influence of site (Figure 3a) indicated that *G. hyalleloides* was associated specifically with WS, this association was influenced by the fact that *G. hyalleloides* was the only species found in substantial numbers at that site. However, the partial RDA examining the pure effects of site found that CAN, ES, or WS were not significant predictors for the occurrence and abundance of all species (Figure 4a). In the partial RDA examining the pure effects of site, the density of *G. hyalleloides* exhibited a significant response, but did not show a clear association with any specific site location (Figure 4a). Although *G. hyalleloides* was the only taxon found at all sites and represented a specific “community type” at the WS and ES sites, further analyses indicated that within individual sites it was strongly associated with sand and gravel substrates (Figure 5a, 5b).

Hydrobiid spring snails such as *T. cheatumi* and *P. texana* have been shown to passively disperse via external factors such as currents, flooding, and waterfowl but are typically unable to travel significant distances across land (Bovbjerg, 1952; Boag, 1986; Figuerola and Green, 2002; Kappes and Haase, 2012). Active aquatic dispersal of hydrobiids is also limited and Wilmer, et al. (2008) estimates the active short-range
aquatic dispersal for hydrobiids to be \( \leq 300 \) m, with anything further requiring facilitation by the external processes listed above. *T. cheatumi* was found to be highly associated with SSP and concrete but was still found in significant populations in CAN in Balmorhea State Park and in substantial numbers at PHA. Dispersal from SSP to CAN is passive transport via the flow path originating in SSP and flowing through CAN. *P. texana* was found to be highly associated with PHA (encompassing highest population estimates and densities) but was present at all sites except for SSC. Thus, once populations of the hydrobiids have been established at a given site location, it is likely that dispersal rates to additional site locations are limited and low, unless there are incidental and active means for them to be dispersed via external processes.

The site-specific associations of *T. cheatumi* and *P. texana* can be attributed to numerous factors and processes, including the sequence of community assembly (i.e., the order in which species arrive in a community location via immigration), the effects of interspecific interactions within a given location, changes in abiotic or environmental conditions at a site, and stochastic (random) processes affecting population persistence (Vellend, 2010). Studies have shown that the sequence of species arrival into aquatic communities greatly affected the final community structure and that initial colonization greatly influences final community composition even when the regional species pool is held constant (Robinson and Dickerson, 1987; Drake, 2001; Fukami and Morin, 2003). Interspecific competition within hydrobiids has not been thoroughly studied, but some studies suggest that heterogeneity in habitat structure may contribute to the ability of different hydrobiids to co-occur (Hershler, 1984; Taylor, 1987; Brown et al., 2007). Environmental filtering and species sorting are unlikely to be influencing the density distribution of hydrobiids due to their low dispersal ability, but the role of dispersal in the
two species examined this study has not been assessed. Previous studies have shown that habitat type at particular locales are not as important to species densities as site location (Bogan et al., 2014; Stanislawczyk et al., 2018). This study suggests that it is likely that different species respond differently to habitat associations. While *T. cheatumi* and *P. texana* did not show mesohabitat associations across sites, both species exhibited strong mesohabitat associations within sites. *G. hyalleloides* exhibited mesohabitat associations across sites (regional) while the hydrobiids exhibited higher site-specific associations (local) which is likely due to the difference in dispersal abilities between amphipods and hydrobiids.

