University of Nevada, Reno

Ecohydrogeology of Owens Valley, California Spring Systems: Relationships between Geochemistry, Benthic Macroinvertebrates, and Microbial Communities

A dissertation submitted in partial fulfillment of the
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by
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Abstract

Integrating hydrogeology and aquatic ecology has intrigued ecologists and hydrologists. The challenges and importance of integrating these disciplines in groundwater-dependent ecosystems (e.g., springs) have been recognized and studied in mesic regions where these systems are at the headwater of streams. Although ecologists and hydrogeologists have studied aridland springs in terms of taxonomic data, habitat classifications, landscape placement, and sampling techniques, the current state of the science (i.e., integration of hydrogeological parameters and spring ecology) is still in its early stages.

This dissertation provides insight into the association between hydrochemistry, invertebrate ecology, and microbial ecology in aridland springs in the southwestern Great Basin. Water chemistry, benthic macroinvertebrates (BMI), and benthic microbial samples were collected from undisturbed rheocrene (flowing into a channel) springs emerging along the Sierra Nevada Frontal Fault Zone in Owens Valley, California. We also sampled one regional spring on the eastern side of Owens Valley. Samples were collected during the summers of 2016 and 2017. First, we examined the temporal variability of benthic macroinvertebrate (BMI) communities before and after 17-years of hydrologic disturbances (frequent droughts and scouring floods) in 2000 and 2017 (Chapter 2). The results showed that persistent desert springs act as refugial aquatic habitats and protect the benthic communities, especially crenobiontics (obligatory spring dependent), during frequent drought and flood in arid regions. These findings motivated us to study the potential factors in shaping these stable communities. To do so, we compared the spatial distribution of BMI communities with the environmental characteristics of spring waters

(Chapter 3). The results revealed that the geochemical fingerprint of spring water has a prominent role in shaping the structure of BMI communities in undisturbed aridland springs. Finally, we compared the ecological patterns of BMI and microbial communities found in these springs. Multivariate statistical and co-occurrence analyses were applied to both BMI and microbial communities to understand the relationship between these biological organizations (Chapter 4). The results indicated a relatively similar ordination pattern among BMI and microbial communities. We also highlighted the hydrochemistry as the primary driver of characterizing both microbial and BMI communities.

I dedicate this dissertation to my wife, Rose, who has inspired and supported me through this journey

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Chapter 1
Introduction

1.1 Aridland Spring Systems

Springs are aquatic and riparian systems where groundwater discharges at or near the land surface (Meinzer 1923, Springer and Stevens 2009). Aridland springs as biodiversity hotspots provide the majority of reliable water for supporting aquatic organisms in arid and semi-arid regions (Shepard 1993, Ponder 2002, Murphy et al. 2015, Cartwright et al. 2020). They occur in a variety of landscapes, and each has its own unique geochemistry, size, degree of isolation, and biota (Stevens and Meretsky, 2008). Relatively stable physicochemical conditions of persistent aridland springs (because of continuous groundwater recharges into spring) provide a refugial habitat for aquatic organisms to overcome hydrological disturbances during previous climate changes (Murphy et al. 2015, Cartwright et al. 2020).

Aridland springs in the Great Basin are critical ecological features supporting a variety of aquatic organisms, as well as the highest number of endemic taxa in North America over both climatic and tectonic timescales (Shepard 1993, Abell et al. 2000, Springer et al. 2008, Stevens and Meretsky 2008, Keleher and Sada 2012). The Great Basin as one of the most arid regions in the Northern Hemisphere, encompasses a series of elongate, north-south oriented endorheic basins that have been isolated from adjacent drainages since the late Tertiary (Phillips 2008, Grayson 2011). Since the late Miocene/early Pliocene, tectonic development has been a major driver of aquatic habitats isolation and therefore aquatic organism speciation in the southern Great Basin (Echelle 2008; Hershler and Liu 2008; Phillips 2008). In addition to tectonic development, glacial-interglacial climate cycles played a critical role in fragmenting aquatic habitats over shorter timescales (Phillips 2008). As the climate dried and the fluvial connection between basins ceased, isolated springs became vital remnant aquatic habitats in the region (Keleher and Sada, 2012).

To date, most ecological studies on aridland springs have focused on identifying new species (Hershler and Sada 1987, Hershler 1989, Soucek et al. 2015), the biogeography of fishes and macroinvertebrates (Hershler and Sada 2002, Polhemus and Polhemus 2002, Smith et al. 2002, Hershler and Liu 2008), identifying endangered species (Minckley and Deacon 1968, Williams et al. 1985, Myler et al. 2007), the physiology of fishes living in harsh environments (e.g., Feldmeth et al. 1974, Shrode and Gerking 1977), autecology of an individual taxon (Sada 2001, 2008), and genetic divergence of species because of their dispersal ability (Thomas et al. 1997, Myers et al. 2001). However, studies on the microbial ecology and the spatial distribution of benthic macroinvertebrate (BMI) communities in aridland springs have received comparatively less attention. This knowledge gap is addressed in this dissertation.

1.2 Biogeographic Patterns:

Ancestral affinities of endemic aquatic species, as well as relationships between persistent aquatic habitats, ancient climates, and landscape evolution in the Great Basin, have been active research topics for many years. For example, several studies have focused on biogeography patterns of fishes, springsnails, and amphipods and have integrated geology and divergence time of aquatic organisms to examine paleo-dispersal avenues

(Blackwelder 1948, Hubbs and Miller 1948, Hershler et al. 2002, Reheis et al. 2008, Witt et al. 2008). Hubbs and Miller (1948) were the first to integrate climate, habitat isolation, and ancient interbasin connectivity to investigate the relationship between geological evidence and fish lineage in the Great Basin. Their work was followed by many other studies, which clarified these relationships for the northern Great Basin (e.g., Johnson 2002, Unmack et al. 2014); however, these relationships are only weakly known for the southern Great Basin (summarized by articles in Reheis et al. (2008)). These relationships in the southern Great Basin remain an enigma because of the lack of geological evidence to support the genetic evidence about interbasin connectivity between northeastern Mexico and the Death Valley system of the southern Great Basin (Echelle 2008, Hershler and Liu 2008).

In the southern Great Basin, spring system isolation is mainly associated with tectonic development (history of geological events) and climate change (glacial/interglacial periods). Tectonic development gradually alters groundwater flowpaths and the structure of subsurface fault zones over a long period of time. However, the impact of tectonic activities has been minimal compared to glacial/interglacial cycles in a short time scale. In this area, paleo-drainage systems supported various perennial aquatic ecosystems that were connected to adjacent basins (e.g., Lake Manly) during the ancient pluvial period (Phillips 2008). At that time, the ancestors of aquatic organisms (i.e., springsnails and pupfishes) entered the southern Great Basin from the adjacent basins and persisted in isolated springs as paleo-streams dried (Hubbs and Miller 1948, Hershler and Sada 2002, Hershler and Liu 2008).

After isolation of aquatic habitats in the southern Great Basin, endemic crenobiontic taxa were differentiated genetically from their ancestors. Differentiation can only occur in persistent springs with a relatively stable environment that can be tolerated by each crenobiontic taxon. Crenobiontic species with limited vagility (only have a submerged dispersal ability and cannot travel between springs) have been restricted to stable and permanent physicochemical conditions that were minimally disturbed by natural or human events (Taylor 1985). Southern Great Basin crenobiontic populations have undoubtedly varied (maybe widely) over time, but they have been resilient and persisted long enough to allow speciation to occur. Therefore, the presence of endemic crenobiontic taxa in a spring system indicates that the spring has been persistent over millennia.

Although these studies highlight the importance of history on biogeographic patterns of aquatic organisms in the southern Great Basin, contemporary processes (such as physicochemical conditions, degree of disturbances, and passive dispersal) can also play an essential role in shaping the spatial distribution of BMI communities in aridland springs. For example, the relationship between the hydrographic history of the region and patterns of molecular diversification was not observed among the *Hyalella* lineages (Witt et al. 2008). Polhemus and Polhemus (2002) indicated that most *Hyalella* taxa could not tolerate cold environment and extirpate from cooler habitats. In addition, some researchers showed the role of passive dispersal (i.e., avian transport) in the distribution of springsnails among spring systems (Liu et al. 2003, Hershler and Liu 2008). The role of physicochemical characteristics of spring waters on shaping the spatial distribution of BMI communities is examined in chapters 3.

1.3 Impact of Disturbance (Climate Change and Human activity) on shaping the BMI communities

Most BMIs have adapted to the physicochemical characteristics of their habitat and cannot survive in springs that dried periodically or have experienced some extreme disturbances. Each BMI taxon tolerates a particular range of environmental conditions and this has been characterized by Mandaville (2002), who assigned a value indicating their ability to tolerate harsh environments (range between 0 and 10). Intolerant species have a low tolerance value (TV<3), and tolerant species have a high tolerance value (TV>7). Most springs in the Great Basin have been influenced by anthropogenic disturbances (groundwater pumping, livestock use, and spring modification) (Williams et al. 1985, Shepard 1993, Sada and Vinyard 2002, Unmack and Minckley 2008). In surveys of more than 2500 Great Basin springs, Sada and Lutz (2016) observed that historical human activities had altered most springs in the Great Basin and the Mojave Desert. In some cases, these disturbances have an irreversible impact on spring systems by altering the functional and structural characteristics of biotic communities and/or eliminating endemic taxa (Sada and Nachlinger 1996, Sada and Vinyard 2002, Sada et al. 2005, Keleher and Rader 2008). For example, Sada and Nachlinger (1996) showed decreases in total richness and increases in the abundance of tolerant taxa in disturbed springs in the Spring Mountains (southwestern Great Basin). Another study focused on eastern Great Basin springs showed a distinction between the BMI community composition of undisturbed and severely disturbed springs (Keleher and Rader 2008). Because of the impact of past disturbance on the structure of BMI communities (the degree of impact depends on frequency, duration, and magnitude of disturbance), only reference springs (undisturbed or naturalized) were sampled for the ecological analysis in this dissertation.

1.4 Study Area

The southern Great Basin is comprised of diverse topography from high elevations in the Sierra Nevada and the Spring Mountains to valley floors. Valley floor elevations range widely from below sea level in Death Valley to more than 2,100 meters above sea level (masl) in the northern Owens basin. The White Mountains, the Spring Mountains, and the Sierra Nevada are the main mountain ranges in the study area with a maximum elevation of 3,632 masl. Studies of paleo-geology for the northwestern portion of the southern Great Basin indicate that the southern Great Basin extension resulted in a creation of a series of north-south oriented ranges with axial fluvial drainage systems (Phillips 2008, Henry et al. 2012). Scattered spring systems and two rivers (the Owens River and the Amargosa River) are the only permanent surface water sources in the area.

The southern Great Basin was selected for this project for several reasons: (1) Several team members had conducted long-term research focused on the geology, hydrology, and ecology of spring systems in the area; (2) it was the focus of the Reheis et al. (2008) volume, which resulted from a major symposium examining evidence of this area's geology and aquatic biogeography; (3) the area has a diverse topography, with different types of aquifer provinces; and (4) Dr. Donald Sada had collected BMI data from more than a thousand springs throughout the study area and has provided primary information for these springs such as location coordinates and spring health conditions.

In total, 85 springs were sampled for isotope hydrology and geochemistry analysis. Approximately 40 reference (undisturbed and naturalized) springs were selected for ecological analysis (Figure 1.1). We sampled sixteen springs along the Owens Valley, eight springs in Death Valley and Ash Meadows, five springs in the Spring Mountains, ten springs in the Panamint Range, and one spring in the White Mountains. The different sets of springs in the study area were selected to cover a wide range of topography, hydrogeological units, geochemistry, residence time, and BMI taxa. All are permanent springs with relatively stable conditions. Most of the springs flow into a channel at their emergence point (Rheocrene), and several springs, especially in Ash Meadow, emerge in large pools before flowing into channels (Limnocrene). Since the primary focus of my research was on answering the underlying questions of each chapter in a valley scale, I only analyzed the Owens Valley springs (all sampled springs in Owens Valley are Rheocrene) in this dissertation (Figure1.1- highlighted area)

1.5 Organization of the Dissertation

This dissertation is part of the National Science Foundation (NSF) Integrated Earth Systems (IES) project. The IES project was designed to improve the understanding of linkages between regional geology, groundwater hydrology, and biotic systems in isolated southern Great Basin aquatic systems. Although the main focus of the IES project is on the spring systems in the southern Great Basin, the ultimate results and findings can be used for understanding aridland springs on a global scale.

The main objectives of this dissertation are (1) to evaluate the temporal stability of BMI communities over 17 years to understand the role of spring systems as refugial habitats (Chapter 2), and (2) to quantify the geochemistry of the spring waters to gain insight into its influence on the ecology of isolated aquatic habitats including the structure of BMI communities, microbial communities, and presence of crenobiontic organisms (Chapters 3 and 4).

These objectives were investigated by collecting water chemistry, BMI, and microbial samples in undisturbed permanent spring systems. The relationship between the collected data was examined using a variety of multivariate and basic statistical methods (i.e., hierarchal cluster analysis, nonmetric multidimensional scaling, and co-occurrence methods, Kruskal-Wallis test.). Each chapter has an independent story seeking to answer an underlying question(s), which builds a foundation for the next chapter.

1.6 Figures

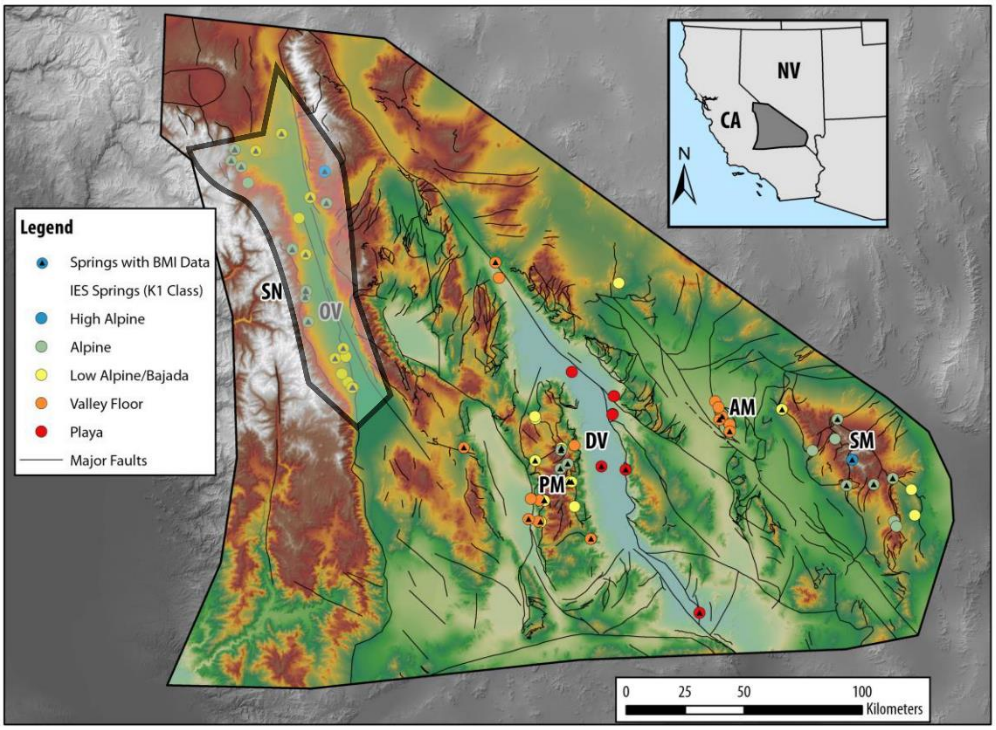

Figure 1.1. Map of the study area. All sampled springs (circles) color-coded by their landscape position classification. Undisturbed springs with associated BMI data are indicated by the black triangles within the circles. Three chapters of this dissertation are based on springs in Owens Valley (Highlighted area). This figure is adapted from (Meyers 2020).

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Chapter 2

The Temporal Stability of Benthic Macroinvertebrate Communities in Persistent Springs before and after hydroclimatic extremes over 17 Years in Owens Valley, California

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2.1 Abstract

Aridland springs are isolated water sources that often provide crucial habitats for wildlife and harbor unique aquatic life forms in dry regions. In this study, we compared the temporal variation of benthic macroinvertebrate (BMI) communities in 12 undisturbed springs located in the southwestern Great Basin in 2000 and 2017. Within these 17 years, three prolonged droughts occurred, followed by extreme wet years in between. The longest drought occurred between 2012 and 2016, which was distinguished as the driest period on record. Several studies have shown the effect of human disturbance on BMI communities in aridland springs, but the ecological consequences of hydroclimatic extremes on BMI communities in spring ecosystems are largely unknown. Environmental metrics and BMI samples were collected near the source of undisturbed springs in Owens Valley, California, during the summers of 1999 and 2000, and were compared with similar samples collected in 2017. The structure of BMI communities near the source of each spring was relatively stable (more than 85 percent similarity for each spring) before and after several hydroclimatic periods (hydrologic disturbances) over 17 years. Moreover, the presence and the relative abundance of crenobiontic (obligatory spring dependent) taxa were similar in both sampling events. These findings suggest that undisturbed aridland springs act as refugial aquatic habitats and preserve the benthic communities, especially non-insect crenobiontics, during frequent droughts and floods in arid regions.

2.2 Introduction

Aridland springs in the Great Basin and the Mojave Desert are biodiversity hotspots that support a variety of aquatic communities, as well as the highest number of endemic taxa in North America (Shepard 1993, Abell et al. 2000, Springer et al. 2008, Stevens and Meretsky 2008, Keleher and Sada 2012). Although the regional hydrology of these systems has been well studied (e.g., Kreamer and Springer 2008, Springer et al. 2008, Hershey et al. 2010), little is known about their ecology. The ecological studies that have been conducted focused on the biogeography of fishes and springsnails (e.g., Hershler and Sada 2002, Smith et al. 2002, Hershler and Liu 2008), and the physiology of fishes living in harsh environments (e.g., Feldmeth et al. 1974, Shrode and Gerking 1977). The extinctions and extirpations of crenobiontic fishes and springsnails in North America's desert springs over the past several decades, as well as efforts to support the conservation of these endangered organisms, have increased the interest in studying spring ecosystems in arid regions (Williams 1985, Sada and Vinyard 2002, Stevens and Meretsky 2008, Unmack and Minckley 2008, Williams and Sada *in press*). This contrasts with mesic regions where there is a long history of interest in groundwater and springs because of their importance as water sources for humans (see La Moreaux and Tanner 2001). Our understanding of spring ecology mostly comes from mesic regions where springs are typically in headwater streams that are connected to lotic systems (e.g., Danks and Williams 1991, Ferrington 1995, Botosaneau 1998).

In the few ecological studies of freshwater springs in North America, geological and evolutionary history, dispersal limitations, or physicochemical characteristics of spring habitats were highlighted as the main factors controlling the spatial distribution of benthic macroinvertebrate (BMI) communities (Glazier 1991, Williams and Williams 1998, Rader et al. 2012, Stanislawczyk et al. 2018, Sada and Thomas *in press*). Some emphasized on contemporary processes such as temperature, electrical conductance (EC), and alkalinity as the most influential factors in structuring BMI communities (Glazier 1991, Myers and Resh 2002, Sada and Thomas, *in press*). Alternatively,(Keleher and Rader 2008a) and Rader et al. (2012) found that metaphyton and BMI communities of springs in western Utah (eastern Great Basin) were attributed to dispersal limitations. Similarly, Stanislawczyk et al. (2018) showed that despite the physicochemical variations among desert springs in the southern Chihuahuan Desert, geographical distance controls the BMI community composition.

Much of our knowledge about the impact of extreme hydroclimatic events on temporal variation of BMI communities is based on studies in stream systems (Resh et al. 1988, Stanley and Fisher 1992, Boulton 2003, Lake 2003, Fritz and Dodds 2004, Wood and Armitage 2004, Bogan et al. 2015, Herbst et al. 2019). In these studies, drought and high precipitation scouring the stream channel were highlighted as the influential factors altering the BMI communities in running waters. One study on mid-elevation mountain streams in the Sierra Nevada, California, showed that droughts have a much larger impact on the structure of BMI communities than wet year floods (Herbst et al. 2019). The response of BMIs to extreme events is characterized as the ability of BMI taxa to resist disturbances (resistance) and/or their capacity to recolonize the habitat after disturbances (resilience) (Stanley et al. 1994). Previous studies showed high invertebrate community resilience to flood events (Lake 2011, Herbst et al. 2019). However, low resistance and resilience were observed for BMI communities in headwater streams to frequent, prolonged droughts (Boulton 2003, Lake 2011, Bogan et al. 2015).

Most springs are classified as “steady-state” systems (Hynes 1970), which implies that they are exposed to less temporal variations in discharge, temperature, and water chemistry than stream systems (Mitsch and Gosselink 2007). However, “steady-state” may not apply to many aridland springs because some of these springs dry periodically (seasonally, annually, or unpredictably), are scoured by periodic floods, and/or affected by fires. Although several summaries about spring ecology have been published (Ferrington 1995, Botosaneau 1998, Scarsbrook et al. 2007), there have been few studies on the impact of extreme hydrologic events on BMI communities in persistent (“steady-state”) aridland springs. A long-term study on cold springs in the Sierra Nevada reported that springs with minimal fluctuation in discharge and temperature have higher species richness (Erman 2002). Similarly, Stanislawczyk et al. (2018) showed little changes in the seasonal variation of BMI communities among springs in the southern Chihuahuan Desert. They proposed that seasonal stability in desert springs is most likely associated with non-insect taxa. Therefore, the temporal stability of spring environments can be predicted by the stability of their BMI taxa composition and richness (Erman and Erman 1995).

The first step in examining the temporal variation of BMI communities in aquatic ecosystems is understanding the history of disturbances in the area (Lake 2003). The historical natural and human disturbances that influenced Owens Valley springs were mainly droughts, scouring floods, and groundwater pumping. The hydrology and ecology of Owens Valley were significantly affected by the Los Angeles Aqueduct (LAA), which was constructed in 1913 to transfer water from Owens Valley to Los Angles. Diversion of the Owens River for the aqueduct created a noticeable stressor for surface water and groundwater of the area, which resulted in the drying of Owens Lake by 1924 (the City of Los Angeles, Department of Water and Power and County of Inyo, 1990). Despite the ecological and hydrological effects of the LAA on Owens Valley, the Los Angeles Department of Water and Power (LADWP) constructed the second LAA in 1970. They increased groundwater pumping from Owens Valley to provide water for the aqueduct (the City of Los Angeles, Department of Water and Power and County of Inyo, 1990). Between 1970 and 1990, numerous groundwater management strategies, scientific studies, and legal battles were conducted to evaluate the impacts of the second LAA on the ecology and hydrology of the region. The final Environmental Impact Report (EIR) was published in 1991, which is known as "the 91 EIR." In our study, none of the springs are known to have been affected by past groundwater use, none are near existing well fields, and only one (IES26: Reinhackle Spring) is on the valley floor and is affected by the LAA. The 91 EIR determined that groundwater pumping was reducing the Reinhackle Spring discharge, so they managed the pumping from certain wells in the area to eliminate the effect of pumping on this spring. For this study, we focused on reference springs (undisturbed and/or naturalized from past disturbance) to eliminate the effect of anthropogenic disturbances and examined the prominent hydrologic disturbances (drought or scouring floods) occurring at selected spring systems.

We compared the BMI communities and physicochemical characteristics of persistent springs that were sampled in 1999/2000 (hereafter referred to as 2000) and again in 2017. A comparison between BMI communities was used to assess their stability or temporal variability in select spring systems over a period that spans ten years of severe droughts and five years of very high winter precipitations. Moreover, we determined the most influential physicochemical variables influencing BMI community compositions in aridland springs. The results of this study provide insights into the role of springs as refugial habitats, which allow BMIs to cope with hydrologic disturbances (droughts and scouring floods) in arid regions.

2.3 Study Area

2.3.1 Area Description

The Great Basin and Mojave Deserts encompass approximately 560,000 km^2 of the United States and include the nation's hottest regions (Pavlik 2008, Grayson 2011). The study area is located in Owens Valley, California (Southwestern Great Basin; Figure 1). It is bounded on the east by the Inyo-White Mountains, on the west by the Sierra Nevada, and on the north by transverse ranges that separate it from the Walker River Basin. To the south, water from the valley flowed into Death Valley during pluvial periods (Knott et al. 2008, Phillips 2008). Its climate is temperate with alternating wet and dry seasons. The average high temperature is 34.8 °C in the valley during summer (*https://wrcc.dri.edu/summary/Climsmcca.html*). Annual snowfall is more than 400 cm in the Sierra Nevada, which is the primary recharge zone for the sampled springs (*Lake Sabrina, CA; Western Regional Climate Center, elevation: 2,800 m above sea level [masl])*, and less than 15 cm in the valley (*Bishop Airport, CA; NOAA Station; elevation: 240 masl).*

2.3.2 Endemic Taxa

Mountain streams and lakes, the Owens River, and isolated springs create a diverse array of aquatic habitats in Owens Valley. Four native fishes (*Cyprinodon radiosus*, *Catostotomus fumeiventris*, *Siphateles bicolor snyderi*, and *Rhinichthys osculus* spp.) found in Owens Valley springs are fluvial taxa and endemic to the valley (Smith et al. 2002), and some of the gastropods (*Pyrgulopsis owensensis*, *P. perturbata*, and *P. wongi*) are either locally or regionally endemic (Hershler 1989). Three fishes colonized Owens Valley from the Walker River Basin during the Pleistocene, and *C. macularius* of the lower Colorado River is the closest ancestor to *C. radiosus*. Mitochondrial DNA studies indicate that these two lineages diverged from one another during the late Miocene/early Pliocene (Smith et al. 2002, Echelle 2008). *Pyrgulopsis* snails are crenobiontic (obligatory spring dependent) taxa, ancestral forms of *P. owensensis* and *P. perturbata* occupy coastal habitats in west-central California, and ancestral forms of *P. wongi* are in the northern Great Basin (Hershler and Liu 2008). It is not known whether these taxa arrived via passive or active transport, but *P. wongi* is believed to have diverged from its ancestral form 0.52 ± 0.12 (minimum) mya, and the other species diverged from their ancestors an estimated 2.76 ± 0.51 (maximum) mya (Hershler and Liu 2008). Since the late Tertiary, tectonic development and glacial/interglacial periods created hydrologic barriers, dried paleodrainages, and caused Owens Valley to become endorheic (Phillips 2008).

2.3.3 Hydrologic Condition

High seasonal and annual variations in temperature and precipitation, as well as groundwater pumping in the area, have been the main disturbances influencing Owens Valley from 2000 to 2017. High degrees of drought classifications (moderate drought or severer) occurred in the study area for more than two-thirds of the years of the period (https://droughtmonitor.unl.edu/Data/Timeseries.aspx; Figure 2-A). The monthly precipitation was highly variable seasonally and annually over the last two decades at the NOAA weather station at Bishop airport (Figure 2-B). The winter precipitation was also extremely high for five years of the study period. There was a wide variation in temperature between 2000 and 2017, which was < 2 SE for five of these years and > 2 SE for four of these years (http://www.climate.gov; https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca0822). The Bishop Creek discharge gauge (USGS: https://waterdata.usgs.gov/nwis, site # 10270872) near Lake Sabrina at the highest elevation of the Bishop Creek middle fork (2760 m above NGVD29) also recorded below-average discharge for most of the months of the study (Figure 2-C). Although the creek discharge was near and below the 30-year monthly average between the 2007-2008 and 2012-2016 water years, it experienced very high discharges (greater than 2.5 m^3/s) during wet years. The discharge of the only gauged spring (IES26: Reinhackle Spring) in the study area followed the hydrologic patterns by reaching the highest discharge between 2005 and 2007 and the lowest discharge (less than 0.04 m^3/s) between 2013 and 2015 (Figure 2-D). These hydrologic conditions pinpoint frequent, prolonged drought as the primary hydroclimatic disturbance in the area between 2000 and 2017.

2.3.4 Site Selection

Sada and Herbst (2001) sampled environmental parameters, and BMIs in 105 Owens Valley springs along the mountain-front fault zones, on bajadas, and the valley floor. They recorded that only 37 percent of the springs were in reference condition (undisturbed or naturalized from past disturbances). Twelve of these reference springs were rheocrene (groundwater emerges into a channel) and sampled again in 2017 to determine the temporal variability in their environments and the structure of their BMI communities (Table 1). Eleven springs are along the Sierra Nevada mountain front in Owens Valley, and one is a regional spring on the eastern side of the valley (IES31: Warm Spring A) (Figure 1). Only reference springs were selected for this study to minimize the effects of human disturbance on environments and BMI communities, as described by Keleher and Rader (2008b) and summarized by Keleher and Sada (2012). Keleher and Rader (2008b) showed that BMI community composition was distinct between undisturbed and severely disturbed sites in the eastern Great Basin. There was evidence of past disturbances (e.g., diversion, livestock use, and channelization) at many of sampled springs. However, recent disturbances were not evident during the 2000 and 2017 sampling events, which indicates that these springs have recovered from any past disturbances. We categorized the sampled springs as reference springs based on the spring inventory and monitoring protocols (Sada and Pohlmann. 2006, Stevens et al. 2016).

