SPRINGS GEOMORPHOLOGY INFLUENCES ON PHYSICAL AND VEGETATION ECOSYSTEM CHARACTERISTICS, GRAND CANYON ECOREGION, USA

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ABSTRACT

SPRINGS GEOMORPHOLOGY INFLUENCES ON PHYSICAL AND VEGETATION ECOSYSTEM CHARACTERISTICS, GRAND CANYON ECOREGION, USA

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The Grand Canyon Ecoregion (GCE) represents the entire landscape that drains into Grand Canyon. This region encompasses a wide array of environments and corresponding plant communities of biological interest. Springs are numerous in the GCE and play a multitude of roles in this generally arid land. Springs serve as critical sources of water and support many endangered and endemic species, many of which are springs-dependent species including *Flaveria mcdougalii, Epipactis gigantea,* and *Eleocharis palustris*. I conducted a statistical community analysis of 352 springs in the Grand Canyon Ecoregion across four spring types – helocrene wet meadows; hanging gardens; rheocrene flowing springs; and hillslope springs – and examined their physical traits and floral assemblages. Mann-Whitney tests were used to detect differences between spheres of discharge and correlation and multiple regression were used to determine relations of physical and geomorphic traits with plant species diversity. An astounding species packing was demonstrated with nearly 1000 species recorded across all springs, representing over 45% of the region's entire flora in less than one square kilometer of springs habitat area. Geomorphic microhabitat diversity was positively related to springs diversity ($p<0.00001$; multiple linear regression).

All springs types were distinguished by differences in physical site characters which in turn were associated with plant community structure and specific species. Geomorphic features including microhabitat features and substrate composition were important in distinguishing springs types. There were also different physical characteristics distinguishing springs types including elevation and water chemistry. These features were correlated strongly with plant assemblages at springs and sets of indicator species were associated with each spring type.

Multivariate regression analysis identified suites of variables related to springs biodiversity metrics explaining nearly half of the variation in species richness between springs. Microhabitat richness, area, and elevation were most important in explaining species richness. Grazing intensity did not have any discernable impact on species richness but did have a negative relation to the percentage of native species found at springs.

In this study, I identified key differences between spring types; however, springs are highly individualistic and each spring needs to be understood in an individual context. Stewardship efforts should aim to protect geomorphic microhabitats and restore them to natural conditions. Their concentrations of biodiversity warrant further conservation and additional inventory and study will prove useful in furthering understanding of springs of the GCE.

Acknowledgements

This thesis wouldn't have been possible without the support of numerous individuals over the past two years. First, I'd like to thank the Springs Stewardship Institute for the incredible work they've done to improve data collection on springs and highlight their importance. Doing field work with SSI over the summer was instrumental in my understanding of the data collection process. Without their support and effort none of these analyses would have been possible. I thank Larry Stevens for his expertise on springs ecology. Jeri Ledbetter was crucial in helping with database access and quality control support; she helped turn a daunting task into just several mornings of database query development. Thank you also to Jeff Jenness for providing GIS support. Numerous other individuals contributed to the data collection of the hundreds of springs surveys that were analyzed during this thesis.

I also like to thank my advisor Abe Springer for his unwavering support and patience. Your easygoing attitude, plentiful advice, and big picture outlook made has made graduate school a positive and relatively smooth process. Numerous chats about recent running workouts and races has been a nice break from reviewing manuscripts and discussing analyses.

I'd like to thank my fellow graduate students for making the whole process bearable and becoming great friends and helping with frequent comic relief when deadlines loomed. Our research group, including Alex Wood, Adrienne Soder, Natalie Jones and Stefan Christie, gave me invaluable advice on communicating and presenting my results. I'd like to thank my family for their continual support. Finally, I thank my girlfriend Isabel Caldwell for agreeing to move to Flagstaff and put up with my busy school schedule these past two years. I couldn't have made it through without you.

Preface

This thesis was first developed as a manuscript targeted at the journal *Oecologia*. This manuscript is presented as Chapter 2 and was styled to the journal's requirements. Thus, to meet thesis formatting guidelines some sections may be slightly repetitive. The most intriguing results are presented in the manuscript in Chapter 2 while additional methods, analyses and general discussion are presented in later chapters. The manuscript is entitled, *Geomorphology Influences on the Springs of the Grand Canyon Ecoregion, Arizona, USA*, and was coauthored with my committee members, Abe Springer and Larry Stevens.

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Chapter 1 – Introduction

1.1 Purpose and Objectives of Study

This study's purpose was to conduct a comprehensive investigation of the differences in geomorphology of the Grand Canyon Ecoregion (GCE) springs' ecosystems and examine the effects of geomorphic diversity on plant diversity.

Two main objectives included: (1) differentiating current geomorphic classes of springs based on their physical and biological characteristics and (2) identifying key geomorphic and physical characteristics of springs related to plant diversity and plant community composition. These two objectives were combined to develop an improved understanding of interactions between springs geomorphology and ecology. Results from this study will improve springs stewardship and conservation by developing a process to determine which springs provide the largest conservation benefit and the potential implications of various management actions on springs communities and biodiversity. A better understanding of the geomorphic differences between spring types and connections to springs species assemblages can allow for prioritization of management needs specific to each spring type and help determine which springs might serve as potential habitat for species of concern.

This study represents a large-scale analysis of springs of the GCE that incorporated both the physical traits of springs and their plant communities and is the first of its kind. The large number of spring ecosystem inventories with complete data available for analysis combined with the known ecological importance of springs in the GCE made it an obvious choice of a region for analysis.

1.2 Significance of Springs as Ecosystems

Springs provide important ecological functions and act as paleorefugia, hotspots of diversity, and keystone ecosystems (Perla and Stevens 2008, Springer et al 2015, Stevens et al. 2016b). Despite these functions, springs have been generally understudied and the lack of high-quality and comprehensive inventory has limited our understanding of springs ecosystems. Springs ecosystems are rare (less than 0.01% of the landscape) in arid lands yet they support a wide array of life and provide critical functions and have been reported to support much higher species densities than surrounding uplands. Springs geomorphology varies widely across the landscape although past studies have often ignored the role of geomorphology, focusing only on species diversity or water chemistry. It is hypothesized that the high levels of biodiversity at springs can be partially attributed to diverse and complex geomorphology at springs that produce many microhabitat types and varied niches.

Springs not only provide a multitude of ecological functions but have been central to human development. Springs serve important roles as focus points for culture and human activity (Stevens and Meretsky 2008), support recreational activities with significant economic value (Bonn and Bell 2003) and provide critical water sources and hydrogeological information about the aquifers that supply them (Toth and Katz 2006). Mueller et al. (2017) identified positive economic value associated with many functions related to springs of the Grand Canyon including habitat for endangered species, places of cultural significance, and backcountry sources of water. Not only are these functions important, but the public has demonstrated a willingness-to-pay to protect and conserve springs – even when they don’t anticipate visiting any in person. However, human uses of springs often threaten their ecological functions.

Despite these many important attributes, springs are globally threatened by development, overgrazing by livestock and groundwater pumping, climate change and contamination. The future of many springs is uncertain with up to 90% showing signs of human impacts and degradation (Grand Canyons Wildlands Council 2002). Due to these threats, the need for better spring stewardship has been acknowledged and springs have recently received much attention for management and restoration; but a comprehensive understanding of springs ecosystems is still lacking.

1.3 Study Area

The 35,000 km^2 Grand Canyon Ecoregion (GCE), defined as the entire physiogeographic region draining into the Grand Canyon (Figure 1), was selected for this study because it is a topographically diverse landscape that supports a wide array of many springs ecosystem types. Although the southern Colorado Plateau is a mega-ecotone, mixing Madrean and Rocky Mountain biota (Stevens 2012), the GCE is geographically, hydrologically and latitudinally constrained. The springs of the GCE have been relatively intensively mapped and occur from 350 – 3500 m elevation (Springs Online 2018). The region's springs have been intensively studied in several hydrogeological contexts, including connate and meteoric hydrogeology, karst hydrology, and water supplies and contamination studies (Campbell 1968, Huntoon 1974, Crossey et al. 2012, Jones et al. 2017, Tobin et al. 2017). The geology and geomorphology of the region are likely important controls on the locations of springs source areas of springs that result in spatial patterns of water chemistry and discharge magnitudes. Regional hydrogeology (Figure 2) consists of local perched aquifers (often on volcanic units) as well as regional Coconino "C" aquifer and Redwall-Muav "R" aquifer (Flynn and Bills 2002).

The flora of the GCE has been intensively inventoried (MacDougall 1976, Phillips et al. 1987, Stevens and Ayers 2002, Rink and Licher 2017), and GCE springs ecosystem ecology also has received some attention (e.g., Perla and Stevens 2008, Ledbetter et al. 2016). The region largely consists of wildlands and rangelands and is managed by several federal agencies and Native American Tribes and has relatively little private or urban land ownership. Springs constitute important riparian and wetland habitats in this generally arid area.

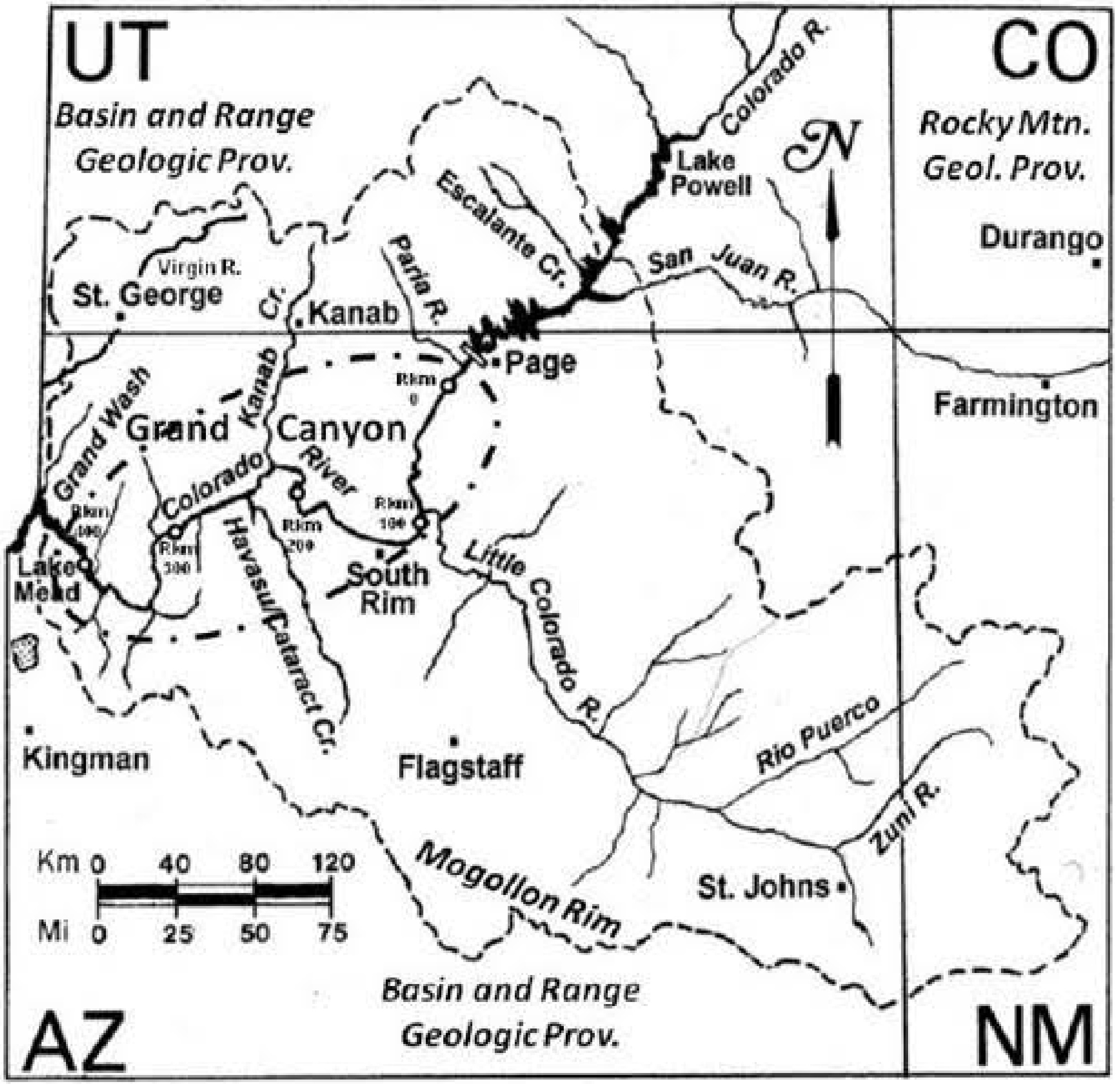

Figure 1. Map of the Grand Canyon Ecoregion showing major drainages and physiogeographic features; adapted from Stevens (2012)

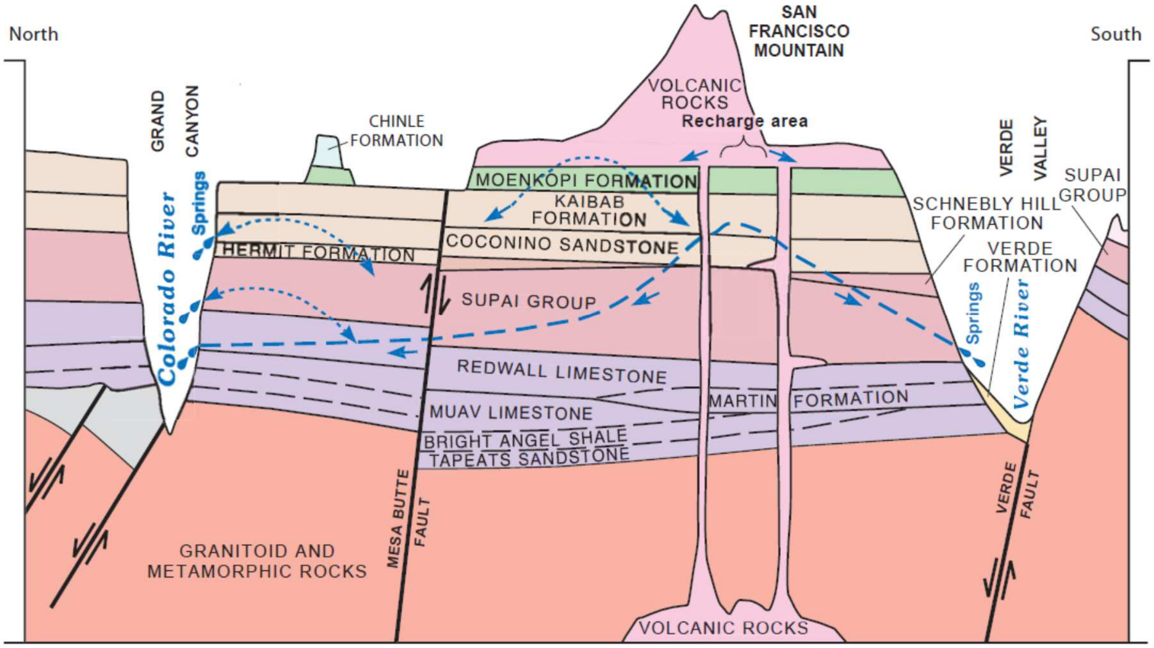

Figure 2. Generalized hydrogeologic cross-section of the Grand Canyon Ecoregion. Major geologic units and faults are drawn with recharge areas perched water tables, regional aquifers, major springs, and regional groundwater flow (shown in blue); adapted from Bills and Flynn (2002).

1.4 Spheres of Discharge

Spheres of discharge describe the way a springs source emerges onto the landscape. Meizner (1923) proposed nine spheres of discharge that were simplified to three (helocrene, rheocrene, limnocrene) by Hynes (1970). However, Springer and Stevens (2009) expanded this to 12 active spheres, not counting paleosprings. These have been recognized and used by hydrogeologists and ecologists, owing to the complexity and diversity of many springs and the need for a common lexicon to compare and describe them (e.g., US Forest Service 2012). Classification of springs by sphere of discharge is needed to help scientists and managers understand links between springs geomorphology and biodiversity. The complex environment around a springs source often creates many microhabitats to support a large diversity of life (Stevens and Springer 2009). They note the classification scheme will become more useful for ecologists and resource managers as more data are acquired:

> *As additional comprehensive inventories of the physical, biological, and cultural characteristics are conducted and analyzed, it will eventually be possible to associate spheres of discharge with discrete vegetation and aquatic invertebrate assemblages, and better understand the habitat requirements of rare or unique springs species. This is an especially important relationship to understand given the high number of rare organisms and the intense species packing at springs.* (Springer and Stevens 2009)

The 12 spheres of discharge are as follows (1) springs that emerge in caves, (2) exposure springs where the water table is exposed on the landscape but water does not flow, (3) artesian fountains from confined aquifers, (4) hot water geysers, (5) gushets from cliff walls, (6) hanging gardens emerging from geologic contacts along cliffs, (7) helocrene wet meadows, (8) springs emerging on steep hillslopes, (9) hypocrene springs buried below the surface, (10) limnocrene lentic pools, (11) mound form springs, and (12) rheocrene flowing channels (Springer and Stevens 2009). Additionally, anthropogenic springs may be recognized when springs are so severely altered so that their original sphere of discharge is no longer identifiable. A table showing the twelve spheres of discharge can be seen in Table 1.

Table 1. The twelve spheres of discharge as described by Springer and Stevens (2009).

Sphere of Discharge	**Emergence Setting**
Cave	Emerges in a cave in mature to extreme karst with sufficiently large conduits
Exposure	Cave, rock shelter fractures, or sinkholes where unconfined aquifer is exposed near the land surface
Fountain	Artesian fountain with pressurized CO_2 in a confined aquifer
Geyser	Explosive flow of hot water from a confined aquifer
Gushet	Discrete source flow gushes from a cliff wall of a perched, unconfined aquifer
Hanging Garden	Dripping flow emerges, usually horizontally, along a geologic contact along a cliff wall of a perched, unconfined aquifer
Helocrene	Emerges from low gradient wetlands; often indistinct or multiple sources seeping from shallow, unconfined aquifers
Hillslope	Emerges from confined or unconfined aquifers on a hillslope (30-60 degree slope); often indistinct or multiple sources
Hypocrene	A buried spring where flow does not reach the surface; typically due to low discharge or high evapotranspiration
Limnocrene	Emergence of confined or unconfined aquifers in pool(s)
Carbonate Mound-form	Emerges from a mineralized mound, frequently at magmatic or fault systems
Rheocrene	Flowing spring, emerges into one or more stream channels

However, to ensure sufficient sample sizes for statistical analysis of sphere of discharge, a minimum of 30 springs in the GCE were necessary in the analysis. Only four spring types occurred over 30 times: hanging gardens, helocrene springs, hillslope springs, and rheocrene springs. These spring types all have unique geomorphic and hydrogeologic properties that are important to the ecosystems they support. Hanging gardens are common in canyons and emerge along geologic contacts in cliff walls (Figure 3). Helocrene springs form low-gradient wet meadows, are often called cienegas and are usually fed by perched aquifers and develop thick organic-rich soils (Figure 4). Hillslope springs are often fed by unconfined aquifers where the water table emerges along a steep slope often with multiple sources or can emerge along a contact or fracture along a hillside (Figure 5). Rheocrene springs are lotic, flowing springs with a defined channel and often support riparian areas downstream (Figure 6).

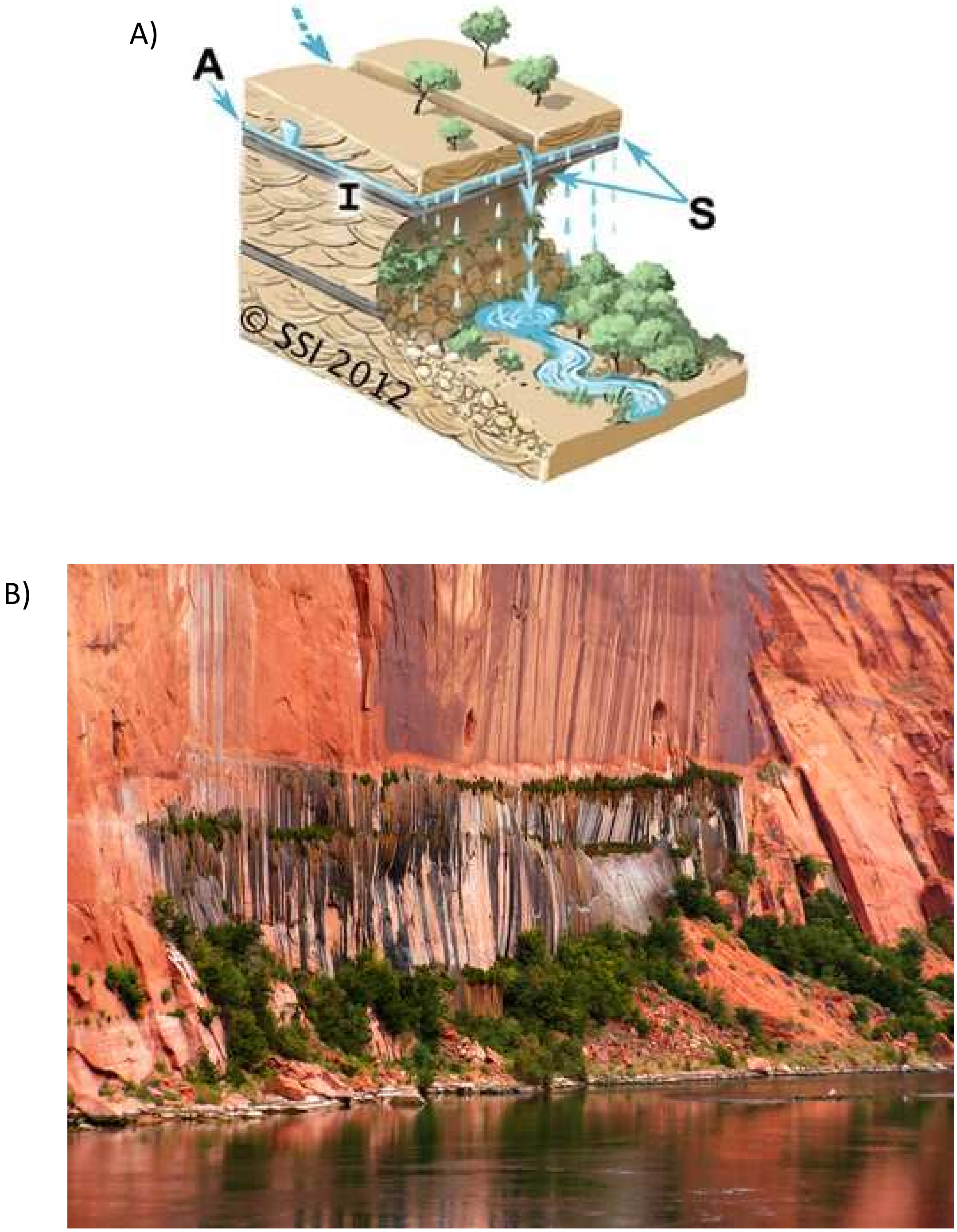

Figure 3. Diagram and example of hanging garden springs. Hanging gardens emerge along geologic contacts and seep, drip, or pour onto underlying walls. A) Sketch of hanging garden spring type courtesy of SSI. A=aquifer; I=impermeable stratum; S=spring source. B) Glen Canyon Dam, Hanging Gardens. Photo by Rich Rudow.