Previous research indicates that *Gammarus* is often associated with more structurally complex benthic substrates, including rocky cobbles and organic materials (Dahl and Greenburg, 1996). More complex substrates with larger interstitial spaces provides protection from predation along as well as access to possible food sources (Rabeni and Minshall, 1977). The sites in Balmorhea State Park contain >89% of the regional *G. hyalleloides* population and ~93% of the aquatic surface area surveyed, further suggesting the more cosmopolitan nature and greater relative dispersal abilities of *G. hyalleloides*. Relative to hydrobiids, *Gammarus* can disperse more easily upstream in high or low flow conditions, allowing for two-way movement throughout a system (Dahl and Greenburg, 1996). Indeed, surface habitat-associated amphipods have been shown to disperse to previously uncolonized habitats via shallow groundwater pathways (Harris et al., 2002). Although *Gammarus* have still relatively low dispersal abilities when compared to other organisms such as fish or emergent aquatic insects, *G. hyalleloides* seems to have much greater dispersal abilities compared to the hydrobiids (Gervasio, et al. 2004).
In contrast with site and mesohabitat associations of the native focus taxon, this study found that two non-native snail species were widely distributed across sites and exhibited high densities at some site locations. *M. tuberculata* and *T. granifera* were found across all sites in this study with the noted exception of the East and West Sandia sites. Both *M. tuberculata* and *T. granifera* were associated with and occurred at their highest densities at HC, SSC, and CAN (Figure 3a). Additionally, *M. tuberculata* was associated with silt and gravel while *T. granifera* showed no clear association with habitat (Figure 4b). The negative ecological effects of *M. tuberculata* especially have been widely documented since its introduction to the United States in the 1960s (Ladd and Rogowski, 2012). Invasive snails pose problems for both native fish communities and native invertebrate communities. *M. tuberculata* has been documented to be an intermediate host to several species of trematode which can negatively affect fish populations (Ladd and Rogowski, 2012; McDermott, et al. 2014). These non-native invasive aquatic snails are generalist grazers and have been documented consuming fish eggs, suggesting that they might also consume eggs of other snail species (Ladd and Rogowski, 2012). They may also affect native snails by being a larger and more-efficient grazers, resulting in the shift of available food sources available for native species, which can lead to increased competition and shifts in entire trophic structures; especially in spring-fed systems with low biodiversity (Ladd and Rogowski, 2012; Larson, 2016). Spring systems are particularly vulnerable to invasion by tropical non-native taxa because consistent temperature and water quality conditions buffer these tropical taxa from environmental variation (Karatayev et al., 2009). I strongly recommend that in any future studies or field work in this region, it is of the utmost importance that every precaution be taken to not introduce *M. tuberculata* and *T. granifera* to East or West Sandia, especially
since all three endangered species were found at these sites.

Conservation Issues

Persistence of the focal endangered species in these spring environments is related to the presence of water (water quantity). However, water quality also likely plays a critical role. Across the studied sites, pH varied the least (7.10-7.61) while temperature (20.32-25.24°C), specific conductivity (3243-4263 µS/cm), and dissolved oxygen (4.35-8.72 mg/L) were more variable (Table 2). No studies have determined environmental tolerance ranges of these species to date. Without environmental tolerance data, a proper risk assessment is impossible (Mace and Lande, 1991; Raimondo et al., 2008). The U.S. Fish and Wildlife Service has attempted to establish preliminary refugia populations of the three focal species at an external facility, the San Marcos Aquatic Resources Center (SMARC). SMARC staff initially had substantial difficulty keeping individuals alive. This suggests that these species are sensitive to changes in environmental conditions although this facet of their ecology warrants future exploration. More recently, SMARC staff have had reproductive success with T. cheatumi, P. texana, and G. hyalleloides, but have not been able to raise juveniles to adulthood, which would be required before any environmental tolerance study can be completed (R. Gibson, personal communication). Because groundwater pumping can reduce spring discharge in an entire region, regional water conservation and planning is of utmost importance to preserve the presence and quality of spring flow, which is required for the conservation of the endangered species in this region (Unmack and Minckley, 2008; Fensham et al., 2011). With agricultural demands and oil and gas development increasing in the Trans-Pecos region, the risk of groundwater over-pumping and contamination is substantially higher.