2.4 Materials and Methods

2.4.1 Spring Environments

The environmental characteristics of each spring (near the source) were measured when BMIs were collected. Environmental metrics were calculated as the mean of five water velocity, water depth, and wetted width measurements. Environmental variables also included several estimated metrics such as substrate composition, percent of bank vegetation cover, emergent vegetation, and overhead shade at each sampling area. Water depth and current velocity were measured in the center of each 120 cm^2 quadrats, which were used for BMI sampling. The current velocity was measured in cm/sec using a Marsh-McBirney Model 2000 current meter. Wetted width was measured as the mean of five equally spaced measurements across each transect. Wentworth (1922) grain size classification was used for the estimation of substrate composition. Electrical conductance and temperature were measured at spring emergence using a YSI Model 30 meter. Springbrook length was estimated in meters.

2.4.2 Benthic Macroinvertebrates

All samples were collected during the summer, but other sample methods differed between 2000 and 2017. In 2000, BMIs were collected from all habitat types (e.g., riffle, backwater, and pool.) within the upper 25 m of each springbrook using a 250µm D-frame mesh net. All BMIs were identified and enumerated for each spring (Sada and Herbst 2001). During 2017, BMIs were collected in 120 cm^2 quadrats that were placed along five equally spaced (3 m apart) transects (that were perpendicular to the thalweg and spanned the wetted width) within the upper 15 m of each springbrook. For these samples, collections were made using a modified surber sampler that was fitted with a 500µm mesh net. During both sampling periods, collections were made by gently rubbing the substrate to detach BMIs and allow them to be captured in the nets immediately below the sample area. The BMIs were preserved in 90 percent ethyl alcohol and returned to the lab for sorting, identification, and enumeration. All 2000 samples were processed and identified by D.B. Herbst, and the 2017 samples were processed and identified by Rhithron Analytical Laboratory, Missoula, Montana. The structure of the BMI communities in 2000 was determined by identifying and enumerating all organisms collected in each sample, and the structure of the BMI communities in 2017 was determined by identifying and enumerating 300 randomly selected BMIs. Spring snails were identified to species; other aquatic insects and mites to genus; nemata (phylum), ostracodes (class), oligochaetes (class), and tricladida (family) were identified no further. The structure of the BMI communities was determined by identifying and enumerating a minimum of 300 randomly selected individuals, which Vinson and Hawkins (1996) showed was sufficiently large to document the BMI community structure.

2.4.3 Data Analysis

We compared environmental parameters (i.e., water depth, wetted width, current velocity, water temperature, and EC) and the structure of BMI communities within each sample between 2000 and 2017 collections using basic statistics (non-parametric Kruskal-Wallis test). All invertebrate taxa in springs were used for the comparison and calculating the community parameters. Proportionate values were arcsine transformed before analyses.

The multivariate data matrix consisted of the relative abundance of non-rare invertebrate taxa (occurring in more than 10 percent of all samples) within all springs in two sampling events. The impact of rare taxa in the multivariate analysis should be minimized because they occur infrequently and do not provide significant information about the structure of communities (Peck 2010). Canonical correspondence analysis (CCA), hierarchical cluster analysis (HCA), and weighted Spearman’s rank correlation were used to examine the temporal variation between BMI samples collected in 2000 and 2017. Prior to each multivariate analysis, BMI data were subjected to a fourth-root transformation to reduce the effect of the dominant taxa in Bray-Curtis similarities between samples. Moreover, each sample was normalized by its total BMI abundance to eliminate the sensitivity of the analysis to sample size (McMurdie and Holmes 2014, Oksanen et al. 2015).

Hierarchical cluster analysis assigns springs to different clusters based on the similarity of their BMI communities in 2000 and 2017 (Peck 2010). In HCA, each sample is considered an individual cluster, and similar clusters are combined into groups using the group average linkage method. This linkage method is independent of sample size and compatible with Bray-Curtis distance values. Furthermore, CCA was used to examine relationships between physicochemical environments and BMI communities. A CCA is generally used to complement HCA to more thoroughly identify the environmental characteristics of habitats that are most influential in structuring BMI communities. Primer® version 6.0 was used to calculate HCA, and the weighted Spearman's rank correlation (Clarke and Gorley 2006), CANOCO version 4.5 was used to calculate CCA (ter Braak and Verdonschot 1995).

2.5 Results

2.5.1 Spring Environments

The temporal variation between mean values for several physicochemical metrics varied widely between 2000 and 2017, but non-parametric Kruskal-Wallis indicated that the temporal variability of the samples ($n = 12$) was not significant for any metric ($p > 0.05$, non-parametric Kruskal-Wallis test, Table 2). This suggests that environmental conditions were relatively similar in 2000 and 2017. Although there was no consistent increase or decrease among metrics to indicate that discharge differed between the sample dates, decreases were observed in wetted width and water depth. Mean water velocity and EC were higher in 2017, but the water temperature was lower (Table 2).

A CCA examining pooled data from both years and the manual forward selection was used to test each environmental metric (i.e., elevation, EC, temperature, depth, wetted width, current velocity [CV], bank cover, head cover, and substrate size). Only EC (p = 0.002), CV (p = 0.044), and temperature (p = 0.004) were found to be the most significant environmental variables influencing the structure of the BMI communities (Figure 3).

2.5.2 Benthic Macroinvertebrates

The total number of identified and enumerated BMIs was much greater in 2000 than in 2017 (8,839 versus 2,655) (Table 3-A). This was because of the identification and enumeration of all BMIs in the year 2000 collections and only 300 BMIs in each spring in 2017. Even with the difference in sample methods and processing, differences between BMI metrics calculated for each year were minimal (Table 3). Total richness (75 taxa), the mean number of taxa per sample (~14 taxa), and percent dominant taxa (~ 55 percent) were relatively similar in both sampling events (Table 3-A). Non-parametric Kruskal-Wallis test also showed that the differences of diversity indices (Evenness, Shannon-Wiener, and Simpson) between the two years, paired by sites (n=12), were not significant (p >0.05, Table 3-B). All these results suggest the similarity of the BMI communities in the two sampling years.

Table 4 represents non-rare taxa found in more than one spring in either 2000 or 2017. Non-insect taxa—*Pyrgulopsis*, *Hyalella*, and *Pisidium*—were present at the same springs with relatively high abundances in 2000 and 2017; the stability of the macroinvertebrate communities in the sampled springs over time can be associated with these taxa. Intolerant insect taxa such as Ephemeroptera (*Baetis* and *Ironodes*), Plecoptera (*Isoperla*), and Trichoptera (*Ochrotrichia*, *Lepidostoma*, and *Gumaga*) were found in fewer springs in 2017. Alternatively, tolerant insect taxa such as Hemiptera (*Argia*), Diptera (*Tipula* and *Brilla*), and Coleoptera (*Optioservus*) were found in more springs in 2017 than in 2000. Although these findings may suggest a potential shift in the communities from intolerant taxa to tolerant taxa, the related Hilsenhoff Biotic Index (HBI; Hilsenhoff 1987) metrics of BMI communities show that the percentages of tolerant (20 percent) and intolerant taxa (20 percent) were similar in 2000 and 2017 (Table 3-C). Moreover, the overall community tolerance value was around 4.5 (scales from 0 [intolerant] to10 [tolerant]) for both sampling years.

Gastropoda, Trichoptera, Diptera, and Amphipoda were the most common BMI groups in Owens Valley springs during both years. Non-insects (gastropods and amphipods) constituted approximately 50 percent of the pooled communities in 2000 and 2017 samples (Figure 4). Crenobiontic gastropods (*Pyrgulopsis wongi* and *Pyrgulopsis owensensis* [family: Hydrobiidae]) were present in most (8 out of 12) of the springs from both sampling events. Common insect orders (Trichoptera, Diptera, and Coleoptera) collectively totaled approximately 20 percent and 30 percent of the community composition in 2000 and 2017, respectively (Figure 4).

The basic non-parametric statistical test (one-tailed Kruskal-Wallis test) failed to reject the null hypothesis that the BMI communities are similar in 2000 and 2017 (Kruskal-Wallis test, P= 0.25). This means that there is no statistically significant difference between BMI communities in these two sampling events. The mean weighted Spearman's rank correlation comparing the 2000 and 2017 BMI communities in each spring was > 0.83, indicating more than 83 percent similarity between BMI communities across years. Results of the HCA graphically shows that communities were categorized into two distinct clusters and that most springs were clustered close to one another in both sampling years (Figure 5). Three springs differed from this pattern: (1) Warm Springs A (IES31) communities were similar during both years, but they did not cluster with other springs during either year; (2) BMI communities in Red Mountain Spring (IES29) and Lubken Canyon Spring1 (IES21) differed from one another in 2000 and 2017 (Figure 5). The distinctiveness of the IES31 communities is likely because of the geothermal characteristics of this spring. Differences between the IES29 and IES21 communities during these years are more puzzling and may be attributed to differences in the spring environments during these years.

2.6 Discussion

Although most studies in western US spring systems focused on an individual taxon (e.g., Hershler and Sada 1987, Hershler 1989, Polhemus and Polhemus 2002, Sada 2008), this is one of the first studies to evaluate the temporal stability of BMI communities in Great Basin springs. The results of our study show that (1) the structure of the BMI communities within each spring was relatively stable before and after 17 years of hydroclimatic changes (i.e., ten years of severe droughts and five years of high precipitations) in 2000 and 2017; and (2) the relative abundance of crenobiontic taxa (*Pyrgulopsis*) was relatively similar in 2000 and 2017, and they were found in the same springs in both sampling years. These results suggest that the source area of aridland springs act as reliable refugial habitats for benthic communities to overcome the effects of frequent drying and high precipitation periods in arid regions.

Previous studies on lotic systems showed a significant impact of frequent droughts on the structure of BMI communities, which highlights the vulnerability of these communities to hydrologic disturbances in headwater streams (Boulton 2003, Lake 2003, Sponseller et al. 2010, Herbst et al. 2019). For example, Herbst et al. (2019) found that the richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa declined, and even sometimes disappeared, in mountain streams of the Sierra Nevada during a severe drought. Although our findings show that the relative abundance of intolerant insect taxa (EPT) declined near the source of spring systems in 2017 compared with 2000, the changes were not significant enough to alter the community structures in these systems. These findings are also consistent with the study by Death and Winterbourn (1994) in New Zealand, in which they found that the seasonal stability of the entire fauna was higher in spring-fed streams than catchment-fed streams. It can be concluded that because of the “steady-state” condition of persistent springs (especially near the source), springs can be more effective in protecting BMI communities during frequent drought and flood events that stream systems in arid environments.

Taylor (1985) noted that crenobiontic gastropods occur in habitats that are stable and unaffected by drought. This suggests that the presence of springsnails is an indicator of spring persistence and a steady-state environment that is minimally influenced by stochastic factors (e.g., drought and flooding). The persistence of some Owens Valley springs is indicated by the presence of springsnails (*P. perturbata* and *P. owensensis*) that are endemic to Owens Valley and diverged from ancestral forms approximately 2.76 mya, and *P. wongi* that diverged approximately 0.52 mya (Hershler and Liu 2008). As with all crenobiontic macroinvertebrates, they are most abundant near spring sources, where environmental variation is lowest, and the presence of a persistent aquatic habitat is greatest (McCabe 1998). These species occurred in 8 of the 12 springs studied, and they were found in relatively high abundance in all eight springs. Desert springs in the southern Chihuahuan Desert and the Bonneville Basin also contain higher gastropod abundances compared with insect abundances (Rader et al. 2012, Stanislawczyk et al. 2018). The other four springs, which are not occupied by gastropods, are all cold springs (IES38, IES28, IES42, and IES43) at the base of the Sierra Nevada and fed by aquifers supported by snowmelt (as indicated by low temperatures and low EC). Geological and evolutionary history (Williams and Williams 1998, Hubbell 2001, Stanislawczyk et al. 2018), dispersal limitation (Rader et al. 2012), or the physicochemical characteristics of spring habitats (Poff 1997, Malcom et al. 2005) can explain the absence of springsnails from these springs.

Potential natural and human disturbances affecting environmental characteristics of Owens Valley springs include droughts, scouring floods, fire, and groundwater use. Portions of Owens Valley burned between 2000 and 2017, but none of the sampled springs were involved. Photographs, field notes, and statistical analyses indicated that there was little difference between the environmental characteristics of springs during the two collection periods. The most notable difference was the EC values in IES29 and IES21, where readings were much higher in 2017 than in 2000. These two springs showed a greater change in the composition of their BMI communities between the sampling years. The reason for the change in EC in these springs is enigmatic, but based on studies in mesic regions documenting the influence of water chemistry on BMIs (e.g., Danks and Williams

1991, Ferrington 1995, Botosaneau 1998, Pond and McMurray 2002) and the CCA results of this study, which highlight EC as one of the influential factors in structuring BMI communities (Figure 2), we suggest that this jump in EC between sampling years could be the reason for the shift in the composition of the BMI communities in these two springs. These findings can be compared with the regional studies of springs in the Great Basin (Myers and Resh 2002, Sada and Thomas, *in press*), which identified temperature and EC as the governing factors in structuring BMI communities. Similarly, temperature and EC were highlighted as the leading environmental variables to explain BMI distribution patterns in rheocrene springs in Italy (Bottazzi et al. 2011).

Discharge in most springs naturally varies daily, seasonally, and annually in response to recharge, temperature, precipitation, evaporation, and transpiration through phreatic vegetation. The effects of these factors on spring discharge in Owens Valley is unknown, but the effects of drought years on the depth to groundwater in the valley (Danskin 1998) suggests that differences in spring discharge during drought and wet years may be significant (see Figure 2). In a field experiment examining the effects of incremental decreases in spring discharge, Morrison et al. (2013) found that springbrook wetted width and habitat diversity reached a tipping point when discharge was decreased by less than 20 percent. There is little information to provide insight into the effects of decreased discharge on aquatic life in springs. In our study, discharge is monitored only in Reinhackle Spring (IES26) (Figure 2-D) by LADWP. Although there is some evidence to support the influence of the LAA on the Reinhackle Spring, the 91 EIR restricted pumping from several wells in the area to protect the flow of Reinhackle Spring from anthropogenic disturbances (the City of Los Angeles, Department of Water and Power and County of Inyo

1990). The presence of crenobiontic springsnails in Reinhackle Spring suggests that this spring existed prior to the aqueduct and that aqueduct water may not have significantly changed the water features of the spring. Therefore, discharge fluctuation at this spring over the years is mimicking hydroperiod and hydrologic cycles. Hydroclimatic changes in the area caused near-historic drought and wet periods between 2000 and 2017. The distance between the pumping fields and springs sampled in 2000 and 2017 and the stability of the BMI communities between these years suggests that discharge from these springs was either unaffected by groundwater use or that the effects were short-lived.

Aridland springs as biodiversity hot spots support high levels of endemism (Myers and Resh 1999), and therefore they are identified as high priority systems for conservation. The extirpation and extinction of the crenobiontic Great Basin and the Mojave Desert fishes and BMIs is evidence of the effects of human activity on spring systems (Williams et al. 1985, Sada and Vinyard 2002, Williams and Sada, *in press*). Meyers (2020) compared the extent of riparian vegetation associated with Owens Valley springs during drought years and wet years and concluded that the extent of riparian vegetation was less in springs with longer residence times (>100 years) than in springs with shorter groundwater flowpath. They showed that vegetation at Reinhackle Spring experienced the highest slope of peak NDVI (normalized difference vegetation index) compared with any other spring over the prolonged drought because of the effect of the LAA on this spring. They concluded that vegetation around Reinhackle Spring suffered more than any other spring during the 2012-2016 California drought. Although this prolonged drought had a significant impact on the vegetation around the spring systems, thanks to the long-term monitoring program and management of groundwater in Owens Valley, the ecohydrological condition of

Reinhackle Spring did not cross a critical threshhold (complete loss of water) during the 2012-2016 drought and could protect crenobiontic springsnails from extirpation.

Temporal variation in the physicochemistry and structure of BMI communities in Owens Basin springs was minimal. This suggests that spring systems that are unaffected by natural and human disturbance are “steady-state” systems. Samples collected twice in 17 years provided insight into the resilience of communities over this period, but not their resistance. These springs are supported by recharge from snow falling at high elevations in the surrounding mountains. The variation in the discharge of the studied springs is unknown, but the similarity in benthic communities during 2000 and 2017 suggests that climate-driven changes in hydrology over this period had minimal long-lasting effects on spring ecology. These observations suggest that either: (1) BMI communities are minimally affected by environmental condition and variation; (2) communities were disturbed by drought event(s), but conditions during wet years rapidly recovered the communities to their pre-disturbance condition; or (3) environmental variation over this period was insufficient to influence the structure of BMI communities. All of these scenarios highlight the role of aridland springs as climatic refugia and their importance in conserving endemic and endangered flora and fauna in the region.

2.7 Figures

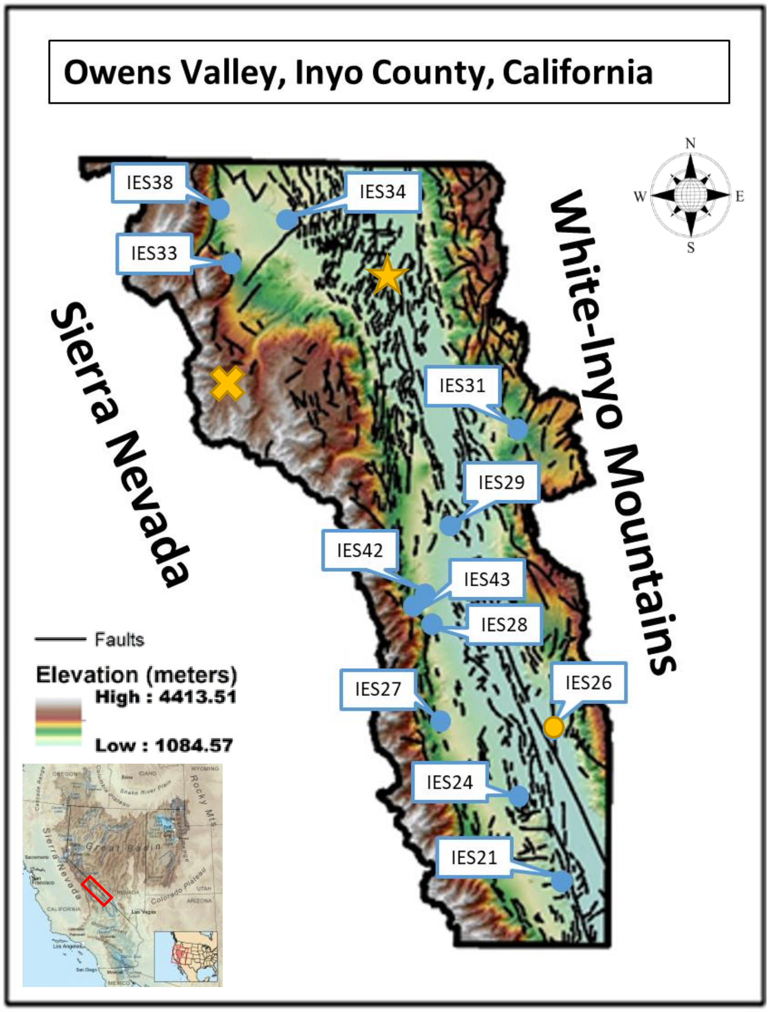
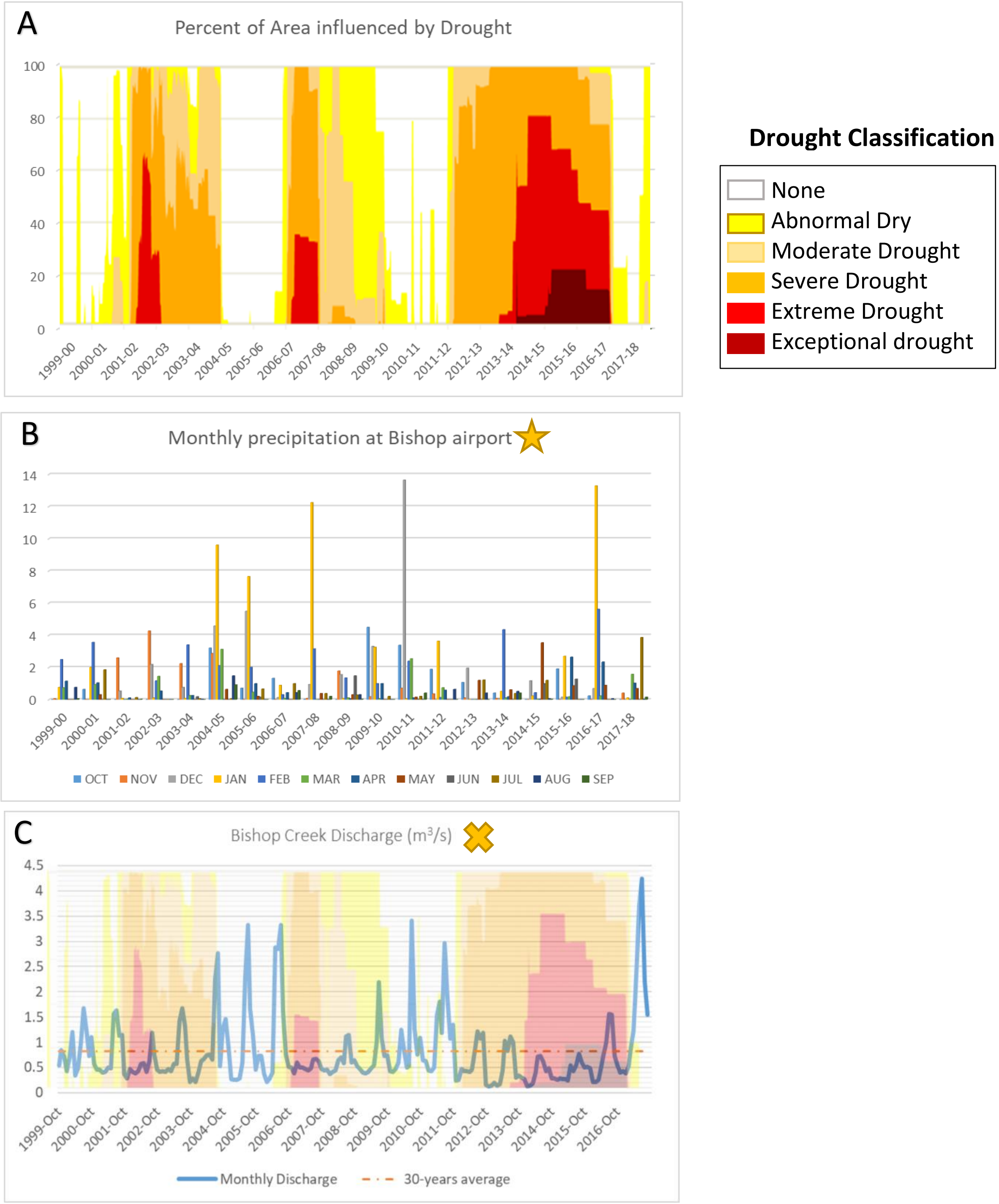


Figure 2.1. Study area boundary in Owens Valley, California, USA. Blue circles represent sampled springs: IES 38- Wells Meadow B Spring; IES 34- Birchim Canyon Spring B; IES 33- Elderberry Canyon Spring; IES 31- Warm Spring A; IES 29- Unnamed Spring north of Red Mountain (Red Mountain Spring); IES 42- North Harry Birch Spring; IES 43- South Harry Birch Spring; IES 28- Grover Anton Spring; IES 27- Boron Spring A; IES 26- Reinhackle Spring; IES 24- Hogback Creek Spring; IES 21-Lubken Canyon Spring 1. The yellow cross represents the Bishop Creek Discharge Gauge (USGS site# 10270872); the yellow star represents the NOAA precipitation collector at the Bishop airport; the yellow circle represents Reinhackle Spring.


A
Percent of Area influenced by Drought
100
80
60
40
20
0
1999-00
2000-01
2001-02
2002-03
2003-04
2004-05
2005-06
2006-07
2007-08
2008-09
2009-10
2010-11
2011-12
2012-13
2013-14
2014-15
2015-16
2016-17
2017-18
Drought Classification
None
Abnormal Dry
Moderate Drought
Severe Drought
Extreme Drought
Exceptional drought
B
Monthly precipitation at Bishop airport
14
12
10
8
6
4
2
0
1999-00
2000-01
2001-02
2002-03
2003-04
2004-05
2005-06
2006-07
2007-08
2008-09
2009-10
2010-11
2011-12
2012-13
2013-14
2014-15
2015-16
2016-17
2017-18
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
C
Bishop Creek Discharge (m³/s)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1999-Oct
2000-Oct
2001-Oct
2002-Oct
2003-Oct
2004-Oct
2005-Oct
2006-Oct
2007-Oct
2008-Oct
2009-Oct
2010-Oct
2011-Oct
2012-Oct
2013-Oct
2014-Oct
2015-Oct
2016-Oct
Monthly Discharge
30-years average

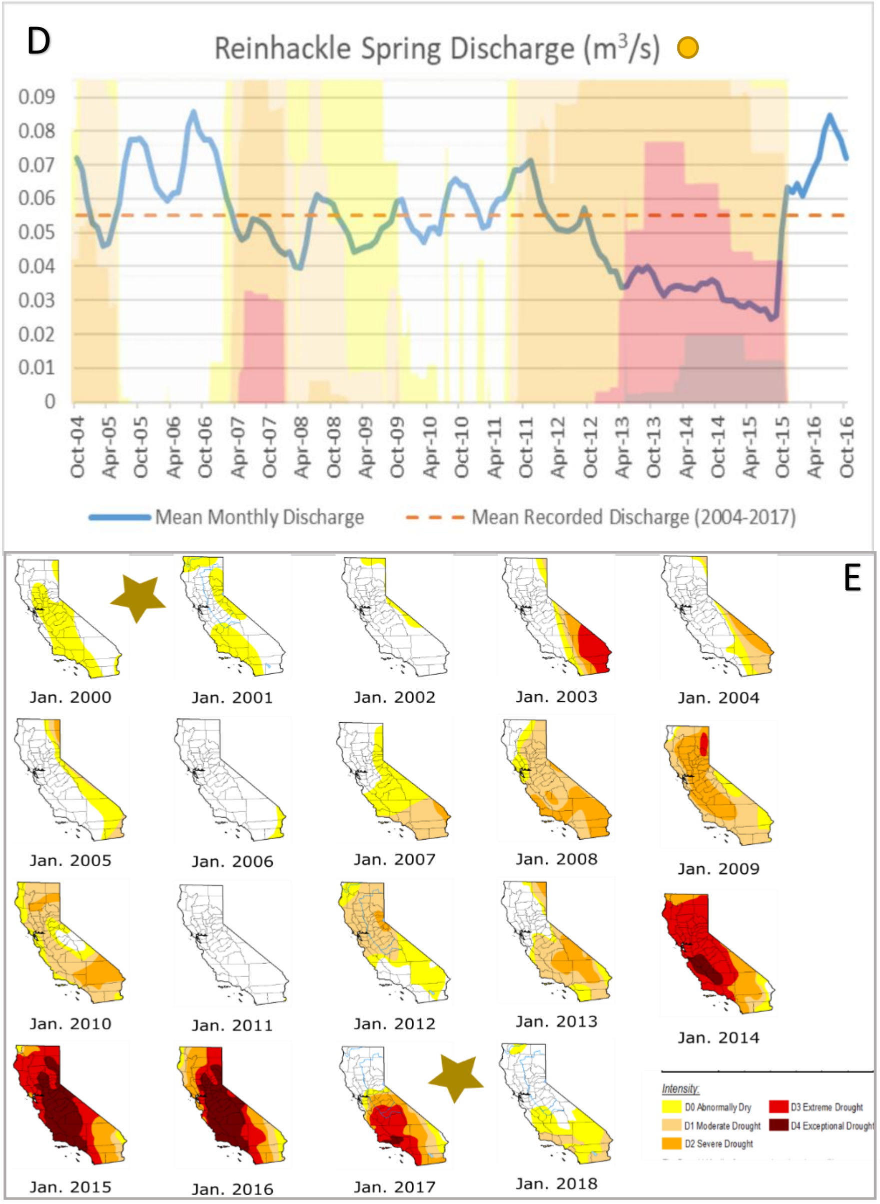


Figure 2.2. Hydrological drought, monthly precipitation, bishop creek discharge, and Reinhackle Spring discharge between 1999/2000 and 2016/2017 water years. (A) Percent area of Inyo County impacted by different classifications of droughts over the study period (Imported from The National Drought Mitigation Center). (B) Monthly precipitation (cm) at the NOAA precipitation station at Bishop airport. (C) Monthly discharge (m3/s) of Bishop Creek near the Lake Sabrina. USGS gauge at the 2761 m elevation from NGVD29 reference altitude (Orange dashed line is the mean discharge of recorded data between 1986 and 2017). (D) Reinhackle Spring discharge between 2004 and 2016 water years (Blue line is spring average daily flow [m3/s] for each month from LADWP Gauge; Orange dashed line is the mean discharge between October-2004 and October-2017). (E) California drought (intensity and coverage) between 2000 and 2018; Two stars represent the BMI collection years. See figure 1 for the locations of Bishop airport, bishop creek, and Reinhackle Spring.