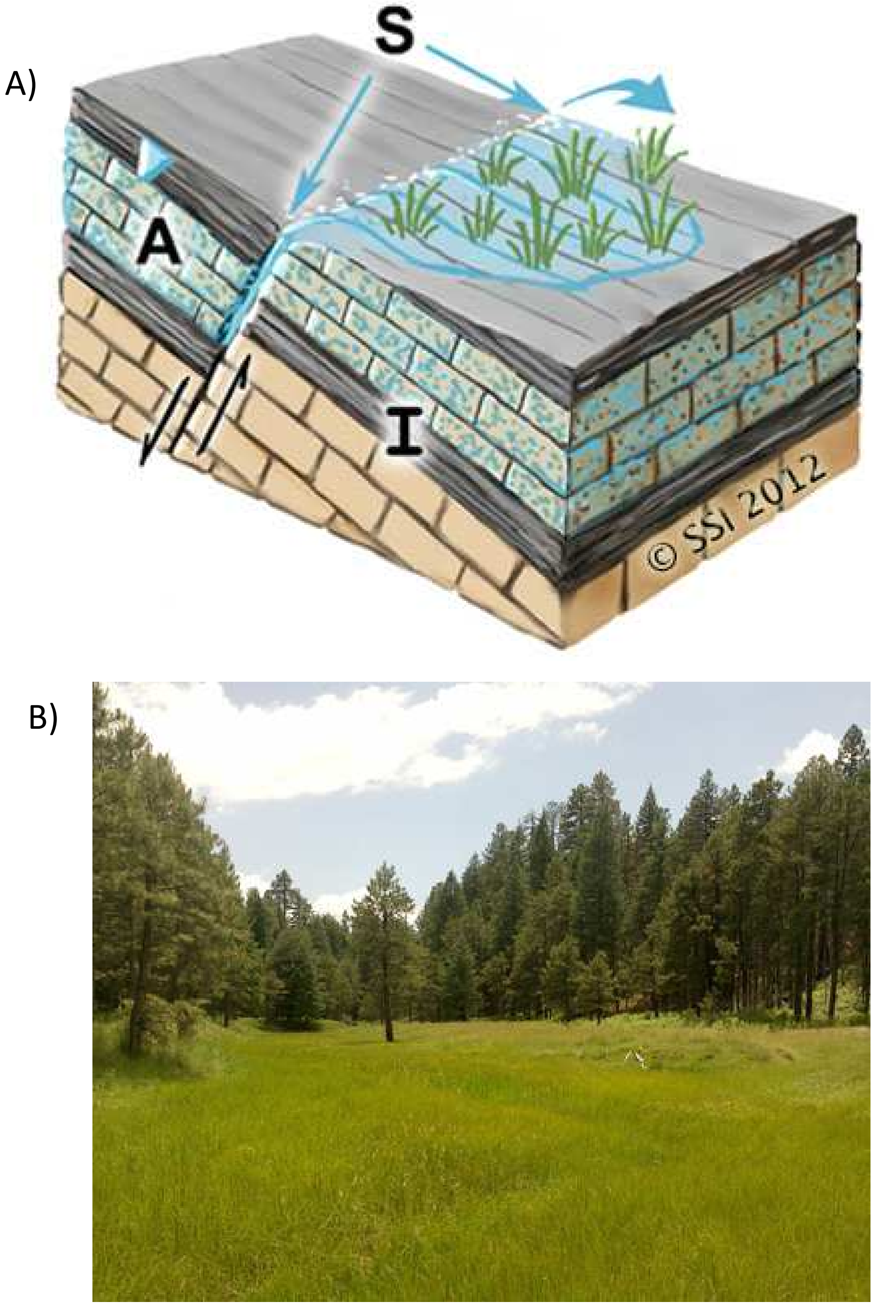

Figure 4. Diagram and example of a helocrene spring. Helocrene springs emerge diffusely in a marshy, wet meadow setting rather than having a discrete source. A) Sketch of helocrene spring type courtesy of SSI. A=aquifer; I=impermeable stratum; S=spring source. B) Helocrene spring, Apache Sitgreaves National Forest, Arizona. Photo courtesy of SSI.

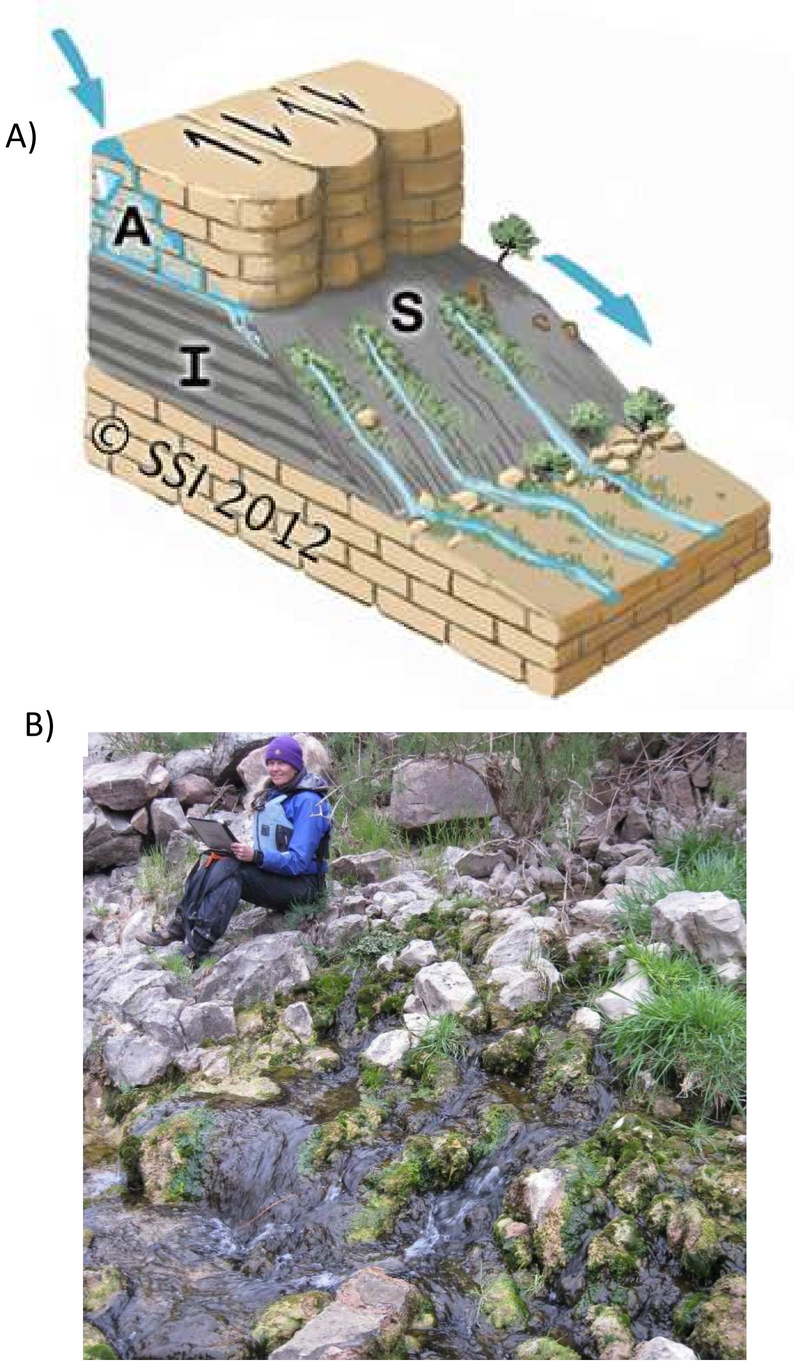

Figure 5. Diagram and example of a hillslope spring. Hillslope springs emerge from confined or unconfined aquifers on a hillslope (~30-60 degree slope), often with indistinct or multiple sources. A) Sketch of hillslope spring type courtesy of SSI. A=aquifer; I=impermeable stratum; S=spring source. B) Fence Fault Garden Spring on the bank of the Colorado River, Grand Canyon National Park. Photo courtesy of SSI.

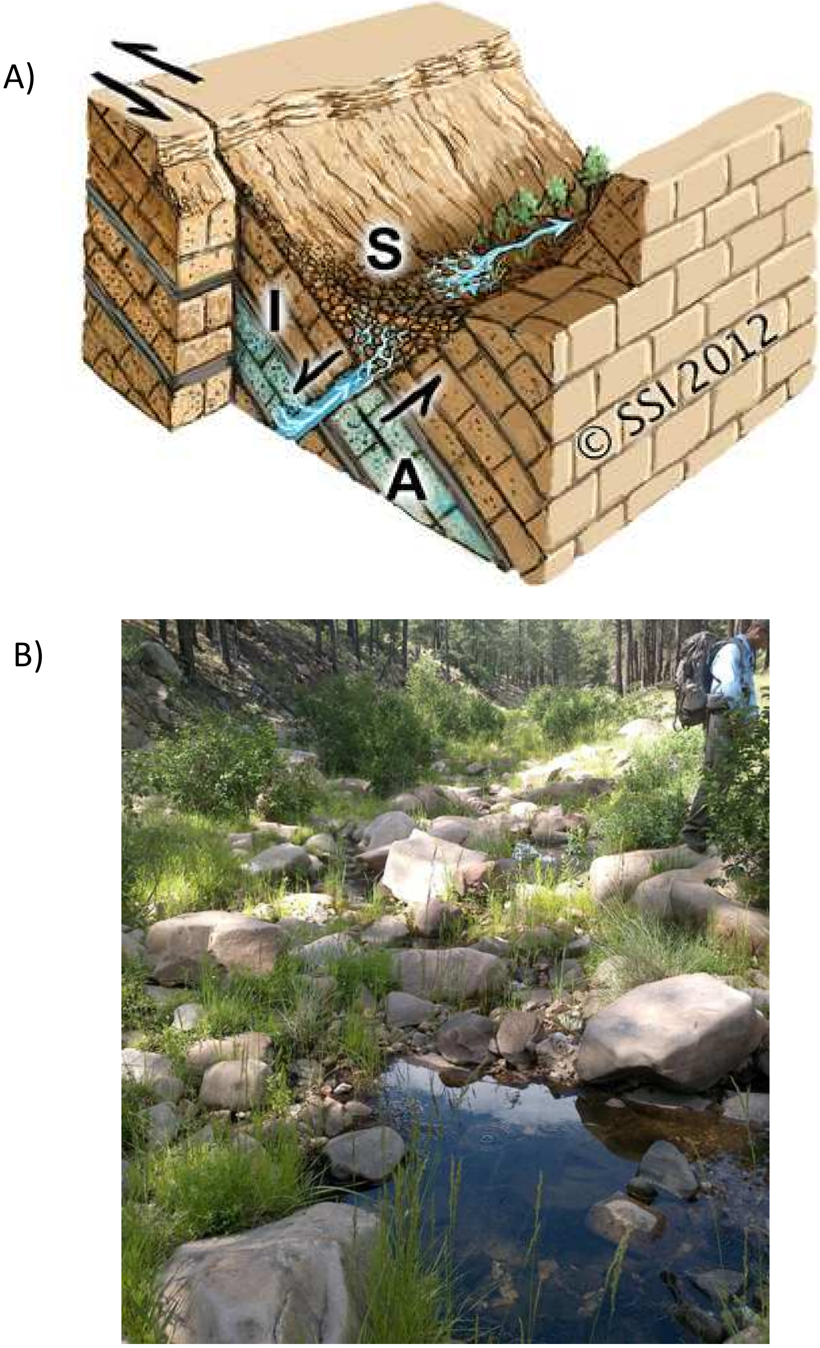

Figure 6. Diagram and example of a rheocrene springs. Rheocrene springs are where flowing water discharges into one or more defined channels. A) Sketch of rheocrene spring type. A=aquifer; I=impermeable stratum; S=spring source. B) Buckeye Crossing spring in the Apache-Sitgreaves National Forest. Photo courtesy of SSI.

1.5 Literature Review

There has long been debate over the sources of biodiversity in the landscape. With the theory of island biogeography, MacArthur and Wilson (1967) proposed that biodiversity is the result of differential colonization and extinction among habitat patches over time and identified species-area relationships that vary depending on rates of extinction and colonization. However, researchers have also identified microhabitat diversity as an important source of diversity. Hutchinson (1958) in his "Homage to Santa Rosalia" stressed the mosaic nature of the environment as the key to biodiverse systems. Local variations in environment, at a range of scales, allow for the development of specific niches. By having a multitude of environments no one species can become totally dominant and biodiversity ensues. This relationship has been discussed and investigated often in a number of different ecosystems and at varying scales (e.g., Simpson 1948, Hutchinson 1953); however, it has seldom, if ever, been investigated at springs ecosystems, specifically.

It is important to note that positive heterogeneity-diversity are not universal and other factors play important roles in the development of biodiversity. Some studies have failed to discover positive relationships (e.g., Rohde 1992, Lundholm 2009) and debate the generality of the relationship. Additional factors such as productivity and disturbance (Huston 1979), human influences (Nabhan 2008), resource availability and interspecific interactions.

An investigation by Tews (2004) found that heterogeneity-diversity relationships vary depending on taxa and scale. However, a meta-analysis by Stein et al. (2014) claims near universality of the positive relationship when studies properly account for other drivers of diversity. They cite three important drivers of the relationship: environmental heterogeneity (1) increases environmental gradients creating more niche space, (2) creates refuges and shelters from environmental conditions or climate change, and (3) separates areas of similar habitat leading to isolation and increased speciation. Springs are important to study in this context given their demonstrated values as paleorefugia and their high rates of endemism (Perla and Stevens 2008).

The high biodiversity at springs has been attributed to the co-occurrence of multiple geomorphic microhabitats created by the emergence environment and steep ecological gradients in moisture, productivity, and nutrient availability (Springer and Stevens 2009, Springer et al. 2015). Stevens and Springer's (2004) conceptual model of springs ecosystem ecology recognized several common geomorphic microhabitats (Table 2) that form heterogeneous habitat mosaics at springs. Many distinct microhabitats can occur at each spring and are often associated with specific spheres of discharge. Microhabitat diversity is proposed as a significant driver of differences in diversity between spring types and strong influences on species assemblages at springs.

Table 2. Common geomorphic microhabitats encountered at springs (Stevens et al. 2016a).

Microhabitat Surface	Abbreviation
Adjacent Uplands	AU
Backwall	BW
Cave	C
Channel	CH
Colluvial slope	CS
High-gradient cienega	HGC
Low-gradient cienega	LGC
Unfocused madiculous	MAD
Organic ooze	ORG
Pool	P
Plunge Pool	PP
Sloping bedrock	SB
Spring mound	SM
Terrace	TE
Tunnel	TU
Other	OTH

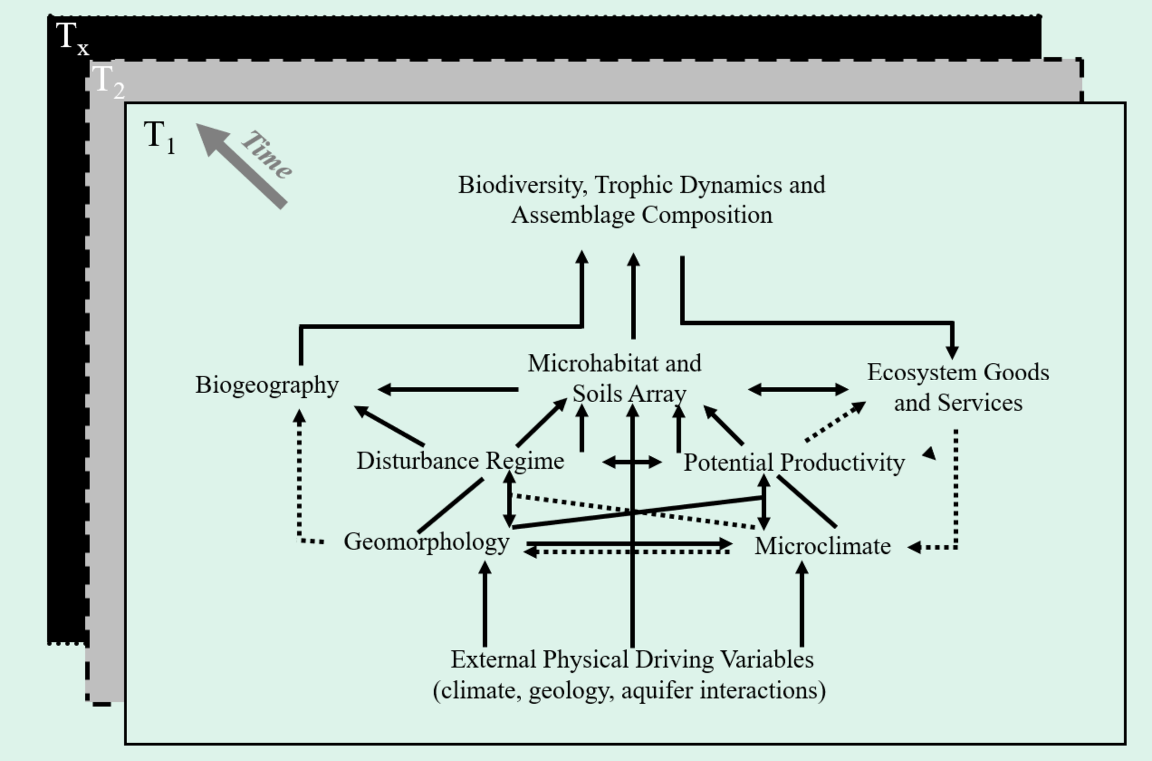

Figure 7. Springs ecosystems conceptual model adapted from Springer and Stevens (2004). Many aspects of springs ecology including physical factors, geomorphology, microclimate, productivity, biogeography, disturbance, ecosystem services, and microhabitats. The net of all these interactions yields species assemblage and biodiversity of a springs ecosystem.

The conceptual model of springs recognizes that springs are complex systems with many feedbacks. It acknowledges geomorphology as an important control on microhabitats and biogeography and eventual driver of springs diversity and species assemblages (Figure 7). Not only are springs highly productive ecosystems, but they exhibit strong environmental gradients (e.g., moisture, light, temperature), have complex biogeographic and climatic histories, create isolated habitat patches, have highly variable microhabitats (e.g, substrate, aspect, slope), and distinct patterns of disturbance (floods, fire, grazing, human uses). Thus, springs are highly multivariate ecosystems. Only by analyzing and controlling for these many interactions can the true link between environmental heterogeneity and biodiversity at springs be discovered.

Several studies have examined the relationship between plant species richness and landform heterogeneity, but few have done this at springs specifically. Dufour et al. (2006) reported a positive correlation between plant species richness and landform diversity in a study in the Swiss Jura Mountains. In a study of deciduous plant communities in Rhode Island, Burnett et al. (1998) found species richness of trees and shrubs to be higher in areas of higher geomorphic heterogeneity. A review by Lundholm (2009) supported the relationship between landscape diversity and species richness at scales ranging from less than a square meter to over a square kilometer. These studies suggest that geomorphic heterogeneity may be an important indicator of species diversity at springs, but little work to test this hypothesis has been completed.

Geomorphology and plant species can interact with each other in many ways. While geomorphic microhabitats provide niches for many plant species, the presence of plants can also influence processes at springs to produce additional microhabitats.

Woodbury (1933) showed that mosses and algae populating backwalls at hanging garden springs can create habitat for ferns whose roots will subsequently erode cracks in the backwall providing additional habitats. Furthermore, plants accelerate weathering and provide organic matter to develop rich soils that support wider arrays of life (Woodbury 1933).

Several studies have examined variations in diversity at springs ecosystems specifically. A previous study in Germany, (Martin and Brunke 2012) compared macroinvertebrate taxon richness with spring characteristics at four classes of spring (helocrene, rheocrene, helo-rheocrene, limnocrene). They found that macroinvertebrate richness was most closely associated with water hardness and conductivity. Further, limnocrene springs had lower macroinvertebrate richness compared to other spring types. Thus, water chemistry was deemed the most important driver of diversity. However, environmental heterogeneity was not explicitly included in their analysis. Limnocrene springs with their large areas of open water often have lower species richness given their area (Springer et al. 2015). This may be related their lower microhabitat diversity.

Geomorphology is not the only parameter controlling diversity in spring ecosystems. Audorff et al. (2011) compared the roles of both water quality and spatial factors on plant species richness at springs in central Europe. They found that nutrient availability and elevation were the strongest predictors. Further, they observed a general decrease in pH and conductivity with altitude and concluded that hydrochemical factors were more influential than spatial or hydrophysical factors in controlling plant community composition. However, overall explanation of variation in species richness was low and no metric of environmental heterogeneity was included their analysis.

Skalicky et al. (2017) examined several springs in the Krokonse Mountains of the Czech Republic. They found elevation, slope, and aspect to be important drivers of springs community composition and identified springs as diversity hotspots supporting many endemic species. This analysis revealed the highest diversity of plant species in areas with the highest elevations and steepest slopes. Soil type was also identified as an influencing factor; but no metric of microhabitat diversity was included in the analysis.

There has been some research specific to the springs of the Grand Canyon. Ledbetter et al. (2016) examined the springs of the Kaibab National Forest. The study focused on analyzing restoration potential of springs on the forest land. A key finding was the importance of groundwater flow paths to water quality. Strong relationships were identified between total dissolved solids and elevation and were related to groundwater flow path length and dissolution of limestone bedrock. More importantly, analysis of natural condition, disturbances, and risks associated with each spring was able to prioritize springs restoration, using a standardized Spring Ecosystem Assessment Protocol (SEAP) (Stevens et al. 2016a).

Tobin et al. (2017) conducted a review of springs in the Grand Canyon. Key findings included wide variety and density of springs in the Grand Canyon. At least 750 springs were recorded, representing 10 out 12 recognized spheres of discharge. Further, discharges ranged over many orders of magnitude and geochemistry was spatially variable. Geologic setting was important in reflecting springs source areas, responses to recharge, and vulnerability to risks such as groundwater pumping and wildfire. While no analysis of springs ecosystems was included these findings, they do highlight the importance of springs in the region and their complexity.

A study of spring inventories from Alberta demonstrated dense plant species packing at springs and suggested that over 25% of the provincial flora was found in just 3.8 ha of springs habitat (Springer et al. 2015). They found that microhabitat richness was positively related to floral species richness. Water hardness, geomorphic microhabitat richness, and anthropogenic impacts were important controls on springs vegetation among springs types. Further, large interregional differences were found between springs across Alberta. These interregional differences could be attributed to variations in climate factors, spheres of discharge, and human uses across regions. The high rate of biodiversity and the documentation of human impacts at many springs highlighted the need for increased springs conservation efforts.

1.6 Research Thread

This study is the third in a research thread investigating the relationship between springs geomorphology and plant biodiversity at springs. The focus has been on identifying if there is a direct link between landscape heterogeneity and springs biodiversity and identifying any differences in this relationship between spheres of discharge. First, Hallam (2010) focused on a set of spring inventories in the Spring Mountains of Nevada. Hallam's study was limited by its sample size due to confined geographic extent. Across 57 springs, she failed to find that geomorphic diversity was related to biodiversity at the springs. However, she did find that geomorphic diversity was related to overall vegetation cover at springs. Further, she concluded that small sample sizes and inadequate geographic extent limited her analysis.

Sparks (2014) expanded on Hallam's work using a larger sample size and greatly expanded the geographic extent. The study analyzed relationships between plant diversity and geomorphology at 341 springs throughout Western North America, dominantly the US Southwest and Alberta, Canada. The study found that a sample of at least 30 springs for each sphere of discharge was needed to make any statistical conclusions between the role of geomorphology on biodiversity. Analysis was limited to four spheres of discharge with sufficient sample sizes for analysis. These were helocrene, rheocrene, limnocrene and hillslope springs.

Sparks' research highlighted several relationships. Species richness was significantly, positively related to geomorphic diversity at helocrene, hillslope, and rheocrene springs. Structural diversity was significantly, positively related to geomorphic diversity only at hanging garden springs. Functional diversity was significantly, though weakly, positively related to geomorphic diversity at hillslope and rheocrene springs. Physical and water quality parameters important for predicting plant diversity and richness were area, microhabitat richness, latitude, percent solar radiation summer, water temperature, flow, and specific conductance. Significant differences of several parameters between springs types supports the validity of the classification of hanging garden, helocrene, and rheocrene springs. No parameters were significantly different for hillslope springs, which suggests the need for further classification of hillslope springs.

These findings identify potential relationships between geomorphology and diversity at springs and support the use of spheres of discharge by distinguishing several spheres of discharge by differences in physical parameters. The relationships found were partially obscured by strong interregional variations in biodiversity. Alberta springs had much higher diversity probably due to increased moisture availability and the convergence of several regional biota. By focusing on a single ecoregion, this study better illuminates relationships between geomorphology and plant biodiversity.

1.7 Hypotheses

This study tackles two key hypotheses with a suite of statistical techniques to better understand and identify relationships between springs geomorphology and plant diversity while accounting for the highly complex and multivariate nature of springs. I proposed:

1. Each type of springs has distinct geomorphic, physical, geochemical, floral characteristics that distinguish it from the other classes of springs.
2. Species richness is positively related to geomorphic diversity at springs and varies by spring type.

Chapter 2 – Manuscript

2.1 Abstract

The Canyon Ecoregion (GCE) represents the entire landscape that drains into Grand Canyon. This region encompasses a wide array of environments and corresponding plant communities of biological interest. Springs are numerous in the GCE and play a multitude of roles in this generally arid region. Springs serve as critical sources of water, and support endangered and endemic species with over 1000 springs-dependent species identified in the GCE. This study conducted a statistical community analysis of 352 springs in the GCE among four spring types – helocrene wet meadows; hanging gardens; rheocrene flowing springs; and hillslope springs. All springs types were distinguished by differences in physical site characters which in turn were associated with plant community structure and specific species. Plant species packing was substantial with almost 1000 species recorded across all springs. All in all, this assemblage represented over 45% of the region's entire flora in less than square km of habitat area. Geomorphic microhabitat diversity was positively related to springs diversity. This study identified key differences between springs types, each spring needs to be understood in an individual context. Stewardship efforts should aim to protect varied microhabitats. Each spring type supported high plant species richness and further different assemblages of plants were associated with each spring type. The high concentration of biodiversity at springs warrants further conservation, and additional inventory and study will prove useful in further understanding of springs of the GCE.