Existing management activities of these sites are important to consider. Currently
the surface flow at PHA is sustained by a pump and is regulated by the United States Bureau of Reclamation. Due to the strong site-specific association of the regional population of *P. texana* with PHA, surface water habitat at Phantom Lake Spring is important but is essentially an *in situ* refugia. It is important to continue efforts to cultivate a refuge population for this species and the other federally listed taxa. In addition, the water level in SSP is drawn down annually and the concrete surface is pressure-washed to remove algal growth for the safety of swimmers and visitors to Balmorhea State Park. Density and population estimates for *T. cheatumi* at SSP are orders of magnitude greater than estimates for *T. cheatumi* at the other sites sampled, with the downstream canal site (CAN) having the second largest population estimate. This suggests that despite the mortalities caused by annual pressure washing activities, a large population of *T. cheatumi* persists at SSP. An analysis of density data by sample date shows densities collected were substantially lower the month following pressure washing (dropping from ~16,000 individuals/m² on the concrete prior to pressure-washing down to ~1,800 individuals/m² after pressure-washing), with numbers increasing in subsequent sampling events. The large density and population estimates for *T. cheatumi* at SSP as compared to other springs in the area indicates Balmorhea State Park is an important habitat to the long-term conservation of the species, which should be taken into consideration in natural resource management decisions.

In addition to habitat and site conservation efforts, genetic analysis needs to be performed between the regional populations of (what are assumed to be) the same species found across sites. There has been some work measuring gene flow between isolated populations of desert fishes. These studies have shown little to no gene flow between isolated populations indicating loss of genetic variability and no recolonization of site
extinction events, both resulting in local divergences at sites (Meffe and Vrijenhoek, 1988). Since *G. hyalleloides* is part of the larger cryptic *G. pecos* species complex, it is important to determine the degree of gene flow between populations, especially with the new population at WS identified (Gervasio et al., 2004). Some genetic analyses have been completed across the *G. pecos* species and gene flow between populations of *G. hyalleloides* have shown substantial genetic flow between the populations from Balmorhea State Park and ES, but minimal gene flow between either of those populations and PHA (Gervasio et al., 2004; Seidel et al., 2009). WS is a new locale for *G. hyalleloides* so these individuals should also be sampled and analyzed. There has been no study found analyzing the degree of gene flow between populations of *T. cheatumi* and *P. texana* within all the studied sites. I recommend genetic analysis of all endangered species across all sites in this study to determine level of gene flow between populations and to estimate population sizes to compare to the quantitative data in this study. Restricted gene flow between isolated populations can lead to higher rates of inbreeding, geographically unique populations, and eventually further speciation (Colgan and Ponder, 2000; Perez et al., 2005). Although the dispersal of the focal endangered species in this study has not been studied specifically, it can be assumed to be relatively poor, so the odds for substantial gene flow between regional populations is low but still unknown.
Figure 1. Maps of study sites: (a) Map of the Texas, USA with major rivers labeled and study area indicated in red; (b) Study sites in and around Balmorhea, Texas including East Sandia, West Sandia, Balmorhea State Park (encompassing San Solomon Pool, a canal, and two cienegas), and Phantom Lake Spring.
<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude, Longitude</th>
<th>Surface Area (m²)</th>
<th>Site Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Sandia (ES)</td>
<td>30.990978, -103.729036</td>
<td>603</td>
<td>Series of isolated pools/cienegas</td>
</tr>
<tr>
<td>West Sandia (WS)</td>
<td>30.986838, -103.736359</td>
<td>339</td>
<td>Narrow canal, flowing water</td>
</tr>
<tr>
<td>Balmorhea State Park</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Solomon Pool (SSP)</td>
<td>30.944279, -103.788395</td>
<td>5,556</td>
<td>Large swimming pool, partially concrete</td>
</tr>
<tr>
<td>Canal (CAN)</td>
<td>30.944538, -103.785917</td>
<td>2,859</td>
<td>Concrete banks, flowing water, connects SSP to HC then to SSC</td>
</tr>
<tr>
<td>Hubbs Cienega (HC)</td>
<td>30.945479, -103.786001</td>
<td>1,050</td>
<td>Cienega fed by CAN, smaller surface area, no riparian cover</td>
</tr>
<tr>
<td>San Solomon Cienega (SSC)</td>
<td>30.945138, -103.784405</td>
<td>4,340</td>
<td>Cienega fed by CAN, larger surface area, some riparian cover</td>
</tr>
<tr>
<td>Phantom Lake Spring Spring (PHA)</td>
<td>30.935005, -103.849613</td>
<td>120</td>
<td>Origins within cave, surface water artificially supplied by pump</td>
</tr>
</tbody>
</table>
Table 2. Mean water quality parameter at each site across sampling dates ± 1 SD.