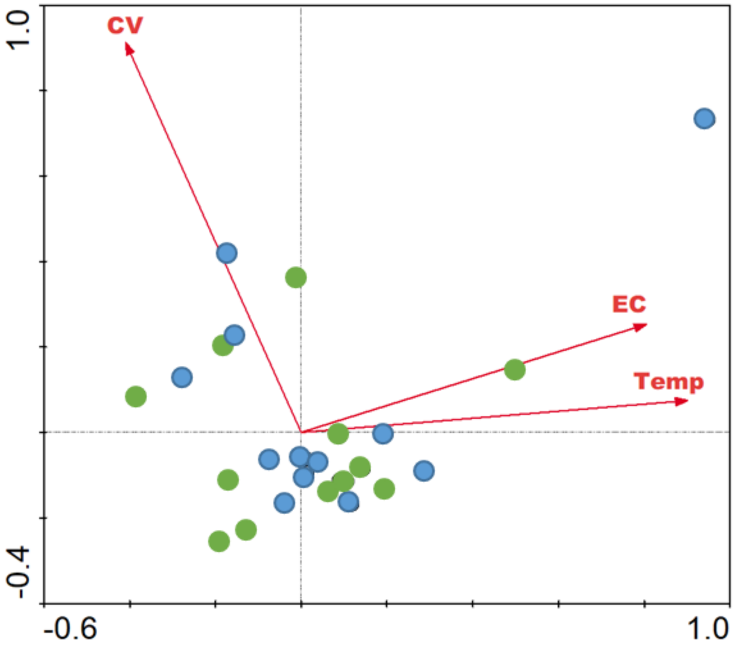


Figure 2.3. Canonical correspondence analysis (CCA) biplot of inter-sample associations between physicochemical characteristics and structure of benthic macroinvertebrate communities using pooled data from both years. Current velocity (CV), electrical conductance (EC), and temperature were statistically significant (P-value < 0.05) environmental variables. Green circles: sample springs in 2000. Blue circles: sample springs in 2017.

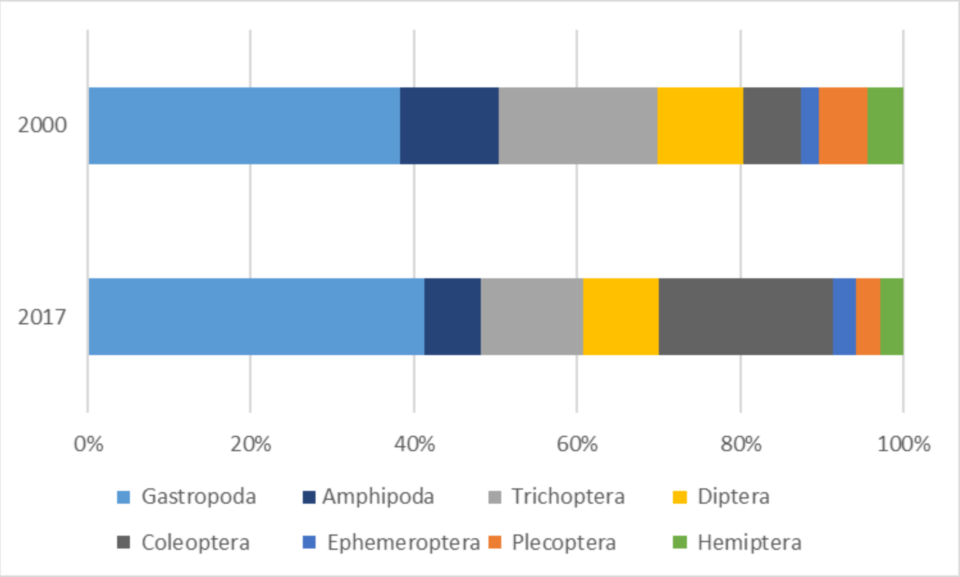


Figure 2.4. Relative abundances of main benthic macroinvertebrate groups (order or class levels) in 2000 and 2017.

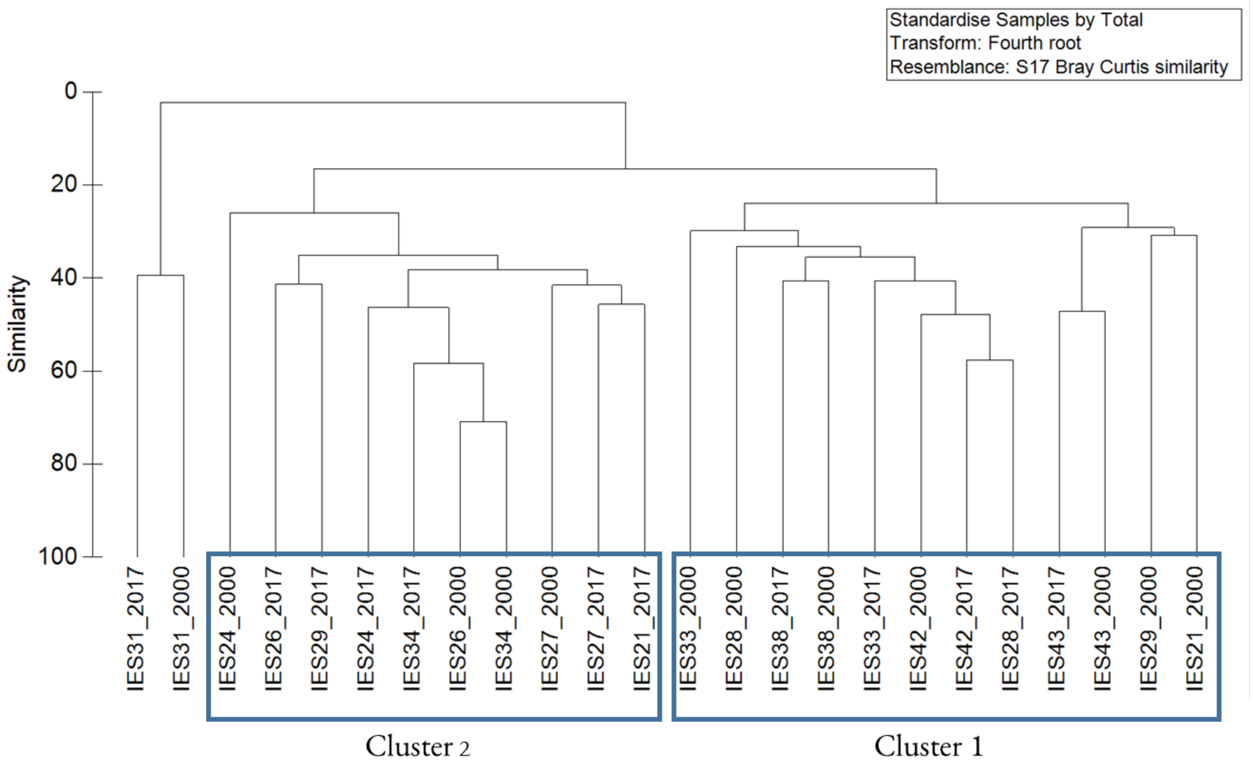


Figure 2.5. The dendrogram of hierarchical cluster analysis (HCA) represents the group average cluster mode with Bray-Curtis distance between benthic macroinvertebrate communities in samples collected in 2000 and 2017.

2.8 Tables

Table 2.1. Spring ID, Name, location, and elevation

Sample ID	Spring Name	UTM 11 S		Elevation
		E	N	(m)
IES-021	Lubken Canyon Spring 1	405405	4044353	1240.623
IES-024	Spring along Hogback Creek A	397732	4056750	1393.323
IES-026	Reinhackle Spring	401193	4061012	1144.635
IES-027	Boron Springs A	386328	4072314	1601.774
IES-028	Grover Anton Spring	385165	4082719	1593.781
IES-029	Unnamed spring north of Red Mountain	385305	4100901	1417.509
IES-033	Elderberry Canyon Spring	357900	4138046	1596.346
IES-034	Birchim Canyon Spring B	364114	4144865	1482.941
IES-038	Wells Meadow B	355222	4145412	1614.472
IES-041	North Fuller Spring	379636	4102941	1981.63
IES-042	North Harry Birch Spring	385273	4085645	1486.732
IES-043	South Harry Birch Spring	385440	4085284	1497.738

Table 2.2. Mean (± 1 SE) of physicochemical features in 12 Owens Valley springs sampled in 2000 and 2017. Non-parametric Kruskal-Wallis test, $\alpha = 0.0.5$, was calculated to compare environmental metrics of all springs (n=12) in 2000 and 2017. Differences were not significant for any parameters between 2000 and 2017 years (P-value>0.05).

Physicochemical characteristics	2000	2017	P-value
Water depth (cm)	6.9 (3.1)	5.34 (1.6)	0.7
Wetted width (cm)	529.85 (440.4)	238.47 (152.3)	0.6
Water velocity (cm/sec)	14.92 (5.4)	19.2 (4.7)	0.4
Water temperature (°C)	16.6 (0.8))	16.2 (1.2)	0.8
EC (µS/cm)	205 (27.2)	308.23 (51.6)	0.1

Table 2.3. Community parameters such as richness, abundance, percent dominant taxa, diversity indices, and HBI metrics in 2000 and 2017. Differences between Community parameters across the 12 study springs among 2000 and 2017 collections were calculated using the non-parametric Kruskal-Wallis test, α = 0.05. (A) Taxonomic richness, total abundance, and mean percent dominant taxa (±1 SE). (B) Mean (±1 SE) of Evenness, Shannon-Wiener Index, and Simpson Index. (C) Mean (±1 SE) of Hilsenhoff Biotic Index (HBI) Metrics. Differences were not significant for any metrics between 2000 and 2017 years (P-value >0.05).

Metrics	2000	2017	P-value
A			
Total # of taxa in all samples	75	75	---
Total # of organisms in all samples	8839	2655	---
Mean of # of taxa per sample	15 (0.06)	13 (0.05)	0.43
Mean percent dominant taxa	47.3 (0.08)	47.9 (0.07)	0.9
B			
Evenness	0.51 (0.07)	0.56 (0.05)	0.51
Shannon-Wiener	1.3 (0.2)	1.5 (0.17)	0.47
Simpson	0.56 (0.08)	0.62 (0.05)	0.50
C			
Percent tolerant taxa	20 (0.03)	20 (0.03)	0.90
Percent intolerant taxa	20 (0.03)	20 (0.04)	0.97
Community tolerance value	4.4 (0.5)	4.5 (0.2)	0.85

Table 2.4. List of common taxa (occurred in more than one spring (>10 percent of all springs) in 2000 or 2017). The number of springs that a specific taxon was found in 2000 and 2017 is shown.

Order	Taxon	# of springs in 2000	#of springs in 2017
Ephemeroptera	*Baetis*	3	2
	Ironodes	3	2
Plecoptera	*Malenka*	4	4
	Hesperoperla	2	2
	Isoperla	3	1
Trichoptera	*Ochrotrichia*	3	2
	Lepidostoma	7	6
	Gumaga	7	4
Coleoptera	*Optioservus*	5	6
	Hydraena	2	1
Diptera	*Brillia*	3	4
	Micropsectra/Tanytarsus	7	2
	Corynoneura	3	1
	Heleniella	2	1
	Metriocnemus	3	2
	Parametriocnemus	4	2
	Larsia	3	1
	Pentaneura	2	2
	Dixa	2	2
	Simulium	5	4
	Tipula	1	3
Hemiptera	*Argia*	8	9
Crustacea	*Hyalella*	3	3
Gastropods	*Pyrgulopsis*	8	8
Bivalves	*Pisidium*	3	3

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Chapter 3

Geochemical Fingerprints Explain Spatial Distribution of Benthic Macroinvertebrate Communities in Aridland Springs in Owens Valley, California

Khaled Pordel, Zachary Meyers, Donald Sada, Marty Frisbee, Laura Rademacher

3.1 Abstract

Aridland springs are groundwater-dependent ecosystems supporting aquatic organisms of both insect and non-insect taxa in arid regions. Our understanding of the relationship between hydrogeological parameters and the ecology of these systems is in its early development stages. In this study, we examined the association between benthic macroinvertebrate community composition (ecology) and geochemical fingerprint of the spring waters (hydrogeology), which is a function of the interactions between groundwater and rock (minerals) in the aquifers. The geologic heterogeneity of the eastern Sierra Nevada in Owens Valley provides a unique standpoint to identify the factors explaining the composition of benthic macroinvertebrate (BMI) communities in a valley-scale study. Twelve mountain front springs along the eastern Sierra Nevada frontal fault zone were sampled for environmental characteristics and BMI taxa. Spring selection was limited to undisturbed rheocrene springs.

Local variations in the geology of the eastern Sierra Nevada exert primary control on the geochemical composition of spring waters. Mountain front springs in Owens Valley are clustered into three groups according to their geochemical characteristics, which corresponds with the geology of their likely recharge areas and water-rock interactions. Group 1 consists of spring waters with a granitic weathering signature. The geochemical

compositions of Group 2 waters are characterized by weathering of granitic plutonic and volcanic rocks, but they are also influenced by surface water features. Group 3 waters are influenced by a metasedimentary roof pendant, as well as granitic/granodioritic plutons. The relative abundances of BMI taxa in the three geochemical groups indicate that springs in Group 3 are faunistically distinct from Groups 1 and 2. Nonmetric multidimensional scaling (NMDS) shows that springs within each geochemical group have a higher degree of BMI community similarity relative to springs in the other geochemical groups. Nonparametric Kruskal-Wallis test and analysis of similarity (ANOSIM) confirms the NMDS interpretations by indicating that the distinction between the structure of BMI communities among geochemical groups is statistically significant. The result reveals that weathering-derived geochemical signals of spring waters may better explain the BMI community composition than other environmental parameters in persistent aridland springs. This finding is only valid in undisturbed/naturalized springs (reference springs).

3.2 Introduction

In a very recent study, ecologists and hydrologists discussed the importance of integrating ecology and hydrogeology fields for studying the groundwater-dependent ecosystem (Cantonati et al. 2020). Hydrogeologists mostly study groundwater flowpaths from the recharge zone to the discharge zone (in this case, spring emergences), whereas ecologists start their studies from the spring emergence. Although accessibility to spring systems has allowed ecologists and hydrogeologists to study these systems in terms of taxonomic data, habitat classifications, landscape placement, and sampling techniques (Hershler 1994, Sada and Pohlmann. 2006, Cantonati et al. 2006, Springer et al. 2008, Soucek et al. 2015), the current state of the science (i.e., integration of hydrogeological parameters and spring ecology) is still in its early development stages.

Aridland springs are biodiversity hotspots that occur where groundwater discharges at the land surface (Springer and Stevens 2009). Springs in arid regions are key ecological features because of the limited availability of surface waters (Myers and Resh 2002). Relatively stable physicochemical conditions of aridland springs (because of continuous discharge into a spring) provide a refugial habitat for aquatic organisms to overcome environmental disturbances over thousands to millions of years (Shepard 1993, Murphy et al. 2015). To date, most biological studies on aridland springs have focused on identifying new species (Hershler and Sada 1987, Hershler 1989, Soucek et al. 2015), the biogeography of fishes and macroinvertebrates (Hershler and Sada 2002, Polhemus and Polhemus 2002, Smith et al. 2002, Hershler and Liu 2008), identifying endangered species (Minckley and Deacon 1968, Williams et al. 1985, Myler et al. 2007), the physiology of fishes living in harsh environments (e.g., Feldmeth et al. 1974, Shrode and Gerking 1977), autecology of individual taxa (Sada 2001, 2008), and genetic divergence of species because of their dispersal ability (Thomas et al. 1997, Myers et al. 2001). However, studies on the spatial distribution of benthic macroinvertebrate (BMI) communities in aridland springs have received comparatively less attention.

Studies on patterns of BMI community compositions in spring systems have been divided into three different spatial scales: along the spring run, local scale (springs discharging from one aquifer province), or regional scale (springs discharging from several aquifer provinces). Previous studies documented that both hydrology (e.g., discharge and temperature) and BMI community of spring systems change along the springbrook

(McCabe 1998, Dumnicka et al. 2013, Morrison et al. 2013); however, environmental and BMI community permanency is greatest near the spring sources (McCabe 1998). To understand the factors controlling the spatial distribution of the BMI communities between spring sources, studies at larger spatial scales and across landscape features (local and regional scales) are necessary. The slow dispersal rate of BMI taxa, geographical proximity of springs, and history of the geologic events may result in the similarity of BMI communities in springs within a valley and variation of that between valleys (Rader et al. 2012, Stanislawczyk et al. 2018). Moreover, contemporary processes were also highlighted as governing factors influencing the spatial distribution of BMI communities. Contemporary processes include temperature and elevation (Myers and Resh 2002); alkalinity and pH (Glazier 1991); discharge and substrate composition (von Fumetti et al. 2006); temperature, electrical conductivity (EC), water velocity (Barquín and Death 2006); and, colonization and interspecies dynamics (Myers et al. 2001). In a recent study, Sada and Thomas (*in press*) suggest a strong association between the structure of BMI communities and aquifer provenance (mountain, local, and regional). They found that the number and proportion of intolerant taxa are higher in springs with short groundwater flowpaths and lower EC (mountain), and they are absent from regional springs with elevated EC. On the other hand, percent tolerant taxa increase along an environmental gradient from mountain (benign) to bajada, valley, and playa (harsh) aquifers with longer groundwater residence time and higher temperature and EC (Sada and Thomas, *in press*). However, because of the lack of empirical data, none of these studies examined the association between geochemical fingerprints of spring waters and their BMI communities. We studied this association and addressed the knowledge gap in this chapter.

Spring geochemical fingerprints are a product of groundwater-rock interactions, which are a function of aquifer mineralogy, mineral solubility, groundwater residence times, groundwater flowpath, and geology at the recharge zone (Güler and Thyne 2004). Different water-rock interactions result in distinct geochemical signatures on spring waters (Springer and Stevens 2009, Hershey et al. 2010, Warix et al. 2020, Meyers et al. in review). Additionally, structural geologic features (faults and contacts) may allow groundwater flow through multiple geologic units or provide preferential flowpaths (Gleason et al. 2020). For example, previous studies on the south-central Great Basin showed that groundwater flow through carbonate aquifers typically results in a high concentration of Ca^{2+}, Mg^{2+}, Sr^{2+}, and HCO^{3-}. In contrast, a geochemical signature of springs discharging from felsic-volcanic aquifers is Na^{+}-K^{+}-HCO^{-3} (Winograd and Thordarson 1975, Koonce et al. 2006, Hershey et al. 2010). These dissimilar geochemical compositions of springs waters may influence the spatial distribution of BMI communities.

Zoobenthos in spring systems mainly comprises insect and non-insect BMI taxa (Cantonati et al. 2006). Insects are the most diverse BMI group in spring systems in terms of biodiversity and morphology (Ferrington 1995). All insect taxa (i.e., stoneflies, caddisflies, and most dipterans) have an emerging adult stage with an aerial dispersal ability to migrate between aquatic habitats (Bogan and Boersma 2012). Although some are strong fliers and have excellent adaptability to a broad range of habitats, some are poor dispersers with narrow habitat requirements and prefer to stay near their larval habitat (Myers et al. 2001). On the other hand, the active dispersal mode of non-insect taxa (i.e., springsnails) is limited within their habitats because of their submerged adult stage (Taylor 1985). Thus, their occurrence is mainly limited by dispersal boundaries (Rader et al. 2012).

Both insect and non-insect BMI taxa are mainly adapted to their habitat environment and remain close to their natal habitat (Danks and Williams 1991). Consequently, the structure of BMI communities has been used as a reliable bio-indicator of the habitat characteristics in freshwater habitats (e.g., Harper et al. 1994, Barbour et al. 1999, Hodkinson and Jackson 2005).

Crenobiontic snails (obligatory spring dependent) are one of the well-known non-insect taxa in Owens Valley, CA, which are abundant near spring emergences with a stable environmental condition (McCabe 1998). Owens Valley endemic crenobiontic taxa, *Pyrgulopsis owensensis, and P. perturbata*, diverged from ancestral forms approximately 2.76±0.51(maximum) mya (Hershler and Liu 2008). Another regionally endemic springsnail (*P. wongi*), which is widely distributed in the Owens and Amargosa River basins, diverged from its ancestral form nearly 0.52 ± 0.12 (minimum) mya, (Hershler and Liu 2008). These endemic crenobiontic taxa were found in most of the undisturbed springs in Owens Valley (Hershler 1989, Pordel et al. *in prep*). These crenobiontic taxa cause the similarity of BMI community composition in spring systems over time and their dissimilarity over space because of their limited dispersal ability (Rader et al. 2012, Stanislawczyk et al. 2018, Pordel et al. *in prep*).

Two recent hydrological and ecological studies on aridland springs along the Sierra Nevada frontal zone motivated this study (Meyers 2020 and chapter 2). In the hydrogeology study, the geologic units and geochemical processes responsible for the aqueous geochemical fingerprint of mountain-front springs are identified using water temperature, stable isotopes, and water chemistry. The geologic heterogeneity (namely, different geologic units in the recharge zone of different springs) of the eastern Sierra

Nevada is recognized as a primary factor controlling spring water geochemical compositions (Meyers 2020). A long-term ecological study on the subset of these springs indicates that Owens Valley mountain-front springs are occupied by a variety of BMI organisms with relatively similar community structures in 2000 and 2017 (Pordel et al. *in prep*). They conclude that perennial aridland springs act as refugial habitats and protect the benthic communities, especially crenobiontics, during frequent hydrologic disturbances. Based on these studies, we hypothesize that BMI community composition is primarily controlled by the geochemistry of the spring water, which is, itself, a reflection of the geology and mineralogy of the rock units through which the groundwater supporting the springs has flowed. We studied undisturbed rheocrene (flows into a channel) springs along the Sierra Nevada frontal fault zone in Owens Valley, California, to (1) identify the main environmental factors shaping the structure of BMI communities, and (2) explore the associations between geochemical fingerprints and macroinvertebrate communities in aridland springs.

3.3 Study Area

3.3.1 Area Description

Owens Valley is a deep extensional graben situated between the eastern Sierra Nevada (west) and White-Inyo Mountains (east). Physiographically, Owens Valley is located on the southwestern boundary of the Great Basin in an area known as the Walker Lane Transition Zone. Elevations along the basin floor decrease southward from ~1,265 meters above sea level (masl) near Bishop to ~1,130 masl south of Lone Pine (Figure 1). Mountain crests (>4,200 masl) are juxtaposed against the basin floor on both sides of the valley. Owens Valley drains internally via an axial fluvial system, the Owens River, which terminates in Owens (Dry) Lake. The majority of surface flow into the valley comes from the headwaters of the Owens River (i.e., Mono Lake) and streams draining the eastern Sierra Nevada. The primary source of groundwater recharge is snowmelt (i.e., mountain-block and mountain-system recharge) and infiltration of surface runoff on the alluvial fans from the eastern Sierra Nevada (mountain-front recharge; Danskin 1998). The White-Inyo Mountains are in a rain shadow from the Sierra Nevada. Therefore, they receive much less precipitation and provide less groundwater recharge to the Owens basin, and have fewer perennial tributaries that drain into the basin. At high elevations, average annual precipitation is over 400 cm (NOAA Stations at Lake Sabrina, CA: elevation around 2,800 masl). Comparatively, average precipitation in Owens Valley is approximately 13.5 cm annually (based on data from the NOAA station at Bishop Airport, CA: elevation 240 masl).

3.3.2 Site Selection

Thirteen springs were sampled (including 12 undisturbed or naturalized springs and one moderately disturbed [IES041] spring) along the Sierra Nevada frontal fault zone in Owens Valley, California during 2016 and 2017 (Table 1). These 13 were selected from the list of 20 springs included in the hydrogeological study of Owens Valley springs (Meyers 2020), as the reminder exhibits signs of disturbance. The sampling was limited to the eucrenal (spring source) area in this study. Additionally, because frequent severe disturbances play an essential role in altering BMI community composition in aridland springs (Sada and Nachlinger 1996, Sada and Vinyard 2002, Keleher and Rader 2008), springs with evidence of recent human disturbances were removed from the ecological investigation. The classification of spring systems based on their level of disturbance was conducted based on observations (photographs, field notes), spring inventory, and monitoring protocols (Sada and Pohlmann. 2006, Stevens et al. 2016). Although we observed a moderate impact of livestock disturbance on IES041 (North Fuller Spring, See Figure S1), we sampled this spring because the disturbances have occurred recently, and they were not observed during our first inventory trip in 2016. We did not include this spring in our hydrological and ecological analyses throughout this chapter, but the results (including IES041) are presented in the supplementary (Figure S1).

The sampled springs were selected to capture a broad spatial distribution and encompass the geologic heterogeneity in Owens Valley. Except for two springs (IES026 and IES034), all springs are supported by recharge from snowmelt in the higher elevation of the Sierra Nevada (Meyers 2020). Groundwater flow in the mountain block and alluvial aquifer intercept the impermeable barriers associated with the Sierra Nevada frontal fault zone and reach the surface as spring discharge (Harrington 2016). Sampled springs are persistent rheocrene springs isolated from other lotic and lentic aquatic systems with no hydrological connections between springs in the area.

3.3.3 Main Geologic units in Spring Contributing Areas

A simplified geologic map of the region showing the major petrologic groups within Owens Valley watershed is shown in Figure 2. This map was first presented in Meyers (2020) and concatenated many geologic quadrangles that use a similar symbology definition based on geologic unit age and geochemical compositions (Moore 1963, 1981, Bateman and Moore 1965, Bateman et al. 1965, Nelson 1966, Lockwood and Lydon 1975, Stone et al. 2000). The majority of plutons comprising portions of the eastern Sierra Nevada within Owens Valley watershed divide are in the petrologic range of quartz monzonite to granodiorite (Figure 2). However, the heterogeneity of the batholith is substantial, and there are localized sections of highly felsic rock (e.g., alaskite and anorthosite) and mafic rocks (e.g., diorite, quartz diorite, and hornblende gabbro). In the north-central region of the study area, sections of Bishop Tuff cover the valley floor and make up the volcanic tableland. Paleozoic (Penn-Permian) metasedimentary roof pendants are found at high elevation regions throughout the eastern Sierra Nevada and are correlated with similar rocks in the White-Inyo Mountains. The mineralogy varies significantly between roof pendants, but the most common metasedimentary facies are marble, calc hornfels, pelitic hornfels, micaceous quartzite, and biotite schist (Moore 1963) associated with the eruption of the Long Valley Caldera. Other notable features within the watershed include basalt flows and cinder cones (e.g., Red Mountain) associated with the Big Pine Volcanic Field and dropped down blocks along the basin floor (e.g., the Alabama Hills and the Poverty Hills). Several springs in this study emerge at faults around the Alabama Hills and downgradient from the Mt Whitney intrusive suite, a series of nested granodiorite plutons west of Lone Pine (Hirt 2007).

3.3.4 Spring geochemical framework

Meyers (2020) presented a detailed geochemical and hydrogeologic framework for 20 springs within Owens Valley based on major ion compositions, environmental tracers, and geochemical modeling. Because of concerns about the effect of disturbance on

BMI community structure, our analysis only incorporates 12 of these springs. A summary of the results from Meyers (2020), focused on the springs analyzed for BMI community structure in this study is presented in the following section.

The sampled springs cluster into three distinct geochemical groups based on geochemical bivariate plots (Figure 3) and their possible groundwater-rock interactions along the flowpath from recharge zones (snowmelt at high elevation) to discharge at a spring. Group 1 consists of five springs with a granitic (alaskite, quartz monzonite, granodiorite) weathering signature that is typical of interaction with the Sierra Nevada plutonic rocks. This group shows the highest variation in geochemical signatures because of differences in plutonic compositions across the Sierra Nevada. The local geology of the contributing spring area among springs in this group can result in identifying some subgroups. For example, two springs (IES054 and IES024) near Alabama Hills can be considered as a subgroup of the Group 1 because they are emerging from the biotite monzogranite of the Alabama Hills with a relatively distinct evolutionary path relative to other Group 1 springs. Group 2 is comprised of two springs (IES026 and IES034) with elevated sodium and potassium concentrations compared to springs of Group 1. The geochemical signature of these springs is interpreted to be influenced mainly by weathering of plutonic rocks as well as the contribution from volcanic weathering or surface water features (e.g., the Los Angeles Aqueduct or the Owens River). Spring waters in this group have enriched stable isotopes (δ^{2}H and δ^{18}O), which indicate rainfall as opposed to snowmelt as the primary source for the spring recharge (Meyers, 2020). Other evidence that supports the possible impact of surface water on the geochemical fingerprint of these two springs are having spatial proximity and geochemical similarity with Owens River and

LA aqueduct (LAA), and their modern/short residence times despite the long spatial distance between springs location and their likely recharge area (Meyers, 2020). Group 3 consists of five springs with an influence of Paleozoic metasedimentary roof pendant bounded within granitic rocks at their contributing recharge area, which exerts control on the geochemistry of down-gradient springs. These springs have geochemical compositions that reflect a mixture of roof pendant and granitoid dissolution. The geologic maps show the spatial proximity of these metasedimentary roof pendants with springs in Group 3 (Figure 2).