2.2 Introduction

Microhabitat diversity has long been regarded as an important factor contributing to biological diversity. Simpson (1948) and Hutchinson (1953) emphasized positive relationships between the mosaic nature of the environment and species richness because complex habitats provide more niche space and opportunities for resource diversity and exploitation. However, positive heterogeneity-biodiversity relationship are not universal, varying among taxa and across spatial scale (Tews et al. 2004), in relation to resource availability and environmental severity (Yang 2015), disturbance-productivity interactions (Huston 1979), and human influences (Nabhan 2008).

Springs ecosystems provide a wide array of natural and anthropogenic services, and critical environmental functions (Kreamer et al. 2015). Springs are paleorefugia, hotspots of diversity, and function as keystone ecosystems (Perla and Stevens 2008, Springer et al 2015). They are focal points of human cultural and socio-economic interest, providing rural and for some communities, urban water supplies, as well as recreational activities with significant economic value (Bonn and Bell 2003, Stevens and Meretsky 2008, Mueller et al. 2017). Also, springs provide important hydrogeological information about aquifer integrity and change (Toth and Katz 2006, Kresic and Stevanovic 2009). Despite these many important attributes, springs are globally threatened by development, groundwater pumping, climate change, contamination, overgrazing, and invasive species (Stevens and Meretsky 2008). Thus springs warrant more scientific and stewardship attention.

Many studies have examined relationships between plant species richness and landform heterogeneity (e.g., Burnett et al 1998, Dufour et al 2006, Lundholm 2009), but few have compared habitat heterogeneity impacts on terrestrial plant species richness within and among different springs types. Geomorphology and plant species richness may interact reciprocally: while geomorphic microhabitats provide niches for some plant species, the presence of cornerstone or keystone plant species, such as trees, can influence physical processes (e.g., weathering, rockfall, sediment deposition) and produce additional microhabitat heterogeneity (e.g., Woodbury 1933). The development of canopy vegetation also can shade understory habitats and potentially reduce understory productivity and species richness. Thus, complex physical and biological feedbacks may facilitate or reduce plant species richness at springs.

High levels of biodiversity at springs have been attributed to the co-occurrence of multiple geomorphic microhabitats created by the emergence environment and steep ecological gradients in moisture, productivity, and nutrient availability (Springer and Stevens 2009, Springer et al. 2015). Stevens and Springer's (2004) conceptual model of springs ecosystem ecology recognized 13 common geomorphic microhabitats that can form a heterogeneous habitat mosaic. A few studies have explored controls on springs plant assemblage structure. Martin and Brunke (2012) compared faunal taxon richness with spring characteristics among four spheres of discharge (helocrene, rheocrene, helo-rheocrene, and limnocrene). They found that aquatic macroinvertebrate species richness was most closely associated with water hardness and conductivity and further reported reduced faunal diversity at limnocrene springs compared to all others. Audorff et al. (2011) compared the roles of water quality and spatial factors on plant species richness at springs, finding that nutrient availability and elevation were the strongest predictors of richness. They observed a general decrease in pH and conductivity with altitude, concluding that hydrochemical factors were more influential than spatial factors in controlling plant community composition. Hallam (2010) studied vegetation at a small set of springs in the Spring Mountains of Nevada, reporting a positive relationship between geomorphic diversity and species richness. Sparks (2014) examined vegetation at hillslope, helocrene, hanging garden, and rheocrene springs across western North America. She reported differences among all but hillslope springs, and a positive relationship between geomorphic microhabitat diversity and species richness. In addition, spring area, latitude, percent solar radiation summer, water temperature, flow, and specific conductance were significantly related to floral species richness. A study of spring inventories from Alberta demonstrated dense species packing at springs: over 25% of the provincial flora was found in just 3.8 ha of springs habitat (Springer et al. 2015). They found that microhabitat richness was positively related to floral species richness. Water hardness, geomorphic microhabitat richness, and anthropogenic impacts were important controls on springs vegetation among springs types.

This paper presents analyses of relationships among springs types and geomorphic variation on plant assemblage composition, structure, and function in the GCE. We asked several questions. (1) What physical and biological traits differentiate GCE springs by sphere of discharge? (2) What ecosystem variables, such as microhabitat diversity and geochemistry, control vascular plant assemblage richness and structure at GCE springs? These analyses can be used to identify hot spots of plant species concentration, improve understanding of plant assemblage patterns in response to ecological gradients and anthropogenic impacts, and inform springs ecosystem inventory and management across large landscapes.

2.3 Methods

2.3.1 Springs Classification

The concept of spheres of discharge was developed to classify and better describe springs as hydrogeologic features, and refers to the geomorphological environment where groundwater is exposed and often flows onto the Earth's surface (Meizner 1923). Springer and Stevens (2009) updated and expanded this concept, cataloging a comprehensive list of 12 distinctive spheres of discharge, including natural and anthropogenic types. Their terminology has been adopted by hydrogeologists and ecologists, improving description, comparison, and measurement of physical and biological attributes among widely varying types and complexity (e.g., USDA Forest Service 2012). This classification scheme will become more useful for ecologists and resource managers as more data are acquired and analyzed. Springer and Stevens (2009) hypothesize that biological characteristics vary among spheres of discharge to create "discrete vegetation and aquatic invertebrate assemblages." We test that concept here, recognizing these if discrete associations are associated with different springs types, such information will help the public and the scientific, managerial, and policy communities, better understand stewardship needs and options. The information compilation effort spearheaded by the Spring Stewardship Institute now allows this hypothesis to be tested.

2.3.2 Study Area

The 35,000 km^2 Grand Canyon Ecoregion (GCE) was selected for this study because it is a topographically diverse landscape that supports a wide array of many springs ecosystem types (Figure 8). Although the southern Colorado Plateau is a mega-ecotone, mixing Madrean and Rocky Mountain biota (Stevens 2012), the GCE is geographically and latitudinally constrained. The springs of the GCE have been intensively mapped and occur from 350 – 3500 m elevation (Springs Online 2018, springsdata.org). The region's springs have been intensively studied in several hydrogeological contexts, including connate and meteoric hydrogeology, karst hydrology, and water supplies and contamination studies (Campbell and Green 1968, Huntoon 1974, Crossey et al. 2012, Jones et al. 2017, Tobin et al. 2017). The flora of the GCE has been intensively inventoried (MacDougall 1947, Phillips et al. 1987, Stevens and Ayers 2002), and GCE springs ecosystem ecology also has received some attention (e.g., Perla and Stevens 2008, Ledbetter et al. 2016). The region largely consists of wildlands and rangelands. Management is by several federal agencies and Native American Tribes, with relatively little private or urban land ownership. Springs constitute important riparian and wetland habitats in this generally arid area, and GCE springs have demonstrated positive non-market economic values (Mueller et al. 2017).

2.3.3 Data Sources

This study employed spring inventory data collected following the Level 2 Springs Inventory Protocol (SIP). This protocol has been adapted and adopted by the U.S. Forest Service, the U.S. Fish and Wildlife Service, several National Park Service units, and many others. Springs inventory data are archived into an online database (SpringsData.org) through the Springs Stewardship Institute (Ledbetter et al. 2014). The SSI protocol includes the complete details and sample data collections sheets (Stevens et al. 2016a). The Level 2 inventory protocols are comprehensive and designed to be carried out by a team of expert scientists over the course of several hours at a site. Data from at least 30 springs per type with Level 2 inventory data were analyzed to determine vegetation patterns. Permission was obtained from regional land managers for these analyses, and care was taken to protect any sensitive data.

The SSI Level 2 inventory data used for these analyses included both physical site characteristics, geomorphic survey data, water chemistry and plant surveys. Physical site characteristics included spring sphere of discharge, geology, solar radiation balance, location and elevation. Elevation is used as a climate variable because temperature and precipitation are closely linked to elevation in the GCE. However, local spring topography often influences microclimate at springs. Geomorphic data included detailed microhabitat data, as well as evaluation of disturbance regime impacts. Discrete geomorphic microhabitats polygons were identified at each springs ecosystem, and microhabitats were mapped based on surface types. Polygon dimensions were measured and mapped to scale in the field. Aspect, slope, moisture condition and visually estimated percent cover (VE%C) of substrate classes were recorded for each microhabitat. Cover measurements included litter, woody debris, chemical precipitate and various grain sizes classes (clay, silt, sand, pea gravel, large gravel, small boulders, large boulders, bedrock). Water chemistry included field temperature, dissolved oxygen, specific conductance, alkalinity, pH, and dissolved oxygen concentration [DO]. Spring discharge was also measured using a variety of methods (i.e., volumetric methods, weirs, flumes, current meters) depending on site characteristics and discharge. Site-specific monthly solar radiation balance was measured with a Solar Pathfinder (Solar Pathfinder 2011) and converted to a percentage of the maximum possible for a flat unshaded site in the summer (PSRS). PSRS is related the amount of photosynthetically active radiation (PAR) available in the growing season. All plant species at the site were identified to species level for each survey. The VE%C was visually estimated for species in each microhabitat by stratum: aquatic, nonvascular, basal (wood emerging from the ground), deciduous ground cover, shrub cover (woody, 0-4 m tall), middle canopy (woody, 4-10 m), and tall canopy cover (woody, >10 m). Species nativity and wetland status were taken from SSI's database. Wetland status was refined from the USDA wetland indicator categories (USDA 2017), and included aquatic, wetland, wetland-riparian, riparian, facultative, and upland categories.

The Level II inventory is used to inform SSI's Springs Ecosystem Assessment Protocol (SEAP), which was designed to facilitate conversations and inform management decisions around springs restoration (Springer et al. 2015, Ledbetter et al. 2016, Paffett et al. in press). The SEAP assesses springs condition and risk with 42 questions across six categories including aquifer and water quality, geomorphology, habitat, biology, human influences, and administrative context (Stevens et al. 2016a). Springs condition are scored between 0 (low condition) and 6 (pristine condition) and risks are assessed 0 (no risk) to 6 (high risk). The SEAP has been shown to be an effective management tool with springs showing moderate conditions and high risks most responsive to restoration action (Paffett et al. in press).

2.3.4 Analyses

Data were examined for outliers and transcription errors before analysis. When suspect data were found they were investigated by reviewing survey notes, crosschecking photos, sketches and site or referencing additional surveys of the same spring. If missing or suspect data could not be rectified, then that spring site was excluded from further analysis.

Data were imported to R (R Core Team 2017) for analysis and displayed in bivariate plots and histograms to examine distributions and relationships between variables. Covariance and correlation matrices were calculated to understand linear relationships. The Shannon Diversity index, H', (Shannon and Weaver 1948) was used to calculate diversity metrics

$$H' = -\sum_{i=1}^{S} p_i ln\, p_i \qquad (1)$$

where S is total entity richness and p_i is relative cover of entity i. Diversity metrics included: (1) overall floral diversity using species relative cover, (2) geomorphic diversity using the relative area of microhabitats, (3) structural diversity of relative cover by stratum level, and (4) functional diversity of relative abundance of functional plant groups by wetland status. Summary statistics were calculated for each physical variable by springs type. Significant differences between springs spheres of discharge were determined using non-parametric Mann-Whitney Tests using the *'wilcox.test'* function in R, and boxplots were constructed to illustrate the most insightful of these differences. Differences were considered significant only if $P<0.001$ due to the larger number of comparisons made.

A linear discriminant analysis (LDA) was conducted to test if physical springs traits could differentiate springs by type using the '*lda*' function in R from the *'MASS'* package (Venables and Ripley 2002). Variables were transformed prior to analysis if it improved normality assumptions and all variables were standardized to a mean of 0 and standard deviation of 1. Plots of discriminant axes were compared to interpret results and the highly weighted variables along each axis were compared to results of Mann-Whitney Tests.

Indicator species analyses were conducted to determine if specific species were associated with specific spring types. The indicator value of each species was calculated with *'indicspecies'* package in R with the *'multipatt'* function (De Cacares and Jansen 2016). Combinations of groups were not considered. The indicator value is a combination of specificity and sensitivity of a species to a specific group (Dufrene and Legendre 1997). Significance for each indicator species was assessed with 1000 random permutations.

A multiple linear regression model was applied to predict plant species richness at each spring. All physical, geomorphic and water quality parameters were included in these analyses. Transformations were applied when indicated by bivariate and histogram analysis. Spring type was included as a predictor allow to have different intercepts for each sphere of discharge. A stepwise procedure was performed in R using the “step” function inside the “*stats”* package (R Core Team 2017). Best models were selected using the Akaike Information Criterion (AIC), which is based on likelihood and penalizes overly complex models (Sakamoto et al. 1986). Permutation tests were used to assess the importance of each variable to overall model accuracy. Covariance of predictors were examined for multicollinearity and to aid in interpretation. Residuals were plotted and examined to assess model fit and any outliers.

Non-metric multidimensional scaling was used as a non-parametric technique to visualize community structure. NMDS was executed in R with the *‘metaMDS’* function from the *‘vegan’* package (Oksanen et al. 2017). NMDS was run with 2 axes and 100 random trials to ensure a global stress minimum was reached. Species percent covers were square root transformed prior to analysis and Bray-Curtis Distance was used as distance measure. Rank correlations of axis scores with physical variables were used to understand the ecologic gradients related to community structure. Significant differences between springs types were assessed through an analysis of similarity using the *‘anosim’* function in *‘vegan’* (Oksanen et al. 2017).

2.4 Results

Three-hundred and fifty-two springs were used in the analyses, including 47 hanging gardens, 49 helocrene springs, 147 hillslope springs, and 99 rheocrene springs (Figure 8**).** There was an insufficient sample size available for analysis of limnocrene,

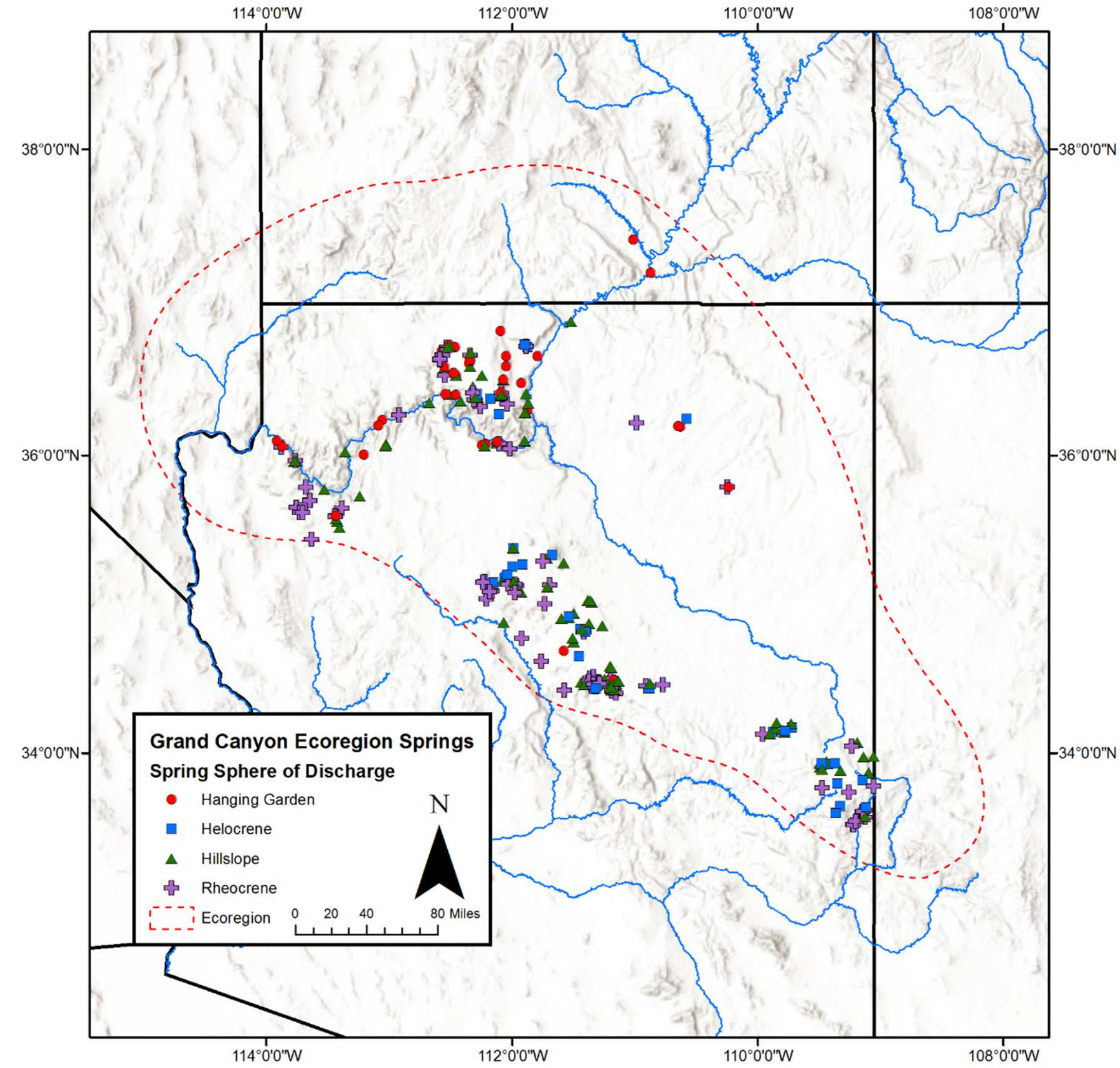

Figure 8. Map showing the Grand Canyon Ecoregion (GCE) and the 352 springs included in this study. The GCE represents the entire region draining into the Grand Canyon with over 3,000 meters of relief. All springs in the region with complete Level 2 springs inventories were included in analysis. There were 47 hanging gardens, 49 helocrene springs, 157 hillslope springs, and 99 rheocrene springs.

gushet, or cave springs. The 352 springs encompassed a total habitat area of 0.42 km^2 and 987 distinct taxa were identified to the species level. Current estimates of the total flora of the GCE is approximately 2200 species (Stevens 2012). Thus, we documented approximately 45% of the regional flora at springs that make up less than 0.01% of the regional land area.

Mann-Whitney tests identified significant differences between physical traits and diversity metrics (Figure 9). Complete tables of the data are included in Appendix C. Spring types occurred in different elevation ranges with helocrenes occurring at the highest elevations followed by rheocrenes, hillslopes, and hanging gardens each at the lower elevations, respectively. Helocrene springs had significantly higher wetted areas, while hillslope springs had more northern facing aspects. Helocrene springs received lower PSRS and helocrenes received higher PSRS than other spring types.

Significant differences in geochemistry were found among springs types (Figure 10). Hanging gardens had higher pH, dissolved oxygen and specific conductance while helocrenes were significantly lower in these three parameters. Hillslopes had lower water temperatures while rheocrene springs had significantly higher water temperatures.

SEAP ecosystem scores revealed that hanging gardens tended to have the highest ecological integrity, with higher scores for aquifer condition, geomorphic functionality, biological condition, water quality, and road, fence, and herbivory impacts (Figure 11). Rheocrene springs had significantly higher flow naturalness than did other types. Helocrene springs had significantly lower aquifer condition, water quality, and fence impacts than other spring types.

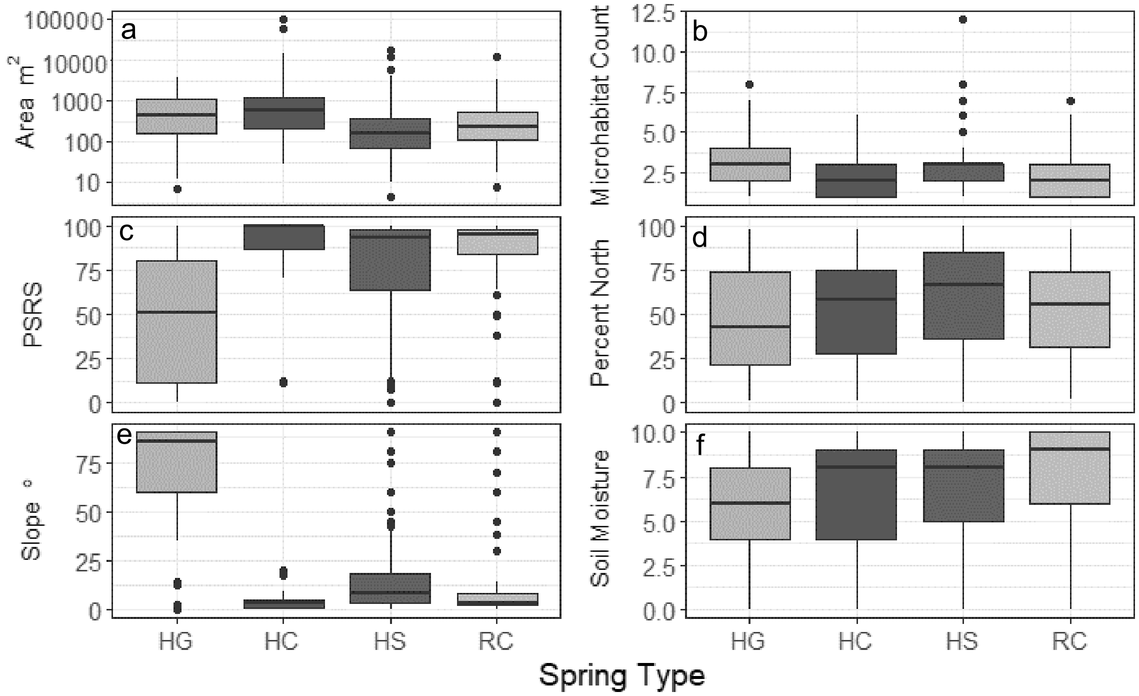

Figure 9. Boxplots showing variations of physical site characteristics by spring type: a) spring area; b) microhabitat richness; c) percent solar radiation summer d) percent north; e) slope; f) soil moisture. Center line shows median; box shows interquartile range; whiskers show an additional 150% of the interquartile range; and points represent outliers. Spring types are abbreviated: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

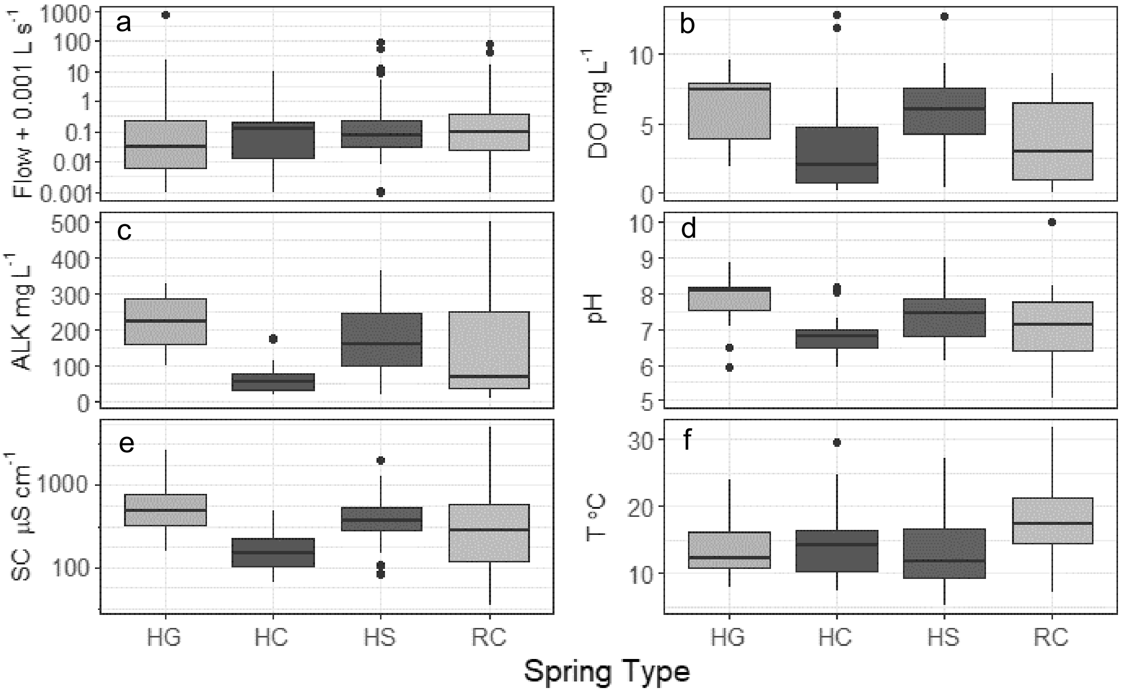

Figure 10. Boxplots showing variations of hydrologic parameters by spring type: a) flow; b) dissolved oxygen; c) alkalinity; d) pH; e) specific conductance; f) water temperature. Center line shows median; box shows interquartile range; whiskers show an additional 150% of the interquartile range; and points represent outliers. Spring types are abbreviated: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

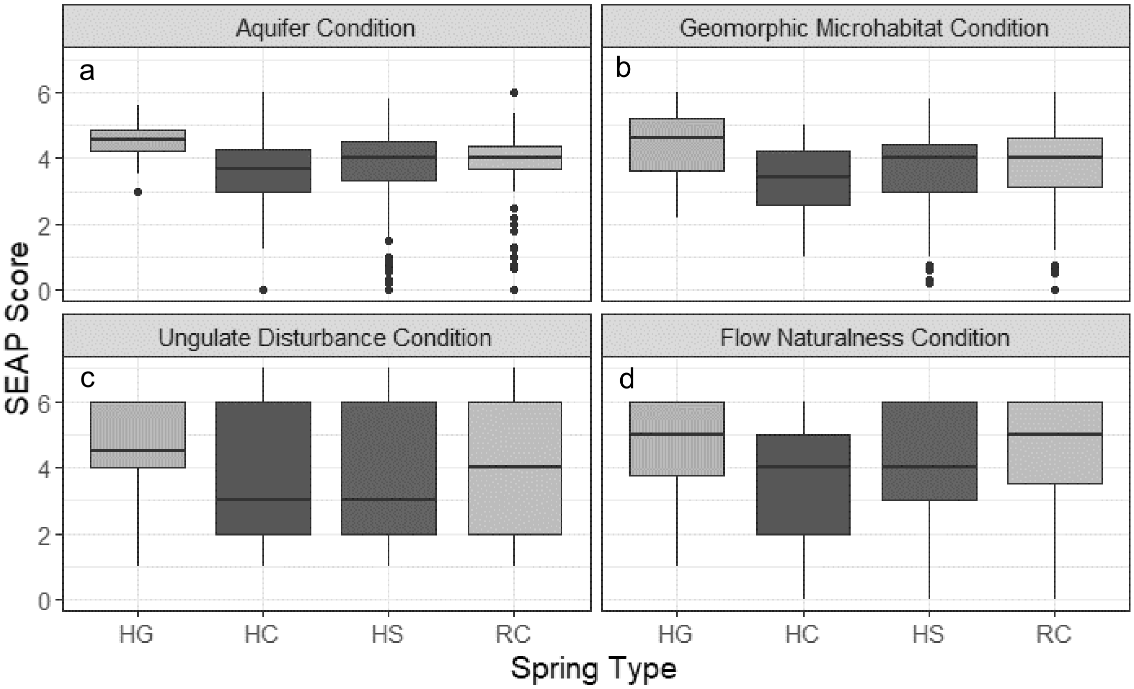

Figure 11. Boxplots showing variations of Springs Ecosystem Assessment Protocol (SEAP) condition scores: a) aquifer condition; b) microhabitat condition; c) ungulate disturbance condition; d) flow naturalness condition. SEAP scores qualitative and used to prioritize management. Scores range 0 through 6 with 6 indicating pristine conditions and lower scores indicating increasing disturbance. Center line shows median; box shows interquartile range; whiskers show an additional 150% of the interquartile range; and points represent outliers. Spring types are abbreviated: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene.