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Specific Conductivity (µS/cm)</th>
<th>Dissolved Oxygen (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>20.32±1.46</td>
<td>7.10±0.20</td>
<td>4263±156</td>
<td>6.62±0.79</td>
</tr>
<tr>
<td>WS</td>
<td>20.63±0.46</td>
<td>7.24±0.19</td>
<td>4044±129</td>
<td>5.42±1.33</td>
</tr>
<tr>
<td>SSP</td>
<td>24.69±0.40</td>
<td>7.15±0.15</td>
<td>3248±147</td>
<td>4.35±1.36</td>
</tr>
<tr>
<td>CAN</td>
<td>25.24±0.81</td>
<td>7.25±0.81</td>
<td>3267±163</td>
<td>5.89±1.19</td>
</tr>
<tr>
<td>HC</td>
<td>24.78±1.54</td>
<td>7.48±0.12</td>
<td>3245±160</td>
<td>8.62±1.98</td>
</tr>
<tr>
<td>SSC</td>
<td>23.45±3.67</td>
<td>7.61±0.14</td>
<td>3243±207</td>
<td>8.72±2.12</td>
</tr>
<tr>
<td>PHA</td>
<td>23.41±1.54</td>
<td>7.37±0.16</td>
<td>3534±84</td>
<td>4.94±1.24</td>
</tr>
</tbody>
</table>
Figure 2. Principal component analysis (PCA) ordination of water physiochemistry and mesohabitat data, (a) PCA axes I and II and (b) PCA axes II and III.
Figure 3. Biplots of redundancy analyses (RDA), (a) site specification and (b) environmental factors and the response variable of each species; (c) Global RDA including a and b as predictors.
Figure 4. Partial redundancy analysis (RDA) biplots; showing (a) pure effects of significant (p<0.05) site locations controlling for environment factors and (b) environment factors controlling for site location.
Figure 5. Mesohabitat association redundancy analysis (RDA) biplots; showing mesohabitat associations of focal species with mesohabitats in each site, only sites with significant $(p<0.05)$ predictor and response variables shown; (a) ES, (b) SSP, and (c) PHA.
Table 3. Population estimates per species per site. Mean, followed by min - max possible range.