The separation of the spring waters based on the groundwater- rock interaction along the flowpath was determined by plotting Mg^{2+}:K^{+} molar ratios against Ca^{2+}:Na^{+} molar ratios (Figure 3-A). The reference lines represent the expected values for biotite weathering (horizontal line at Mg^{2+}/K^{+} ratio ~3) and plagioclase weathering (two vertical lines of Ca^{2+}/Na^{+} ratios for Owens Valley granitoids weathering at ~0.4 and 0.6 (White et al. 1999; Meyers 2020)). Two springs have Mg^{2+}/K^{+} ratio (IES 041 and IES 024) elevated above the expected ratio for biotite weathering, which suggests the possible weathering of hornblende along the flowpath. Although Group 1 springs evolve from granitic plutonic rocks, five out of six show a higher Ca^{2+}/Na^{+} ratio than the bounded values, ranging from 0.41 to 1.05, which highlight the carbonate dissolution along the flowpath. Five springs (Group 3) are distinguished by Ca^{2+}/Na^{+} molar ratios (1.35-1.69) higher than of what would be expected by the dissolution of the Sierra Nevada plagioclase alone, indicating an excess source of calcium. Springs in Group 2 show a Ca^{2+}/Na^{+} ratio around the minimum of the expected value for plagioclase weathering with an average of 0.37. In the dominant anions ($HCO^{3-} + SO_4^{2-}$) versus alkaline earth cations ($Ca^{2+} + Mg^{2+}$) plot, all spring waters clustered below the 1:1 line for carbonate and silicate weathering (Figure 3-B). This highlights the dominance of silicate weathering in all spring waters, but those springs with a higher Ca^{2+}:Na^{+} ratio fall closer to the 1:1 line. All springs are also plotted on mole percent Ca^{2+} versus mole percent dissolved SiO_2 and mole percent Na^{+} in figures 3C and 3D, respectively (adapted from Pretti and Stewart (2002)). In figure 3C, three reference lines representing 1:1 line for weathering of plagioclase to kaolinite, plagioclase to smectite, and plagioclase to calcite dissolution. This figure illustrates the separation between three geochemical groups. In figure 3D, the mole percent Na^{+} versus Ca^{2+} plot shows that plagioclase with "An" above An_{50} has to be weathered to explain observed Ca^{2+} and Na^{+} concentrations in Group 3. Because the average range of plagioclase compositions for Owens Valley springs are between An_{28} and An_{36}, and plagioclase compositions of An_{50} or greater are not present at the recharge area of Group 3 springs, Meyers (2020) identified metasedimentary roof pendant as a source of additional calcium for the springs in this group.

3.4 Materials and Methods

3.4.1 Field Measurement

Environmental metrics such as temperature, pH, EC, and dissolved oxygen (DO) were measured with a YSI Professional Plus (Quatro) multiparameter probe in the spring emergence at the time of water chemistry sampling. The YSI probe also calculated total dissolved solids (TDS) and specific conductance (SPC) from EC values. All parameters were calibrated at the start of the sampling campaign. Conductivity and pH were also calibrated daily.

Current velocity, water depth, wetted width, and spring brook length were also recorded at the time of BMI sampling (Table S2). Wetted width was measured as the mean width of five evenly spaced transects that were oriented perpendicular to the channel. Current velocity and water depth were measured as the mean of five measurements at the center of quadrats where BMIs were collected. A Marsh-McBirney Model 2000 current meter was used to measure current velocity. Bank vegetation cover, emergent vegetation, substrate composition, and shaded area were also qualitatively estimated at each spring. All probes, equipment, and tubing were sanitized with quaternary ammonia between sampling locations to prevent cross-contamination.

3.4.2 General Chemistry

Major cations and anions of water (general chemistry) can be used as a forensic tool to infer solute sources and likely water-rock interactions for spring waters (Hem 1985). Water samples for general geochemical analysis were collected in spring 2016. One additional sample (IES054) was collected in spring 2017. Springs were sampled as close to the area of spring emergence as possible using Masterflex platinum-cured silicone tubing placed in the spring emergence. Samples were collected using a GeoTech peristaltic pump and filtered using 0.22 μm pore size, polyethersulfone membrane Sterivex-GP pressure filter units. General chemistry samples were collected in pre-rinsed 250 ml high-density polyethylene (HDPE) bottles and refrigerated upon collection until sent for analysis. Major cations and anions were measured at the New Mexico Bureau of Geology and Mineral Resources Chemistry Lab. Cations were measured using inductively coupled plasma optical emission spectrometric (ICP-OES) techniques, according to EPA 200.7. Anions were measured using ion chromatography (IC) according to EPA 300.0. Duplicates were run on every tenth sample. Cations had reporting limits from 0.0005 mg/L for Strontium to 0.05 mg/L for Calcium, Magnesium, and Potassium. Reporting limits for anions were between 0.1 mg/L for Bromide, Fluoride, Nitrate, and Nitrite, and 1.0 mg/L for chloride and Sulfate.

3.4.3 Benthic Macroinvertebrates

Aquatic habitat metrics and BMIs were collected during a single sampling campaign in summer 2017. Benthic macroinvertebrate sampling was not repeated because of the potential irreversible damage that could be caused by repeated sampling of small springs (Kubíková et al. 2012). At each spring, samples were collected no more than 15 m from the source because spring discharge near the source represents the most stable environment (i.e., constant water chemistry and temperature) for BMI taxa (Glazier 1991, McCabe 1998). We placed 120 cm^2 quadrats, and 10 cm x 12 cm, 500 µm mesh D-frame net along five equally spaced (3m apart) transects to collect a composite of five subsamples from various aquatic microhabitats. We collected each subsample by orienting the quadrat and the mesh net perpendicular to the thalweg, then gently roiling the substrate within each quadrat to release BMIs and allow them to float downstream into the mesh net. Each sample was collected by placing the quadrat at the center, right bank, center, left bank, and center of the springbrook. All samples were preserved in 90 percent ethyl alcohol and delivered to Rhithron Analytical Laboratory, Missoula, Montana, for processing, identification, and enumeration. Crenobiontic taxa were identified to species, other aquatic insects and mites to genus, and ostracodes, tricladida, oligochaetes, and nematodes were identified no further. The structure of BMI communities was determined by identifying and enumerating a minimum of 300 randomly selected individuals, which Vinson and Hawkins (1996) showed was sufficiently large to quantify community structure.

3.4.4 Data Analysis

Hierarchical clustering analysis (HCA), a hard-clustering method commonly employed for partitioning water chemistry data, along with a Piper diagram were used to cluster the 12 springs into geochemical groups. All major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} Cl^{-}, $SO_4{}^{2-}$ and HCO_3^{-}) were included in the clustering analysis. Prior work has shown that traditional processing (i.e., log transformation and standardization) of geochemical data prior to HCA can obfuscate potential geochemical relationships and overweight the magnitude of solutes in relation to the relative proportion of solutes (Dreher 2003). Therefore, each major cation and anion was converted to milliequivalents and then normalized relative to the sum of all cations and anions, respectively (percent meq/kg). These variables expressed as fractions, form the matrix for HCA. HCA was performed in MATLAB using Ward's Linkage and Euclidean distance, the standard approach for water chemistry studies to produce the most distinctive groupings (Templ et al. 2008).

We then used the nonparametric Kruskal-Wallis test to compare the relative abundances of common invertebrate taxa among different geochemical groups. Nonmetric multidimensional scaling (NMDS) was also applied to examine the degree of similarity between the sampled sites according to their BMI community compositions (Clarke and Gorley 2006). Analysis of similarities (ANOSIM) was employed to evaluate the significance of differences among the structure of BMI assemblages in the different geochemical groups that were indicated by NMDS. The tightness of, and differences between, groups were determined using a pairwise test that calculates statistical differences between groups and within each group (R-values) (Clarke and Gorley 2006). A pairwise R-value near zero indicates a strong overlap of two groups, which means the groups barely differ. However, a high R-value (i.e., greater than 0.5) shows completely different (no overlap) characteristics between two clusters (Clarke and Gorley 2006). Furthermore, canonical correspondence analysis (CCA) was used to examine relationships between environmental parameters (water chemistry and physical metrics) and BMI communities. A CCA is generally used to identify the metrics of habitats that are most significant in structuring communities (ter Braak and Verdonschot, 1995). The similarity percentage (SIMPER) method was then applied to identify BMI taxa that are responsible for the similarities within each group and the dissimilarities between groups. This allowed us to determine the role of each BMI taxa in the similarities or differences between two samples (Clarke and Gorley 2006). All multivariate analyses were carried out on the Bray-Curtis resemblance matrix of the fourth root transformed BMI data. All proportionate data were arcsine-transformed before analysis. We also minimized the effect of rare taxa by removing the taxa that occurred in less than 10 percent of the samples. The relative abundances of common invertebrate taxa were used in all statistical analyses; and all taxa in the community were used in creating bar plots and pie charts. Primer v6 was used to calculate NMDS, ANOSIM, and SIMPER (Clarke and Gorley 2006). CANOCO version 4.5 was used to calculate CCA (ter Braak and Verdonschot 1995).

3.5 Results

3.5.1 Spring Environment Data

The spring waters are relatively dilute with specific conductance between 83 and 515 µS/cm, and hardness between 25 to 126 mg $CaCO_3$/l (Table 1). The springs have circumneutral pH (range from 6.8 to 7.9) and moderate cold temperatures (range from 11.2 to 18.7 °C).

The geochemical compositions of spring waters vary across the study area in two primary geochemical groups based on the Piper diagram: sodium-bicarbonate and calcium-bicarbonate waters, with low concentrations of other major cations (Mg^{2+} and K^{+}). Sulfate (SO_4^{2-}) and Si (as SiO_2) were also present in spring waters. Mineral-derived solutes vary considerably throughout the study area, including calcium (9.25 to 44.9 mg/l), magnesium (0.38 to 8.15 mg/l), potassium (0.92 to 4.13 mg/l), and sodium (3.74 to 44.8 mg/l) (Table S1). All spring waters analyzed for general chemistry have charge balance errors less than 5 percent.

Current velocity, channel depth, and wetted width were measured at each sampling location and are in the ranges of 0.0 to 51.4 cm/sec, 1 to 23.4 cm, and 8.30 to 2,210 cm, respectively (Table S2). Environmental differences among springs by different geochemical groupings were not statistically significant ($P<0.05$, Kruskal-Wallis one-way ANOVA) for any parameters (Table S3).

3.5.2 Geochemical Clustering

The spring geochemical groupings from Meyers (2020) were primarily based on solute biplots (Figure 3) and inverse geochemical models. For our analysis, the graphical approach (Piper diagram) is combined with a multivariate statistical technique (e.g., HCA) to examine if these results and geochemical groupings hold in a multivariate analysis of major ions to integrate the geochemical clusters into the ecological results. The resulting dendrogram from the hard clustering of spring water classified the sampled springs into three groups at the linkage distance between 0.3 and 0.46 (Figure 4). Classification of springs into three geochemical groups is a subjective interpretation of dendrogram, and a higher or lower number of groups could also be identified by changing the cutting line to the upper or lower linkage distance values. To further interpret the dendrogram, the groups suggested by the HCA dendrogram are compared with external data and frameworks that are not directly used in calculating the dendrogram (Forina et al. 2002). Thus, all springs are symbolized by the groupings obtained from geochemical biplots (Figure 3) and Meyers (2020) framework. The dendrogram result shows that the cluster on the right (blue squares) corresponds to spring waters in Group 3 with higher Ca^{2+}/Na^{+} ratio, and the cluster on the center (red circles) matches with Group 1 springs. Two springs on the far-left branch of the dendrogram (pink triangles) represent springs in Group 2, samples that are distinguished from other springs by higher sodium and potassium concentrations. Differences between Group 1 and 2 are subtle; springs in these groups have a lower Ca^{2+} and higher Na^{+} concentrations than springs in Group 3. HCA also confirms the overlap between geochemical characteristics of springs in Groups 1 and 2; springs in these two groups can be classified into a single group at higher linkage distances (> 0.46).

Furthermore, the Piper diagram was used to examine the relative concentration of the major ions and the overall chemical character of spring waters within each cluster (Piper 1944). The central diamond plot indicates that sampled springs are largely calcium-bicarbonate waters, but three springs are sodium-bicarbonate type waters (including all springs of the Group 2 and one spring of the Group 1). The two trilinear plots of the Piper diagram confirm (1) the separation between the chemistry of Group 3 waters and the other two groups (cations), and (2) overlap between the water chemistry of Groups 1 and 2 (anions).

Although these three groups are distinct based on their geochemical fingerprints, there is some overlap between the elevation, SPC, pH, hardness, and temperature among springs in different groups (Table 2). Differences between springs (n=12) are not significant for any of these metrics (Kruskal-Wallis one-way ANOVA, $p > 0.05$). Springs in Group 1 show the highest variation among these five parameters. The spring elevations of Group 3 (1486-1614 m) fall into the elevation range of Group 1 (1259-1863m). The temperature was relatively similar in Group 1 and 2, while Group 3 has the lowest temperature of 13.8°C (±0.8 standard error (SE)). The pH is near neutral for all three groups. Springs in Group 2 show the highest value of SPC 471 µS/cm (±44 SE), and SPC values in Group 1 and 3 show overlap in some sites. Waters in Groups 1 and 3 have the highest variation in hardness (Table 2).

3.5.3 Benthic Macroinvertebrates

A total of 70 taxa (including one crenobiontic taxon, *pyrgulopsis wongi*) were identified from 2,300 BMIs collected from all springs. The number of individuals per source spring ranged from 26 to 325 (Mean 173 ± 38 SE), and the number of taxa ranged from 7 to 23 (Mean 12 ± 2 SE). Only 37 taxa were non-rare and occurred in at least two (>10 percent) of 12 springs (Table 3). Pooled BMI data from all springs show that insects (with aerial dispersal ability) are the most diverse group in this study, constituting more than 75 percent of the BMI taxonomic richness. On the other hand, the population (number of organisms) of non-insect taxa (with submerged dispersal ability, like springsnails) is relatively higher than insects in Owens Valley springs and constitutes 67 percent of all individuals (Figure 5). Insects account for around 33 percent of the BMI population, which comprises 39 percent *Optioservus,* 10 percent *Gumaga,* eight percent *Argia,* and six percent *Lepidostoma* (Figure 5). Although the crenobiontic species (*P. wongi*) was absent from four springs (IES038, IES028, IES042, and IES043), it comprises near half of the BMI population in the study area (49 percent; Figure 5). Other non-insect taxa make up around 18 percent of the population; *Hyalella,* Tricladida, *and Pisidum* form more than 94 percent of non-insect taxa group (Figure 5).

3.5.4 Associations between BMIs and Geochemical Classifications

The NMDS analysis is used to examine the degree of similarity between the sampled springs according to their BMI community composition. The springs in the NMDS plot are color-coded based on the three environmental parameters that were highlighted in previous studies as the main controlling factors for the spatial distribution of BMI communities (i.e., specific conductance, temperature, and hardness) (e.g., Glazier 1991, Myers and Resh 2002, Sada and Thomas, *in press*). The NMDS result does not show a relationship between the structure of BMI communities and any of these parameters (Figure 6, A-C). This finding is also supported by CCA (Figure 7).

Relationships between environmental variables and BMI communities are studied by CCA (Figure 7). The manual forward selection and Monte Carlo permutation test of the inter-sample distance were used to examine the significance level of each environmental metric. The result shows that from all environmental parameters (field measurements and major ions) and geochemical signals (Ca^{2+}: Na^{+} and Mg^{2+}: K^{+}), only Ca^{2+}: Na^{+} ratio (p-value = 0.002), and current velocity of spring discharge (p-value = 0.04) are statistically significant factors associating with the structure of BMI communities. Apart from current velocity, the CCA biplot does not show any significant relationship between BMI samples and physicochemical metrics; however, it highlights (Ca^{2+}: Na^{+}), which is a metric associated with water-rock interaction, more specifically presence (high Ca^{2+}: Na^{+}) or absence (low Ca^{2+}: Na^{+}) of metasedimentary roof pendants in the recharge zone for the spring.

Furthermore, the NMDS plot is also symbolized with the associated geochemical classifications to test the hypothesis and investigate the possible association between the spatial distribution of BMI communities and the geochemical composition of spring waters (Figure 6, D). The combination of the NMDS and ANOSIM indicates that the ordination pattern of the BMI community structure in sampled springs follows the same pattern as the geochemical groups (Group 1, Group 2, and Group 3). The NMDS result reveals that the structure of BMI communities in Group 3 differs statistically from the other groups, and springs in Groups 1 and 2 share some common BMI taxa. The result indicates that although insect taxa have the ability to travel between springs and occupy all springs in the study area, they only occupy springs with particular geochemical signatures and create the observed pattern in the NMDS plot. Furthermore, springs within each cluster of similar BMI communities are not necessarily close to one another spatially, but they have similar geochemical characteristics.

The low-stress value (0.105) of the NMDS plot and ANOSIM calculations (Global R=0.74, p-value = 0.001) means that the BMI clustering is tight in each geochemical group. The pairwise R-value of the ANOSIM analysis emphasizes that the structure of BMI communities is similar within the springs in geochemical Group 3 and has a unique BMI community structure compared to the structure of communities in Groups 1 and 2 (pairwise R = 0.98, p-value = 0.048, and pairwise R = 0.82, p-value = 0.008, respectively). The NMDS plot also shows the separation between the structure of BMI communities in Group 1 and 2, and the differences are notable, but not significant in ANOSIM (pairwise R = 0.33, p-value = 0.09). The possible explanation for lack of statistical significance for the separation between Group 1 and 2 in ANOSIM (which is designed for multivariate responses) could be the small sample size (n=2) of Group 2 and/or the high variation in Group 1. However, differences in BMI community compositions between Group 1 and 2 were statistically significant using a nonparametric Kruskal-Wallis test (Kruskal-Wallis H= 9.19, p-value:0.01).

All springs in this study have corresponding BMI samples collected in 2017. The relative abundances of the nine most abundant BMI orders in the three geochemical groups indicate that springs in these three groups are faunistically distinct from one another (Figure 8). Springs in Group 3 are occupied by the highest number of insect taxa, with respect to both richness and population (Table 3). Ephemeroptera, Plecoptera, and Trichoptera collectively constitute more than 35 percent of the relative abundance of BMIs in Group 3, followed by Diptera (25 percent) and Coleoptera (15 percent) (Figure 8, A). *Gumaga* (order: Trichoptera), *Argia* (order: Odonata), *Malenka* (order: Plecoptera), and *Lepidostoma* (order: Trichoptera) are non-rare insect taxa with high populations in springs within this group (Table 3). Although Amphipoda and Bivalvia are absent and Gastropoda constitute only 15 percent of the relative abundance of BMIs in Group 3, these non-insect BMIs form more than 60 percent of the communities in Groups 1 and 2 (Figure 8-A). Crenobiontics are dominant in Groups 1 and 2, constituting 53 percent and 71 percent of the invertebrate communities, respectively (Figure 8-B). However, these spring specialists account for only 15 percent of the BMI community in Group 3. Overall, the result shows that insects are dominant in Group 3 (74 percent), whereas non-insect taxa are dominant in Group 1 and 2, 77 percent and 84 percent, respectively.

Similarity percentage analysis (SIMPER) highlights the differences in assemblage compositions between geochemical groups. The result indicates that the average Bray-Curtis dissimilarity between Groups 1 and 3 is 80.36 (0-100 scale), and the average dissimilarity between Groups 2 and 3 is approximately 89. However, spring in Groups 1 and 2 have a lower degree of dissimilarity (66) between them. The SIMPER analysis also provides information about the contribution of each BMI taxon to the similarity of springs within each group. In Group 1, *Pyrgulopsis* (class: Gastropoda), *Argia* (order: Odonata), and *Pisidium* (class: Bivalvia) comprise up to 70 percent of the similarity between the springs. However, *Lepidostoma* (order: Trichoptera), *Gumaga* (order: Trichoptera), and *Malenka* (order: Plecoptera) are the typical taxa of Group 3. Three BMIs (*Pyrgulopsis* (class: Gastropoda), Tricladida, and *Ochrotrichia* (order: Trichoptera)) explain 100 percent of the similarities of the springs in Group 2.

The results of this study also indicate that geographic distance between springs does not necessarily lead to a higher degree of similarities in BMI communities among springs. For example, four springs on the northern part of the study area with distinct geochemical signatures are located geographically close to one another (distance between springs is around 5 to 8 km, Figure 9). One spring is from Group 1 (granitic-influenced springs) without roof pendant units in its recharge area and has Ca^{2+}/Na^{+} ratios less than 1.0 (IES039). The other two springs are influenced by the roof pendants in their recharge areas and have Ca^{2+}/Na^{+} ratios higher than 1.0 (IES038 and IES033 from Group 3). The last spring in the geographic group discharges from Bishop Tuff (volcanic unit) with a higher concentration of sodium than calcium (IES034 from Group 2). The BMI community compositions of these four springs are statistically distinct, with a high degree of similarity between springs in the same geochemistry group. Results show that despite the geographical proximity of springs, the structure of BMI communities is determined by the geochemical signals of spring waters. Furthermore, springs within each cluster with relatively similar geochemistry and BMI community are not necessarily close to one another spatially.

3.6 Discussion

In previous local-scale studies, geological homogeneity was the main limitation in evaluating the connection between geochemical fingerprints and the structure of BMI communities (e.g., von Fumetti et al. 2006). However, geologic heterogeneity of the eastern Sierra Nevada in Owens Valley provides a unique standpoint to examine the relationship between the structure of BMI communities and geochemical signatures of spring waters in a valley-scale study. Our findings show (1) although felsic plutonic bedrock is abundant, springs that have metasedimentary roof pendants (which include various facies like marble, calc-hornfels, and biotite schist (Moore 1963)) in the high-elevation recharge zones have distinct geochemical signatures compared to the geochemical composition of the remaining springs. The geochemical composition of the remaining springs reflects groundwater interaction primary with the felsic plutonic rock units. These geochemical differences between springs highlight the geologic heterogeneity of the Sierra Nevada as the primary factor driving the geochemical characteristics of mountain front springs in Owens Valley. (2) These weathering-derived geochemical signals are a better predictor of the variation among BMI communities than other environmental variables in persistent aridland springs. For example, springs in Group 3 (influenced by metasedimentary roof pendants) have distinct geochemical fingerprints, and therefore, the degree of similarity between BMI communities is higher within springs in Group 3 than between the springs in other groups. Consequently, we conclude that regardless of the geographical distance between springs, the structure of BMI communities is primarily determined by the geochemical signals associated with surrounding geology.

3.6.1 Geochemical Signature of Spring Waters Control BMI Communities

Previous studies on understanding the controlling factors of BMI communities near the source of spring systems are mostly divided into two main groups: regional-scale studies (springs discharging from different aquifer provinces), or local-scale studies (springs discharging from a single aquifer province). In regional-scale studies, differences in BMI communities have been attributed to factors associated with hydrogeology focusing on EC, temperature, pH, or alkalinity (Glazier 1991, Myers and Resh 2002, Sada and Thomas *in press*). Alternatively, Rader et al. (2012) identified the evolutionary history of the region and dispersal limitations as the governing factors of community distribution. Local-scale studies find no significant correlation between environmental conditions and

BMI community compositions in spring systems (Sada et al. 2005, Stanislawczyk et al. 2018). The results of our study, as a local-scale study, show that the geochemical signature of spring waters has the most influential role in shaping the BMI community compositions in undisturbed springs in Owens Valley, California.

In a valley-scale study on spring systems in the Chihuahuan Desert, Stanislawczyk et al. (2018) identified that the geographical distance between springs and dispersal limitation had a much larger effect on the BMI community composition among desert springs than did environmental metrics. All five sampled springs in their study were occupied by a high abundance of non-insect taxa (gastropods and amphipods) with limited dispersal abilities; thus, increases in spatial distances between two springs raise the degree of dissimilarity between macroinvertebrate community compositions (Stanislawczyk et al. 2018). In contrast, our ecological analysis indicates that springs with geographic proximity do not necessarily have similar BMI community compositions. For example, some springs in Group 3 (influenced by roof pendant) are not spatially close to one another, and there are some springs with distinct geochemistry and BMI communities in between springs of Group 3 in the study area. But because springs in this group have similar geochemical characteristics, they all are occupied by a particular group of taxa (insects). Moreover, these insect taxa with an aerial dispersal ability can travel between springs and occupy all springs, but they are absent or occurred with low densities in other springs. Thus, our findings suggest that geochemical characteristics of spring waters, which depend heavily on the surrounding geology, may better explain the structure of the BMI communities than dispersal limitation or geographic distance (distance–decay relationship).

The findings reported here can also address some knowledge gaps in the previous regional-scale studies. For example, Myers and Resh (2002) classified the 28 Great Basin springs into three main groups based on temperature and elevation. They found that BMI communities in warm-water springs (15.9 °C with low elevation 1,794 m) were different from cold springs (< 8.5 °C with elevation > 2,300 m). Myers and Resh (2002) found that amphipods (*Hyalella)* and gastropods (hydrobiid) were the two most abundant groups in warm springs, but these groups were absent from the cold springs, thus exerting a strong influence on the classification of springs based on BMIs. All, but one of the springs investigated in our study (Temp >11 °C with elevation 1495 ±53 m (Mean ±SE)) would be classified as warm water springs according to Myers and Resh (2002) (IES039 is higher than 1,794 m). The average temperature of the studied springs is 14.8 ±0.6 (Mean ±SE), which is closer to the temperature for the warm springs in Myers and Resh study. Based on the findings of Myers and Resh (2002), Owens Valley springs sampled for our study should be dominated by gastropods and amphipods, but these taxa were absent from all the springs in Group 3 except one (IES033). Consequently, we propose that elevation and water temperature could be the controlling factors in regional-scale studies (Sada and Thomas, *in press*), but in a valley-scale study, the geochemical characteristic of the spring water is the most significant factor defining the distribution of BMI taxa in aridland springs. Although our finding highlights the importance of geochemistry in determining patterns of BMI communities, other factors (e.g., past disturbances or dispersal limitations) can also play a role in the absence of non-insect taxa (e.g., gastropods) from some springs (Sada and Vinyard 2002, Rader et al. 2012).

Moreover, in another regional-scale study on temperate cold springs in North America, Glazier (1991) proposed that insect taxa tend to live in soft acidic water (low alkalinity), while gastropods and amphipods dominate in hard waters (they did not specify the range for their definition of soft versus hard waters). The spatial distribution of BMI communities in our study does not support Glazier's (1991) hypothesis. Three springs in Group 3 (influenced by metasedimentary roof pendants) have high hardness (>80 mg $CaCO_3$/l) with pH values in the range of 7.2 to 7.8 (not acidic), but insect taxa are dominant in these springs, and gastropods are absent. Additionally, although IES039 spring in Group 1 has the lowest hardness (25 mg $CaCO_3$/l) among all springs in all groups, gastropods and amphipod assemblages are dominant in this spring, similar to other springs in this group. Results from our study highlight the Ca^{2+}: Na^{+} ratio (an indicator of geochemical separation of spring waters based on mineral dissolution along the flowpath) as a better predictor of the spatial distribution of BMI communities than hardness (alkalinity), pH, or other physicochemical variables.

In the mountain-front springs in Owens Valley, CA geochemical characteristics and physicochemical stability of springs associated with granitic and volcanic geological units provide a favorable habitat for non-insect taxa (i.e., gastropods and amphipods). Although environmental parameters (e.g., temperature, calcium concentration, and hardness) may limit the gastropod distributions, their role in explaining the relative abundances and densities of gastropods in most freshwater habitats is challenging (Malcom et al. 2005, Brown and Lydeard 2010). For example, most gastropods are restricted to freshwater habitats with a calcium concentration of more than 3 mg/l; moreover, freshwater snails can handle a broad range of temperatures (0-40 °C), but they grow and reproduce faster at higher temperatures (Brown and Lydeard 2010). Malcom et al. (2005) showed a positive association between springsnails (hydrobiid) densities and water temperature of 14-22 °C in one spring in southeastern Arizona. Amphipods (*Hyalella*) also tend to live in warmer waters with neutral pH (Covich et al. 2010). Thus, the high density of non-insect invertebrates within springs in Groups 1 and 2 can be associated with the ecophysiological responses of non-insect BMIs to the geochemical characteristics and environmental conditions of springs in these groups.

Biological interactions between insects and non-insect taxa may also play a role in shaping the BMI communities in aridland springs. Regarding intense resource competition between non-insect and insect BMIs, and predation of unprotected eggs and dormant larvae of insects by non-insect BMIs (Macan 1977, Glazier 1991, Cantonati et al. 2006), insect taxa may be excluded from habitats with a high density of non-insect taxa. They may instead colonize habitats (Group 3) with a lower population of amphipods, gastropods, and flatworms.

Alternatively, geochemistry, temperature, and environmental condition of spring waters in Group 3 (influenced by metasedimentary roof pendants) may provide a favorable habitat for insect taxa. All insects are aerobic organisms and cannot complete their life cycle without oxygen; they prefer habitats with higher dissolved oxygen concentrations. Since oxygen solubility is temperature-dependent and solubility increases as water temperature decreases, most aquatic insects prefer cool water temperature (Hershey et al. 2010a). Temperature also may have a direct impact on insects by regulating their metabolic rates (Hershey et al. 2010a). Springs in Group 3 have cooler temperatures because the metasedimentary units at the recharge area of these springs allow a contribution of cold groundwater with short residence time (<70 years) to the spring discharge (Meyers 2020). Additionally, previous studies on cold springs showed that insect taxa are dominated in springs in glaciated regions. The higher Ca^{2+} concentration in Group 3 spring waters (from the roof pendants) may act similarly to higher Ca^{2+} in cold-springs influenced by the glacial zones; thus creating favorable habitat for insect taxa (Williams and Williams 1998, Fureder et al. 2001). Although it is well known that extremes in water chemistry metrics (i.e., high salinity or very low pH) alter insect communities, little is known about physiological adaptations of insect taxa to various geochemical signals. The structure of BMI communities in the spring systems of our study is probably tied to the combination of these ecological, physiological, and hydrological factors.