Substrate composition also helped differentiate spring types (Figure 12**).** Hanging gardens were characterized by higher proportions of bedrock and carbonate precipitate, as well as low proportions of gravel, woody debris, litter, and organic soil. Helocrene springs were dominated by organic soil and litter cover with little bedrock. Hillslope springs had significantly higher cover of gravel and woody debris while rheocrene had more varied substrates.

Diversity metrics trends indicated significant differences among some spheres of discharge (Figure 13). Hanging gardens had lower species richness (mean = 16 species), while other spring types supported an average of 22-24 species. However, hanging gardens had higher proportions of native species, averaging 84%, while helocrene springs had the lowest percent nativity at 66%. Hanging gardens also had the highest geomorphic and structural diversity levels, while helocrene springs had significantly lower structural diversity.

The linear discriminant analysis differentiated among springs types. Using three discriminant axes, 245 (70%) of the 352 springs analyzed were correctly classified (Table 3). The majority of the separation occurred on the first two axes, explaining 86% of between group variance (Figure 14). The first axis mostly separated hanging gardens and highly weighted elevation (+) and slope (-). The second axis most strongly weighted biological condition (-), longitude (+), log wetted area (-), and species richness (+). The third axis separated hillslope and rheocrene springs, and weighted lands condition (-), woody debris cover (-), geomorphic diversity (-).

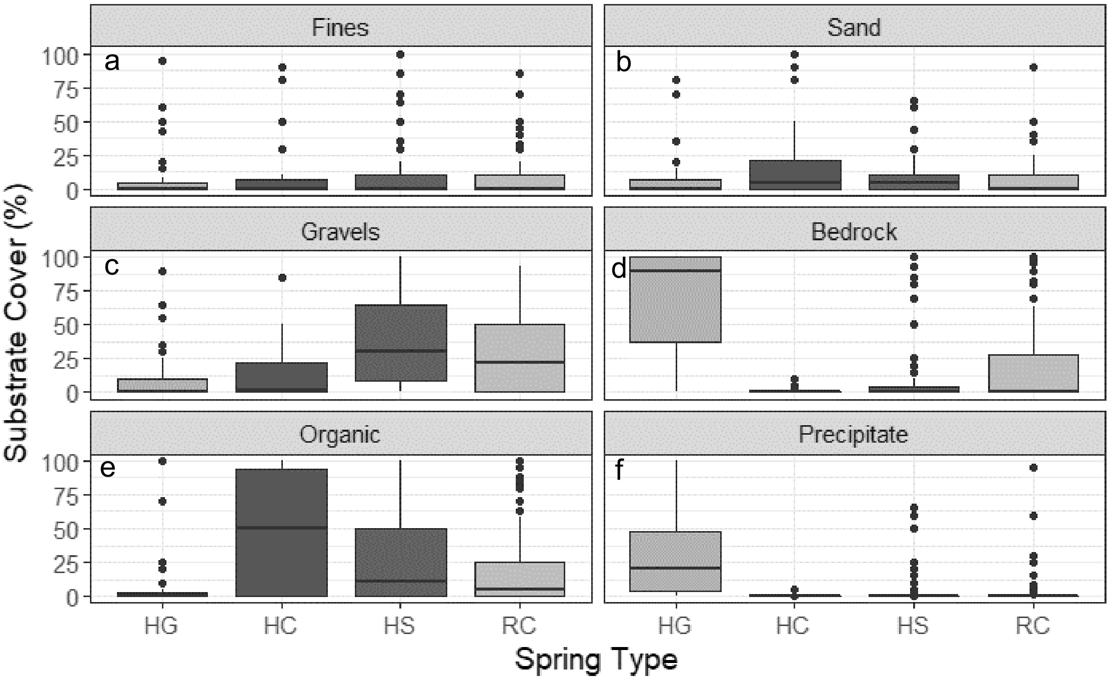

Figure 12. Boxplots showing variations of substrate composition by springs type: a) fines (clay and silt); b) sand; c) gravel and cobbles; d) bedrock; e) organic soil; f) precipitate. Center line shows median; box shows interquartile range; whiskers show an additional 150% of the interquartile range; and points represent outliers. Spring types are abbreviated: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

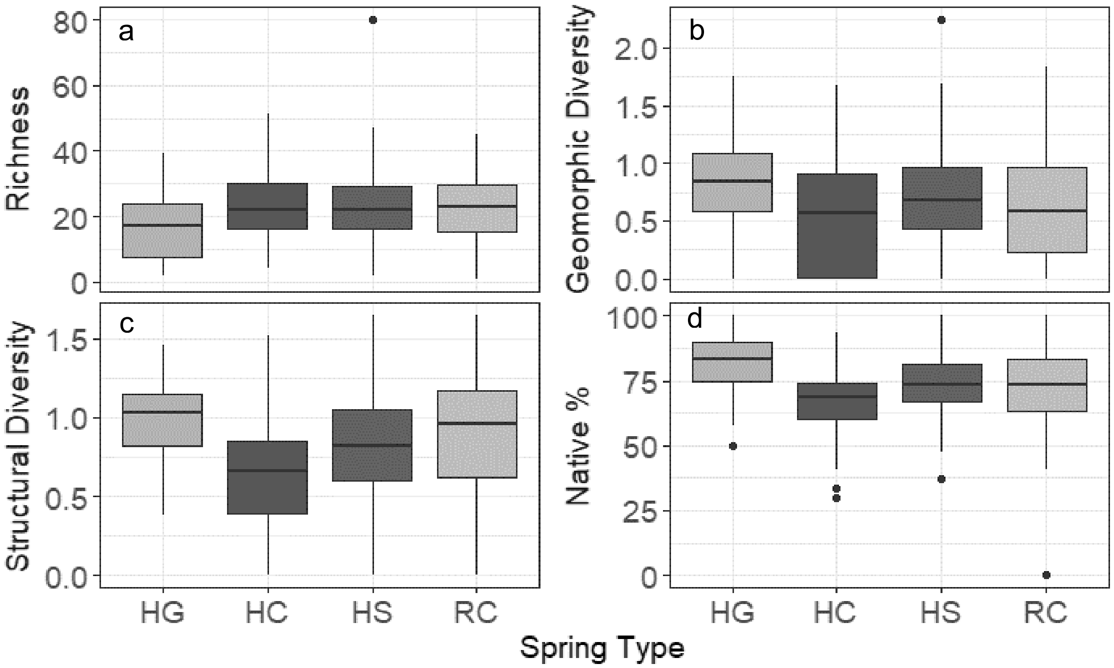

Figure 13. Boxplots showing variations of diversity metrics by spring types: a) floral species richness; b) geomorphic diversity; c) structural diversity; d) percent native plant species. Center line shows median; box shows interquartile range; whiskers show an additional 150% of the interquartile range; and points represent outliers. Spring types are abbreviated: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

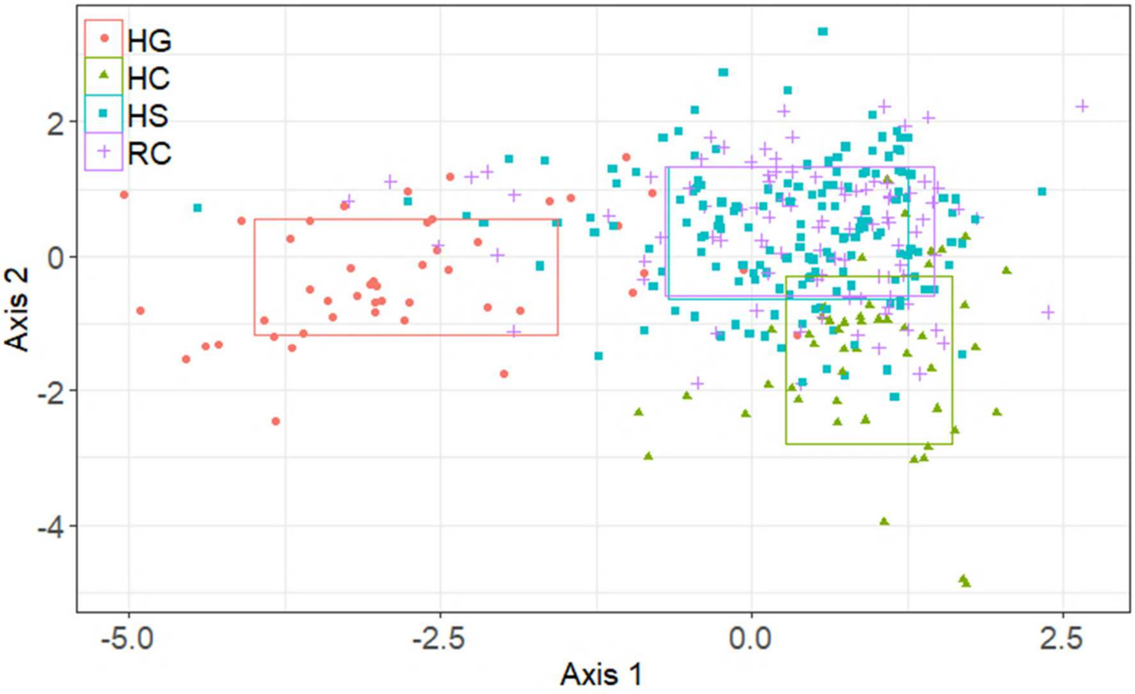

Figure 14. Plot showing ordination of spring sites based on LDA results. Boxes represent means ± standard deviation for each spring type. These first two axes explained 86% of variation between springs type and the third axis explained the remaining 14%. Axis 1 predominantly reflects slope (-), elevation (+) and alkalinity (-). Axis 2 strongly weights area (-), longitude, and biologic condition (-). The LDA successfully discriminated 70% of the springs. Points are coded by spring type: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

Table 3. Results of validation of linear discriminant analysis based on 352 springs. Predicted springs type are shown on rows and actual spring types are columns. Diagonal shows percent of springs in each class correctly identified.

	Hanging Garden	**Helocrene**	**Hillslope**	**Rheocrene**
Hanging Garden	81%	0%	4%	6%
Helocrene	0%	57%	4%	8%
Hillslope	15%	29%	81%	33%
Rheocrene	4%	14%	11%	53%

Overall Success = 70%

Indicator species analysis identified several species associated with each springs type (Appendix F). Hanging garden springs supported the largest group of significant indicators: 44 significant indicator species were identified, and 96% of hanging gardens had at least one indicator species present. The best indicators were *Adiantium capillus-veneris*, *Andropogon glomeratus*, and *Epipactus gigantea*. Helocrene springs had 35 significant indicator species, of which at least one occurred in 94% of the helocrene springs. However, the strongest indicators were non-native *Poa pratensis*, *Taraxacum officiniale*, *Cirsium vulgare*, and *Ranunculus hydrocharoides*. Hillslope springs were characterized by 8 indicators species, but only 55% of hillslope springs had at least one indicator present. The strongest indicators were *Pteridium aquilinum, Veronica americana*, and *Rudbeckia laciniata*. Rheocrene springs had 10 significant indicator species, with at least one indicator species identified at only 46% of those springs. The strongest indicators were native *Salix goodingii* and *Fraxinus velutina* as well as non-native *Melilotus officinalis* and *Carex kellogii*. Low values of indicator species arose because all species but non-natives have discrete elevation ranges while spring types are less constrained by elevation.

Linear modeling of springs state variables and diversity metrics revealed significant correlations. Elevation, wetted area, and geomorphic diversity were significantly positively related to species richness (r=0.16, 0.29, and 0.29, respectively) and negatively related to specific conductance (r=-0.29) (Figure 15). Water chemistry variables (pH, specific conductance, DO, alkalinity) were positively related among themselves, and negatively correlated with elevation (Figure 16). The proportion of native species was positively related to the proportion of invasive species (r = 0.37).

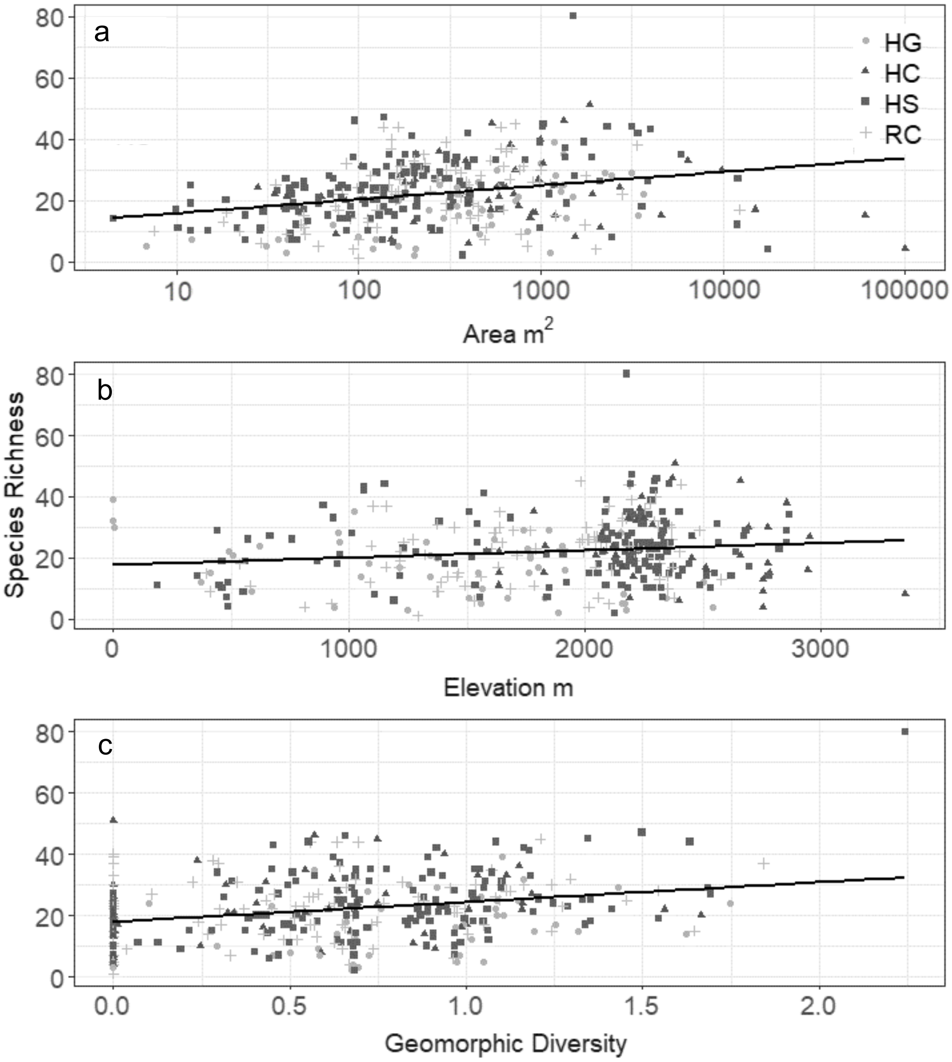

Figure 15. Scatterplots of physical parameters versus floral species richness with linear correlations: a) spring area (r = 0.29); b) elevation (r = 0.14); c) geomorphic diversity (r = 0.29). All three were significantly positively correlated with species richness. Points are coded by spring type: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

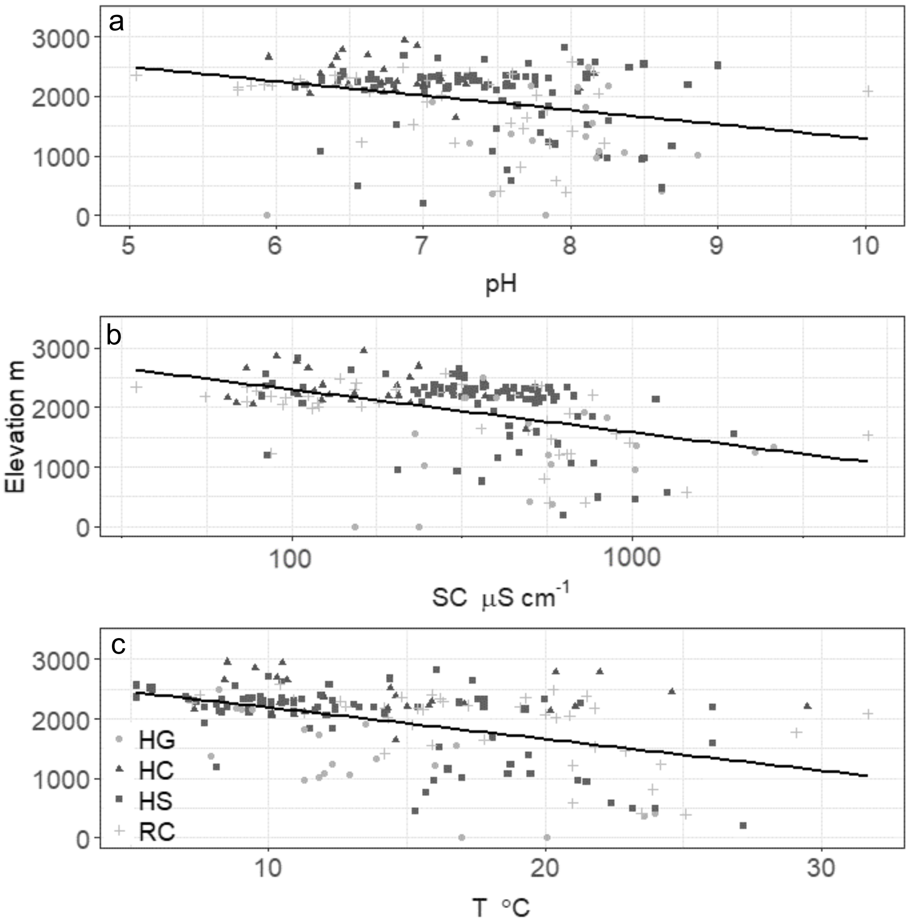

Figure 16. Scatterplots of water quality parameters versus elevations with linear correlations: a) pH (r = -0.30); b) specific conductance (r = -0.35); c) water temperature (r = -0.46). All water quality parameters were negatively correlated with elevation. Points are coded by spring type: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene

Multiple regression diversity metrics identified key ecological variables associated with plant diversity. The best model selected to predict species richness used 9 predictor variables and achieved an R^2 of 0.39. Listed in order of importance to the overall model R^2, the predictors were: (1) microhabitat richness, (2) wetted area ($\log_e$), (3) elevation, (4) spring type, (5) pH, (6) soil moisture, (7) woody debris cover, (8) PSRS, and (9) limestone bedrock presence. The model had a residual standard error of 8 species (Table 3).

Nonmetric multidimensional scaling identified important ecological gradients influencing assemblage composition. Successful ordination was achieved with 2 axes of assemblage structure (stress = 0.15) (Figure 17). Axis 1 was most closely related to elevation, with a negative relationship between elevation and axis score. There also were positive relationships of the axis 1 score with microhabitat richness, specific conductance, dissolved oxygen, and percent cover of precipitated. Axis 2 was positively related to PSRS and negatively related to assessment scores for water quality condition and fence condition. Bedrock cover, spring area, structural diversity, and polygon area were related to a combination of both axes. Some clustering of spring types by assemblage was evident in the NMDS results, and significant differences were identified between spring types (ANOSIM $R = 0.1011$; $P = 0.001$).