<table>
<thead>
<tr>
<th>Site</th>
<th><em>Tryonnia cheatumi</em></th>
<th><em>Pyrgulopsis texana</em></th>
<th><em>Gammarus hyalleloides</em></th>
<th><em>Melanoides tuberculata</em></th>
<th><em>Tarebia granifera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>137,153</td>
<td>95,771</td>
<td>3,973,888</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(35,471 - 1,671,847)</td>
<td>(42,565 - 27,800,665)</td>
<td>(1,632,829 - 27,800,665)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td>0</td>
<td>1,130</td>
<td>848,630</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(0 - 4,520)</td>
<td>(282,500 - 1,906,130)</td>
<td>(282,500 - 1,906,130)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SSP</td>
<td>55,594,725</td>
<td>750,060</td>
<td>8,452,065</td>
<td>8,847,930</td>
<td>21,369,765</td>
</tr>
<tr>
<td></td>
<td>(6,847,770 - 327,477,585)</td>
<td>(305,580 - 24,890,880)</td>
<td>(2,972,460 - 34,072,170)</td>
<td>(6,028,260 - 24,890,880)</td>
<td></td>
</tr>
<tr>
<td>CAN</td>
<td>6,000,326</td>
<td>2,129,955</td>
<td>21,013,650</td>
<td>3,559,455</td>
<td>44,157,255</td>
</tr>
<tr>
<td>HC</td>
<td>178,043</td>
<td>41,087</td>
<td>1,072,826</td>
<td>12,622,826</td>
<td>10,011,522</td>
</tr>
<tr>
<td></td>
<td>(13,696 - 1,255,435)</td>
<td>(0 - 310,434)</td>
<td>(123,260 - 5,770,434)</td>
<td>(3,839,348 - 38,735,869)</td>
<td>(483,913 - 51,121,304)</td>
</tr>
<tr>
<td>SSC</td>
<td>30,456</td>
<td>0</td>
<td>14,649,404</td>
<td>36,867,158</td>
<td>84,645,228</td>
</tr>
<tr>
<td></td>
<td>(22,842 - 137,052)</td>
<td>(0 - 109,286)</td>
<td>(395,929 - 89,289,790)</td>
<td>(12,113,930 - 133,443,579)</td>
<td>(3,129,368 - 440,654,667)</td>
</tr>
<tr>
<td>PHA</td>
<td>679,286</td>
<td>7,883,571</td>
<td>451,286</td>
<td>575,143</td>
<td>21,000</td>
</tr>
<tr>
<td></td>
<td>(0 - 4,636,286)</td>
<td>(111,428 - 6,106,714)</td>
<td>(0 - 29,143)</td>
<td>(0 - 3,099,428)</td>
<td>(0 - 109,286)</td>
</tr>
<tr>
<td></td>
<td>(1,515,429)</td>
<td>(31,515,429)</td>
<td>(31,515,429)</td>
<td>(31,515,429)</td>
<td>(31,515,429)</td>
</tr>
</tbody>
</table>
Table 4. Density estimates per species per site. Mean followed by min – max possible range shown in individuals/m².

<table>
<thead>
<tr>
<th>Site</th>
<th>Tryonia cheatumi</th>
<th>Pyrgulopsis texana</th>
<th>Gammarus hyalleloides</th>
<th>Melanoides tuberculata</th>
<th>Tarebia granifera</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES</td>
<td>227</td>
<td>159</td>
<td>6,590</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(59 – 12,772)</td>
<td>(71 – 2,502)</td>
<td>(2,707 – 46,103)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WS</td>
<td>0</td>
<td>3</td>
<td>2,503</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(0 – 13)</td>
<td></td>
<td>(833 – 5,623)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSP</td>
<td>10,006</td>
<td>130</td>
<td>1,521</td>
<td>1,593</td>
<td>3,846</td>
</tr>
<tr>
<td></td>
<td>(1,233 – 58,941)</td>
<td>(55 – 355)</td>
<td>(535 – 4,480)</td>
<td>(1,085 – 6,132)</td>
<td>(1,418 – 4,480)</td>
</tr>
<tr>
<td>CAN</td>
<td>2,099</td>
<td>745</td>
<td>7,350</td>
<td>1,245</td>
<td>15,445</td>
</tr>
<tr>
<td></td>
<td>(1,466 – 5,323)</td>
<td>(560 – 1,393)</td>
<td>(3,259 – 17,980)</td>
<td>(626 – 3,963)</td>
<td>(8,569 – 34,497)</td>
</tr>
<tr>
<td>HC</td>
<td>170</td>
<td>39</td>
<td>1,022</td>
<td>12,060</td>
<td>9,535</td>
</tr>
<tr>
<td></td>
<td>(13 – 1,196)</td>
<td>(0 – 296)</td>
<td>(117 – 5,496)</td>
<td>(3,657 – 36,891)</td>
<td>(461 – 48,867)</td>
</tr>
<tr>
<td>SSC</td>
<td>7</td>
<td>0</td>
<td>3,375</td>
<td>8,495</td>
<td>19,504</td>
</tr>
<tr>
<td>PHA</td>
<td>5,660</td>
<td>65,969</td>
<td>3,760</td>
<td>4,793</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>(0 – 38,635)</td>
<td>(929 – 262,629)</td>
<td>(0 – 50,889)</td>
<td>(243 – 25,829)</td>
<td>(0 – 911)</td>
</tr>
</tbody>
</table>
LITERATURE CITED