The results of this study are only valid in undisturbed/naturalized (reference) aridland springs. Previous studies highlighted the role of disturbances in changing the BMI community compositions in spring systems (Sada and Nachlinger 1996, Sada and Vinyard 2002, Keleher and Rader 2008). Sada and Nachlinger (1996) showed decreases in total richness and increases in abundance of tolerant taxa in disturbed springs in the Spring Mountain (southwestern Great Basin). Another study on eastern Great Basin springs showed a distinction between the BMI community composition of undisturbed and severely disturbed springs (Keleher and Rader 2008). In our research, North Fuller Spring (IES041) was sampled and categorized as a moderately disturbed spring (with evidence of livestock disturbances). We removed this spring (IES041) from our ecological interpretations because it hosts an entirely distinct BMI community compared with other springs (Figure S1). One possible explanation for this distinction could be that the effect of disturbance on BMI communities of this spring was significant enough to overwhelm the impacts of geochemical signals, leading to the development of dissimilar BMI communities. Since this interpretation is made based on only one disturbed spring, further research is necessary to examine the impact of livestock disturbances on mountain front spring systems.

Developing the field of ecohydrogeology by enhancing the collaboration between ecological and hydrogeological disciplines may enhance the efficiency of the management and monitoring of groundwater-dependent ecosystems. In the big picture, this study provides insight into the relationship between hydro-geochemistry and ecology in groundwater-dependent ecosystems. The geology of the recharge area and geology of the aquifers along a flowpath control the geochemical characteristics of the spring systems, and the subsequent geochemical signals of the spring waters influence the structure of benthic macroinvertebrate communities. In terms of detail, this valley-scale study shows how geologic heterogeneity in the Sierra Nevada results in distinct geochemical groupings of mountain front spring waters, which plays a significant role in determining the spatial distribution of insect and non-insect taxa communities in reference aridland springs in Owens Valley, California. This relationship, based on our results on this local scale study, suggests that springs with similar geochemical fingerprints are occupied by relatively similar BMI communities. Therefore, the relative abundances of BMI taxa can be used as a proxy for geological heterogeneity and water-rock interaction in persistent aridland springs. However, further studies on BMI communities of mountain front springs are needed to better understand this relationship and to make a definitive conclusion.

3.7 Figures

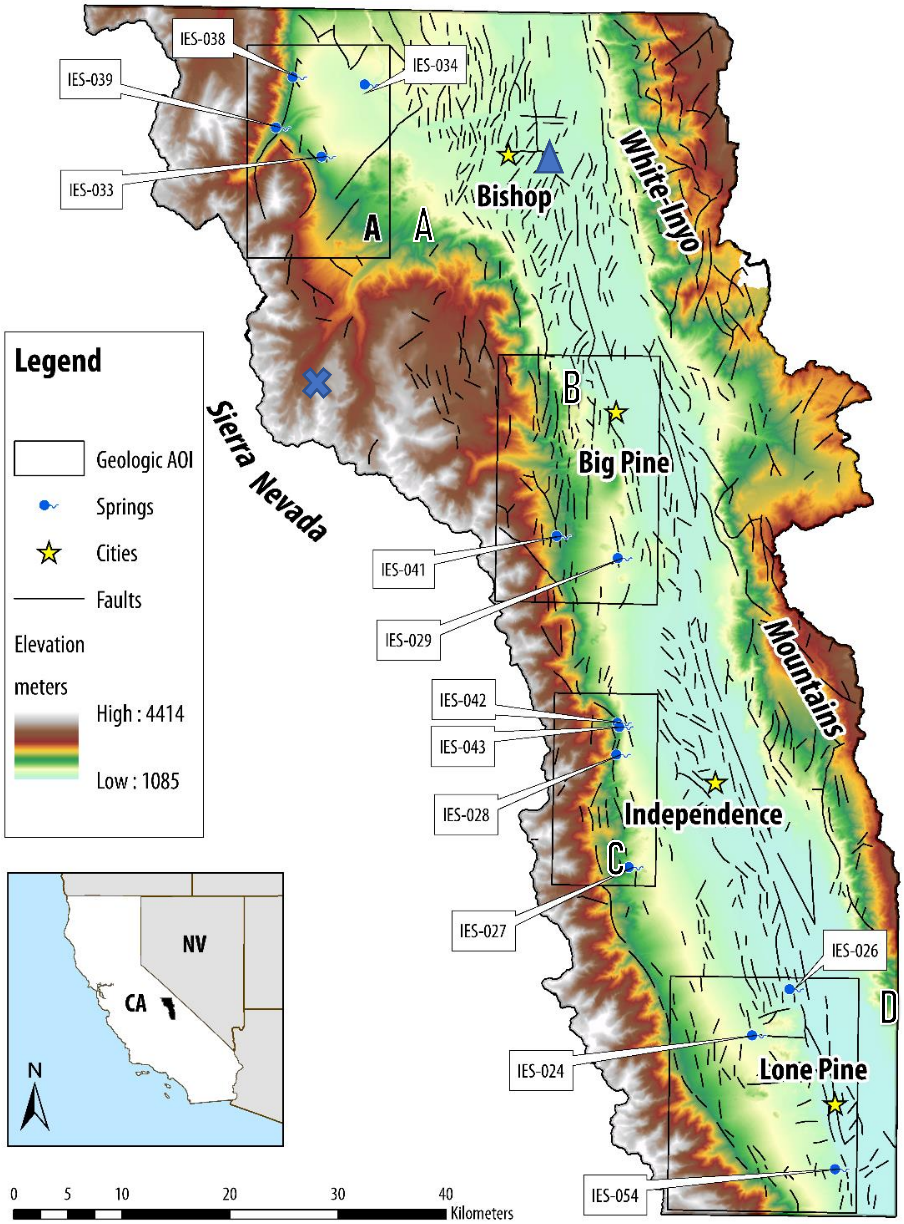

Figure 3.1. Study area boundary in Owens Valley. Blue circles indicate springs that were sampled: ***IES 038***- Wells Meadow B Spring; ***IES 039***- Unnamed Pine Creek Spring; ***IES 033***- Elderberry Canyon Spring; ***IES 034***- Birchim Canyon Spring B; ***IES 041***- North Fuller Spring; ***IES 029***- Unnamed Spring north of Red Mountain; ***IES 043***- South Harry Birch Spring; ***IES 042***- North Harry Birch Spring; ***IES 028***-Grover Anton Spring; ***IES 027***- Boron Spring A; ***IES 026***- Reinhackle Spring; ***IES 024***- Hogback Creek Spring; ***IES 054***- Lubken Canyon Spring 2. Blue triangle shows NOAA precipitation stations at the Bishop Airport. Blue cross shows the location of Lake Sabrina at 2800 masl elevation.

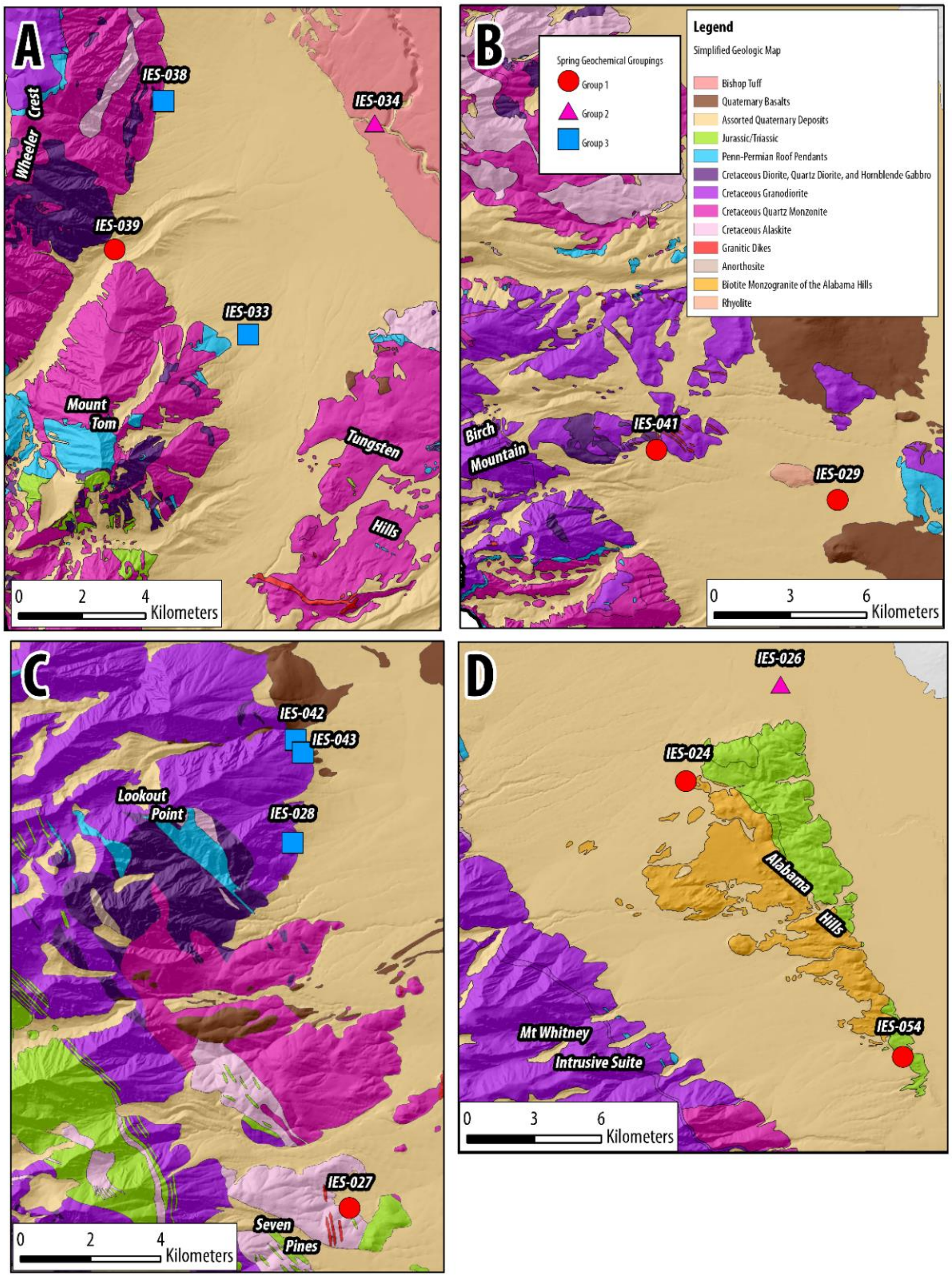

Figure 3.2. Detailed geologic map of spring contributing areas for 12 sampled springs in four different geologic areas of interests (AOI) in the study area. The AOI sections (A, B, C, and D) are indicated in Figure 1. This figure is adapted from Meyers (2020). (A) AOI of Bishop, CA. (B) AOI of Big Pine, CA. (C) AOI of Independence, CA. (D) AOI of Alabama Hills, west of Lone Pine, CA. Springs are symbolized based on their geochemical groupings.

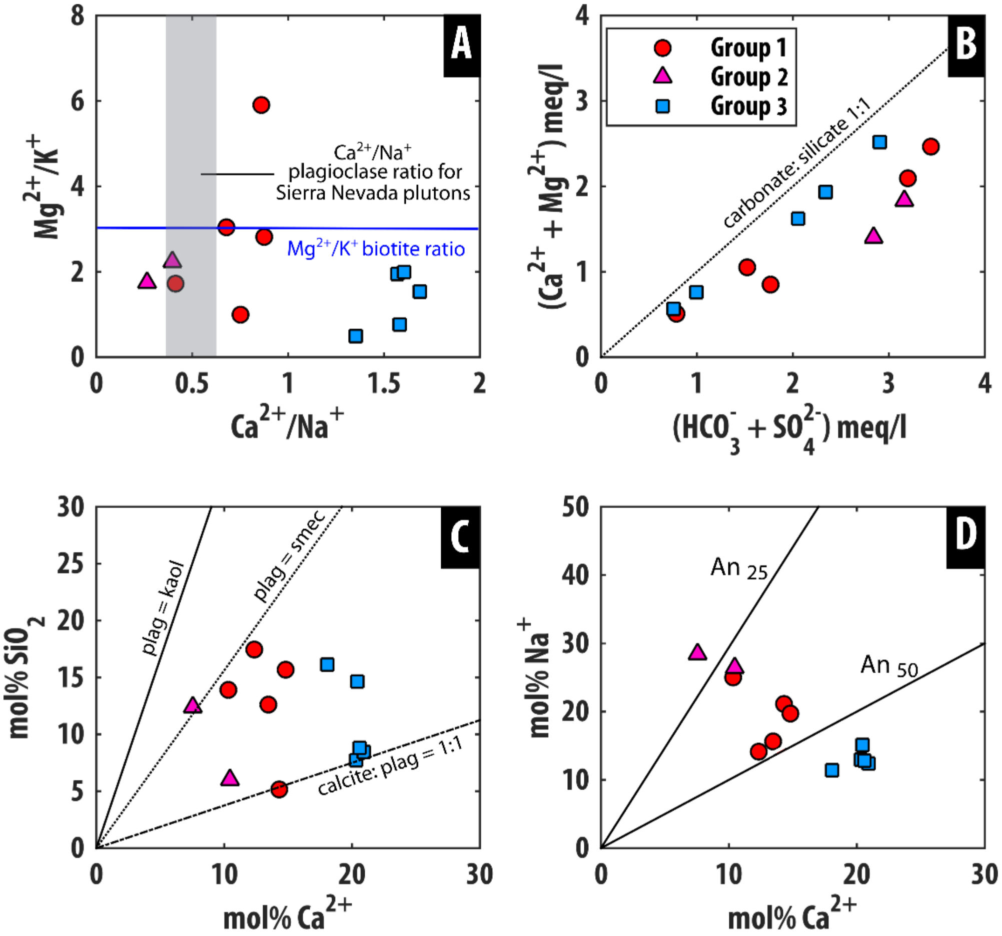

Figure 3.3. Geochemical relationships between sampled springs. This figure is adapted from Meyers (*2020*). (A) Molar ratio plot showing Ca^{2+}: Na^{+} ratio versus Mg^{2+}: K^{+} ratio of the sampled springs in Owens Valley. (B) Dominant cations (Ca^{2+} and Mg^{2+} meq/l) versus anions (HCO_3^- and SO_4^{2-} meq/l). All springs clustered below the 1:1 line highlight the governing of silicate weathering. C) Relationship between mol % SiO_2 and mol % Ca. Solid line: predicted weathering trend of plagioclase to kaolinite. Dotted line: predicted weathering trend of plagioclase to smectite. Dashed and dotted line: predicted trend of both calcite dissolution and plagioclase to smectite weathering in a 1:1 ratio. D) Composition of spring water of mol % Na^{+} versus mol % Ca^{2+}. Solid lines indicate a range of plagioclase dissolution for the Sierra Nevada. Group 3 waters are plotted on the high mol % Ca^{2+} side of the plot, which suggests an excess calcium dissolution. Plots C and D adapted from Pretti and Stewart (2002).

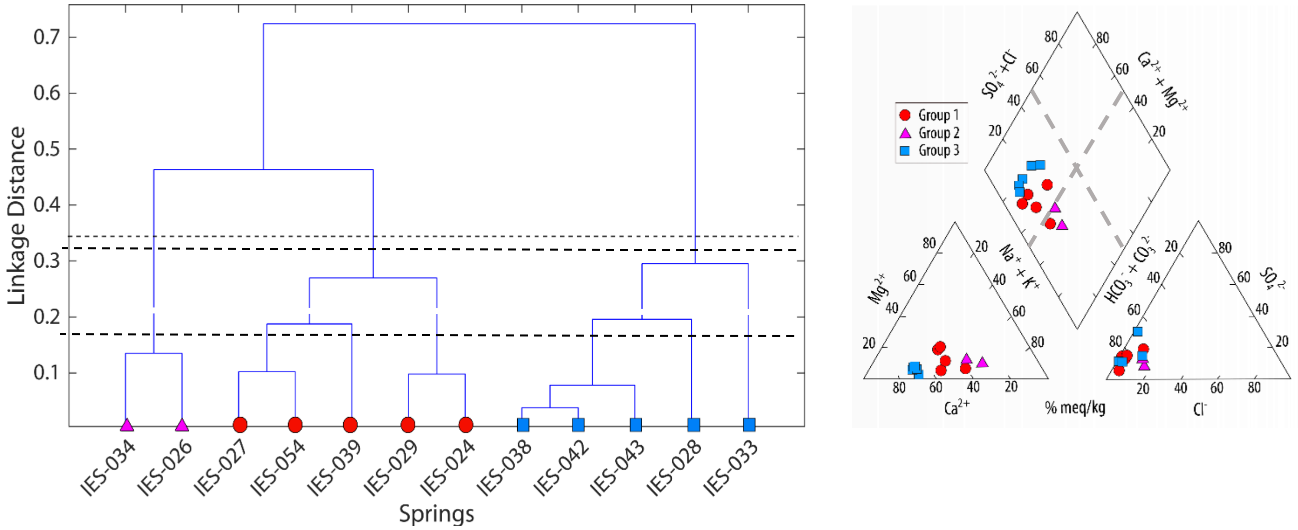

Figure 3.4. Left: Dendrogram from the hierarchical clustering analysis for the 12 spring waters. The dashed lines represent the range of linkage distances (0.3-0.45) for classifying the springs into three clusters. Right: Piper diagram displays the geochemical composition of spring waters. Dashed lines in the diamond plot represent 50/50 lines and divide the plot into four quadrants to determine water types. Springs are symbolized by geochemical groupings.

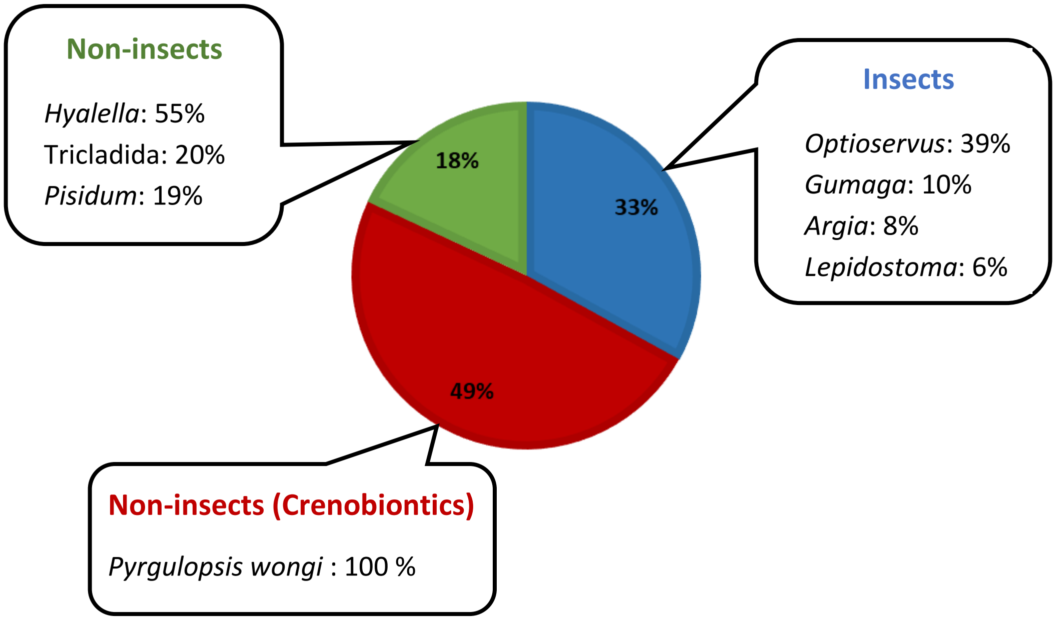

Figure 3.5. Proportion of common insect, non-insect, and crenobiontic taxa in all springs. Relative abundances of benthic macroinvertebrate taxa in each group.

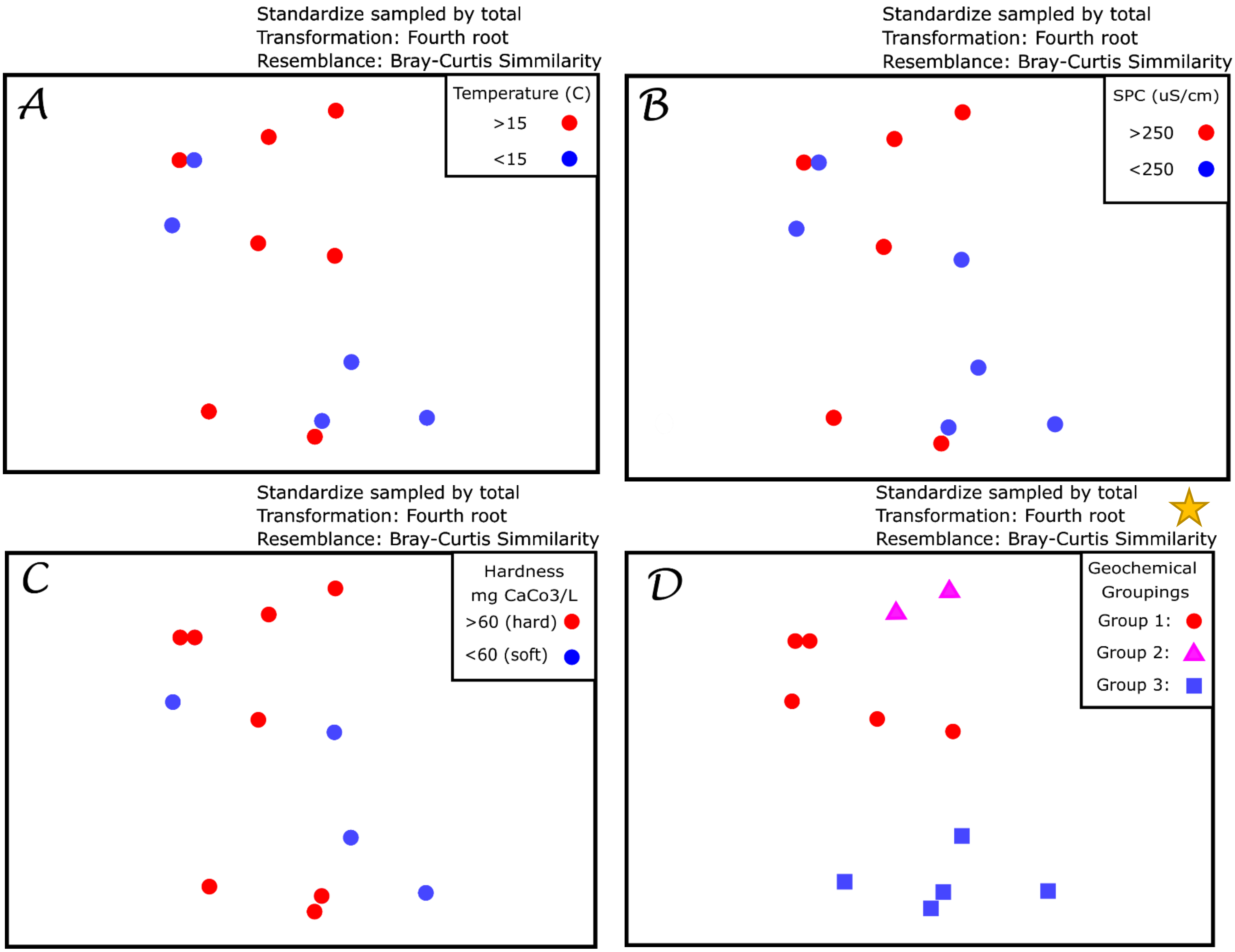

Figure 3.6. NMDS analysis of reference springs in Owens Valley based on their BMI community structure (2D stress value= 0.105). Bray-Curtis similarity was used for this analysis. NMDS was applied to cluster the springs based on the similarity of their BMI community structure. Springs are color-coded according to the A) **Temperature**: <15°C is the cutoff temperature for Mountain Springs in Sada and Thomas (*in press*), and 15.9 °C was a cutoff point for the warm water springs in Myers et al. (2002). B) **Specific Conductance**: Sada and Thomas (*in press*) identified EC value 200 µmhos for Mountain Springs with temperature <15°C, We used the Specific conductance (at 25°C) = (EC/ 1+ 0.0191 * (T-25)) equation and calculated SPC cutoff value of 250 µS/cm, C) **Hardness**: the cutoff point (60 mg $CaCO_3$/l) is justified based on figures in Glazier (1991) and USGS definition of soft vs. hard water "https://www.usgs.gov/special-topic/water-science-school/science/hardness-water?qt-science_center_objects=0#qt-science_center_objects"). Springs are also symbolized based on D) **Geochemistry Groups**: Group classifications are shown in Figures 3 and 4 in this study. ANOSIM results show global R =0.74, P= 0.002; pairwise R-value 0.98 with a significance level of 0.04 between Group 3 versus Group 2; pairwise R-value 0.82 with a significance level of 0.008 between Group 3 versus Group 1; pairwise R-value 0.33 with a significance level of 0.09 between Group 2 versus Group 1.

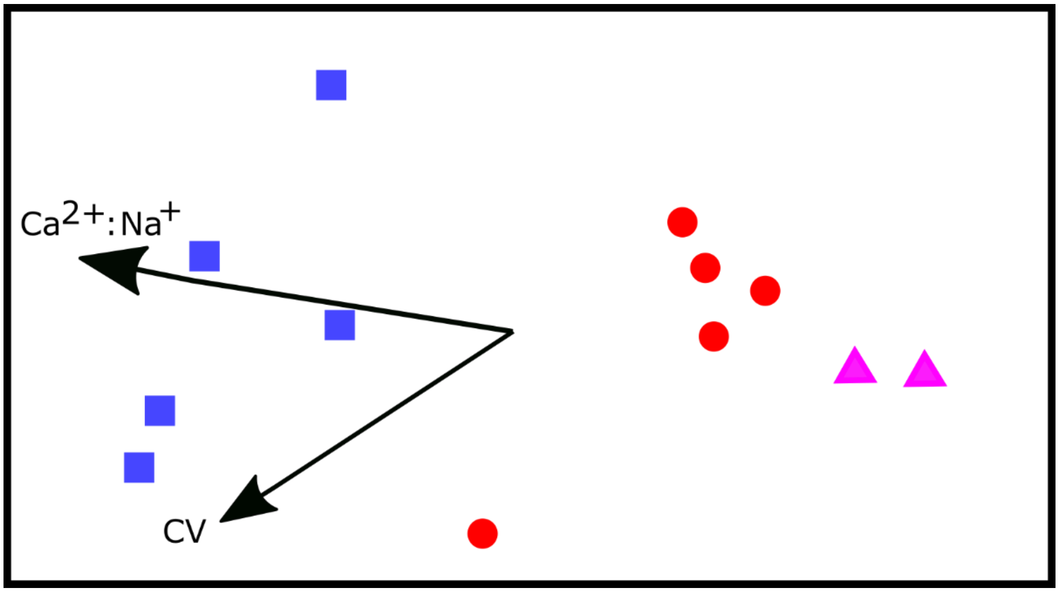

Figure 3.7. CCA biplot of BMI community structure and two statistically significant ($p < 0.05$) environmental variables as determined by CCA (inter-sample relationships). Red circles represent springs in Group 1. Pink triangles represent springs in Group 2. Blue squares represent springs in Group 3.

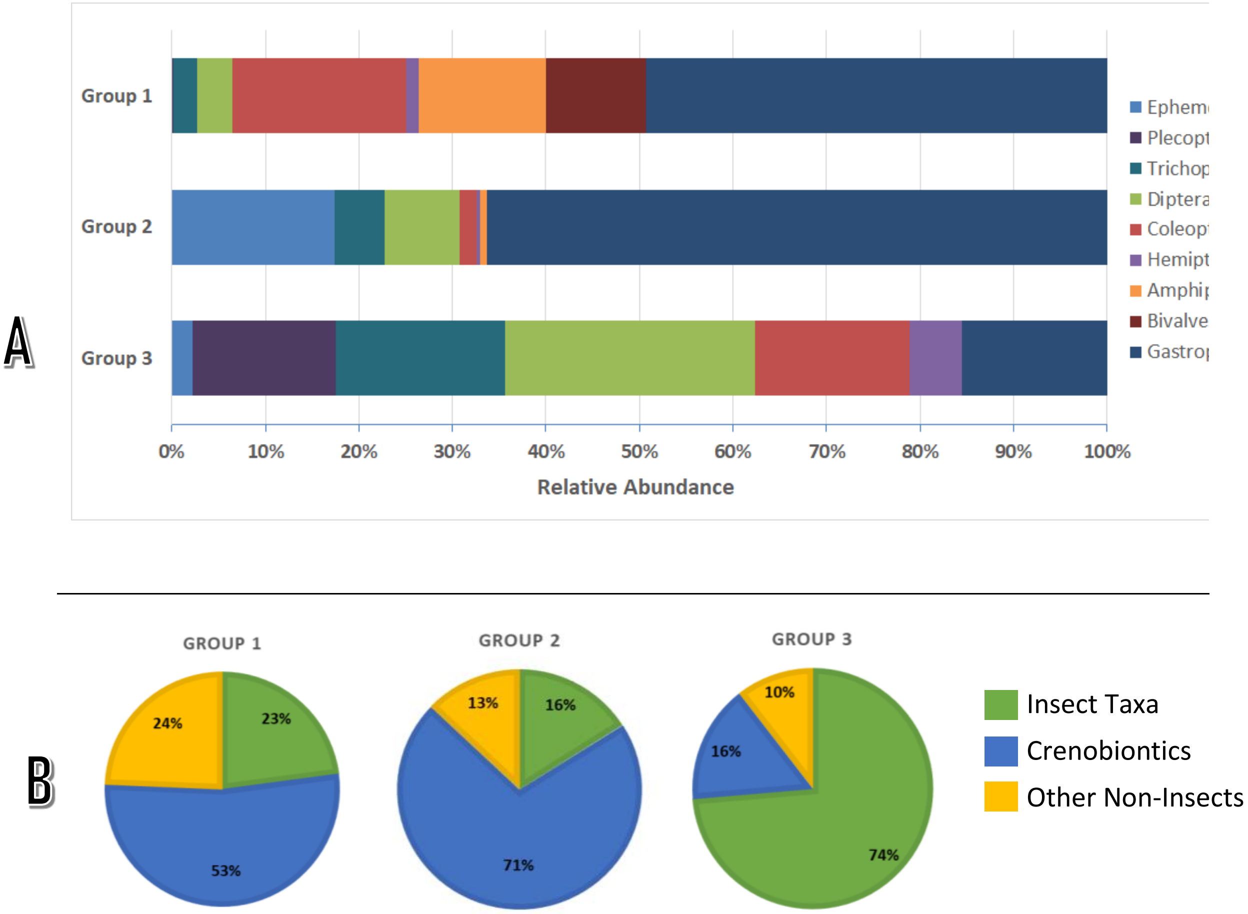

Figure 3.8. (A) Bar plot: Relative abundances of main benthic macroinvertebrates (at order or class taxonomic levels) in each group. (B) Pie chart: Relative abundances of crenobiontics, non-insects, and insects in sampled springs in each group.