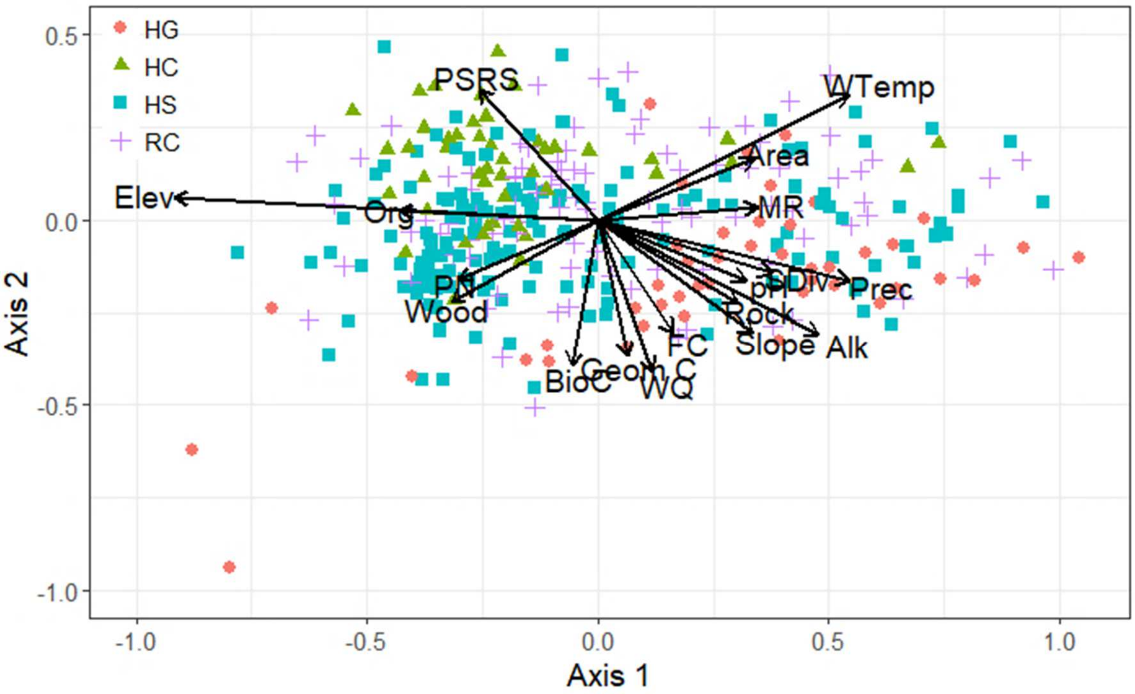

Figure 17. Scatterplot of NMDS axes showing differences in plant communities by springs. Points reflect individual springs coded by spring type as follows: HG = Hanging Garden; HC = Helocrene; HS = Hillslope; RC = Rheocrene. Stress of ordination was 0.15. Arrows represent direction and strength of Spearman correlation with axis scores related to dimensions of community structure; only rank correlations greater than 0.30 are shown. Codes for environmental variables as follows (clockwise from left): Elev = elevation; PSRS = percent solar radiation summer; WTemp = water temperature; Area = spring area; MR = microhabitat richness; SDiv = structural diversity; Prec = precipitate cover; pH = pH; Rock = bedrock cover; Alk = alkalinity; Slope = slope; FC = fence condition; WQ = water quality condition; GeomC = geomorphic microhabitat condition; BioC = biologic condition; Wood = wood cover; PN = percent north; Org = organic soil cover

Table 4. Multiple linear regression predicting floral species richness at 309 springs. Model was chosen with stepwise selection minimizing AIC. Importance column reflects average reduction in model R-squared when the values of the parameter were randomly permuted. Bolded P-values indicate P<0.05

Parameter	Estimate	Std. Error	t Value	P	Importance
Intercept	10.15	6.22	1.63	0.1037	-
log(Area) (m^2)	1.91	0.35	5.39	**<0.00001**	14.5
Microhabitat richness	2.43	0.33	7.27	**<0.00001**	26.7
log(Woody Debris Cover) (%)	0.64	0.21	3.08	**0.0023**	4.2
Elevation (m)	0.003	0.001	3.70	**0.0003**	6.5
Soil Moisture (1-10)	0.46	0.15	3.19	**0.0016**	4.8
pH	-2.13	0.67	-3.19	**0.0016**	4.6
Spring Type	-	-	-	**-**	4.9
Hanging Garden	-2.57	1.28	-2.01	0.0450	-
Helocrene	-1.67	1.21	-1.38	0.1694	-
Hillslope	1.73	0.82	2.11	**0.0360**	-
Rheocrene	2.51	0.86	2.90	**0.0040**	-
PSRS (%)	-0.55	0.23	-2.36	**0.0188**	2.4
Limestone	1.55	1.09	1.43	0.1545	0.5
R^2 = 0.39	**Res. Std. Error = 8.0**				

2.5 Discussion

Inventories across the GCE demonstrated remarkably tight packing of plant species at springs. Despite the small total area of springs in the region, we detected nearly 50% of the regional flora at just 352 springs in 0.48 km^2 of springs habitat. This result is consistent with estimates from previous studies of springs in other regions (Springer et al. 2015, Ledbetter et al. 2016), reaffirming the important biologic role played by springs as refugial hotspots of biodiversity. The intensity of species packing underscores the conservation significance of springs: protection of springs is an important and efficient way to protect a large number of species in a small habitat area, including several endemic, rare, or threatened species (e.g., Hershler et al. 2014, Kreamer et al. 2015).

This study identified key habitat and environmental variables associated with springs plant diversity. Plant species richness was positively related to springs area (Figure 15), as expected in relation to positive species-area relationship in insular habitats (MacArthur and Wilson 1967). Geomorphic microhabitat diversity also was positively related to species richness: more heterogeneous environments support increased plant species richness and diversity (Figure 15). Our data confirm results obtained in previous studies (Hallam 2010, Sparks 2014, Springer et al. 2015). Interestingly, microhabitat richness rather than geomorphic diversity was a better predictor of species richness in the MLR model (Table 4), suggesting that equivalence of area among microhabitats may be less important than absolute richness. Each additional microhabitat was associated with addition of about two plant species present in the springs ecosystem. Elevation was a proxy for climate since elevation is strongly related to temperatures and precipitation in the GCE (Sellers et al. 1985). The negative effects of elevation on plant richness suggests that water availability, air temperature and growing season length limit biodiversity at springs. Soil moisture also positively influenced plant diversity, suggesting soil water may limit plant biodiversity. PSRS had a negative relationship to richness in the model: many springs are light-limited ecosystems (Stevens et al. 2016a). PSRS is strongly effected by aspect and slope and can lead to interactions exacerbating aridity on south-facing slopes but create refugia for boreal species on north facing-slopes. More complex assemblage relationships to climate legacy effects also may exist within these patterns (Stevens 2012), but analyses were not pursued.

Due to complex covariation among variables, interpretation of regression coefficients does not necessarily yield a single best model. Inclusion or absence of specific variables in the final model should not be over-interpreted due to the observational nature of the study. That springs type was an important predictor variable suggests there are remaining differences between springs types, even after accounting for the predictors already included in the model. Thus, there likely are additional fundamental geomorphological differences among spheres of discharge and unexplained assemblage responses to those differences.

Strong relationships were identified between elevation and water chemistry in the dataset. In the largely karstic GCE, regional groundwater generally flows from high elevations, where there is increased precipitation and infiltration to springs inside Grand Canyon or in adjacent low elevation terrains (Kreamer and Springer 2008, Ledbetter et al. 2016, Tobin et al 2017). Therefore, springs at low elevations generally are fed by regional groundwater systems with longer flow paths; while springs at high elevations often are fed by perched groundwater with relatively short flow paths (Springer et al 2017). Longer flow paths and travel times allow increased dissolution of carbonates and other minerals, resulting in higher pH, specific conductance, and alkalinity (Ledbetter et al. 2016).

We successfully differentiated among several major springs types in the GCE both on the basis of physical characteristics and biological characteristics. This affirms that those springs types have different underlying morphologies that influences their physical characteristics, components of their distribution, and the composition, structure, and function of their biological assemblages. The wide array of springs types and associated microhabitats across elevation, aspect, and slope in the GCE is related to springs biodiversity through niche heterogeneity. The focused geographic area and large sample size in our study helps elucidate those relationships, and many of the differences between spring types were expected and are readily understood. For example, hanging gardens are geomorphically unique but highly productive vertical wall ecosystems, conditions that favor unique adaptations and biotic interactions. For example, P.K. Dayton (personal communication) observed that the seeds of rare wall-hanging plant populations, such as *Primula specuicola* are captured in the webs of *Tetragnatha* spiders, and thus are able to germinate and maintain their position on vertical or over-hung hanging garden backwalls.

NMDS results indicated that substantial variation in plant assemblages can be described along two multidimensional gradients of physical factors (Figure 17). The strong correlation between the first NMDS axis and elevation indicates that variation in climate related to temperature, precipitation, and moisture availability exert dominant control over species composition and structure. However, variation in substrata (especially bedrock vs substrate) and water chemistry (pH, alkalinity, specific conductance and water temperature) also are closely aligned with this axis, making it difficult to distinguish the relative effects of substrate, water chemistry, and climate. Nonetheless, all play large roles in shaping springs assemblages. The second NMDS axis was most strongly related to ecological integrity assessment scores. Most of those assessment condition scores were mutually positively intercorrelated, and collectively indicate the overall degree of ecological impairment of the springs ecosystem. Variation among those variables influences plant assemblage composition and structure at springs. Three other variables (PSRS, slope and percent north) also were identified as being strongly related to assemblage structure. These also are related to photosynthetically active radiation (PAR) availability, and are expected to further influence plant assemblages.

Springs plant assemblages generally clustered by springs type, although there was substantial variation and overlap among spring types (Figure 17). This is to be expected because the niche requirements of many plant species are met in many different springs types (but not in adjacent, non-springs uplands). The analysis of similarity test showed that assemblage groupings are significant (R=0.1011; P=0.001). However, R statistics for that test range from 0 (random) to 1 (perfect clustering), so and R statistic of 0.1011 indicates considerable overlap in assemblage composition among spheres of discharge. Hanging garden and helocrene springs had particularly distinctive plant assemblages, while rheocrenes had moderately large variation, and hillslope springs were the most variable.

Hanging gardens were the most distinctive springs ecosystem type in our study. Hanging gardens tended to occur at low-moderate elevations, below the rim of Grand Canyon. This was expected because hanging gardens are defined by emergence along geologic contacts in cliffs, which are numerous in canyons. Hanging gardens had high slopes due to their emergence on near-vertical to over-hanging cliff faces. They also had distinct water quality parameters, such as high pH, specific conductance, and alkalinity, which were generally attributed to long groundwater flow paths and travel times through aquifers, and increasing dissolution of the predominantly limestone bedrock in the region. The extent of precipitate cover at hanging garden springs related to elevated alkalinity. Hanging gardens also tended to have higher ecological integrity assessment scores. This may be related to their geographic isolation in canyons, limiting the extent of anthropogenic disturbance. The high percentage (84%) of native species at hanging gardens reflects their unique geomorphology, limited accessibility and lesser extent of anthropogenic disturbance. Hanging gardens had lower overall species richness, but had higher structural diversity and microhabitat richness (Figure 13). Low species richness reflects slope and microclimate severity, and PAR limitation, all of which strongly influence these ecosystems (Woodbury 1933, Malanson 1980, Welsh and Toft 1981, Spence 2008). Hanging gardens also had the most indicator species, including native *Adiantum capillus- veneris*, *Mimulus cardinalus*, and *Epipactus gigantea*, which are restricted to low –medium elevations in the Grand Canyon, and are tightly associated with springs, seeps, and wet cliff faces. Interestingly, both *Adiantum capillus- veneris* and *Epipactus gigantean* disperse through anemochorously, occur nearly exclusively at springs, and were among the first colonizers of anthropogenically created hanging gardens downstream of Glen Canyon Dam (Figure 3; Larry Stevens personal communication).

Helocrenes were distinctive in terms of physical and biological attributes. While low elevation helocrenes, or cienegas, occur in the Sonoran Desert, they are nearly absent in the GCE due to the limited area of low desert habitat and no low elevation helocrenes were included in this analysis. They are characterized by organic-rich to peat soils underlying low-gradient wetlands or cienegas and often occur in the GCE as wet meadows fed by perched water tables (Figure 12). These observations are supported by the generally high elevations, low slopes and high PSRS, high organic soil cover, and low specific conductance signifying this spring type (Figure 10). Many helocrenes are known to be open to grazing or other human disturbances including nearby roads and flow alterations and this may be responsible for the for the generally lower SEAP scores in some categories and related to the lower percentage of native species present at springs (Figure 11). In fact, a few of the best of the many indicator species associated with helocrene springs were exotic such as *Taraxacum officiniale*, *Poa pratensis* and *Achillea millefolium*. The higher disturbance levels among helocrenes by livestock grazing may increase potential for invasion by exotic species.

Rheocrene springs also had a set of properties that distinguished them from the other spheres of discharge. They occurred at moderate elevations and had moderate slopes (Figure 9). Rheocrene springs also had significantly higher scores for flow naturalness (Figure 11). Rheocrene springs emerge directly into channels and many rheocrenes are naturally subjected to scouring flows as a regular disturbance (Griffiths et al. 2008). As a result, their natural state is inherently less fragile. Rheocrene springs also had higher water temperatures than other spring types (Figure 10). This can be interpreted as their greater connection to regional (not local) groundwater systems. Additionally, rheocrene springs may reemerge below their true source leading to warmer measured water temperatures. Rheocrene springs also had distinctive flora. Many of these are known to be associated with perennial riparian systems such as *Salix gooddingii*, *Fraxinus velutina*, and *Juglans major*. However, a majority of rheocrene springs (54%) did not actually have an indicator species present indicating considerable variability in plant communities at rheocrene springs.

Springer and Stevens (2009) hillslope category type has been the most complex springs type to differentiate on the basis of vegetation (Sparks 2014, Springer et al. 2015). Hillslope springs assemblages were less distinctive in our study as well, but several distinguishing characteristics were identified. Hillslope springs were the most numerous and accounted for 45% of springs included in the study. They occurred at moderate elevations, had moderate areas, and had lower water temperatures (Figure 9). By definition, hillslope springs emerge onto gently to steeply sloping terrain, and thus had significantly higher slopes than did helocrene and rheocrene springs, but had significantly lower slopes than did hanging gardens. Substrata were most useful in distinguishing hillslope springs: they had much higher cover of gravel and woody debris (Figure 12). This may be related to position on hillsides causing fine material to be winnowed away and trapping woody debris in springbrooks. Hillslope springs also supported a suite of indicator species mostly restricted to higher elevations and mesic or shaded sites. These indicators included *Rudbeckia laciniata*, *Veratrum californicum*, *Abies concolor*, and *Pseudotsuga menziesii* var *glauca;* all of which are native species and with the latter two being tree species generally reflecting mesic, sloping landscapes and perennial site shading. However, many low elevation hillslopes springs do not support those indicator species, contributing to the high variability in hillslope springs flora. This is reflected by their scatter in the NMDS plot (Figure 17). Hillslope springs had significantly more north-facing slopes than did other spring types (Figure 9). While this may be a sampling artifact, the dataset included both rims of Grand Canyon, and it may be that physical processes related to shading or weathering causes hillslope springs to occur more on northerly aspects.

The LDA affirmed many of the observed physical characteristics differentiating springs types. The characteristics were detected by the LDA and generally weighted much higher along discriminant axes. The majority of springs of each type were correctly classified by the LDA, with a success rate of 70%**.** This indicates substantial physical differences among spring types. Further, those physical differences were directly related to plant assemblage composition and structure, and resulted in distinctive associations in different springs ecosystem types. Nonetheless, a great deal of unexplained variation exists in botanical attributes among these different spheres of discharge. While spring types have overarching characteristics, the history and legacy of local physical conditions, relationships to adjacent uplands, as well as differential biogeographic colonization, extinction, and anthropogenic alteration confer high levels of individuality to springs ecosystems (Stevens and Springer 2004). Every spring has its own unique geomorphology and biogeographic history, an ecosystem individuality that limits synthetic statistical integration.

Our analyses can help managers improve and enhance stewardship of springs ecosystems; however, management needs to be considered at local, as well as landscape scales. Inventory knowledge of the array of springs within the management area is important to understanding rare springs types and the likelihood of occurrence of rare springs-dependent species. Inventories also are needed to understand the diversity, distribution, and conservation status of springs-dependent (crenobiontic) species. For example, wetland plants, hydrobiid and other Mollusca, nepomorph water bugs, dryopoid beetles, cyprinodontid and other fish, and a broad array of other known and undescribed biota are critically restricted to Southwestern desert springs. Such species warrant concerted conservation attention by collaborating neighbors to achieve sustainable aquifer, habitat and biodiversity management (e.g., Shepard 1993, Unmack and Minckley 2008, Stevens and Polhemus 2008, Hershler et al. 2014). This approach has been adopted in the European Union for protection of travertine-depositing springs, which host an array of endemic plant and invertebrate species (Council of European Communities 1992), and restoration guidelines have been proposed for southwestern desert springs (Stevens et al. 2016b). Our results indicate that restoration planning should include consideration of rare springs types, wetted area, and protection of fragile microhabitats and rare species. Incorporation of these springs characteristics into restoration activity, coupled with detailed monitoring can provide invaluable experimental insight into gradient effect analyses.

Disturbances to springs including grazing, development, exotic species introduction and alteration of hydrology and water quality all affect the ecological integrity of the associated assemblage. Qualitative SSI ecological assessment metrics provide a simple and effective way of describing these disturbances (Paffett et al. in press).

Inventory and monitoring efforts should be continued because the majority of GCE springs have not been surveyed and trend data are limited. There are additional concerns over the identification of all springs across the GCE landscape, as demonstrated for the Death Valley region (Junghans et al. 2016) and in the GCE by Ledbetter et al. (2016). Further, additional attention should be focused on information management to ensure that data assembled thus far remain available to the public and the scientific and managerial communities. The critical status of aridland springs, their high biodiversity and remarkably dense species packing, as well as their cultural and economic significance affirm the need for iincreased scientific attention and improved stewardship for these remarkably unique and threatened ecosystems.

2.6 Acknowledgements

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Chapter 3 – Methods

3.1 Data Acquisition

The data used in this study was obtained through Spring Stewardship Institute's Springs Online Database. SSI has been instrumental in promoting springs conservation, improving awareness for springs ecosystems, creating standardized springs inventory practices, and creating a flexible data repository. The Springs Stewardship Institute developed the Springs Inventory Protocol (SIP) and Springs Online database to facilitate improved management through improved consistency of the springs ecosystem lexicon, data collection and compilation, synthesis, and reporting. The SIP created three standardized levels of spring inventories that can be selected based on management needs.

The protocol has been adapted and adopted by the U.S. Forest Service, the U.S. Fish and Wildlife Service, several National Park Service units, and many others. Springs inventory data are archived into an online database (springsdata.org) through the Springs Stewardship Institute (Ledbetter et al. 2014). The SSI protocol includes the complete details and sample data collections sheets (Stevens et al. 2016a). The database allows for efficient, flexible, and centralized cataloguing and maintenance of springs data. Land or project managers control permissions to access inventories securing sensitive data. Further, the database structure allows for multiple surveys to be linked to each unique spring entry making adding additional surveys, updating data, and long-term monitoring simple. The widespread adoption of the Spring Online Database by land managers in the GCE and use of the SIP make data collection uniform and led to a large quantity of high quality and directly comparable spring inventories, facilitating the analyses in this study.

The study data were collected following the Level II inventory. The data have been collected by teams of experts in biology, hydrogeology, and socio-cultural resources (Stevens et al. 2016a). The inventory data are archived in an online database through the Springs Stewardship Institute (SSI). The structure of the database and methods of data collection are outlined below.

SSI has developed detailed methods for inventorying springs. Their document entitled, *Inventory and Monitoring Protocols for Springs Ecosystems*, includes the details and sample data collections sheets (Stevens et al. 2016a). Springs inventory falls into three levels depending on the level of data collection. Level I springs inventories are primarily for reconnaissance and intended as quick preliminary investigations followed up by more detailed Level II inventories. Level I surveys collect data on site location, access directions, name, description, and photographs and can be carried out by one or two individuals with only minor training. The Level II inventories are comprehensive and designed to be conducted by a team of expert scientists over the course of several hours. Inventory data fall into the categories of Site Description, Biotic Inventory, Geomorphology, Flow, Water Quality, and Cultural Resources. The inventory usually consists of the Level II Springs Inventory Protocol (SIP), which includes the quantitative spring properties, as well as the Springs Ecosystem Assessment Protocol (SEAP), which is a qualitative assessment of the spring's health to inform management and restoration decisions. Finally, the Level III inventory is designed for long-term monitoring and involves repeated measurements of many data collected in Level II surveys. This study's analysis requires detailed information on geomorphology and biotic inventory, so only springs with complete Level II inventories were included in the analysis.

The inventory data are located on the SSI's Springs Online Database (Springs Online; springsdata.org). This database was originally developed as a Microsoft Access Database, but has since migrated to an online SQL format to facilitate its large size and data accessibility for land managers. The data are structured so individual surveys are linked to both a specific spring and project with one-to-many relationships. Each geo-referenced springs can have many different surveys with different data variables collected on each survey data. Each survey is further linked to a larger inventory project. Thus, surveys can be found by searching by spring name, Project, or location. Further permission is required to access surveys to protect sensitive data. Permission is granted by administrators to all springs in either a Project or Land Unit (Ledbetter et al. 2014).

Due to the limited search functionality of the online-interface a SQL Query was developed to gather data on all springs with Level II inventories. The query grouped springs by their spheres of discharge, and required surveys to include geomorphology microhabitat data, and full plant biota inventories to limit the later statistical analysis to springs with sufficient quantity and quality of data. Due to the database structure which stores general site information, survey geomorphic data, water chemistry data, flow data, and plant data as separate tables (Ledbetter et al. 2014), separate queries had to be developed for each table. These queries were then combined into a single entry per springs using spring and survey identification numbers. Further, for springs with multiple inventories, only the most recent or complete analysis was considered. The survey data were exported as .csv files for further statistical analysis in the Statistical Program R (2016).

Detailed methods of all data measured in a Level II inventory are in the SIP manual. Key methods for data collection are repeated here from the protocol manual (Stevens et al. 2016a).

Site-specific geographic information was recorded at each springs site. Global Positioning System (GPS) coordinates and elevation were measured using varying techniques and were recorded in the Springs and Springs-Dependent Species Database (Ledbetter et al. 2014). The azimuth aspect of polygon A (the springs orifice), was recorded and later converted to percent north. Solar radiation was measured using a solar pathfinder (Solar Pathfinder 2013) at the springs orifice. The solar pathfinder measures mean monthly sunrise and sunset times for a given range of latitudes at a spring's source, a point that may be locally shaded by surrounding topography. Those data are used to calculate the mean monthly solar radiation flux and the percent monthly radiation budget at the site. The ratio of actual solar radiation a site receives to the maximum potential solar radiation a site could receive during the months of July, August, and September is defined as "percent solar radiation summer," and is abbreviated as PSRS. This value is used instead of total solar radiation because it focuses on the important growing months for vegetation.

Discharge measurement technique varied depending on the magnitude and concentration of flow. Standard flow measurement techniques included a weir plate, flume, volumetric, and current meter procedures.

The weir plate method is useful for flows of shallow depth and low velocity in channels consisting of loose substrate (Buchanan and Somers 1969). The weir plate itself consists of 10 to 16-gage galvanized sheet iron with a v-notch ranging from 45º to 90º angle. The plate is placed into loose substrate with the top surface horizontal to land surface. Once the flow stabilizes behind the weir plate, the gage height is read 5-6 times within three minutes and the mean value is recorded. The gage height is then converted to discharge using a rating table corresponding to the angle of the weir plate.

The portable flume is another method useful for flows of shallow depth and low velocity (Buchanan and Somers 1969). A flume is preferred over a weir plate in wider channels (>30 cm). Also, the throat of a flume is interchangeable to accommodate a range of flows. A 1-inch throat can read flows of 0.2 to 2.5 L/s while an 8-inch throat can read flows of 2 to 21 L/s. To install a portable flume, the flume is placed in the channel with the top horizontal, and the flume sides are sealed to minimize water escaping. The stage height through the flume must be allowed to stabilize before reading the gage height. The gage height is read multiple times within 5-15 minutes to assure flow stabilization. The gage height is then converted to discharge using a rating table corresponding to the width of the flume throat. In this study, either a 1-inch or 8-inch cutthroat flume was used, when necessary.

Volumetric measurement is used when the flow is concentrated or can be concentrated and diverted into a container (Buchanan and Somers 1969). This is the most accurate technique to measure small discharges. To take a volumetric measurement, flow must be concentrated such that it can be collected in a volumetric container. Once the maximum amount of flow possible has been concentrated, the time required to fill a container of known volume is recorded several times until three consistent measurements are obtained.

A current meter is used for relatively large flows from approximately 0.10 to >1,000 m^3/s (Buchanan and Somers 1969). It is useful in channels of sufficient depth (approximately >20 cm) that are too wide (approximately >1 m) to use a portable flume or weir plate. The current meter consists of a rod connected to rotating cups or a sensor that, when submerged, rotate as a result of the stream discharge. Discharge is calculated by counting the number of revolutions in a given time. For optimal results, the cups of the current meter should be placed at 60 percent depth of flow. Measurements should be taken at frequent and regular intervals along a given cross-section of a channel.

Field water quality parameters are measured as close to the springs orifice as possible. A multi-parameter water quality probe is used for temperature, dissolved oxygen, specific conductance, and pH. The multiprobe is calibrated with standard reference solutions for pH and specific conductance daily. Alkalinity is measured using an alkalinity drop count kit.

Discrete geomorphic microhabitats are identified at each springs ecosystem, and microhabitats are mapped based on surface types (Table 5). Polygon dimensions are measured and mapped to scale in the field on a sketch map (Figure 18) or by walking the perimeter of the site with a GPS unit. Substrate (Table 6) visually estimated percent cover (VE%C), aspect and slope, soil moisture condition, litter depth were recorded for each microhabitat polygon.

Table 5. Substrate cover classes recorded for each microhabitat. Substrate is hypothesized as an important aspect of environmental heterogeneity.

Substrate	**Description**
Clay	Fine sediment <0.2mm in diameter
Silt	Fine sediment 0.02 to 0.1 mm in diameter
Sand	Sediment 0.1 to 1 mm in diameter
Pea Gravel	Sediment 1 to 10 mm in diameter
Coarse Gravel	Sediment 1 to 10 cm in diameter
Small Boulders	Boulders 10 to 100 m in diameter
Large Boulders	Boulders >1 m in diameter
Bedrock	Intact geologic units
Organic	Fine organic matter or peat
Litter	Leaves and twigs <1 cm thick
Wood	Branch and logs >1 cm thick
Precipitate	Chemical precipitate such as travertine

The botanist surveys the entire site and makes a plant species list. Unidentified specimens are collected for later identification. Species names are verified with the USDA PLANTS Database then entered into the Springs and Springs-Dependent Species Database (USDA, the PLANTS Database, 2014; Ledbetter et al., 2014). The visually estimated percent cover VE%C was visually estimated for species in each microhabitat by stratum: aquatic, nonvascular, basal, deciduous ground cover, shrub cover, middle canopy, and tall canopy cover (Table 6). Species nativity and wetland status were taken from SSI's database. Wetland status was refined from the USDA wetland indicator categories (USDA 2017), and included aquatic, wetland, wetland-riparian, riparian, facultative, and upland categories (Table 7).