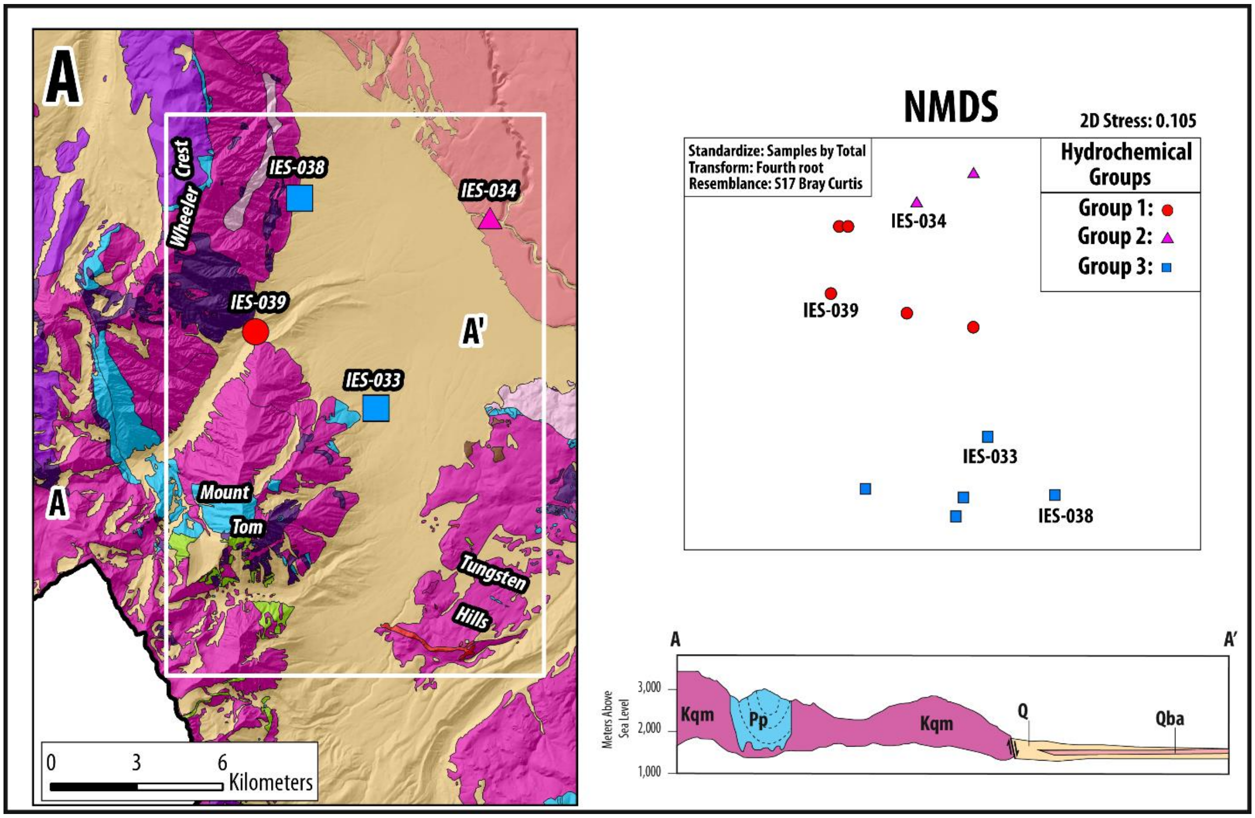

Figure 3.9. An example: geological heterogeneity better explains the pattern of BMI communities than geographical proximity. Figure on the left: Simplified geologic units of the area on the northern part of the Valley (see Figure 1 for the location of the area A); Red circle represents IES039 in Group 1 (influenced from granitic rock units). Blue squares represent IES033 and IES038 from Group 3 (influenced by granitic rocks and metasedimentary roof pendant units). Pink triangle represents IES034 in Group 2 (influenced by volcanic units [Bishop Tuff]). Right-Below: Simplified Geology of cross-section A-A' at section A. Right-Up: NMDS analysis of springs in Owens Valley based on their BMI community structure. Springs are symbolized based on the geochemical groups.

3.8 Tables

Table 3.1. Spring ID, Name, location, main field measurements, and geochemical classification of springs

Spring ID	Spring Name	UTM 11 S		Elev.	Temp.	SPC	pH	Hardness	Groups
		E	N	(m)	°C	µS/cm		mg $CaCO_3$/l	Geochemical Classification
IES 054	Lubken Canyon Spring 2	375010	4152152	1259	17.2	176	6.8	42.6	**1**
IES 024	Spring along Hogback Ck. A	397732	4056750	1393	18.7	397	7.1	123	**1**
IES 027	Boron Springs A	386328	4072314	1601	15.2	470	7.9	105	**1**
IES 029	Unnamed spring north of Red Mountain	385305	4100901	1417	15.6	273	7.8	52.7	**1**
IES 039	Unnamed Pine Creek Spring	353679	4140727	1863	13.5	83	7.3	25	**1**
IES 034	Birchim Canyon Spring	364114	4144865	1482	16.3	427	7.7	70.2	**2**
IES 026	Reinhackle Spring	401193	4061012	1144	17	515	7.5	91.6	**2**
IES 038	Wells Meadow B	355222	4145412	1614	11.2	66	7.8	28.2	3
IES 028	Grover Anton Spring	385165	4082719	1593	15.6	480	7.2	126	3
IES 033	Elderberry Canyon Spring	357900	4138046	1596	12.7	165	7.8	38	3
IES 042	North Harry Birch Spring	385273	4085645	1486	14.3	192	7.7	81.2	3
IES 043	South Harry Birch Spring	385440	4085284	1497	15.3	244	7.4	96.6	3

Table 3.2. Mean (± SE) of elevation, specific conductance (SPC), pH, temperature, and hardness for each group. Differences between geochemical groups are not significant for any of these metrics (nonparametric Kruskal-Wallis, $p > 0.05$).

Metrics	**Group 1**	**Group 2**	**Group 3**
Elevation - m	1507 (±104.4)	1313 (±169)	1557 (±27)
SPC - µS/cm	280 (±70.5)	471 (±44)	229 (±69)
pH	7.4 (±0.2)	7.6 (±0.1)	7.6 (±0.1)
Temp - °C	16 (±0.9)	16.7 (±0.4)	13.8 (±0.8)
Hardness – mg $CaCO_3$/l	69.8 (±18.7)	80 (±10.7)	74 (±18)

Table 3.3. Population of BMI taxa that occurred in at least two source samples (>10%) of the 12 sampled springs. BMI population within each geochemical group is also shown.

Order or (Class)	**BMI taxa**	**BMI population (# of springs)**	**Group 1**	**Group 2**	**Group 3**
Ephemeroptera	*Baetis*	2 (2)	0	0	2
	Ironodes	2 (2)	0	0	2
Plecoptera	*Malenka*	25 (5)	1	0	24
	Hesperoperla	5 (2)	0	0	5
Trichoptera	*Hydropsyche*	7 (3)	6	0	1
	Hydroptila	9 (3)	7	2	0
	Ochrotrichia	13 (3)	6	6	1
	Lepidostoma	29 (8)	5	0	24
	Gumaga	54 (4)	8	0	46
Coleoptera	*Optioservus*	215 (6)	199	10	6
	Zaitzevia	18 (2)	18	0	0
Diptera	*Rheotanytarsus*	5 (3)	3	1	1
	Brillia	5 (3)	0	0	5
	Cricotopus (distinct)	9 (2)	5	4	0
	Eukiefferiella	8 (3)	1	0	7
	Metriocnemus	5 (3)	4	1	0
	Orthocladius	3 (2)	0	2	1
	Parametriocnemus	14 (2)	0	0	14
	Tvetenia	2 (2)	0	0	2
	Brundiniella	6 (3)	4	0	2
	Larsia	3 (2)	2	0	1

	Pentaneura	14 (2)	0	0	14
	Thienemannimyia grp.	10 (2)	1	0	9
	Zavrelimyia	5 (2)	5	0	0
	Dixa	4 (2)	3	0	1
	Simulium	23 (5)	1	10	12
	Tabanidae	4 (2)	0	0	4
	Tipula	3 (3)	1	0	2
Odonata	*Argia*	44 (8)	11	1	32
Gastropoda (Class)	*Pyrgulopsis wongi*	1044 (8)	691	297	57
Amphipoda	*Hyalella*	211 (4)	207	4	0
Bivalvia (Class)	*Pisidium*	72 (3)	72	0	0
Arachnida (Class)	*Arrenurus*	4 (2)	1	0	3
Tricladida	*Order Tricladida*	75 (5)	25	50	0
Nematoda	*Phylum Nematoda*	2 (2)	1	0	1
Lumbriculida	*Lumbriculidae*	2 (2)	1	0	1
Haplotaxida	*Enchytraeidae*	17 (3)	0	0	17

3.9 Supplementary Information

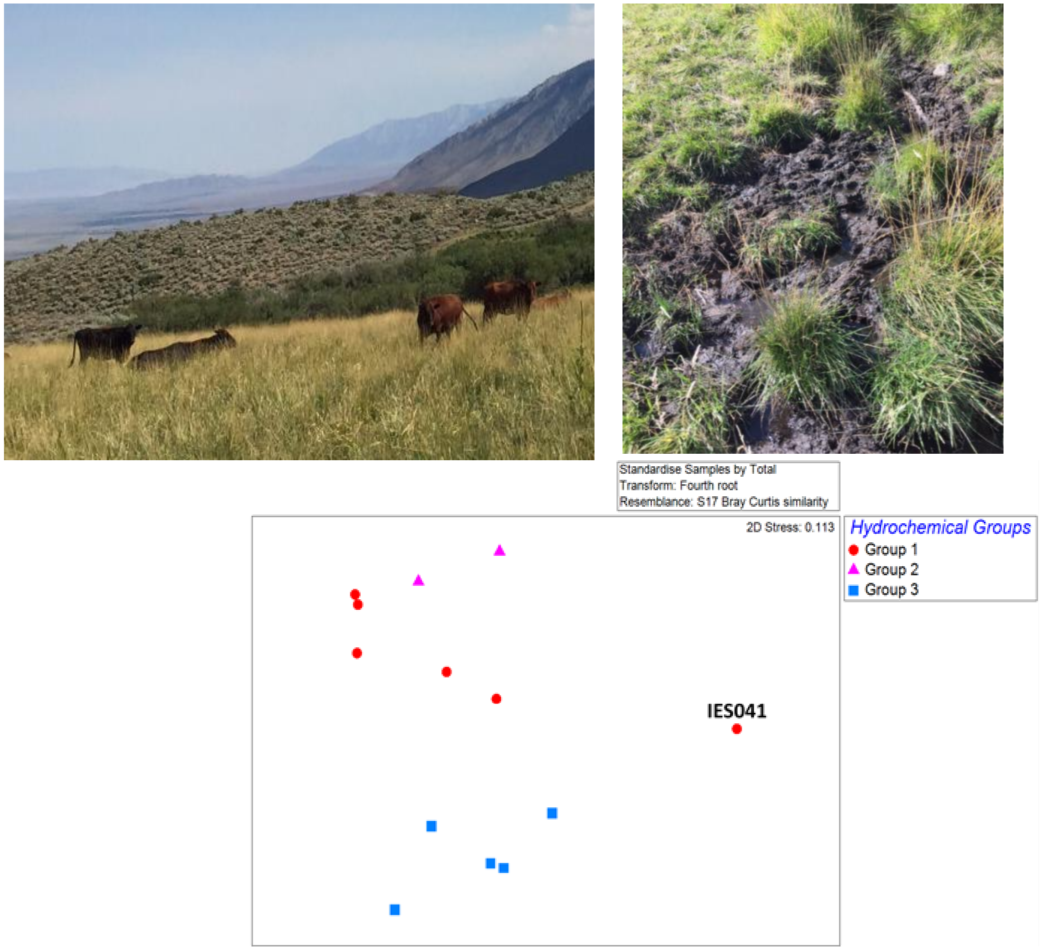

Figure 3.S1. Evidence of recent livestock disturbance at the North Fuller Spring. (A) Presence of livestock (cattle) in the area. (B) evidence of Livestock disturbance at the lower part of the spring run. (C) NMDS analysis of springs in Owens Valley based on their BMI community structure (2D stress value= 0.105). Bray-Curtis similarity was used for this analysis. NMDS was applied to cluster the springs based on the similarity of their BMI community structure. IES 041 is a moderately disturbed spring (North Fuller Spring).

Table 3.S1. Major geochemistry data of Owens Valley springs collected in spring 2016 and 2017. All metrics used in an initial CCA. Mean (±SE) for each group.

Spring ID	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Sr^{2+}	Cl^{-}	NO_3^{-}	NO_2^{-}	PO_4^{3-}	SO_4^{2-}	$CaCO_3$	HCO_3^{-}	Balance	Si as SIO2	DO	DO
							Nutrient									
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	[%]	mg/l	mg/l	%
IES 054	19.7	3.5	2.82	17.9	0.284	5.48	ND*	ND	ND	12.8	75	91	4.9	35.4	7.9	83
IES 024	36	8.15	2.22	24	0.411	5.52	2.71	ND	ND	9.33	162	198	-1.06	50.6	6.8	72.9
IES 027	33.9	4.88	2.58	28.7	0.174	13.6	2.01	ND	ND	32.4	126	154	-2.93	18.3	6.3	63
IES 029	14.9	3.76	2.15	9.77	0.098	1.9	0.74	ND	ND	9.51	66	81	-2.08	31.6	5.8	58.8
IES 039	9.25	0.57	0.92	7.06	0.026	0.44	ND	ND	ND	5.49	34	41	0.46	14.7	3.5	33.8
IES 041	32.9	8.45	1.84	18	0.204	2.25	0.19	ND	ND	2.82	157	191	-1.65	33.4	2.7	25.4
Group 1	**24.44 (±4.6)**	**4.89 (±1.2)**	**2.0 (±0.3)**	**17.57 (±.3.4)**	**0.20 (±0.1)**	**4.87 (±1.9)**	**1.41 (±0.6)**	---	---	**12.1 (±4.3)**	**103.33 (±21.5)**	**126.0 (±25.3)**	---	**30.67 (±5.3)**	**5.50 (±0.8)**	**56.15 (±9.1)**
IES 034	20.7	4.48	4.13	44.8	0.108	19.4	2.35	ND	ND	13.6	128	156	-0.17	51	6.6	70
IES 026	27.4	5.65	4.07	39.7	0.195	16.9	0.52	ND	ND	22.8	134	164	-0.24	23.6	6.7	69.8
Group 2	**24.05 (±3.4)**	**5.07 (±0.6)**	**4.10 (±0.03)**	**42.25 (±2.6)**	**0.15 (±0.04)**	**18.15 (±1.25)**	**1.44 (±0.92)**	---	---	**18.20 (±4.6)**	**131.00 (±3.0)**	**160.00 (±4.0)**	---	**37.30 (±13.7)**	**6.65 (±0.1)**	**69.90 (±0.1)**
IES 038	10.3	0.62	1.3	3.74	0.031	0.28	0.76	ND	ND	4.1	34	41	-2.75	13.8	7.6	69.6
IES 028	44.9	3.38	2.79	16.4	0.188	13.7	0.84	ND	ND	23.8	121	147	-0.45	25.6	7.2	72.3
IES 033	14.6	0.38	1.27	6.19	0.053	0.68	1.34	ND	ND	14.6	34	42	0.55	15.7	8.7	81.8
IES 042	29.7	1.71	1.79	10.1	0.129	1.87	0.54	ND	ND	15.2	87	106	-0.99	18	5.4	53
IES 043	34.7	2.43	1.96	12.4	0.164	2.43	0.11	ND	ND	12.5	104	127	1.68	22.3	2.9	29.5
Group 3	**26.84 (±6.4)**	**1.70 (±0.56)**	**1.82 (±0.28)**	**9.77 (±2.3)**	**0.11 (±0.03)**	**3.79 (±2.5)**	**0.72 (±0.2)**	---	---	**14.04 (±3.15)**	**76.00 (±17.9)**	**92.60 (±21.85)**	---	**19.08 (±2.16)**	**6.36 (±1.0)**	**61.24 (±9.2)**

*ND: Not detected, the value was below the detection limit.

*All spring waters analyzed for general chemistry have charge balance errors less than 5%

Table 3.S2. Median and range of environmental (physical) metrics. These metrics used in an initial CCA.

Metrics	**Units**	**Median (Range)**
Wetted Width (WW)	cm	90.8 (8.30-2210)
Water Depth	cm	2.4 (1.0-23.4)
Current Velocity (CV)	cm/sec	9.7 (0.0-51.4)
Head Cover	proportion	1.0 (0.5-1.0)
Bank Cover	proportion	1.0 (0.8-1.0)

Table 3.S3. Mean (+1 SE) of measured and estimated environmental characteristics in springs among geochemical groups. Differences between geochemical groups are not significant for any of these metrics (nonparametric Kruskal-Wallis, $p > 0.05$).

Metrics	**Group 1**	**Group 2**	**Group 3**
Wetted Width (WW)	175.4 (51.6)	1184 (1025)	63.6 (14.4)
Water Depth	5.2 (2.4)	12.3 (11.1)	4.6 (1.8)
Current Velocity (CV)	13.3 (9.5)	4.9 (1.8)	31.8 (10.7)
Head Cover	0.9 (0.01)	0.95 (0.05)	0.97 (0.03)
Bank Cover	0.98(0.02)	1.0 (0.0)	1.0 (0.0)

Table 3.S4. General taxonomic information for each group. Mean (±1 SE)

Spring Group	Taxonomic richness	Mean no. of taxa per spring	Mean number of individuals per source sample
Group 1	49	16 (±2.2)	262±39
Group 2	27	17 (±6.5)	209±115
Group 3	65	25 (±0.9)	150±25

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Chapter 4

Water Chemistry, Macro- and Micro-fauna in Isolated Aridland Springs: Are There Similar Patterns in the Biological Organization?

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4.1 Abstract

There is increasing interest in multi-disciplinary studies to understand the relationships between hydrological and ecological processes in groundwater-dependent ecosystems. In this study, we examined the relationship between the ecology of aridland springs in Owens Valley, California, and the organization of their benthic macroinvertebrate (BMI) and microbial communities. We also identified the potential factors shaping BMI and microbial communities. Eleven mountain front springs and one regional spring were sampled to characterize their BMI, microbial, and water geochemistry compositions. All sampled springs were classified as “reference springs,” which are springs that have either not been disturbed or have naturalized from past human or natural disturbances.

A free ordination tool, nonmetric multidimensional scaling (NMDS), was used to compare the community assembly of both biological datasets. A Mantel test and co-occurrence heatmap were also used to calculate and visualize the correlation between the two datasets (BMI and microbial community compositions). We found similar ordination patterns and a significant relationship between BMI and microbial communities in undisturbed aridland springs. Although this research did not examine the underlying processes, our findings highlight the geochemistry of spring water as a potential factor in shaping both BMI and microbial communities. Furthermore, because macroinvertebrates

are commonly used to examine the ecological health of aquatic ecosystems, the similar ordination patterns of microbial and BMI communities suggest that microbial communities can also serve bioindicators for evaluating the environmental conditions of spring systems.

4.2 Introduction

Isolated groundwater-dependent ecosystems (GDEs), such as spring systems, are aquatic ecosystems where groundwater reaches and flows on the surface through faults or geologic contacts (Meinzer 1923). Springs occur in different landscapes, and each spring has unique geochemistry, size, degree of isolation, and biota (Stevens and Meretsky 2008). Aridland springs as refugial aquatic habitats provide the majority of the reliable water for supporting aquatic organisms in arid and semi-arid regions (Shepard 1993, Ponder 2002, Murphy et al. 2015). Often, aridland springs are referred to as biological hot spots because many endangered and endemic species occur in these ecosystems (Stevens and Meretsky 2008, Keleher and Sada 2012, Murphy et al. 2015). Almost all ecological studies on aridland springs have focused on macro-organisms (fishes and invertebrates) exclusively, whereas the knowledge of microbial ecology in these systems is largely unknown.

Benthic macroinvertebrate (BMI) communities in small aridland springs mostly consist of insect and non-insect zoobenthos (Cantonati et al. 2006). Both neutral dynamics (i.e., history and dispersal limitations) and contemporary selection (i.e., environmental characteristics) shape BMI community composition patterns in springs (Rader et al. 2012; Stanislawczyk et al. 2018; Pordel et al. in prep; Sada and Thomas in press). For example, temperature, electrical conductance (EC), alkalinity, and pH have been recognized as factors controlling the structure of BMI communities in spring systems (Glazier 1991; Myers and Resh 2002, Sada and Thomas in press). A valley-scale study on the spatial distribution of BMI communities among mountain front springs in Owens Valley, CA, revealed that weathering-derived geochemical signals have a prominent role in shaping the structure of BMI communities (chapter 3). In chapter 3, I showed that although insect taxa with aerial dispersal abilities can travel between springs and occupy all springs, they only select springs with similar geochemical characteristics.

A meta-analysis (survey of 54 studies) of bacteria, archaea, and microbial eukaryotes highlighted both the environmental and historical processes in structuring microbial biogeographic patterns (Hanson et al. 2012). Bacteria tend to co-occur more often than expected by chance, which indicates the importance of underlying factors (i.e., habitat filtering) in structuring their assemblages (Horner-Devine and Bohannan 2006). The phylogenetic clustering of bacterial communities can be associated with the sensitivity of communities to the environmental parameters, or it can be related to their dispersal and colonization propensity. For example, temperature, salinity, and pH are associated with the structure of microbial communities in aquatic systems (Lozupone and Knight 2007, Wang et al. 2013, Cole et al. 2013, Yang et al. 2016, Power et al. 2018).

Research regarding interactions between BMI and microorganisms has mainly focused on the food web (Cummins and Klug 1979, Brett et al. 2017, Ayayee et al. 2018). Benthic macroinvertebrates are the main consumers in aquatic habitats, and their ecological encounters with microorganisms are common. Invertebrates receive much of their nutrition through microorganisms in freshwater ecosystems, either by grazing free-standing microbial biomass or by consuming detrital organic matter colonized by bacterial communities (Bärlocher and Kendrick 1975). Taxa with similar functional feeding groups prefer the same dietary materials (Sinsabaugh et al. 1985). For example, periphyton communities (e.g., attached algae) are critical food sources for scrapers (Russell-Hunter 1970), whereas shredders prefer coarse particulate organic matter colonized by saprophytic microorganisms (Kaushik and Hynes 1971). The BMI taxa within the same functional feeding groups tend to have more similar gut microbial communities, whereas the BMI taxa with different functional feeding groups tend to have significant dissimilarities in gut microbial community assemblages (Ayayee et al. 2018). Alternatively, invertebrates have a greater tendency to use basal nutrients that best match their own biochemical composition (Brett et al. 2017), which highlights the bottom-up control of microorganisms on BMIs in a food web.

Previous studies on the response of the microbial community structure to grazing pressures (top-down control) or increasing primary productivity (bottom-up control) demonstrated that both processes influence bacterial communities. The effect of protozoa grazing pressure on microbial productivity and diversity has been well studied, but little is known about the effect of macroinvertebrate grazing on the structure of microbial communities. Several studies showed that an increase in grazing (both protozoan and macroinvertebrates) activities change the morphological and taxonomic structure of microbial communities (Sommaruga and Psenner 1995, Yeager et al. 2001, Hahn and Hofle 2001). Regarding bottom-up dynamics, relationships were identified between increased primary productivity and bacterial community composition in aquatic habitats (Fisher et al. 2000, Schäfer 2001, Horner-Devine et al. 2003). For example, Horner-Devine et al. (2003) showed that despite the presence of bacterivores, bottom-up processes (primary productivity status) were more critical than top-down processes in shaping the overall abundance and community composition of bacteria in an aquatic system.

Co-occurrence patterns between BMI and microorganisms allow us to understand processes influencing microbial communities. Both macro- and microorganism assemblages show non-random patterns of co-occurrence within their communities (Horner-Devine and Bohannan 2006, Horner-Devine et al. 2007). Ecological studies of macroorganisms revealed that some taxa co-occur less often than expected by chance (Gotelli and McCabe 2002). Although a variety of research has demonstrated the applicability of co-occurrence frameworks to the study of microbial communities (Horner-Devine et al. 2007, Xia et al. 2011, Faust et al. 2015, Guo et al. 2016), comparing these communities is still challenging because of their differences in taxonomy and biological distinctiveness, such as size, reproduction, and life cycle stages. Although challenging, finding connections between very distantly related and functionally distinct organisms informs us about the fundamental factors that influence their distribution.

In aquatic ecosystems, anthropogenic disturbances significantly affect both microbial and BMI communities (Sada and Vinyard 2002, Lear and Lewis 2009). However, in undisturbed ecosystems, BMI communities showed a higher sensitivity to environmental conditions than microbial communities (Lear et al. 2009). The lack of sensitivity of microbial communities to habitat conditions within less impacted sites might be related to the techniques used to define microbial taxa. Recent developments, such as advancements in the quantity and quality of DNA sequencing methods, lower cost of multiplexing and per-base sequencing, and the creation of statistical packages that can handle large datasets have enabled more in-depth surveys of microbial communities than ever before (Caporaso et al. 2010, Bolyen et al. 2019). Because of these advancements, more precise distinctions can be made between the microbial communities present in an ecosystem, which makes it possible to compare the spatial distribution of microbes and macroinvertebrates in undisturbed springs.

The main objective of this study was to define the relationships between the community structure and ecological patterns of BMI and microorganisms in aridland springs. This was achieved by performing relative-abundance-based analyses of both BMI and microorganisms from 12 springs within Owens Valley, CA, and detecting co-occurrence patterns between the two communities. Furthermore, investigating an association between BMI and microbial assemblages provided insight into the potential factors that shape these communities. As far as we know, limited studies have been conducted that directly compare the community structures of BMI and microbial communities. This study is the first to compare the ecological patterns of microbial communities with the well-studied BMI communities in a freshwater ecosystem.

4.3 Materials and Methods

4.3.1 Study Area

To understand the relationship between BMI and microbial communities of aridland springs, twelve "reference springs" (undisturbed or naturalized) were sampled in Owens Valley, CA (Figure 1). Owens Valley is bounded by the Sierra Nevada (west) and White-Inyo Mountains (east). Moving northward, Owens Valley is bounded by mountains that separate it from the Walker River basin. To the south, Owens River historically flowed into Owens (Dry) Lake. Precipitation at high elevations of the Sierra Nevada provides the majority of spring recharge in the area. Average rainfall at high elevations is more than 400 cm annually (Lake Sabrina, CA; Western Regional Climate Center, elevation: 2,800 m above sea level [masl]). Owens Valley is located in a rain shadow of the Sierra Nevada and receives an average annual precipitation of approximately 13.5 cm (Bishop Airport, CA; NOAA Station; elevation: 240 masl).

Eleven of twelve sampled springs were mountain springs located along the Sierra Nevada Frontal Fault Zone with elevations ranging from 1,145 to 1,614 m and an average elevation of 1,461 m (Table 1). Additionally, one regional spring (Warm Spring A (IES 031): elevation 1,254 m) was sampled on the eastern side of Owens Valley.

4.3.2 Field Measurements

Physicochemical characteristics were recorded for each spring system when microbial samples were collected. A YSI Professional Plus (Quatro) multiparameter probe was used to measure electrical conductivity (EC), dissolved oxygen (DO), pH, and water temperature. The YSI probe also calculated specific conductance (SPC) from the EC values. Spring water was collected with a Geopump peristaltic pump (Geotech, Denver, CO) directly from the spring source (or as close to the source as possible) using Masterflex platinum-cured silicone tubing (Cole-Parmer, Vernon Hills, IL) and filtered using 0.2 µm polyethersulfone membrane Sterivex-GP pressure filters (Millipore Sigma, Burlington, MA). These samples were collected in pre-rinsed 250 ml high-density polyethylene (HDPE) bottles and refrigerated upon collection until sent for analysis. Major cations and anions were measured at the New Mexico Bureau of Geology and Mineral Resources Chemistry Lab (Socorro, NM). Cations were measured using inductively coupled plasma optical emission spectrometric (ICP-OES) techniques, according to EPA 200.7. Anions were measured using ion chromatography (IC) according to EPA 300.0. Duplicates were run on every tenth sample. Cations had reporting limits from 0.0005 mg/L for strontium to 0.05 mg/L for calcium, magnesium, and potassium. Reporting limits for anions were between 0.1 mg/L for bromide, fluoride, nitrate, and nitrite, and 1.0 mg/L for chloride and sulfate.

4.3.3 Benthic Macroinvertebrates

Benthic macroinvertebrates were sampled near the source of each spring system during the July 2017 summer sampling event. At each spring, BMIs were collected from 120 cm^2 quadrats that were placed along five, equally-spaced (3 m apart) transects. Quadrats were oriented perpendicular to the thalweg, and placed sequentially along transects at springbrook center, right bank, and left bank. Samples were collected by gently roiling the benthic materials into the quadrate net (opening size, 10 cm x 12 cm; mesh size, 500 µm) within 15 m from the spring emergence. All subsample collections were poured into one container, preserved in 90 percent ethyl alcohol, and sent to Rhithron Analytical Laboratory in Missoula, MT, for processing and enumeration. Most of the collected BMIs were in their early life stages (i.e., larval and pupal forms), so all insects and mites were identified to genus. Springsnails were identified to species, tricladida to family, ostracodes, and oligochaetes to class, and nemata to phylum. The structures of the BMI communities were determined by identifying and enumerating a minimum of 300 randomly selected individuals, which Vinson and Hawkins (1996) showed was sufficiently large to quantify community structure.

4.3.4 Microbial Sampling

To analyze microbial communities in spring systems, benthic samples and planktonic samples were collected in the spring (March) and summer (May) of 2016. Planktonic microbial communities were sampled by pumping spring water (≥ 2 L total) onto 0.2 µm polyethersulfone membrane Sterivex-GP pressure filters, as described in detail in the 'Field Measurements' section. Benthic microbial biomass was gathered using a sterilized shovel by collecting the top >2 cm of spring sediment or benthic microbial mat. Four samples were collected at each spring. Sampling locations were selected based on the substrate diversity (e.g., fine-grain sediment, coarse-grain sediment, or microbial mat) of each spring to maximize sampling of unique habitats at every spring. Excess water was cleared from each filter after sampling with a sterile syringe before sample storage. All samples were frozen immediately after sampling and kept frozen on dry ice in the field, upon returning to the lab the samples were stored in a -80 °C freezer until DNA extraction. The FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA) was used for DNA extraction. The V4 region of the 16S rRNA was amplified and sequenced using the updated bacterial- and archaeal-specific 515F/806R primer set (Apprill et al. 2015, Parada et al. 2016, Thompson et al. 2017). Argonne National Laboratory (Lemont, IL) performed the amplification, library preparation, and sequencing on an Illumina MiSeq platform (2x151 bp) per the Earth Microbiome Project pipeline (https://earthmicrobiome.org/protocols-and-standards/16s). Paired-end Illumina MiSeq reads were quality filtered, aligned, and assigned to amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016) via Qiime2 version 2019.1 (Caporaso et al. 2010, Bolyen et al. 2019). The ASVs were classified in Qiime2 using a naïve-Bayesian classifier (Bokulich et al. 2018) trained on the

V4 region of the Silva NR99 132 alignment (Pruesse et al. 2007). Variants were aligned using mafft (31) through the q2-alignment plugin using default settings. ASVs classified as chloroplast or mitochondrial were removed. Qiime2-compatible archives and scripts will be available at the GitHub repository hedlundb/LP16S.

4.3.5 Data Analysis

A combination of a multivariate (Hierarchal cluster analysis) and graphical techniques (Piper and stiff diagrams) is a powerful tool to classify spring water based on geochemical fingerprints (Meyers 2020). All major cations and anions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} Cl^{-}, $SO_4{}^{2-}$, HCO_3), EC, and temperature were included in the HCA. To categorize spring waters based on their geochemical characteristics, milliequivalents of cations and anions were normalized by the total anions and cations (unit of percent meq/kg). The EC was log-transformed and standardized prior to analysis. No transformation was applied to temperature values. The HCA was calculated in Primer® v6 using single linkage and Euclidean distance.

Alpha-diversity metrics (Observed, Shannon, and InvSimpson) were calculated using the R package phyloseq version 1.30 (McMurdie and Holmes 2013). The taxonomic composition of the two communities was assessed by grouping all taxa at the family-level, calculating the percent relative abundance, and then generating taxonomic bar plots using the R packages phyloseq version 1.30.0 (McMurdie and Holmes 2013) and ggplot2 version 3.3.0 (Wickham 2011).

Multivariate statistical approaches were applied to measure the degree of similarity between BMI and microbial communities in sampled springs. Both BMI and microbial datasets were similarly analyzed with nonmetric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) using Bray-Curtis dissimilarity matrices of the transformed data generated using the R package vegan version 2.5-6 (Dixon 2003). Square root transformation and Wisconsin double standardization were applied on both datasets prior to these analyses. The NMDS ordination was conducted using the R packages vegan and phyloseq version 1.30.0 (McMurdie and Holmes 2013). Next, the Mantel test was performed on a Sørenson dissimilarity matrix to determine the significance of correlations between BMI and microbial assemblages. Pearson correlation coefficient was used to calculate the strength of the correlation between the two assemblages. PC-ORD v6 was used for the Mantel test (McCune and Mefford 2011).

The relationship between microbes and BMIs was visualized using a co-occurrence heatmap. For this analysis, the relative abundances of common BMIs (i.e., those that occur in more than 25 percent of samples) were compared with those of common ASVs (i.e., those that occur in >1 sample and have a total count ≥ 10 within all samples). The filtered common ASVs were then glommed at the family-level for further comparison to the common BMIs. Spearman’s rho correlations were calculated between the two community abundance matrices using the R package Hmisc version 4-4.0. The heatmap was built using pheatmap version 1.0.12; Ward’s linkage method was used to calculate the hierarchical clustering of BMI and microbial data in the heatmap.

Bioassessment metrics were also used to examine differences in functional characteristics of BMI communities across the study area. Five functional feeding groups and five behavioral metrics, as well as four Hilsenhoff Biotic Index-related metrics (HBI; Hilsenhoff 1987), were calculated for the ecological assessment of BMI communities

(Rosenberg and Resh 1993, Barbour et al. 1999). BMI taxa were assigned to different functional feeding groups and tolerance values using tabulated data in Appendix B of Barbour et al. (1999). Tolerance values represent the relative sensitivity of BMI organisms to different types of stressors (Barbour et al. 1999). For example, HBI indicates BMI sensitivity to organic pollution. Each BMI taxon tolerates a particular range of perturbations that are expressed by a valu

e indicating their ability to tolerate harsh environments (range between 1 and 10). Functional feeding groups represent BMI organisms feeding strategies. Generalists (collector gatherers and filterers) consume broader types of food materials than specialized feeders (scrapers and shredders) (Cummins and Klug 1979). Thus, specialized feeders are more sensitive to the availability of certain food sources. BMI taxa in the heatmap were symbolized based on their feeding groups and tolerance values.

4.4 Results

4.4.1 Environmental Characteristics

All springs, except Warm Spring A, emerge at small faults associated with the Sierra Nevada Frontal Fault Zone and are categorized as cold and dilute springs with a temperature of 16.4 ±1.2 °C (Mean ±SE) and an EC of 232.2 ±39.7 µS/cm (Mean ±SE) (Table 1). Warm Spring A is located on the valley floor of eastern Owens Valley, CA, and it has a higher temperature (29.2 °C) and EC (790.1 µS/cm).

Hierarchal cluster analysis was used to classify springs based on their geochemical characteristics. Two main clusters and an outlier group were identified by comparing the resulting dendrogram with the stiff and Piper diagrams (Figure 2). The one outlier spring, Warm Spring A (IES031), is distinct because it is a regional carbonate spring recharged by the White-Inyo Mountain Range, whereas other springs in the study are local springs recharged from the Sierra Nevada, which is predominantly granite. The separation between the geochemistry of springs in Groups 1 and 2 was most likely associated with the Penn-Permian metasedimentary roof pendants at the recharge area of Group 2 springs (Meyers 2020).

4.4.2 Benthic Macroinvertebrates

A total of 2,179 BMIs belonging to 68 taxa were tallied near the source of the study springs. The mean number of BMIs per sample was 168 (range: 25 to 325 individuals), and the mean number of taxa was 11 (range: 3 to 23 taxa). Only 24 taxa are considered common and occurred in more than 25 percent of the samples. The other 44 taxa are rare and found in less than three springs. The family Hydrobiidae (springsnails) were present in eight of the twelve springs, and numerically dominated the communities in Group 1 springs (Figure 3). *Pyrgulopsis owensis* was found in one spring (IES031), whereas *Pyrgulopsis wongi* was present in seven springs (IES024, IES026, IES027, IES029, IES034, IES054, and IES033). Alpha diversity was relatively similar between Group 1 (ranging between 7 and 23) and Group 2 (ranging between 7 and 19). Differences between the alpha diversity (richness) of springs in Groups 1 and 2 were not statistically significant (one-way ANOVA, $P>0.05$). Warm Spring A (IES031) hosted the lowest BMI family richness (four BMI families). Non-insects (families Hydrobiidae and Hyalellidae (shrimp-like)) were the most abundant BMI families in Group 1 springs, whereas insects (families Lepidostomatidae, Chironomidae, and Sericostomatidae) with an emerging adult stage and an aerial dispersal mode were dominant in Group 2 springs (Figure 3). Differences between Groups 1 and 2 were statistically significant for both non insect and insect taxa abundances among Groups 1 and 2 (one-way ANOVA p-value = 0.015 and 0.004, respectively). Midges (family Chironomidae) were found in all springs except IES031. The BMI community in IES031 (Group 3) was comprised of a high percentage of *Microcylloepus* (family Elmidae).

The functional characteristics of BMI communities among psychochemical groupings were examined using bioassessment metrics. The data were not significantly different from normality for any bioassessment metrics using a nonparametric Kolmogorov-Smirnoff test. Differences in metrics between geochemical groups were examined using a one-way ANOVA. Six bioassessment metrics were significantly different (p-value < 0.05) between Groups 1 and 2 (Table 2). For example, the functional feeding group results show that the percent of scrapers (i.e., springsnails) was highest in Group 1 (Table 2). Although shredders (i.e., most of the stoneflies and caddisflies taxa) were absent from most of the Group 1 springs (IES024, IES034, IES026, and IES029) and Warm Spring A (IES031), they constituted nearly 31 ±5.2 (Mean ±SE) percent of the community in the Group 2. Statistically significant differences also occurred for the percent of sprawlers and climbers. Benthic macroinvertebrates with these behavioral metrics were more abundant in Group 2 than Group1. Of the HBI related metrics, percent intolerant taxa and percent intolerant EPT (**E**phemeroptera [mayflies], **P**lecoptera [stoneflies], and **T**richoptera [caddisflies]) differences were statistically significant between Groups 1 and 2 (Table 2). These intolerant taxa were abundant in springs in Group 2.

The differences in some bioassessment metrics were not statistically significant between Groups 1 and 2. For example, collector-gatherers (i.e., mostly midges and mayflies) were present in both Groups 1 and 2. The HBI index also showed a moderate community tolerance value (ranging from 4 to 6) for all springs.

4.4.3 Microbial Diversity and Composition of Benthic Communities

After the quality filtering, 1,005,380 16S rRNA gene sequences were recovered from 46 benthic samples, and the number of sequences per sample ranged from 5,037 to 37,289. After taxonomic classification, 17,347 ASVs were identified, comprising 67 phyla, 240 classes, 674 orders, 1,256 families, and 2,123 genera. Rarefaction curves for all samples plateaued at a reasonable sequence depth (approximately 3,000 to 10,000 sequences), indicating adequate sequencing coverage within our dataset.

Most samples showed high values for the observed counts (richness), Shannon diversity index, and Gini-Simpson index, indicating that the microbial communities in the springs were both species rich and diverse (Table S1). Group 2 springs (IES028, IES033, IES038, IES042, and IES043) demonstrated the highest mean and lowest standard deviation for the observed counts (738.65 ± 149.18 ASVs), Shannon index (5.97 ± 0.27), and Gini-Simpson index (0.99 ± 0.002). Group 1 springs (IES024, IES026, IES027, IES029, IES034, and IES054) had a lower mean but a larger standard deviation for all metrics (observed counts: 671.05 ± 318.41, Shannon: 5.58 ± 1.0, Gini-Simpson: 0.97 ± 0.04). Additionally, the highest values in the dataset for all metrics were observed in Group 1 springs. The single Group 3 spring (IES031) had the lowest recorded mean value but a high standard deviation, which indicates a high variability within that spring (Observed: 478.5 ± 266.22, Shannon: 4.02 ± 1.43, and Gini-Simpson: 0.87 ± 0.13).

After merging all families <25%, the abundances of 44 of the original 1,256 families were plotted for all benthic samples (Figure 4). Strikingly, most of the benthic communities were comprised of a large percentage (>30% of the total community) of families <25%, underscoring the high microbial diversity and evenness observed in the springs. All springs in Group 1 hosted populations of *Burkholderiaceae*, *Hyphomicrobiaceae*, *Chitinophagaceae*, *Saprospiraceae*, *Rhizobiales Incertae Sedis*, and *Sphingomonadaceae*. Several families were variably present across springs in Group 1, such as *Methylomirabilaceae*, *Methylomonaceae*, *Rhodobacteraceae*, *Xenococcaceae*, *Pedosphaeraceae*, *Spirosomaceae*, *Microscillaceae*, and *Anaerolineaceae*. Most springs in Group 1 (except IES029) had abundant populations of *Nitrosopumilaceae, Nitrospiraceae*, and *Nitrosomonadaceae*, which suggests an active oxidative nitrogen cycle. Several samples from IES029 hosted unique and abundant populations of *Nostocaceae* and bacterial ASVs that are unclassified at the phylum level. A less abundant population of *Nostocaceae* was also observed in one sample from IES034. One benthic sample from spring IES027 contained a relatively abundant population of an unclassified class of *Proteobacteria*.

All five springs in Group 2 contained populations of *Burkholderiaceae*, *Hyphomicrobiaceae*, *Chitinophagaceae*, *Saprospiraceae*, *Rhizobiales Incertae Sedis*, *Nitrosopumilaceae*, *Nitrospiraceae*, *Nitrosomonadaceae*, *Xanthobacteraceae*, and *Sphingomonadaceae*. Two springs in Group 2, IES033 and IES038, hosted unique populations of *Leptolyngbyaceae* and an unclassified order of *Oxyphotobacteria* that were not observed in the other springs within this group. Similar to Group 1 springs, certain taxa were prevalent in only one or a few springs, such as *Bacillaceae* (IES028, IES033, and

IES042), *Methylomirabilaceae* (IES028), *Methylomonaceae* (IES043), *Micrococcaceae* (IES028, IES033, and IES038), and *Pseudomonadaceae* (IES028 and IES033).

The microbial composition of the Group 3 spring (IES031) was clearly distinct from the microbial compositions of Groups 1 and 2. Families found in all four samples included *Burkholderiaceae*, *Chitinophagaceae*, *Hyphomicrobiaceae*, *Leptolyngbyaceae*, *Microscillaceae*, *Nitrosomonadaceae*, *Nitrosopumilaceae*, *Nitrospiraceae*, *Nostocaceae*, *Xenococcaceae*, an unclassified order of *Oxyphotobacteria*, and an unclassified class of *Proteobacteria*. Two of the benthic samples demonstrated unique community profiles (S1 and S2), which were dominated by *Leptolyngbyaceae* (S1), *Microscillaceae* (S1), *Nostocaceae* (S2), Unclassified *Oxyphotobacteria* (S2), and Unclassified *Proteobacteria* (S2). The other two samples (S3 and S4) showed very similar community profiles, with slight differences in the abundance of taxa, such as *Xenococcaceae*, *Nitrosomonadaceae*, *Nitrosopumilaceae*, *Nitrospiraceae*, *Burkholderiaceae*, and *Chitinophagaceae*. Although *Leptolyngbyaceae*, *Microscillaceae*, *Nostocaceae*, and Unclassified *Oxyphotobacteria* were found in benthic samples S3 and S4, they were much less abundant than observed in other two samples (S1 and S2); Unclassified *Proteobacteria* was only seen in sample S2.

4.4.4 Association between BMIs and ASVs

Nonmetric Multidimensional Scaling and Analysis of Similarity-

The ordination pattern of BMI communities mirrored the geochemical groupings. The NMDS plot of BMI communities (stress = 0.122), color coded by the geochemical groupings, shows three distinct groups (Figure 5-A). Group 1 consists of six springs (red circles) grouped at the center of the NMDS plot. The five springs (blue circles) on the left part of the plot are classified as group 2. Warm Spring A (IES031) shows an entirely distinct BMI community compared with springs in the two other clusters, and it is located on the far-right section of the plot. The ANOSIM R-value indicated that the BMI community composition was similar within each geochemical group and distinct from the others (ANOSIM, global R = 0.846, P-value = 0.001). Pairwise comparison between Groups 1 and 2 also indicated significant differences between BMI communities (Pairwise R: 0.8, P = 0.002).

The ordination pattern of benthic microbial communities (stress = 0.187) followed the same pattern (Figure 5-B; ANOSIM: global R = 0.58, P-value = 0.002), whereas planktonic communities did not (Figure S2; ANOSIM: global R = 0.2, P-value = 3.5). The NMDS plot of benthic microbial communities is also color coded by the three designated groups observed in the HCA plot of geochemical classification. Similar to BMI communities, a strong relationship was identified between benthic microbial communities and geochemistry. Although the NMDS plot shows minor overlaps between the Groups 1 and 2 communities, the global R-value > 0.5 indicated that ASV communities were similar within each group and distinct between groups. Microbial communities in spring IES031 were well separated from other microbial communities. Different environmental metrics (SPC, temperature, Mg^{2+}, Sr^{2+}, Na^{+}, $CaCO_3$, and K^{+}) were positively associated with both BMI and ASV community compositions in Groups 1 and 3 (Figure 5).

To further investigate BMI and microbial biological organization, taxa vectors representing the BMI (all taxa >10%) and microbial families (all families >25%) were overlaid on their ordination plots (Figure 7). The results showed that *Hyalella* (family Hyalellidae), *Rheotanytarsus* (family Chironomidae), and *Helicopsyche borealis* (family

Helicopsychidae) were strongly associated with springs in Group 1 (Figure 7-A). However, *Tvetenia* (family Orthocladiinae), Enchytraeidae, and *Lepidostoma* (family Lepidostomatidae) were abundant in Group 2. Vectors representing the microbial families *Chitinophagaceae, Xanthobacteraceae, and Chthoniobacteraceae* were dominant in the microbial communities of Group 2 springs (Figure 7-B). However, taxa vectors representing *Xenococcaceae,* UC *Proteobacteria,* UC *Oxyphotobacteria, Leptolyngbyaceae, and Nostocaceae* were highly correlated with samples in Groups 1 and 3. Other microbial taxa in the plot were associated with the variation of microbial data along the NMDS1 axis.

Co-occurrence Analyses-

The potential relationship between BMI and ASV communities in springs was examined using the Mantel test. A Mantel test on the presence/absence-transformed datasets (Sørenson dissimilarity matrices) showed a positive relationship ($R = 0.4$) between BMIs and ASVs. A small statistical significance level (P-value = 0.004) rejected the null hypothesis (no relationship between matrices) and suggested that the observed association is statistically significant.

To better examine the correlation between BMI taxa and microbial families, a co-occurrence heatmap was used to visualize the relationship (Figure 6). Each column of the heatmap represents a common BMI taxon, and each row represents a common microbial taxon grouped at the family level. The functional feeding and tolerance value of each BMI were also shown in the plot. The heatmap illustrated statistically significant associations between the co-occurrence of BMIs and ASVs in dark red. Nearly 6.1 percent of the correlations (95 out of 1548) were statistically significant (P-value < 0.05).

The BMIs (columns) and microbial families (rows) were also clustered based on Ward’s method for linkage between the abundances of BMIs or microbes. Classification analysis showed four distinct BMI clusters (Groups A-D) and three microbial clusters (Groups E-G) (Figure 6). Overall, only four significant associations were observed between intolerant BMIs and benthic microbial families, while the number of significant correlations was higher (near 15 correlations) between tolerant BMIs and benthic microbial families. Most of the significant correlations were observed between midges (Order *Diptera*) and microbial families in Groups E and F. BMI taxa in Cluster C (BMIs in this cluster are mainly predator and collector-gatherers) also showed a higher association with microbial families in Clusters E and F.

4.5 Discussion

Both BMI and microbial communities segregated into three groups that corresponded to the three geochemical groups sampled in this study. Therefore, spring geochemistry appears to be the primary driver of structuring both BMI and microbial communities. The geochemistry of springs is ultimately controlled by the interaction of groundwater with the host aquifer, mineral solubility, recharge elevation, and groundwater residence time. Although we cannot directly determine to what degree the geochemical controls and biological feedbacks are responsible for creating the observed patterns, the statistical approaches suggest water geochemistry is a primary influencing factor in structuring these communities. Alternatively, co-selection of BMI and microbial communities because of trophic interactions may result in the observed similarities between communities. Finally, because macroinvertebrates are commonly used to examine the ecological health of ecosystems, the similar spatial distribution patterns and significant co-occurrence relationships between microbial and BMI communities, suggest that microbial communities could serve as an alternative bioindicator for evaluating the environmental conditions of spring systems.

Non random taxon co-occurrence patterns are common in both macro- and microorganisms (Gotelli and McCabe 2002, Horner-Devine et al. 2007). This means that some taxa tend to co-occur more or less often than expected by chance within macro- and microorganism communities (Horner-Devine et al. 2007). The multivariate statistical approaches in this study showed a similar pattern among microbial and macroinvertebrate communities in spring systems, which confirms the previous findings that these communities are not distributed randomly. Although microbes and BMIs are taxonomically distinct, the observed patterns of co-occurrence make sense in light of known trophic interactions. These results are consistent with previous findings that revealed a strong relationship between cyanobacteria, heterotrophic bacteria, and diatom communities in ephemeral stream habitats (Stanish et al. 2013). Different mechanisms may influence these communities and result in a similar ordination pattern among the communities. Environmental characteristics, dispersal limitations, and biological interactions between these communities are the possible mechanisms for structuring these communities.

The environmental selection hypothesis was first presented by Baas Becking (1934), which highlights the role of contemporary processes in structuring microbial communities. This hypothesis stated that “everything is everywhere, but the environment selects” (Baas-Becking 1934). Endemism at the level of microbial strains, species, or genera is evidence of biogeography, which reveals that the spatial distributions of these communities are non random and caused by some underlying processes (Hedlund and Staley 2004, Martiny et al. 2006a, Hanson et al. 2012). Therefore, the first part ("everything is everywhere") of the Baas-Becking hypothesis is not valid. However, the environmental filtering part ("the environment selects") has been verified by biological and ecological studies on both microbes and macroinvertebrates (Poff 1997; Hanson et al. 2012). Previous studies showed that environmental characteristics of the habitat can place physiological constraints on BMIs and that they affect the structure of BMI communities (Thorp and Covich 2010). For example, the temperature, EC, alkalinity, and geochemical signature of the water was the most influential factor in structuring BMI community composition in spring systems (Glazier 1991, Barquín and Death 2006, Sada and Thomas *in press*, Pordel et al. in prep (chapter 3)). Similarly, other studies on microbial communities showed that environmental variables (i.e., temperature, salinity, and pH) might explain greater variation in microbial composition than geographic distance (Hanson et al. 2012, Wang et al. 2013, Yang et al. 2016, Power et al. 2018). A negative relationship was also observed between temperature and microbial richness (Cole et al. 2013, Righetti et al. 2019). Although these observations demonstrate the prominent role of environmental factors in shaping both BMI and microbial communities, distance-decay relationships were also reported for both BMI (Rader et al. 2012, Stanislawczyk et al. 2018) and microbial communities (Horner-Devine et al. 2004, Hewson et al. 2006, Soininen et al. 2011). In our study, springs with similar BMI and microbial community compositions were not necessarily close to one another spatially, but they had relatively similar geochemistry (See Figure 1). Therefore, in our local-scale study, geochemistry seems to be more influential than a geographic distance in shaping the structure of BMI and microbial communities.

The similarity between the observed patterns in macroinvertebrate and microbial assemblages appeared to be associated with the geochemical signature of the spring water, which is a product of groundwater-rock interaction from recharge areas to the spring emergence. In this study, springs were separated into two main groups based on their BMI and geochemical compositions: one group with higher Ca^{2+}/Na^{+} molar ratios (which is related to the presence of metasedimentary roof pendants at the spring contributing area) in spring waters hosted a higher proportion of intolerant taxa in their communities, and the other with lower Ca^{2+}/Na^{+} molar ratios (which is related to the absence of metasedimentary roof pendants at the spring recharge area) in spring waters dominated by non-insect taxa (See Chapter 3). Therefore, the geochemical fingerprints of spring waters, which are affected by the lithological heterogeneity of the spring recharge areas, are a governing factor of BMI assemblages in a valley-scale study. Our results also demonstrated that microbial composition follows the same ordination pattern as the macroinvertebrate communities in the sampled springs. The co-occurrence analyses also revealed a positive relationship between common BMIs and common microbes in these springs. These co-occurrence patterns could be due to the symbiosis between the two biological organizations. Alternatively, the distinct environment (i.e., geochemical characteristics) of the spring systems could provide a suitable habitat for particular BMI and microbial assemblages. Therefore, spring water geochemistry is a potential factor in determining microbial biogeographic patterns. Similarly, a recent study in Australia found a significant relationship between the water chemistry of pit lakes and both the macroinvertebrate and microbial assemblages (Blanchette et al. 2019).

In contrast, the dispersal ability of microbes and BMIs, as well as dispersal barriers, may act as controlling mechanisms in the spatial distribution patterns of these organisms.

The high proportion of specialist taxa in a community and the genetic differentiation of organisms between sites suggest that the distribution patters of microorganisms are determined by history rather than contemporary mechanisms (Martiny et al. 2006b, Rader et al. 2012). Most microorganisms have limited capacity to disperse between isolated springs in arid regions. Some microbes have the ability to travel between isolated spring systems through the subsurface, and some disperse among springs via passive dispersal mode by the colonization of macroinvertebrate exoskeletons and guts (Leff et al. 1994, McEwen and Leff 2001). This highlights the role insect invertebrates with aerial dispersal capabilities play in shaping microbial composition and diversity among springs (Leff and Lemke 1998, McEwen and Leff 2001). In our study, five springs with a high relative abundance of insects versus non-insect taxa (submerged adult stage) had similar microbial communities. Although this finding suggests that BMIs play a role in the spatial distribution of microorganisms, their role may not be significant in aridland springs because of BMI organisms tendency to remain close to their natal spring habitat (Danks and Williams 1991).

The co-occurrence of microbial and BMI communities could also occur because of their connectivity in the food chain (Kim et al. 2008). Under stable flow conditions in most small spring systems, BMIs become dominant in the food web, because of the absence of higher-level predators, and develop a two-level trophic system (resource-consumer). The biological interaction between macroinvertebrates and microbes is controlled by both bottom-up and top-down dynamics (Yao et al. 2017). Top-down control happens because of BMI-utilizing microorganisms, and bottom-up control highlights the role of bacteria as a food source for BMIs (Cummins and Klug 1979, Yao et al. 2017). Experimental studies showed that heterotrophic bacteria and terrestrial leaf litters have poor food quality because they have a food quality that is ten times lower than phytoplankton for herbivores. For example, *Daphnia* can tolerate a bacteria-dominated diet, but they cannot survive if fed only bacteria (Taipale et al. 2012), and they are near starvation if they depend only on terrestrial leaves as a food source (Taipale et al. 2015). Additionally, Whatley et al. (2014) showed that the variation in aquatic insect communities was significantly associated with the ratio of heterotrophic to autotrophic phospholipid fatty acids (i.e., sulfate-reducing bacteria to algae) in nutrient-rich peatland ditches. In our study, we found a significant difference between the percent of scrappers and shredders between Groups 1 and 2. Shredders prefer using particulate organic detritus colonized by some microorganisms. In contrast, scrapers tend to consume periphyton (attached living algal cells) and have the ability to shear off algae attached to surfaces (Cummins and Klug 1979). The algal diet was the primary food source consumed by the scraping grazers in small headwater streams (Finlay 2001). However, more field data and extensive research are required to improve our understanding of the significance of BMI and microbe interactions within the food chain of small isolated spring systems.

This study provides the first insight into the connection between BMI and microbial communities in freshwater ecosystems by directly comparing these communities in aridland springs. The results of this study should serve as encouragement for further investigations seeking to more deeply understand the mechanisms at work. The main challenge in interpreting the association between microbial and macroinvertebrate assemblages is the lack of data on the sensitivity of specific microbial taxa to environmental factors. In contrast, the effect of different environmental metrics on BMI communities has been well studied. Bacterial communities in stream systems can only distinguish between highly impacted streams and slightly disturbed sites, but they are not sensitive enough to differentiate between undisturbed sites (Lear et al. 2009, 2011). The level of correlation between microbial and macroinvertebrate communities was low in impacted streams (Kim et al. 2008). The results of our study showed a positive correlation between the structure of BMI and microbial communities in undisturbed springs. The similarity of the patterns of these communities and their associations suggest that microbial organisms can be as sensitive as macroinvertebrates to their habitat environment in undisturbed springs. Therefore, microbial communities could be considered as an alternative bioindicator tool for evaluating the health of spring systems.

However, we cannot make a strong conclusion about the underlying processes responsible for the similar ordination patterns of these communities. Further experimental and field studies on these distinct biological organizations are needed to improve our understanding of microbial and BMI symbioses, as well as their survival strategies and physiological adaptations. Further research will also help us examine the implications of these communities in the management of spring ecosystems.