Table 6. Wetland Indicator Statuses used for spring inventories. This varies slightly from USDA nomenclature recognizing riparian species as separate wetland species.

Wetland Indicator Status	**Abbreviation**	**Description**
Aquatic	A	Grows directly in the water
Wetland	W	Occurs almost always in wetland; hydrophyte
Riparian	R	Occurs almost always in riparian areas; hydrophyte
Wetland-Riparian	WR	Occurs in almost always in either wetlands or riparian areas; hydrophyte
Facultative	F	Occurs in both wetland and upland habitats; hydrophyte
Upland	U	Occurs rarely in wetlands; nonhydrophyte

Table 7. Stratum levels used when recording springs vegetation. VE%C is determined for each plant species in each microhabitat by stratum.

Cover Stratum	**Abbreviation**	**Description**
Ground Cover	GC	0-2 m tall, graminoid/herb/non-woody
Shrub Cover	SC	0-4 m tall, woody perennial
Middle Canopy	MC	4 - 10 m tall, woody perennial
Tall Canopy	TC	>10 m tall, woody perennial
Aquatic	AQ	Grows in or on water
Nonvascular	NV	Moss
Basal Vegetation	BV	The portion of the plant that extends into the water

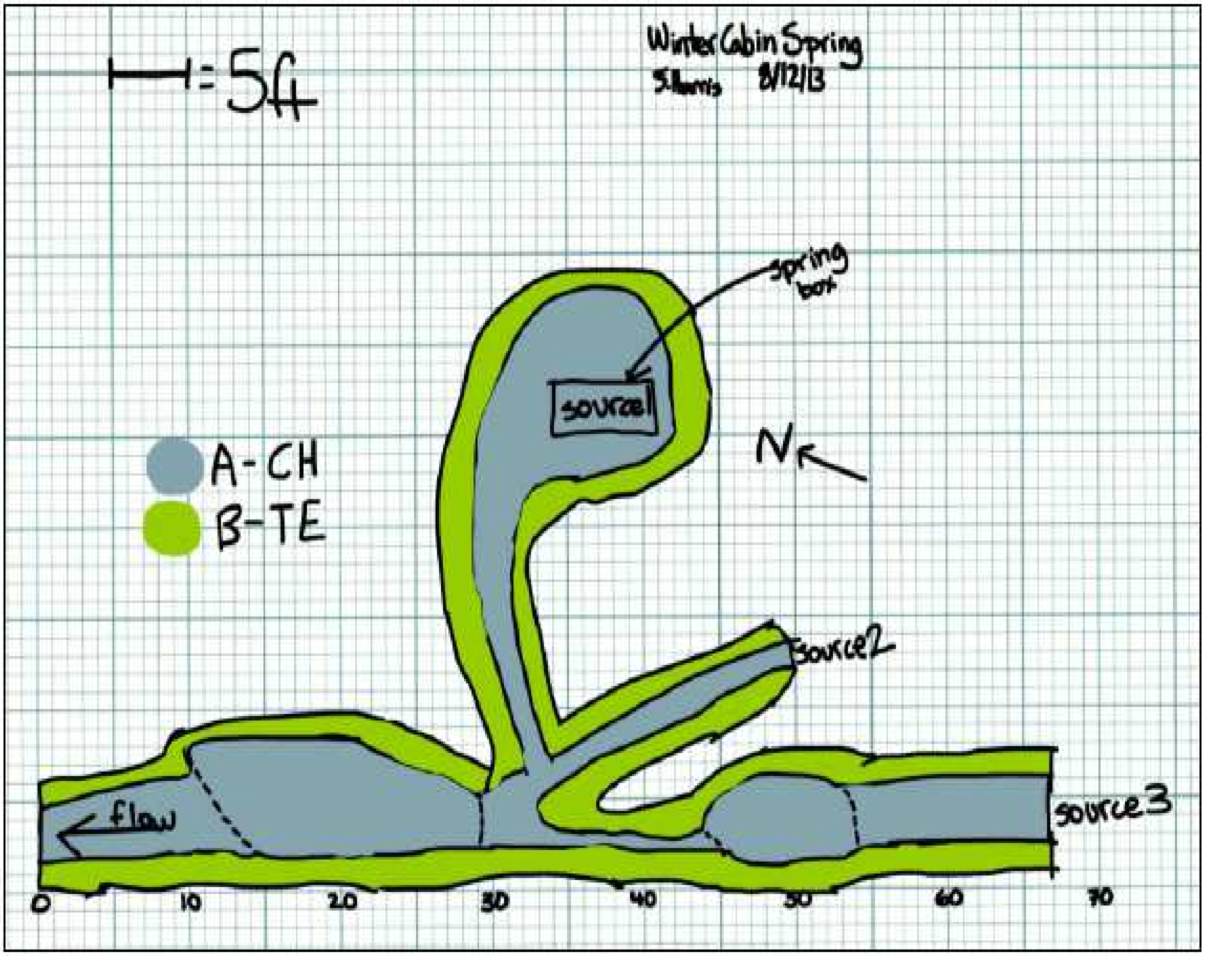


Figure 18. The site sketch map for Winter Cabin Spring. Two microhabitats were identified, the springbrook channel and the surrounding terrace. Each habitat forms distinct habitats for various species of plants depending aspect, slope, moisture availability and substrate

After completing the Level II survey a management focused semi-quantitative Springs Ecosystem Assessment Protocol (SEAP) is followed. It was designed to facilitate conversations and inform management decisions around springs restoration (Springer et al. 2015, Ledbetter et al. 2015, Paffett et al. in press). The SEAP assesses springs condition and risk with 42 questions across six categories including aquifer and water quality, geomorphology, habitat, biology, human influences, and administrative context (Stevens et al. 2016a). Springs condition are scored between 0 (low condition) and 6 (pristine condition) and risks are assessed 0 (no risk) to 6 (high risk). The SEAP has been shown to be an effective management tool with springs showing moderate conditions and high risks most responsive to restoration action (Paffett et al. in press). The SEAP helps translate inventory data into risk and condition scores which are often more pertinent for management decisions. SEAP scores were used in several statistical analyses to understand how disturbance varies between spring types and influences geomorphology and springs plant assemblages.

3.2 Diversity Metrics

To complete the analyses of the study, a metric is needed for calculating diversity. A simple diversity metric often used is entity richness. Species richness is often used for biodiversity studies but ignores differences in evenness (common vs. rare species) which may be important or desired in many contexts. The Shannon Diversity metric is commonly used to calculate a single metric of diversity. It is well-suited to spring survey data since springs are divided into polygons. The index depends on percent-cover rather than simply total species and number of individuals of that species as many other diversity indexes do. The Shannon Diversity index comes from information theory and incorporates both evenness and abundance into a single diversity metric (Shannon and Weaver 1949):

$$H' = - \sum_{i=1}^{S} p_i ln\, p_i \tag{2}$$

Where S is quantity richness or total number of elements, and p_i is the proportional contribution of each of the i elements in a sample. The incorporation of evenness and richness makes the Shannon diversity index an effective, quantitative metric of informational diversity.

Both the richness and Shannon diversity metrics are used to represent diversity in this study. Species richness is used for floral diversity since it is a common and easily interpretable metric and total number of species present is often more important for conservation efforts and management decisions. Both Shannon Diversity and richness are used for microhabitats as a measure of environmental heterogeneity and allow explore relative importance of abundance and evenness in heterogeneity relationships. Shannon Diversity is used to calculate a functional diversity of wetland indicator statuses and structural diversity of plant cover across strata.

3.3 Analyses

Data were examined for outliers and transcription errors before analysis. When suspect data were found they were investigated by reviewing survey notes, crosschecking photos, sketches and site or referencing additional surveys of the same spring. If missing or suspect data could not be rectified, then that spring site was excluded from further analysis.

A spatial of analysis of the distribution of springs was conducted. The spring types, water quality, species richness, and geology were all mapped to understand spatial distribution of springs and patterns related to their distribution. The springs were not randomly selected and spatial correlation of variables was a concern with many statistical analyses. Spatial analysis of springs was used to address distribution selection.

The source microhabitats of each spring type were also summarized. The frequency of each common microhabitat at each sphere of discharge was summarized. The identification of microhabitats associated with spring types helps determine underlying geomorphic differences between spring types that might be associated with physical and ecological differences between spring types. The types and attributes of microhabitats associated with each sphere of discharge may be an important aspect of relationships between environmental heterogeneity.

A linear mixed model was constructed to predict percentage of native species present at springs. All physical, geomorphic and water quality parameters were included as candidate model predictors. Transformations were applied when indicated by bivariate and histogram analysis. Spring type was included as a candidate predictor allowing have different intercepts for each sphere of discharge. A stepwise procedure was performed in R using the "step" function inside the "*stats"* package (R Core Team 2017). Best models were selected using the Akaike

Information Criterion (AIC), which is based on likelihood and penalizes overly complex models (Sakamoto et al. 1986). Permutation tests were used to assess the importance of each variable to overall model accuracy. Covariance of the selected predictors were examined for multicollinearity and to aid in interpretation. Residuals were plotted and examined to assess model fit and any outliers.

Effects of grazing disturbance on biodiversity were also investigated. One of the scores assessed during the SEAP describes negative ungulate impacts including overgrazing, hummocking, and trampling. The effects of grazing were assessed by their impact on residuals from mixed models for floral species richness and percentage of native plants. The grazing effect was allowed to vary by condition level and between spring types. The results were inspected visually with 95% confidence intervals and an F test between models with and without grazing.

Species area effects were also evaluated. Hypothesized models including both logarithmic and semi-logarithmic models and assessed by total variation explained. Both have been argued for in the literature (e.g., Gleason 1922, Wilson and MacArthur 1967). The logarithmic model is

$$log_{10}S = a + c\ log_{10}A \tag{3}$$

and the semi-logarithmic model is

$$S = a + c\ log_{10}A \tag{4}$$

where S is species richness, A is spring area (m^2), and a and c are intercept and slope coefficients, respectively. Single models for all GCE springs were also contrasted with mixed models allowing slope and intercept coefficients to vary with spring sphere of discharge.

Species richness was also compared with elevation as an ecological gradient analysis. Elevation is often used as a proxy for climate in ecological studies (see Korner 2007) and elevation strongly influences air temperature and precipitation in the GCE (Sellers et al. 1985) and subsequently potential evapotranspiration and growing season length. It would be best to model these climate variables directly but due to limited available data and the fact springs often create their own microclimate (Springer and Stevens 2004) this was beyond the scope of this study. Species richness was expected to increase with elevation throughout most of the elevation ranges with CGE do to cooling temperatures (less ET) and increased precipitation (increased water availability) but eventually decline at (very) high elevations due to shortened growing seasons (Larry Stevens personal communication). Both linear and quadratic models were tested and evaluated by proportion of variation explained to assess this relationship.

Species richness environmental heterogeneity relationships were investigated by themselves. Linear mixed models were used to evaluate the effect between environmental heterogeneity using both microhabitat richness and Shannon diversity. Slopes and intercepts were allowed to vary between spring types and the significance of differences between spring type were assessed with F tests. Because the universality of positive heterogeneity-richness relationships may depend on controlling for area (Stein et al. 2014), the species density metric was also compared with microhabitat diversity. Species density was represented:

$$D = \frac{S}{log_{10}A} \tag{5}$$

where D is the species density, S is richness, and A is spring area (m^2).

Correlograms were constructed to explore complex relationships at springs that can make bivariate relationships difficult to interpret. Spearman rank correlation between environmental variables and diversity metrics included in models as well as SEAP condition scores were compared. These results were used to assess previous model results and identify possible lurking or confounding variables that were not considered directly in models.

Since previous studies have often found hillslope springs highly variable and difficult to differentiate from other spheres of discharge (Sparks 2014, Springer et al. 2014). Possible sub-classifications of hillslope springs were considered in this study. Classification methods included separation of hillslope springs into forested and unforested subclasses. Forested springs were hypothesized to have additional shading, litter and woody material influencing springs plant assemblages (Larry Stevens personal communication). This classification was accomplished by flagging springs with any tall canopy cover as forested. Other hypotheses on the high variation reclassification into hillslope-helocrene or helocrene-hillslope depending secondary sphere of discharge (Sparks 2014). An additional possibility included reclassification based on proximity to nearby streams (by elevation or distance) which could strongly impact microhabitats availability and presence or absence of many riparian species (Larry Stevens personal communication).

Chapter 4 – Results and Discussion

4.1 Spatial Analysis

Spatial analysis indicated some clustering of springs types. There were spatial patterns to the distribution of springs included in the datasets. Springs used in the analysis occurred mostly inside the rims of the Grand Canyon, on the Kaibab Plateau, and along the Mogollon Rim. This clustering likely reflects both sampling and actual distribution areas. Springs are likely more common in these areas. Further, due to their locations on NPS or USFS land, increased levels of funding is available leading to more high-quality in these areas. Despite this potential bias, the dataset represents a large sample of the springs of the GCE with a broad spatial distribution throughout the area.

There were distinct patterns in the lithology of the GCE springs (Figure 19). Patterns in springs water chemistry often reflects the geology (Figure 20). Springs dominantly discharged from limestone, sandstones or volcanic units (basalts or rhyolite). Springs along the Mogollon Rim often discharged from basalt or rhyolite. This lithology of discharge reflects the widespread volcanic cover in the region which often forms perched aquifers with local flow systems (Springer et al 2017). Most of the local springs also had low specific conductance. Springs along the Kaibab Plateau mostly occurred in limestone with some springs occurring in sandstone or shale. The water chemistry of springs on the Kaibab Plateau seems to vary strongly with elevation. The elevation correlation with chemistry in this study is similar to the results of Ledbetter et al. (2014) and probably reflects flow path length since groundwater tends to recharge at high elevations (Springer et al 2017). Springs inside the Grand Canyon mostly issued from limestones and sandstones with springs discharging from metamorphic or intrusive igneous rock rare. Specific conductance values of water discharging from springs appears more variable throughout the canyon but tended to be higher than the high elevation springs on the Kaibab Plateau or Mogollon Rim.

Species richness at springs varied widely among springs (Figure 21). There appear to be few spatial relationships between species richness at springs with nearby springs often exhibiting widely different floral species richness. This indicates that each spring is a unique entity with its own biogeographic history and geomorphic characteristics separate from its neighbors. While there are likely spatial patterns in the distribution of each individual species patterns of overall floral species richness don’t seem to reflect this strongly.

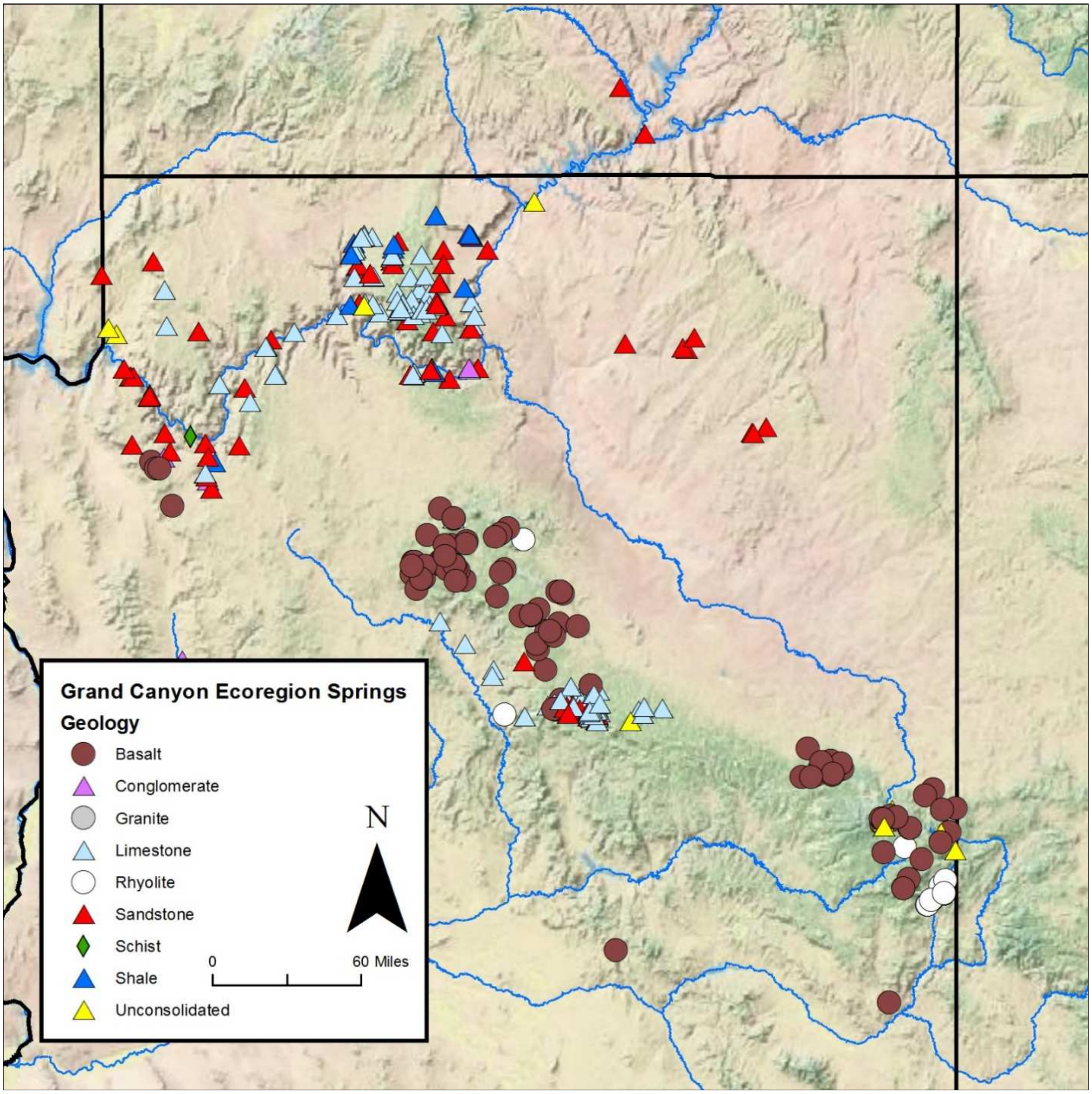


Figure 19. Geology at springs sources throughout the GCE. The source geology reflects the regional geology and the majority of springs issued from sandstone, limestone, or volcanic units.

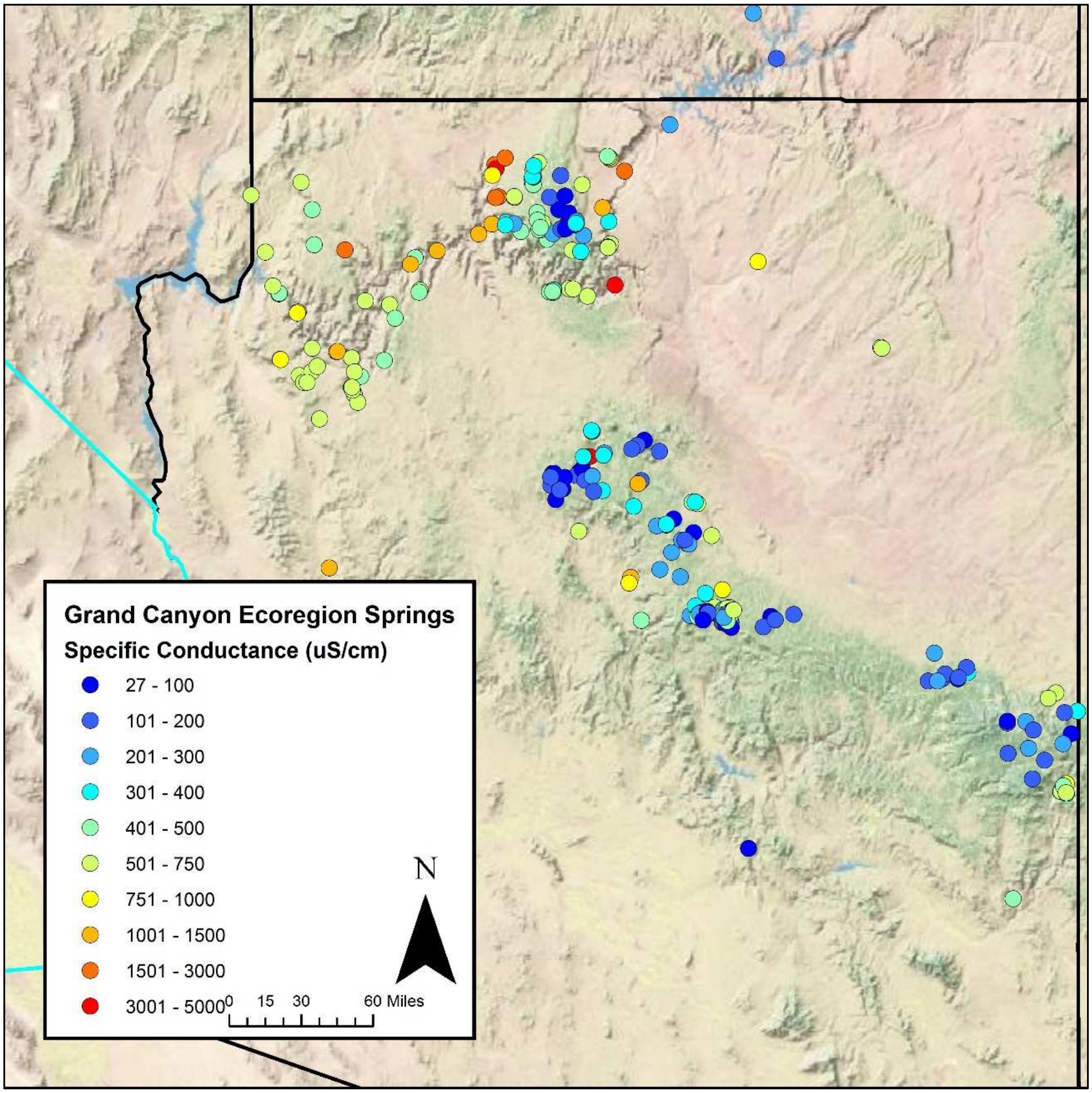


Figure 20. Specific conductance at springs sources in the GCE. The conductance of springs water reflects the total dissolved solids load. Nearby springs tended to have similar conductance and tended to be related closely to elevation reflecting flow path lengths and source aquifers.

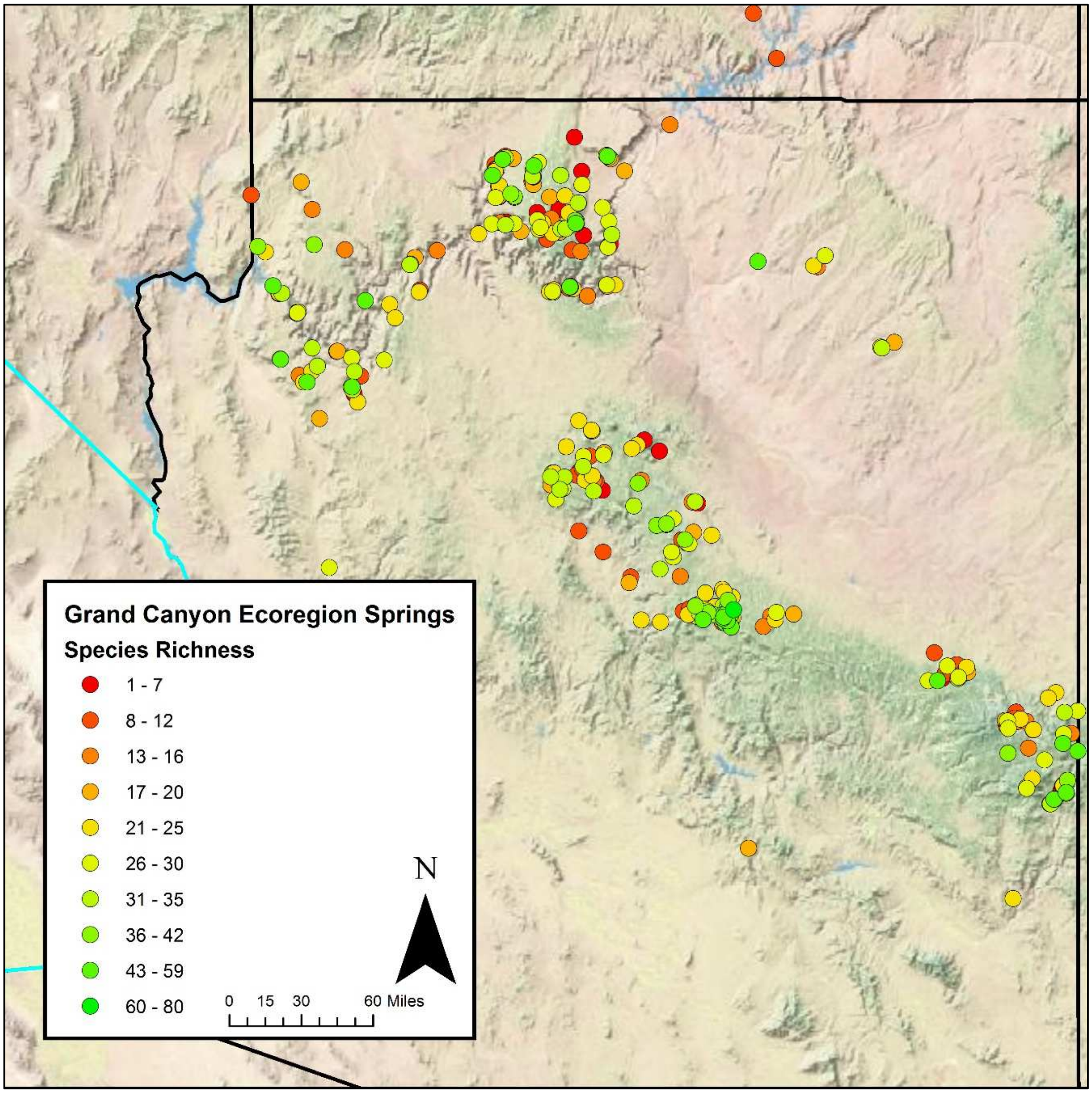


Figure 21. Floral species richness at springs in the GCE. No distinct spatial patterns are readily apparent.

4.2 Microhabitat Differences Between Spheres of Discharge

Differences were found between the types of microhabitats associated with each sphere of discharge. The frequency of occurrence of each microhabitat was tabulated for each sphere of discharge (Table 8). Certain microhabitats were common across all springs types but others were associated specifically with certain spheres of discharge. Backwalls were specifically associated with hanging garden spheres, occurring 81% of the time and only occasionally with hillslope and rheocrene springs, and never at helocrene springs. Channel microhabitats were common at all springs but more so at hillslope and rheocrene springs than helocrene and hanging gardens. Colluvial slopes were found at all spring types but most commonly at hanging gardens. Madicolous microhabitas were rare and mostly constrained to hanging gardens, while plunge pools were only found at hanging gardens. Low-gradient cienegas were most common at helocrene springs. High gradient cienegas were rare but occurred most frequently as helocrene springs. The tendency of specific types of microhabitats to occur with each sphere of discharge indicates the emergence environment of a spring is directly related to the microhabitats that form. Associations of microhabitats with specific spheres of discharge suggests that sphere of discharge is an important geomorphic consideration that will influence the microhabitats available and thus the species assemblages that occur at each spring. The large variety of microhabitats occurring at springs may be the fundamental reason springs serve as hotspots of biodiversity and such a large proportion of the GCE's floral diversity can be found at springs.

Table 8. Frequency of common geomorphic microhabitats by sphere of discharge. Values indicate percentage of springs of that sphere of discharge containing each microhabitat. Bolded values show most common microhabitat at each sphere of discharge.

Sphere of Discharge	Hanging Garden	Helocrene	Hillslope	Rheocrene
BW	**81%**	0%	18%	12%
C	4%	0%	3%	3%
CH	53%	55%	**78%**	**70%**
CS	30%	12%	20%	6%
HGC	6%	8%	7%	2%
LGC	6%	**59%**	17%	7%
MAD	4%	0%	1%	0%
OTH	6%	8%	4%	3%
P	17%	10%	18%	17%
PM	0%	4%	2%	0%
PP	6%	0%	0%	0%
SB	23%	0%	4%	5%
SM	2%	0%	1%	0%
TE	36%	39%	46%	58%
UPL	2%	0%	1%	1%

4.3 Species Area Relationships

There was evidence that floral species richness increased with increasing spring area. A semi-logarithmic model was preferred when analyzing all springs with one group rather than a fully logarithmic model (R^2 0.078 vs. 0.049, respectively). Both models showed significant, albeit weak trends. There was evidence that species area trends varied significantly by sphere of discharge. A model incorporating separate intercepts for each sphere of discharge was preferred to a single-group semi-logarithmic model based on an F-test between models ($P<0.0001$, F-stat = 7.96). A model analyzing different slopes between spheres of discharge was preferred to both previous models based on an F-test ($P=0.0013$, F-stat = 5.3). This model ($R^2 = 0.17$) is shown in Figure 22 and indicates that species area effects vary between spring types.

Relationships between area and floral species richness depended on spring type. Helocrene springs showed a slight negative (95% CI overlapping 0) floral richness–species area relationship while all other springs had positive species area relationships. This result was strongly influenced by several helocrene springs with extremely large areas but relatively low floral species richnesses. It is possible that floral richness doesn't increase with spring area in helocrene springs due to low heterogeneity of large wet meadows. Alternatively, sample effects may be important because it is very difficult to detect all floral species in a large wet meadow during a brief survey and some species may be missed. Hanging garden, hillslope, and rheocrene springs all showed clear increases in species richness with elevation. The estimates for the slopes ranged from an increase of 10 plant species for a 10-fold increase in area at hillslope springs to a 5 plant species increase per 10-fold increase in area for rheocrene springs (Table 9).

Springs area reflects the area where vegetation is distinct from the surrounding upland due to moisture related to the springs. It was interesting that wetted area was related to plant species richness while no significant relationships were found between spring discharge plant species richness. It is possible that different effects would be discovered for macroinvertebrate species, but this study suggests wetted area may be more important than springs discharge to land managers from a plant species biodiversity perspective.

Table 9. Estimates of slopes of species-area relationships for plant species by sphere of discharge. Values represent increase in plant species richness for a 10-fold increase in area using a semi-logarithmic model. Lower CI and Upper CI show the 95% confidence limits on the estimate.

Sphere of Discharge	**Mean**	**Lower CI**	**Upper CI**
Hanging Garden	9.9	5.4	14.3
Helocrene	-1.0	-4.8	2.7
Hillslope	6.4	4.0	8.7
Rheocrene	5.4	1.8	9.1

4.4 Species Elevation Relationship

There were only weak trends between elevation and species richness. A simple model with all spheres of discharge grouped together showed a very weak positive linear relationship between elevation and species richness ($R^2 = 0.02$). There was no evidence for a quadratic effect of elevation on area based on comparison of models with and without quadratic terms ($P=0.76$, $F = 0.09$). There was evidence that elevation richness relationships varied by sphere of discharge. A model with different intercepts for each spring type was preferred to a model that did not consider spheres of discharge ($P=0.02$, $F = 3.2$). Additionally, a model allowing slopes to vary by sphere of discharge was preferred to a single slope model ($R^2=0.10$; $P<0.0001$, $F = 7.3$, Figure 23).

Magnitude and direction of elevation-richness relationships varied significantly between spheres of discharge. This relationship is not surprising given that spheres of discharge were found to have significant differences in the elevations where they occur and the types of plant species that occur there. The abundance of each species will respond to a changing elevation gradient differently. The species elevation gradient is the combination of responses of all plant species responding to the gradient and thus likely not a linear relationship and our model poorly represents the true relationship. Further, since different assemblages of plant species are associated with each sphere of discharge there are different relationships with biodiversity between spring types. At hanging garden springs, floral richness decreases with elevation. This may be related to the many hanging gardens that occur at lower elevations in the GCE. Hillslope and rheocrene springs show positive relationships that may be related to increased productivity with increased moisture availability at moderate and high elevations. Finally, helocrene springs showed limited trends with elevation but are mostly limited to high elevation sites in the GCE.

Estimates and confidence intervals for the slope of species richness elevation relationships were calculated (Table 10). All spheres of discharge show wide 95% confidence intervals indicating weak relationships between elevation and floral richness. Additional complexity is added to elevation richness relationships because of various changes that occur with altitude. Elevation primarily influences air temperatures but secondary effects related to elevation include moisture availability, geology, spring water chemistry, solar radiation budgets, and growing season. These complications may responsible for the non-uniform variations of species richness with elevation across spheres of discharge.

Table 10. Slope of elevation floral richness relationships by sphere of discharge. Values represent expected change in floral richness for a 100-meter increase in elevation. Upper and Lower CI show bounds of 95% confidence intervals.

Sphere of Discharge	**Mean**	**Lower CI**	**Upper CI**
Hanging Garden	-0.64	-1.1	-0.21
Helocrene	-0.16	-0.9	0.59
Hillslope	0.23	-0.02	0.49
Rheocrene	0.71	0.32	1.1

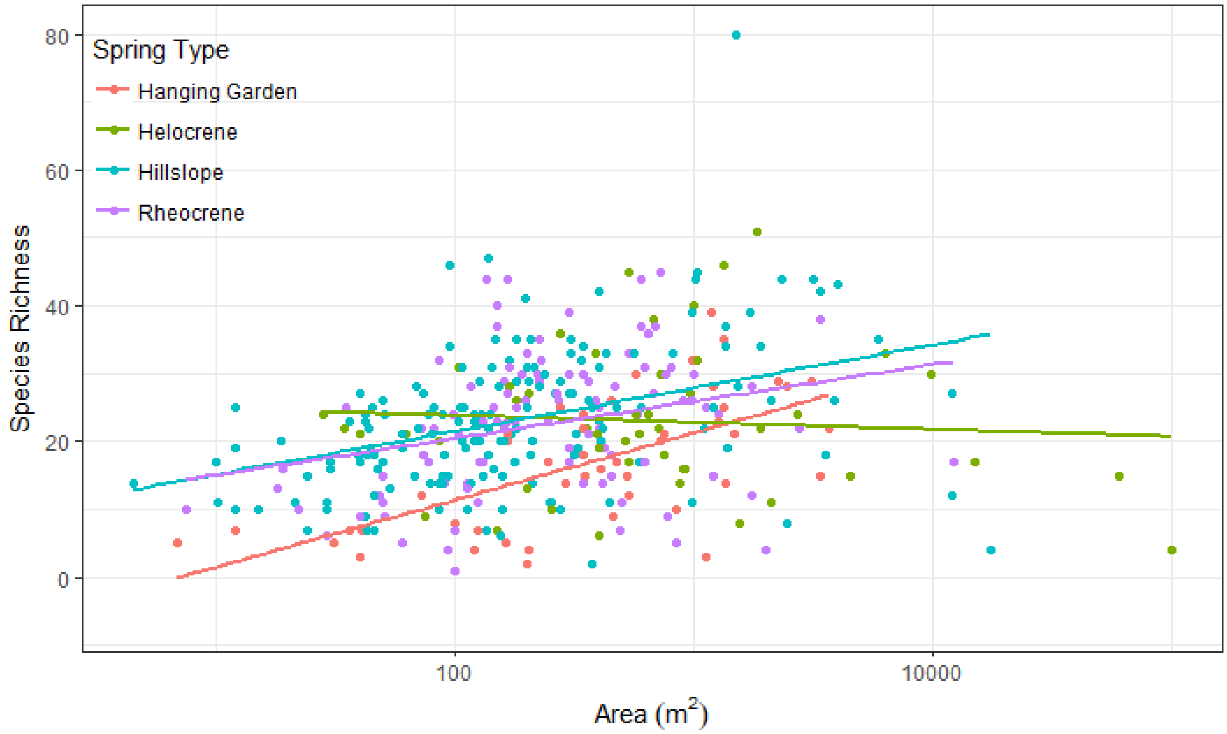


Figure 22. Wetted area versus floral species richness by springs type using a semi-logarithmic model with varying slopes. Lines show best fit for each sphere of discharge. Model fit for all points combined was relatively poor with an R^2 of 0.17.

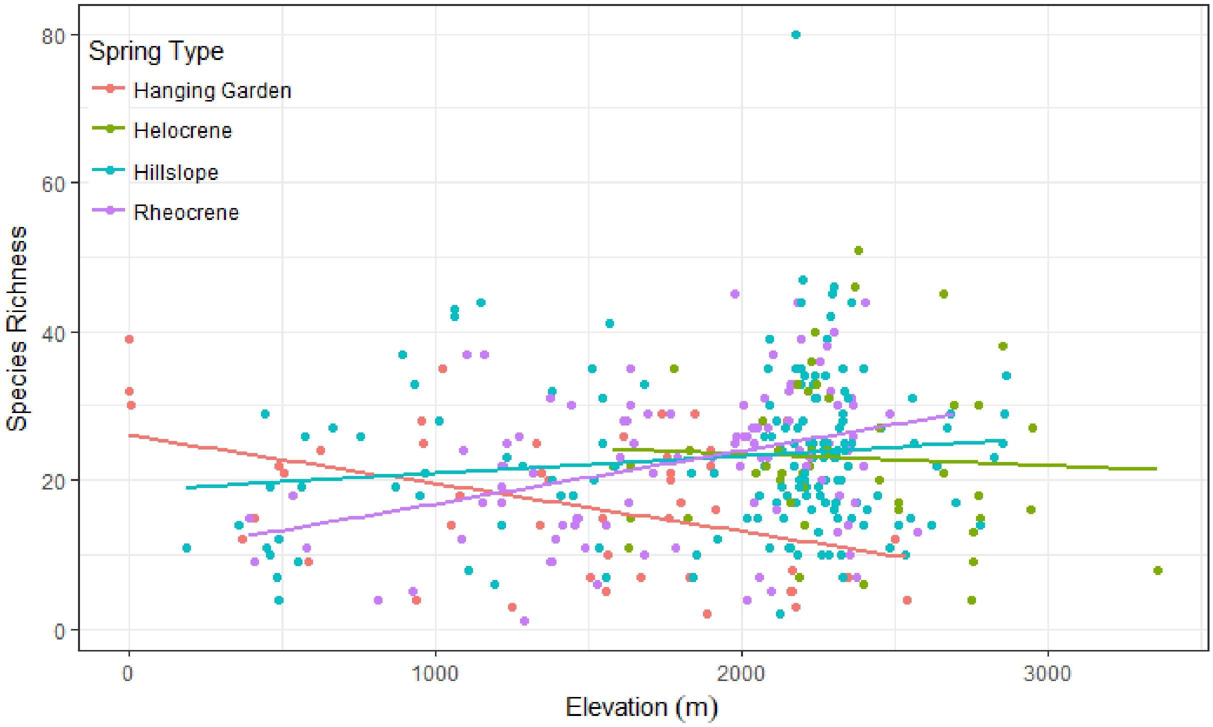


Figure 23. Elevation versus floral species richness by springs type. All relationships were weak and indicated elevation by itself is a poor predictor of floral species richness at springs. The model achieved an overall R^2 of 0.10 although 95% confidence intervals for the slope of the relationship at hillslope and rheocrene springs overlapped with no effect.

4.5 Species Richness-Heterogeneity Relationships

There was evidence of positive relationships between floral species richness and both microhabitat diversity and microhabitat richness. Both geomorphic microhabitat diversity and microhabitat richness were positively related to floral species richness indicating landscape heterogeneity is associated with increased species richness. The relationship was stronger with microhabitat richness rather than microhabitat diversity ($R^2 = 0.10$ vs. 0.075). This indicates that abundance of microhabitats is more important and evenness of microhabitats is less important for floral species richness. It was estimated that each additional microhabitat was associated with an increase of 2.2 plant species (95% confidence interval [1.5, 2.9]).

A positive relationship was also found between microhabitat richness and species density. This relationship was not as strong as the relationship with species richness ($R^2 = 0.015$ vs 0.10). This suggests microhabitat richness is more closely related to overall floral richness than floral species density. Microhabitat richness was found to be positively correlated with springs area. Very small springs may be less likely to contain multiple microhabitats.

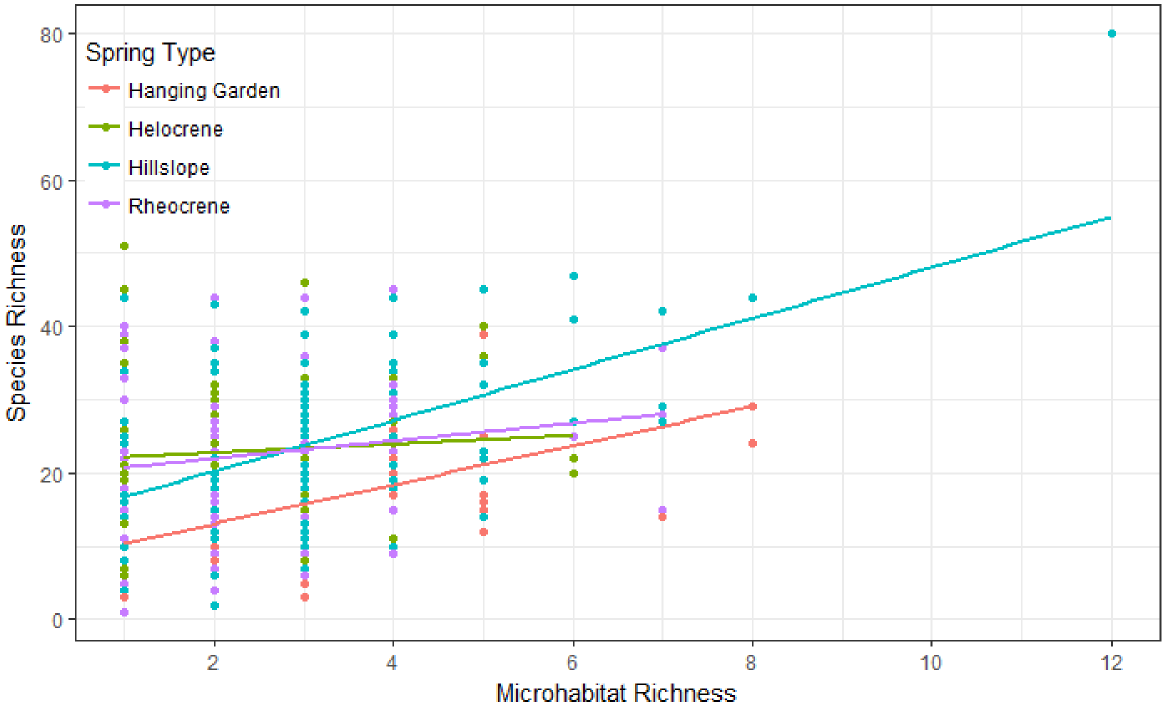


Figure 24. Microhabitat richness versus floral species richness relationships by springs type. All spheres of discharge exhibited positive relationships with trends stronger at hanging garden and hillslope springs. Model fit adequately well with $R^2 = 0.19$ although.

There was evidence that the microhabitat richness, floral richness varied by sphere of discharge. A model allowing different intercepts between spheres of discharge performed significantly better than model that did not include spheres of discharge ($P<0.001$, $F = 9.3$). Additionally, there was evidence that the slope of the relationship varied by spring type. This model was preferred over the single-slope model ($P=0.01$, $F = 3.8$). This model is shown in Figure 24. The model fit adequately well explaining 20% of the total variation in floral species richness amongst all GCE springs. This indicates that microhabitats have a strong relationship with species richness but are not sufficient to explain species richness alone. Environmental heterogeneity, expressed as microhabitat richness, may be a fundamental source of biodiversity at springs but other processes must also be considered important. Hillslope and hanging garden springs showed stronger positive relationships than helocrene and rheocrene springs. This suggests differences in the types of microhabitats associated may affect the relationship. The abundance of microhabitats and the specific nature of each individual microhabitat may both be important. However, the relationship appears to be universally positive at springs in the GCE (Table 11).

Table 11. Slope of microhabitat richness vs. floral richness relationships by sphere of discharge. Values represent expected increase in species richness for each additional microhabitat. Lower and upper CI indicate 95% confidence intervals for estimate.

Sphere of Discharge	**Mean**	**Lower CI**	**Upper CI**
Hanging Garden	2.7	1.0	4.3
Helocrene	0.6	-1.3	2.5
Hillslope	3.5	2.5	4.4
Rheocrene	1.2	-0.2	2.6

4.6 Nativity Model

The best model selected to predict the percent of native species present at various spheres of discharge achieved an R^2 of 0.37 and a residual standard error of 10% (Table 12). The suite of predictors in order of importance to model accuracy included: (1) dissolved oxygen, (2) slope, (3) pH, (4) log(flow + 1), (5) alkalinity, (6) soil moisture, (7) PSRW, (8) organic soil cover, (9) elevation, (10) litter cover, (11) log(Litter Depth + 1), (12) microhabitat richness. After accounting for these predictors, there were no longer significant differences between springs types (*P*=0.65; *F* = 0.54). Water quality parameters tended to be most related to the percentage of native species. Dissolved oxygen and pH had especially strong effects. This may reflect intolerance of some invasive species to extreme conditions. However, due to the correlation of many water quality parameters among themselves and with other factors including sphere of discharge and elevation, interpretation

is difficult. Slope was also important an important factor of analysis in the model. Slope may be important because of the remote locations and difficult access to many springs on steep terrain. PSRS may have been important for similar reasons although it is possible exotic species are less tolerant of these low light habitats. Exotic species may tend to be isolated from invasive species or have less human activity and lower disturbance (which also correlates strongly with percentage of native species).

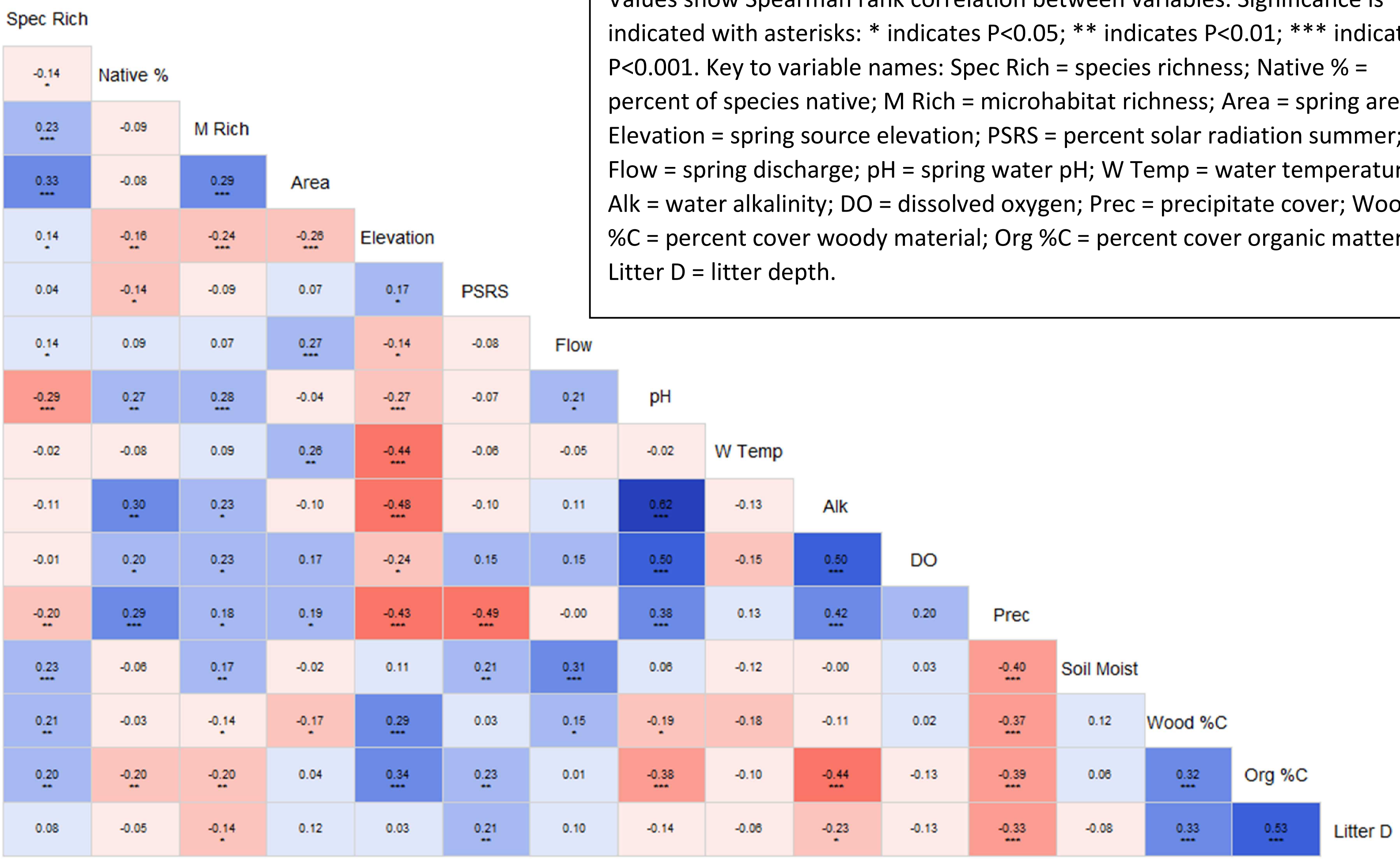
Table 12. Multiple linear regression predicting percent of native plant species present at 309 springs. Model was chosen with stepwise selection minimizing AIC. Importance reflects average reduction in model R-squared when the values of the parameter were randomly permuted. Bolded P-values indicate P<0.05

Parameter	**Estimate**	**Std. Error**	**t Value**	**P**	**Importance**
Intercept	109.90	7.49	14.68	**<0.00001**	-
Alkalinity (mg/L)	0.05	0.01	5.08	**<0.00001**	14.4
Slope (^{0})	0.17	0.03	6.23	**<0.00001**	22.0
pH	-6.81	1.12	-6.11	**<0.00001**	21.0
Dissolved Oxygen (mg/L)	1.60	0.23	7.00	**<0.00001**	27.9
log(Flow) (L/s)	1.28	0.24	5.40	**<0.00001**	16.3
Microhabitat Richness	-0.85	0.41	-2.08	**0.0389**	1.9
Elevation (m)	0.0035	0.0013	2.65	**0.00856**	3.5
Soil Moisture (1-10)	-0.88	0.23	-3.80	**0.00018**	7.8
PSRS (%)	-1.17	0.32	-3.61	**0.000356**	7.0
Litter Cover (%)	-0.05	0.02	-2.23	**0.026573**	2.3
Organic Soil Cover (%)	0.07	0.02	3.11	**0.000204**	5.1
log(Litter Depth) (cm)	-1.06	0.51	-2.08	**0.038169**	2.0
R^2 = 0.37	**Res. Std. Error = 10.1**			**Df = 296**	

4.7 Correlograms

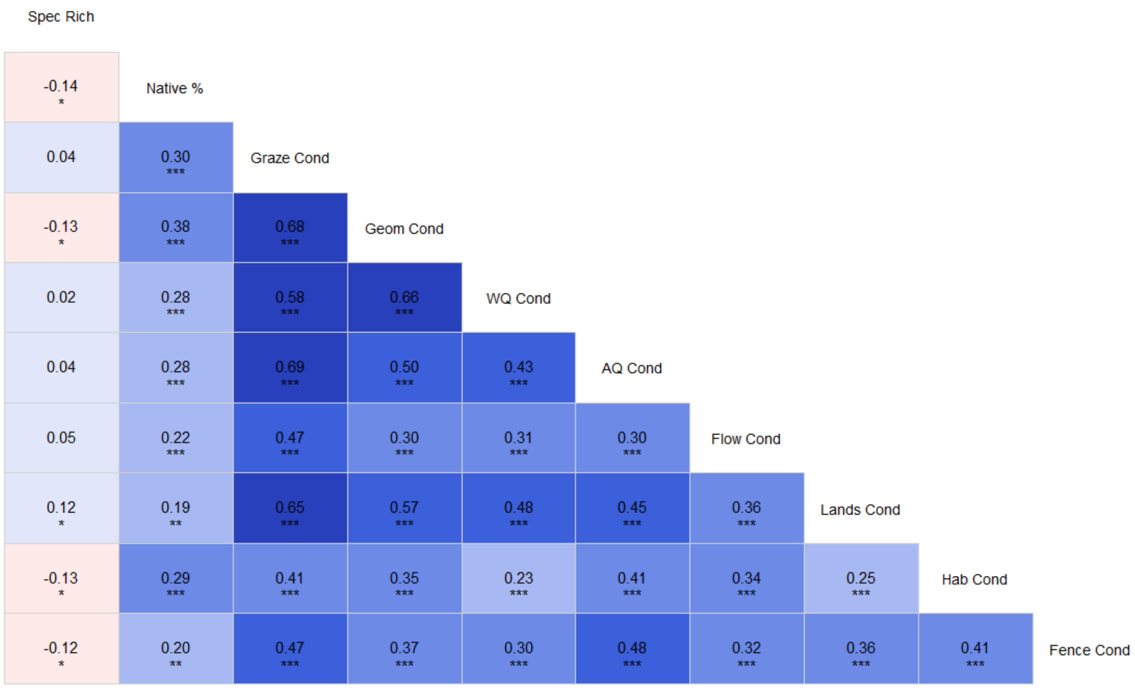
Correlograms illustrated significant correlations important to understanding covariance between springs state variables that need to be considered. Effect estimates in multiple linear regressions represent effects while holding all other parameters constant. However, at springs this seldom occurs because of interrelationships between variables (i.e. alkalinity and pH). Thus interpretation can be confusing and unintuitive for highly correlated variables and can create a multicollinearity problem. Figure 25 shows the correlations of variables included in the models. Many of the water chemistry parameters and elevation were strongly correlated, as well as the percent cover for woody debris, organic matter and litter depth. Because many of these indicators were included in the model for plant nativity results should be interpreted with some caution. It is possible external factors not quantified or included in model selection are important.

SEAP Condition scores all showed high correlation values (Figure 26). This indicates that if that a spring is in good or bad condition for one indicator, it is likely to be in similar condition for other indicators. This suggests degradation and disturbance of springs manifests itself in multiple ways. Thus SEAP scores are good indicators of overall disturbance level of springs. Few of these condition scores correlated well with species richness. This combined with NMDS results (see Chapter 2) indicate conditions scores measured with SEAP differentiates springs plant communities but does not differentiate overall species richness. However, many of the SEAP condition scores were positively correlated with the percentage of native species. This indicates these disturbances and degradations of spring condition does result in invasion of exotic species.



	Spec Rich	Native %	M Rich	Area	Elevation	PSRS	Flow	pH	W Temp	Alk	DO	Prec	Soil Moist	Wood %C	Org %C
Native %	-0.14 *														
M Rich	0.23 ***	-0.09													
Area	0.33 ***	-0.08	0.29 ***												
Elevation	0.14 *	-0.16 **	-0.24 ***	-0.26 ***											
PSRS	0.04	-0.14 *	-0.09	0.07	0.17 *										
Flow	0.14 *	0.09	0.07	0.27 ***	-0.14 *	-0.08									
pH	-0.29 ***	0.27 **	0.28 ***	-0.04	-0.27 ***	-0.07	0.21 *								
W Temp	-0.02	-0.08	0.09	0.26 **	-0.44 ***	-0.06	-0.05	-0.02							
Alk	-0.11	0.30 **	0.23 *	-0.10	-0.48 ***	-0.10	0.11	0.62 ***	-0.13						
DO	-0.01	0.20 *	0.23 *	0.17	-0.24 *	0.15	0.15	0.50 ***	-0.15	0.50 ***					
Prec	-0.20 **	0.29 ***	0.18 *	0.19 *	-0.43 ***	-0.49 ***	-0.00	0.38 ***	0.13	0.42 ***	0.20				
Soil Moist	0.23 ***	-0.06	0.17 **	-0.02	0.11	0.21 **	0.31 ***	0.06	-0.12	-0.00	0.03	-0.40 ***			
Wood %C	0.21 **	-0.03	-0.14 *	-0.17 *	0.29 ***	0.03	0.15 *	-0.19 *	-0.18	-0.11	0.02	-0.37 ***	0.12		
Org %C	0.20 **	-0.20 **	-0.20 **	0.04	0.34 ***	0.23 **	0.01	-0.38 ***	-0.10	-0.44 ***	-0.13	-0.39 ***	0.06	0.32 ***	
Litter D	0.08	-0.05	-0.14 *	0.12	0.03	0.21 **	0.10	-0.14	-0.06	-0.23 *	-0.13	-0.33 ***	-0.08	0.33 ***	0.53 ***

Figure 25. Correlation of springs state variables included in regression models. Values show Spearman rank correlation between variables. Significance is indicated with asterisks: * indicates P<0.05; ** indicates P<0.01; *** indicates P<0.001. Key to variable names: Spec Rich = species richness; Native % = percent of species native; M Rich = microhabitat richness; Area = spring area; Elevation = spring source elevation; PSRS = percent solar radiation summer; Flow = spring discharge; pH = spring water pH; W Temp = water temperature; Alk = water alkalinity; DO = dissolved oxygen; Prec = precipitate cover; Wood %C = percent cover woody material; Org %C = percent cover organic matter; Litter D = litter depth.



	Spec Rich	Native %	Graze Cond	Geom Cond	WQ Cond	AQ Cond	Flow Cond	Lands Cond	Hab Cond
Native %	-0.14 *								
Graze Cond	0.04	0.30 ***							
Geom Cond	-0.13 *	0.38 ***	0.68 ***						
WQ Cond	0.02	0.28 ***	0.58 ***	0.66 ***					
AQ Cond	0.04	0.28 ***	0.69 ***	0.50 ***	0.43 ***				
Flow Cond	0.05	0.22 ***	0.47 ***	0.30 ***	0.31 ***	0.30 ***			
Lands Cond	0.12 *	0.19 **	0.65 ***	0.57 ***	0.48 ***	0.45 ***	0.36 ***		
Hab Cond	-0.13 *	0.29 ***	0.41 ***	0.35 ***	0.23 ***	0.41 ***	0.34 ***	0.25 ***	
Fence Cond	-0.12 *	0.20 **	0.47 ***	0.37 ***	0.30 ***	0.48 ***	0.32 ***	0.36 ***	0.41 ***

Figure 26. Correlation of Springs Ecosystem Assessement Protocol (SEAP) condition scores as well as species richness and percentage of native species. Values indicate Spearman rank correlation. Asterisks indicate level of significance: * indicates P<0.05; ** indicates P<0.01; *** indicates P<0.001. Key to variable names: Spec R = species richness; Native % = percent of species with native status; Graze Cond = grazing disturbance impacts level; Geom Cond = geomorphic microhabitat condition level; WQ Cond = water quality impacts condition; AQ Cond = aquifer impacts condition; Flow Cond = flow naturalness impacts condition; Lands Cond = lands impact condition; Hab Cond = habitat impacts condition; Fence Cond = fence impacts condition. See SEAP document for specific definitions of categories and condition levels.

4.8 Grazing Effects Analysis

Grazing effects were examined by adding SEAP scores for grazing impacts to best models for species richness and percentage of native species. Grazing effects did not significantly improve the model for species richness based on an F-test (P=0.16, F=1.6). However, analysis of estimated effects of each level of grazing disturbance still provides suggestive insights to management worthy of further study. Only the estimate for "Slight Grazing Impacts" was significantly different for zero (Figure 27). Further, there was no discernible pattern to the effects, such as decreasing richness with increased grazing impacts. However, grazing impacts did significantly improve the model for percent of native species. Based on an F-test comparing the models with and without grazing impact levels the model with grazing impacts included was preferred (P=0.03, F=3.7). Effects of grazing monotonically increased with better grazing condition corresponding to a higher percentage of native species (Figure 28). However, the confidence intervals for all but "no grazing impacts" included no effect. These results are suggestive but more data are needed. Together these results suggest that grazing doesn't have an impact on species richness but it may increase the likelihood of invasion by invasive species. Understanding could be improved by examining relationship of grazing intensity and total cover of exotic versus native species.

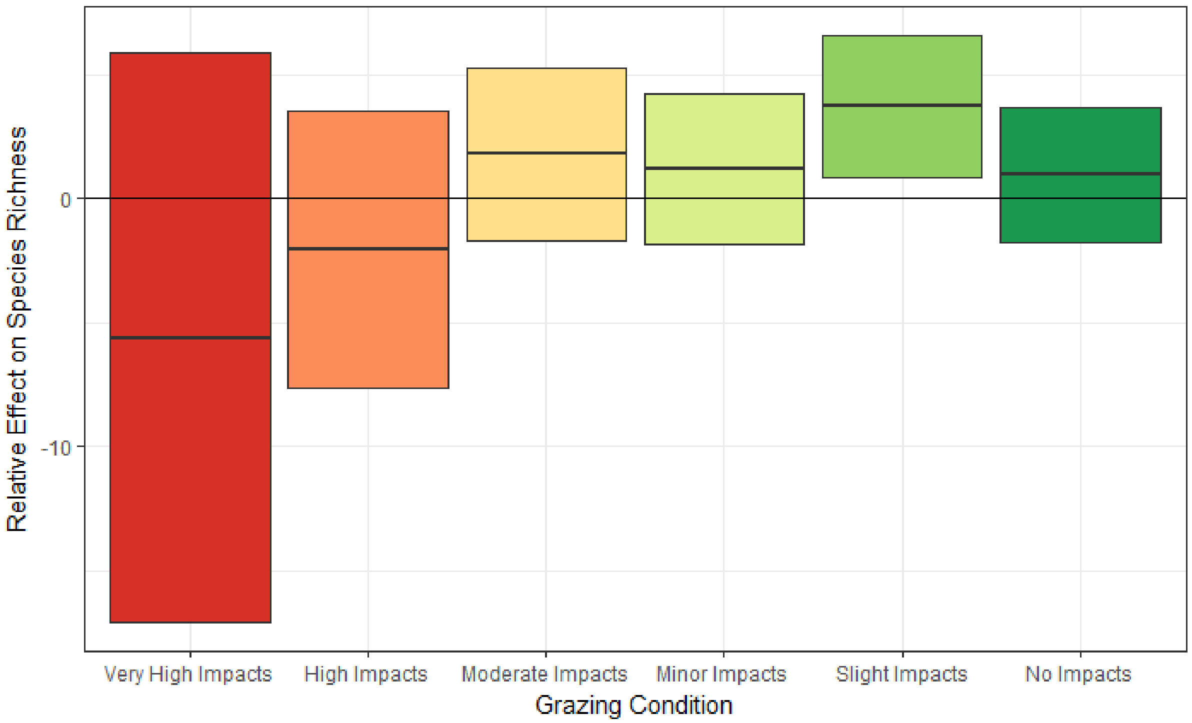
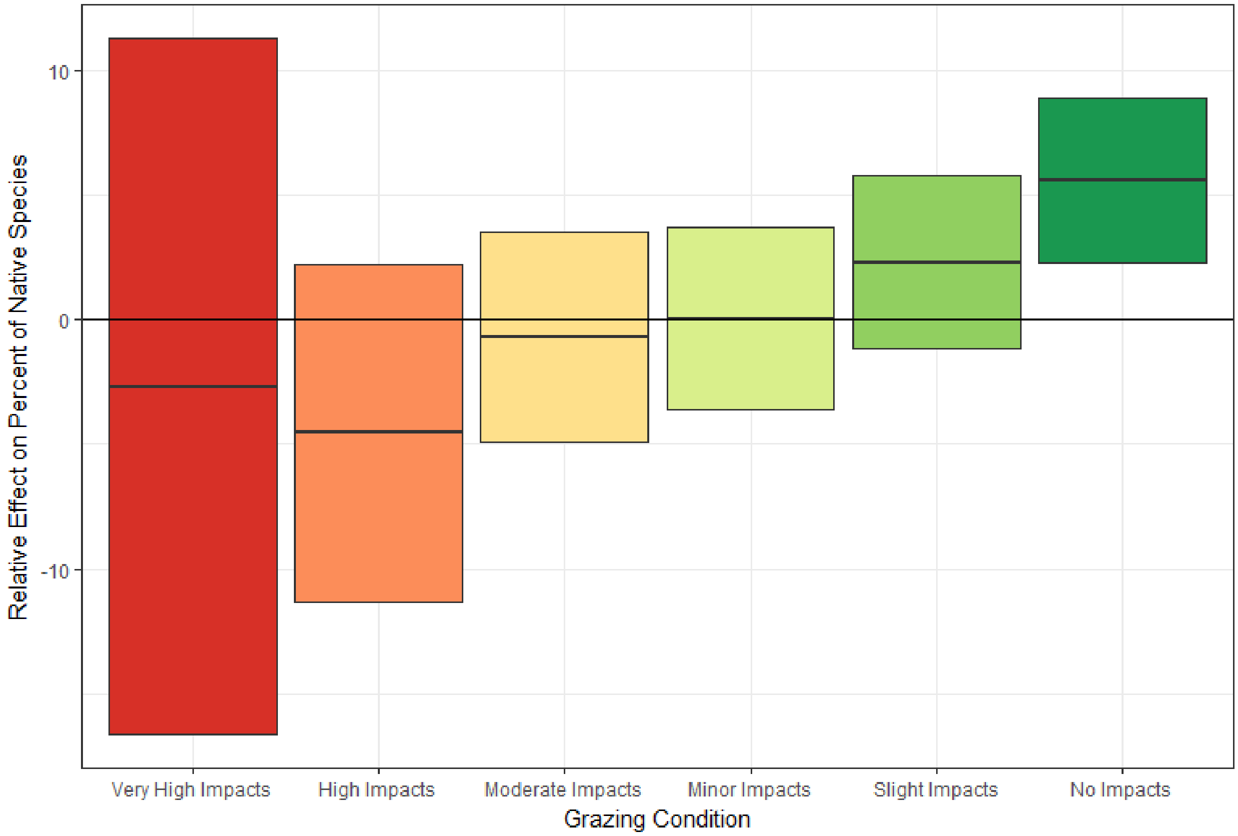


Figure 27. Effects of grazing disturbance level on expected species richness after controlling for other predictors of species richness. Bars show mean and 90% confidence interval of estimate. Model was not significantly improved by the addition of grazing condition and only effect of “slight impacts” was significantly different from zero.

Figure 28. Grazing impacts on percentage of native species after accounting for other important



predictors. Bars indicate mean effect estimate and 90% confidence interval. While most individual effects weren't significant, together they significantly improved the model.

4.9 Hillslope Springs Reclassification

Subclassification of hillslope springs by unforested and forested identified clear differences between each subclass of hillslope springs. Hillslopes springs were split relatively even into each group with 90 springs identified as unforested and 67 springs as forested allowing for statistical differentiation of the subgroups. The forested springs tended to occur at higher elevation (average 2130 meters vs 1815 meters) and had higher species richness (25.0 plant species vs 21.7 species). This suggests that forested springs mostly occur at higher elevations where moisture is more plentiful to support trees. Trees may in turn alter microhabitats with additional shading and by providing litter and woody matter which provide niche space for specific species. These differences are illustrated in Figure 29. However, due to fundamental differences between hillslope springs and other spheres of discharge, including different relationships between elevation and species richness, microhabitat richness and species richness, and different types of microhabitats associated with them (more woody matter and dominantly north facing slopes) reclassification may not be needed. Still, hillslope springs tend to exhibit higher variability that other spheres of discharge. Classification into forested and unforested categories seems to help reduce the variability within the hillslope classification. Skalicky et al. (2017) also suggested differentiating forested and unforested springs and analyze them separately. Classification based on secondary sphere of discharge characteristics or locations relative to stream channels still remains promising, but unexplored.

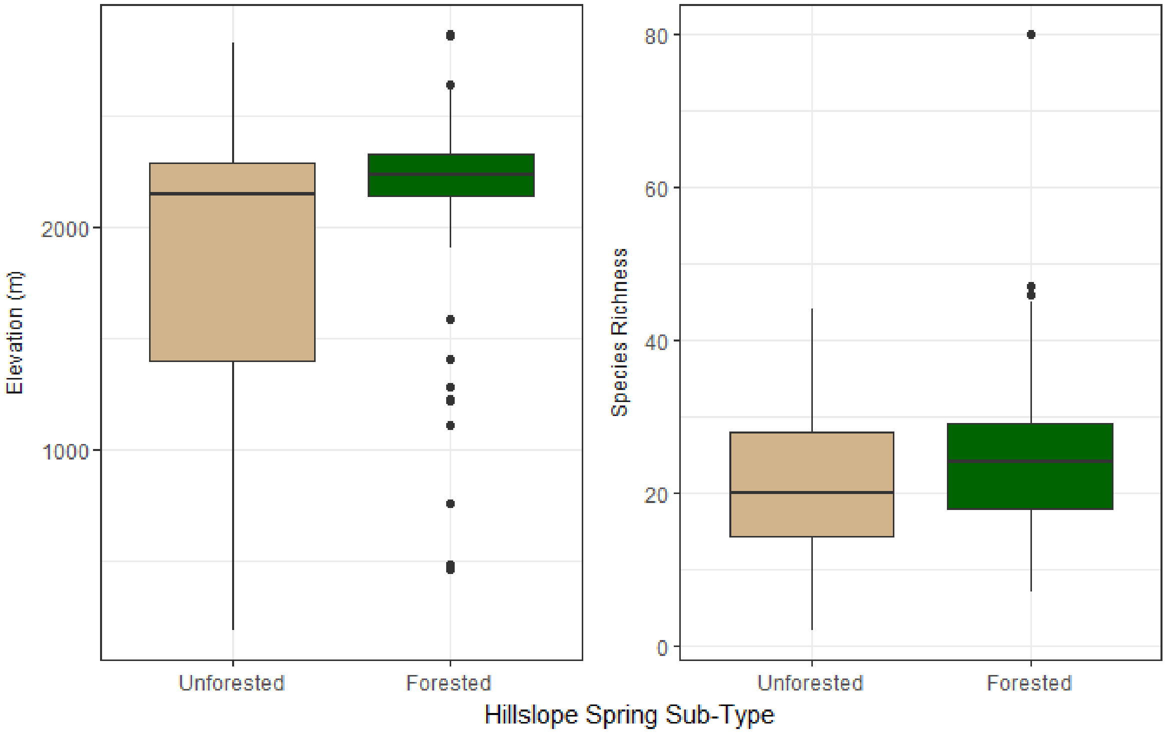


Figure 29. Subclassification of hillslope springs into forested and unforested subtypes show differences in floral species richness and elevation that help resolve the high variation found at hillslope springs and difficulty differentiating them as spheres of discharge.

Chapter 5 – Conclusions

The results of this study highlight the significance of springs ecosystems in the GCE. Springs in the GCE are hotspots of biodiversity and support a large portion of the biota (45%) of the GCE on a small fraction of its land surface. Statistical analyses demonstrate the heterogeneous geomorphology of springs in the GCE as a fundamental source of the high biodiversity and intense species packing at GCE springs. Floral species richness was positively related to geomorphic microhabitat richness across all spring types. Additionally, plant assemblages varied between spheres of discharge. The differences in species richness reflects the geomorphic characteristics associated with each sphere of discharge. Differences in substrate and microhabitat types may be especially important. However, many springs in the GCE show signs of human disturbance with geomorphic microhabitats often degraded or obliterated.

SEAP condition scores were shown to be a simple and useful way to measure disturbance levels at springs. Although SEAP condition scores were not related to floral species richness, low SEAP condition scores were associated with increased occurrence of exotic species. Disturbances such as heavy grazing, flow alterations, and degradation of geomorphic microhabitats may increase the likelihood of invasion by exotic species.

Hanging garden and helocrene springs are particularly unique ecosystems with distinct characteristics related to their plant assemblages. While hillslope and helocrene springs were more variable, they, too, had distinct geomorphic characteristics that differentiated them. All spheres of discharge had sets of indicator species that tended to occur commonly at those springs. These findings emphasize spheres of discharge represent distinct geomorphic spring types that effect the types of species that occur. Continued research on spheres of discharge and use of the terminology will enhance the description and understanding of springs ecosystems.

The study of springs ecosystems highlight their importance as ecosystems. They are highly multivariate which makes statistical analyses difficult. Complex interactions at springs makes straightforward analysis of springs ecosystems difficult. However, despite the inconclusiveness of some analyses, the evidence highlights springs as diverse and critical ecosystems in the GCE. The history and legacy of local physical conditions, relationships to adjacent uplands, as well as differential colonization, extinction, and anthropogenic alteration confer high levels of individuality to springs ecosystems. Every spring has its own unique geomorphology and biogeographic history, an ecosystem individuality that limits synthetic statistical integration.

These analyses can help managers improve and enhance stewardship of springs ecosystems; however, management needs to be considered at local, as well as landscape scales. Inventory knowledge of the array of springs within the management area is important to understanding rare springs types and the likelihood of occurrence of rare springs-dependent species. Inventories also are needed to understand the diversity, distribution, and conservation status of springs-dependent (crenobiontic) species. My results indicate that restoration planning should include consideration of the springs wetted area, protection of fragile microhabitats, and occurrence of rare species. Incorporation of these springs characteristics into restoration activity, coupled with detailed monitoring will provide invaluable experimental insight into springs ecosystems.

Inventory and monitoring efforts should be continued because the majority of GCE springs have not been surveyed and trend data are limited. Additional attention should be focused on information management to ensure that data assembled thus far remain available to the public and the scientific and managerial communities. Because of the observational nature of the study, future research would benefit from experimental design include study effects of direct manipulation of microhabitats or grazing disturbance at a random subset of springs. The critical status of aridland springs, their high biodiversity and remarkably dense species packing, as well as their cultural and economic significance affirm the need for increased scientific attention and improved stewardship for these remarkably unique and threatened ecosystems.

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