4.6 Figures

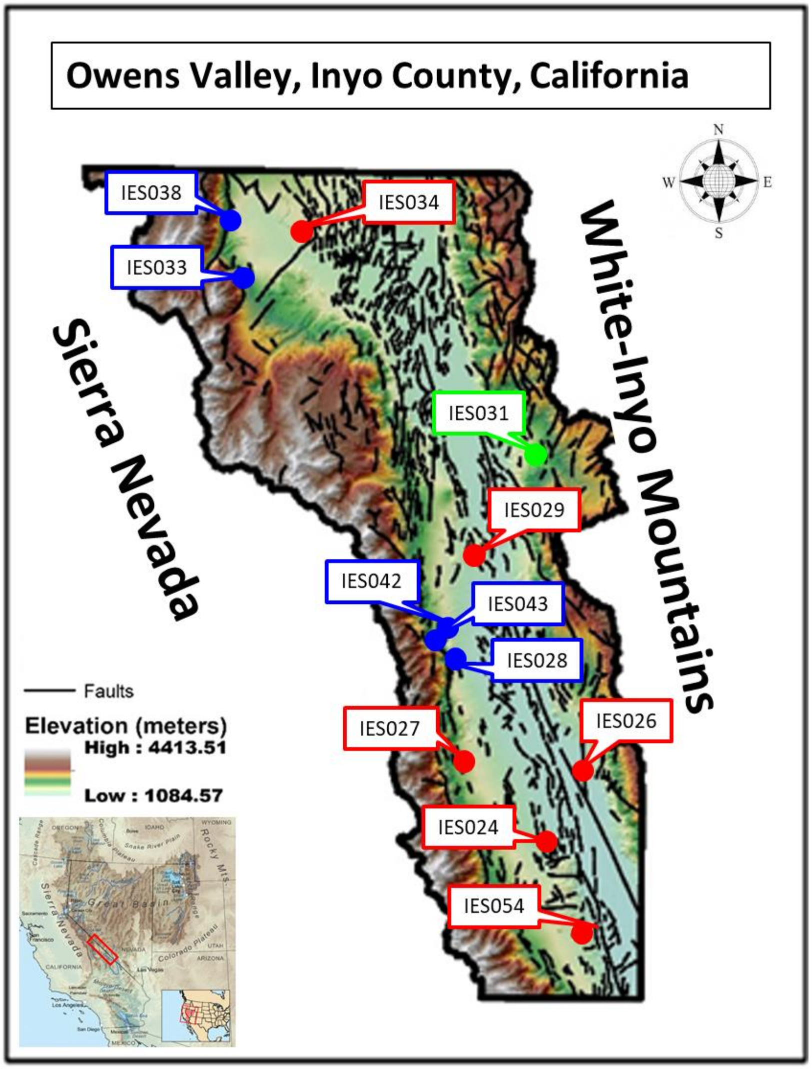

Figure 4.1. Study area boundary in Owens Valley. Blue circles indicate springs that were sampled: *IES038:* Wells Meadow B Spring; *IES034:* Birchim Spring B; *IES033:* Elderberry Canyon Spring; *IES031:* Warm Spring A; *IES029:* Red Mountain Spring; *IES042:* North Harry Birch Spring; *IES043*: South Harry Birch Spring; *IES028:* Grover Anton Spring; *IES027:* Boron Spring A; *IES026:* Reinhackle Spring; *IES024:* Hogback Creek Spring; *IES054:* Lubken Canyon 2. Springs are color-coded based on their physicochemical groupings. Group 1 (red circles): springs with a granitic weathering signature. Group 2 (blue circles): springs influenced by metasedimentary roof pendants enclosed within granitic rocks at their recharge area. Warm Spring A (green circle): a regional spring with distinct geologic units at the spring recharge are.

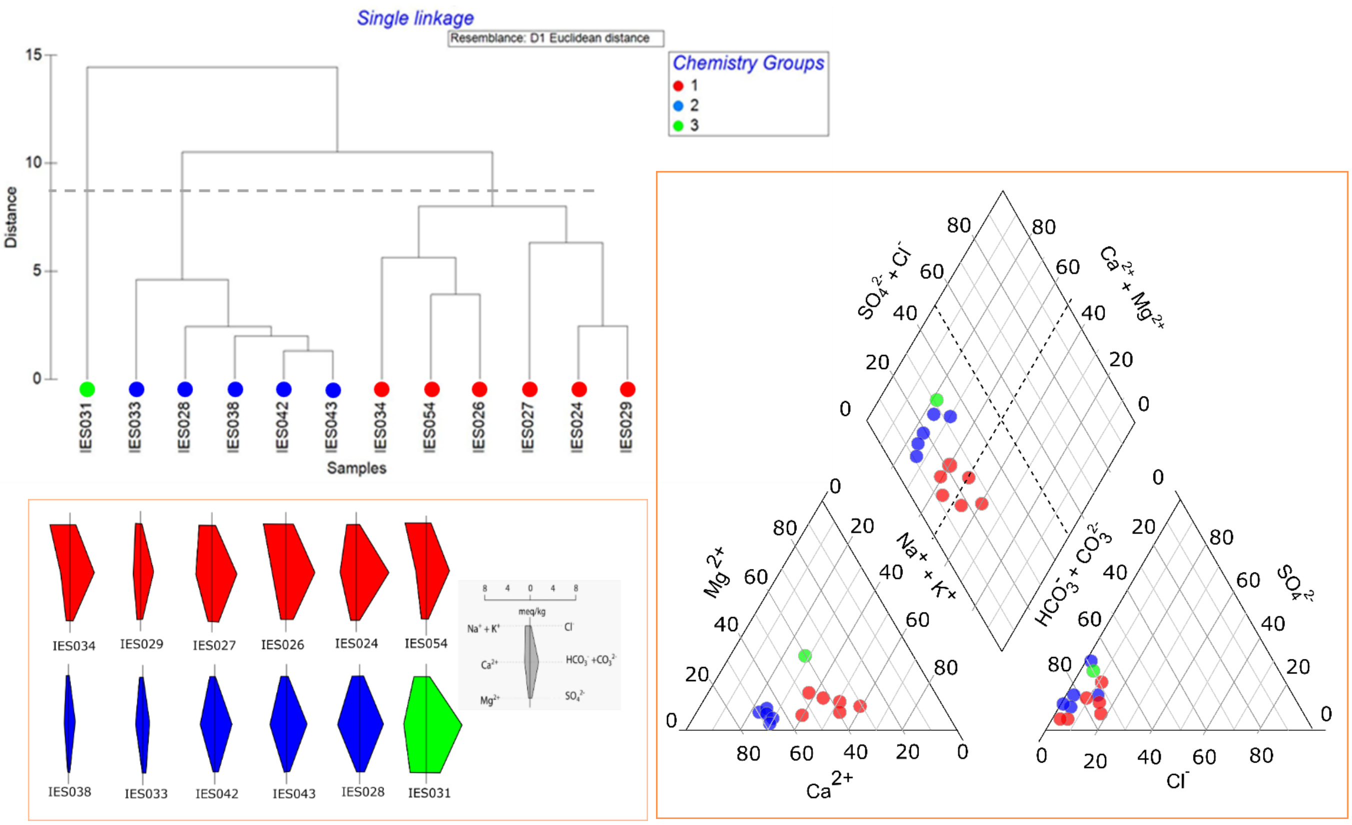

Figure 4.2. Dendrogram from the hierarchical clustering analysis for the twelve spring waters. The dashed line represents the phenom line for classifying the springs into two main clusters and one outlier spring (IES 31). Springs are color coded based on these three geochemical groups in the graphical methods (Piper and Stiff diagrams).

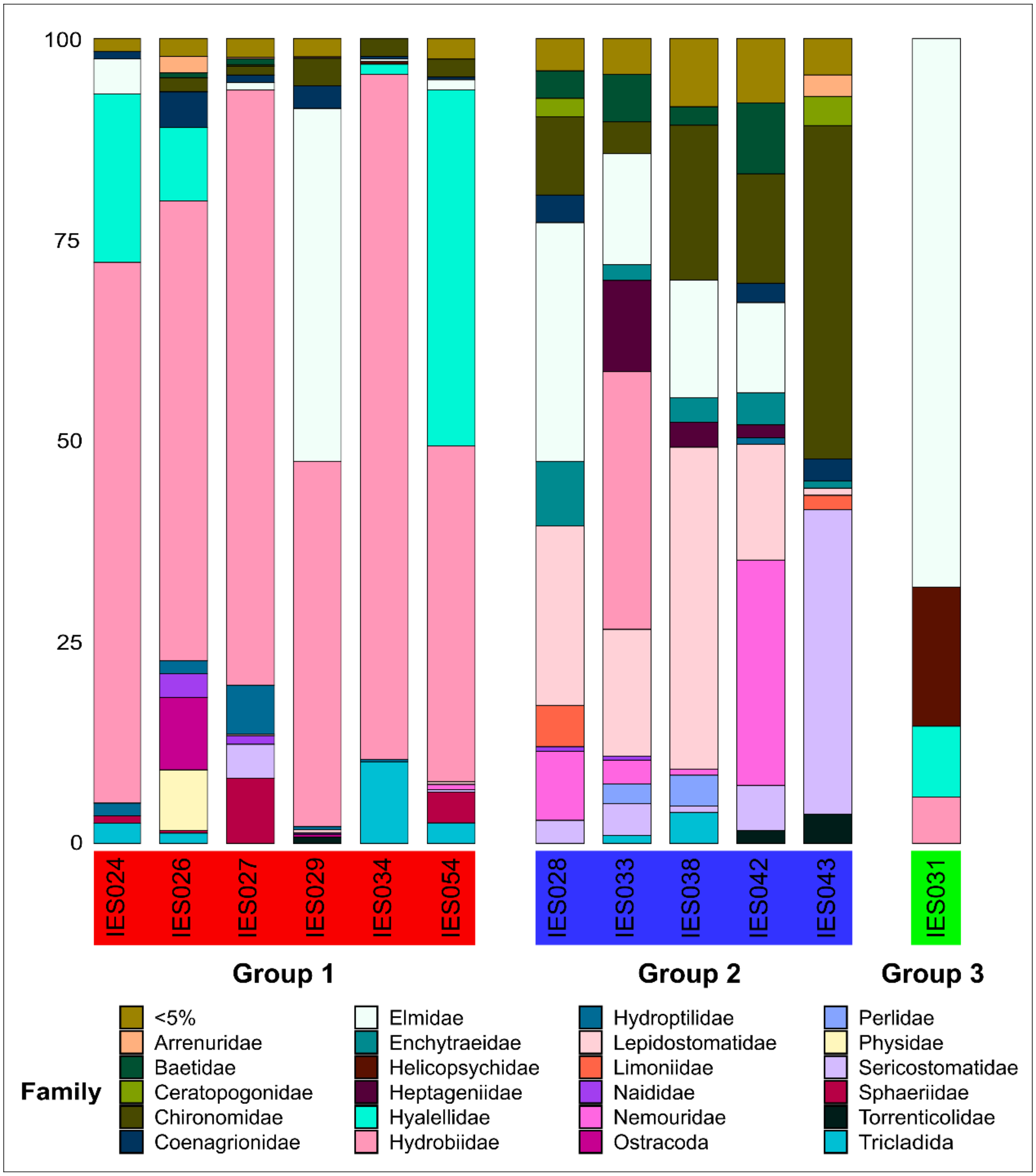

Figure 4.3. Relative abundance of BMI families in the study area.

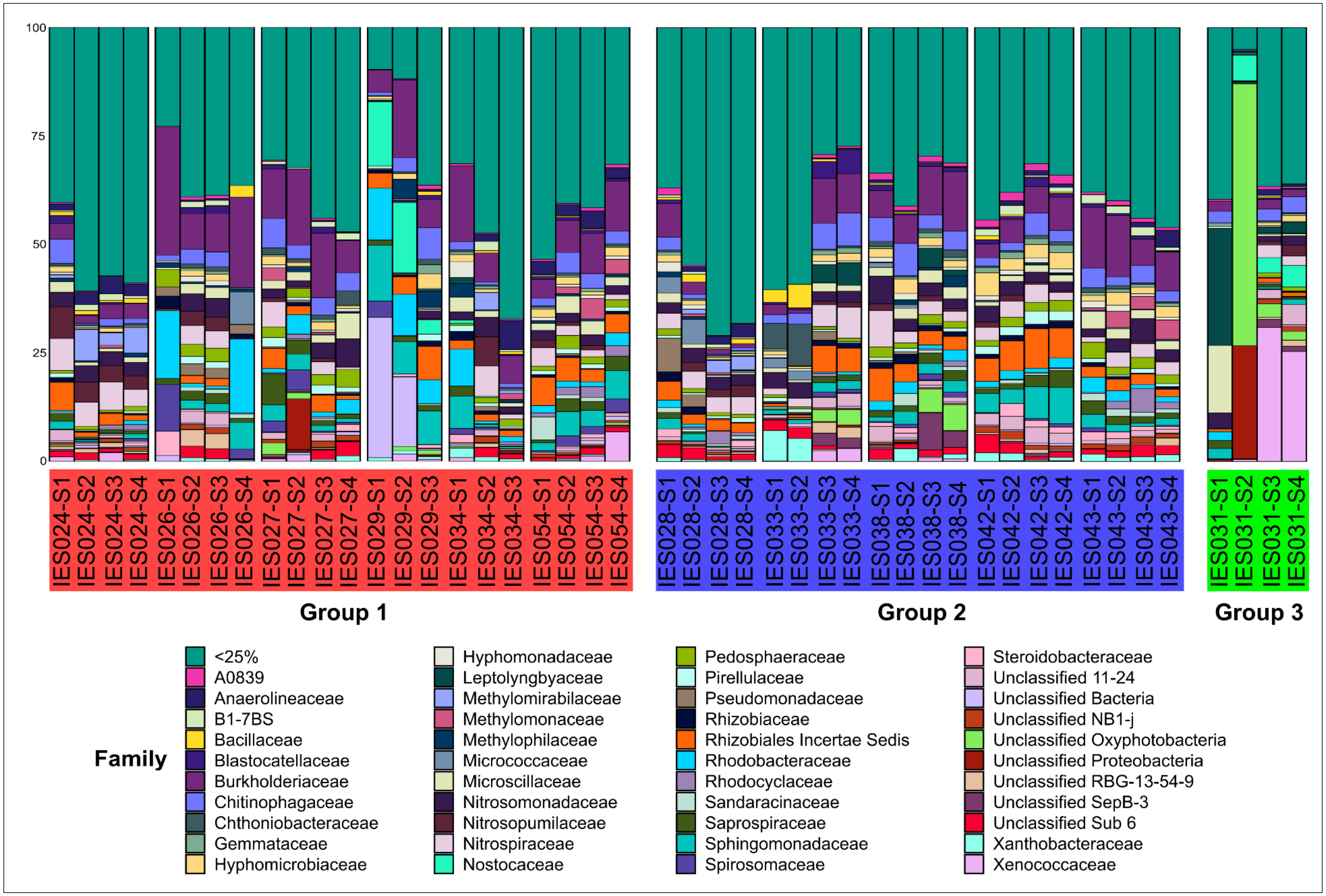

Figure 4.4. Relative abundance of microbial families in the study area.

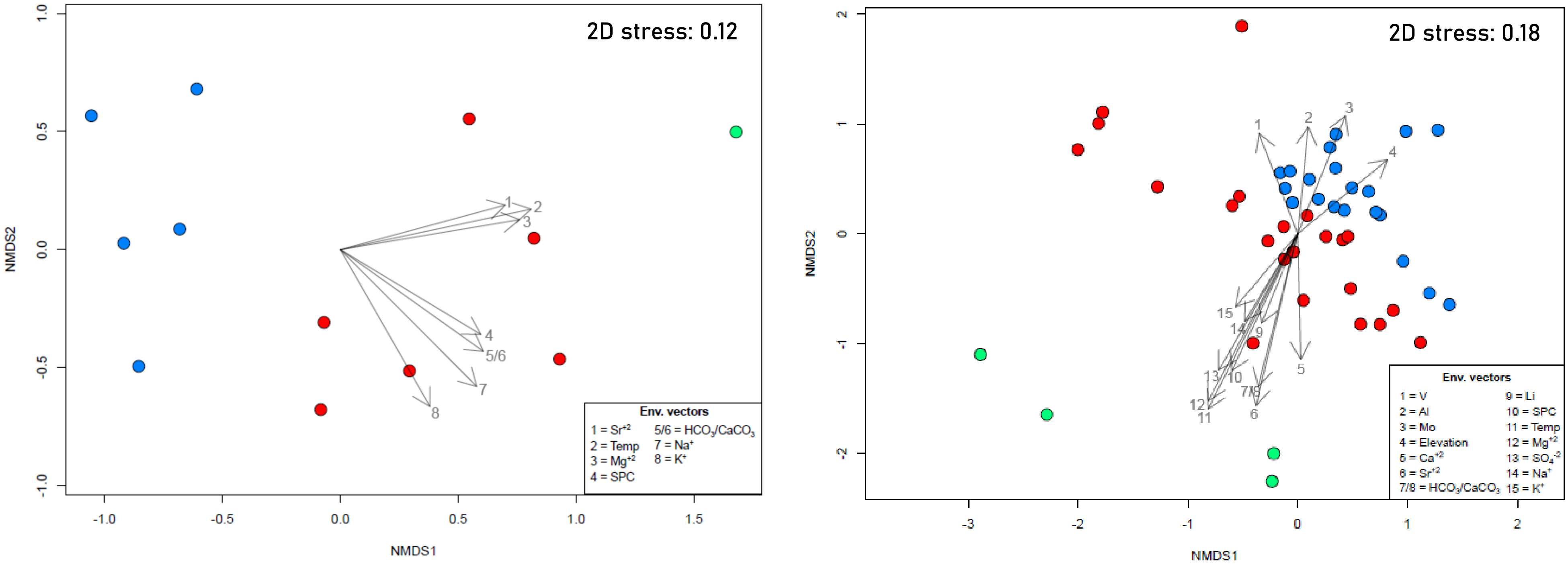

Figure 4.5. NMDS plot of benthic communities (at genus level) and microbial communities (at ASV level) in twelve undisturbed springs. The plot is color coded by geochemical groupings. Group 1: red color, Group 2: blue color, Group 3: green color. (**A)** Based on BMI community composition. ANOSIM showed statistically significant differences between the three clusters (global R = 0.846, a significance level of a sample statistic P-value = 0.001). Differences between Groups 1 and 2 are strong (R-value = 0.86) and statistically significant (P-value = 0.001). (**B)** Based on microbial community composition. ANOSIM showed statistically significant differences between the three clusters. Differences between Groups 1 and 2 are strong (ANOSIM R-value = 0.58) and statistically significant (P-value = 0.002). Environmental vectors are also overlaid on both NMDS plots.

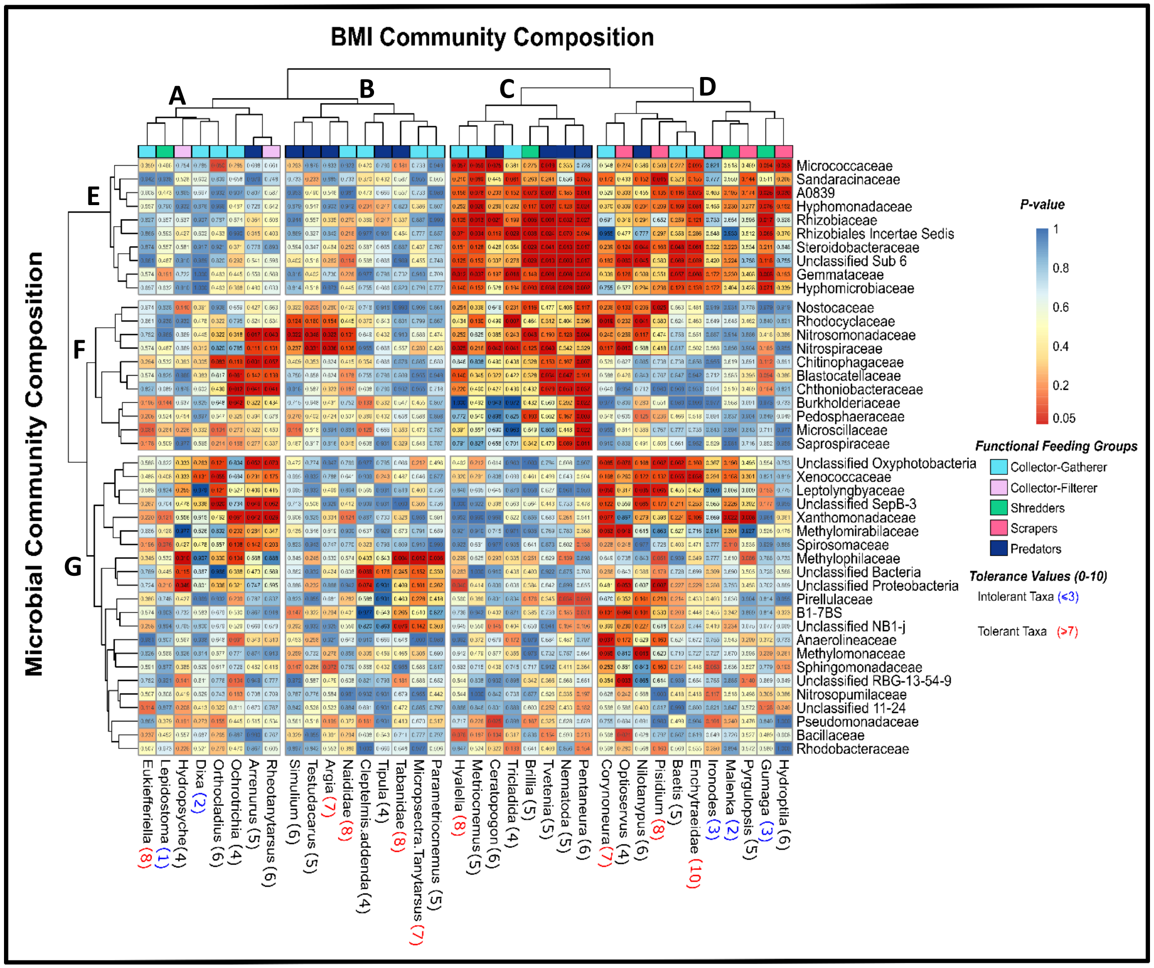

Figure 4.6. Heatmap is demonstrating the analysis of co-occurrence among common BMI (genus level) and microbes (family level) in twelve undisturbed springs in Owens Valley, CA. Hierarchical clustering was calculated (Ward Linkage) on BMIs (columns) and ASVs (rows). The numbers in the parentheses shows the BMI tolerance values.

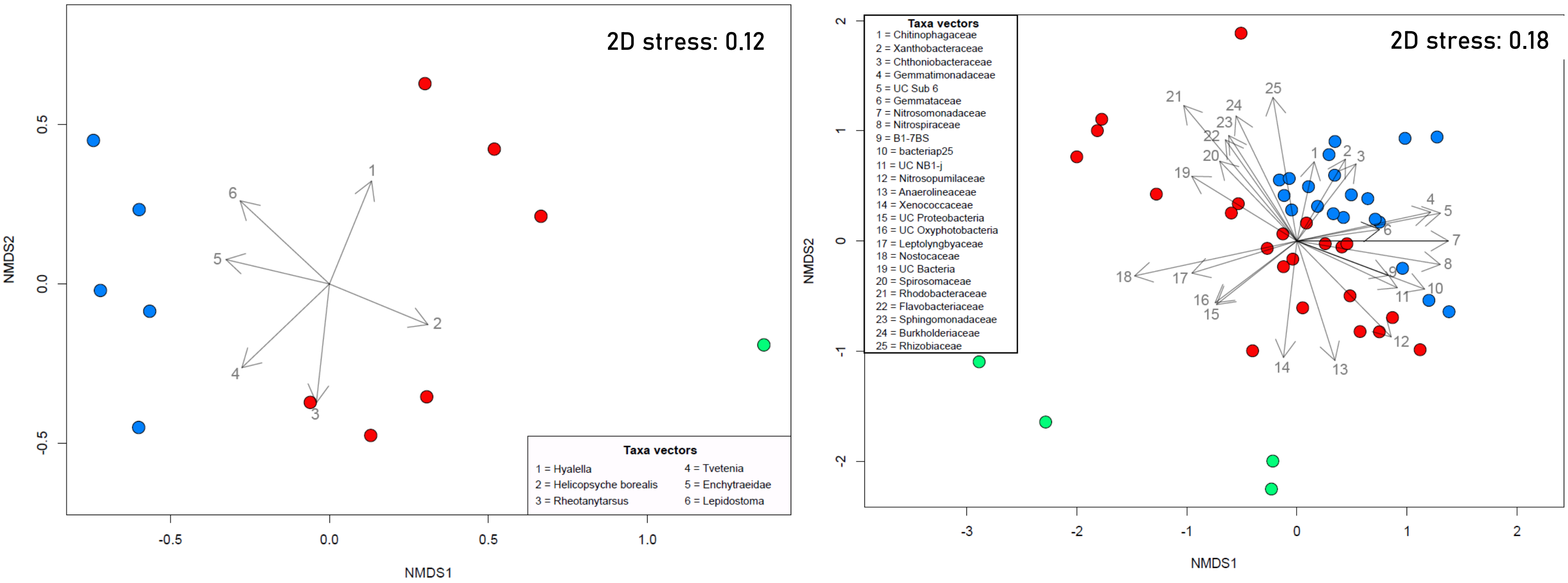

Figure 4.7. NMDS plot of BMI and microbial communities in twelve undisturbed springs. The plot is color-coded by geochemical groupings. The BMI and microbial taxa matrices are overlaid on BMI and microbial ordination plots, respectively. **(A)** NMDS plot based on BMI taxa. ANOSIM showed statistically significant differences between three clusters (global R = 0.846, a significance level of a sample statistic P-value = 0.001). Differences between Groups 1 and 2 are strong (R-value = 0.86) and statistically significant (P-value = 0.001). (**B)** NMDS plot based on ASVs. ANOSIM showed statistically significant differences between the three clusters. Differences between Groups 1 and 2 are strong (ANOSIM R-value = 0.58) and statistically significant (P-value = 0.002).

4.7 Tables

Table 4.1. Sample ID, spring name, location, and main field measurements of springs

Sample ID	Spring Name	UTM 11 S		Elevation	EC	Temperature
		E	N	m	μS/cm	°C
IES 54	Lubken Canyon Spring 2	375010	4152152	1240.623	176.7	26.0
IES 24	Spring along Hogback Creek	397732	4056750	1393.323	307.8	20.2
IES 26	Reinhackle Spring	401193	4061012	1144.635	513.2	15.1
IES 27	Boron Spring A	386328	4072314	1601.774	249.3	15.3
IES 28	Grover Anton Spring	385165	4082719	1593.781	392.8	15.7
IES 29	Unnamed spring north of Red Mountain	385305	4100901	1417.509	146.3	15.5
IES 33	Elderberry Canyon Spring	357900	4138046	1596.346	109.3	12.8
IES 34	Birchim Canyon Spring B	364114	4144865	1482.941	173.4	16.7
IES 38	Wells Meadow B	355222	4145412	1614.472	54.5	11.1
IES 42	North Harry Birch Spring	385273	4085645	1486.732	184.3	14.4
IES 43	South Harry Birch Spring	385440	4085284	1497.738	246.6	18.4
IES 31	Warm Spring A	387270	4125103	1253.598	790.1	29.2

Table 4.2. Bioassessment metrics for twelve reference springs in Owens Valley, CA. Mean (1 SE) bioassessment metrics for each group. Bioassessment metrics calculated using all BMIs identified and enumerated in each spring. Statistically significant metrics are shown in bold (one-way ANOVA, $\alpha < 0.0.5$). Abbreviations: EPT is (Ephemeroptera [mayflies], Plecoptera [stoneflies], and Trichoptera [caddisflies] and HBI is the Hilsenhoff Biotic Index (HBI; Hilsenhoff

Bioassessment metrics	IES24	IES54	IES34	IES26	IES29	IES27	Group 1	IES42	IES43	IES38	IES33	IES28	Group 2	IES31
<u>Functional Feeding metrics</u>														
Percent Shredders*	0.0	8.1	0.0	0.0	0.0	8.1	3.3 (1.7)	33.8	35.1	15.3	24.4	46.2	31 (5.2)	0.0
Percent Scrapers*	59.3	42.7	68.0	35.1	78.5	74.7	59.8 (7.1)	16.4	0.0	0.0	57.4	11.5	17.1 (10.6)	10.0
Percent Collector-Filterers	0.0	8.1	0.0	20.3	0.0	8.1	7.3 (3.3)	16.4	15.3	0.0	0.0	8.1	8.0 (3.6)	0.0
Percent Collector-Gatherers	28.0	42.1	11.5	36.9	10.0	8.1	21.7 (6.1)	31.3	20.3	60.7	15.3	36.3	32.8 (7.9)	80.0
Percent Predator	10.0	10.0	18.4	25.8	0.0	5.7	12.0 (3.8)	22.8	41.6	24.4	8.1	14.2	22.2 (5.7)	0.0
<u>Behavioral Metrics</u>														
Percent Swimmers	28.0	41.0	5.7	33.2	0.0	0.0	16.0 (7.4)	0.0	0.0	10.0	11.5	0.0	4.3 (2.6)	0.0
Percent Clingers	61.3	42.7	81.9	56.8	84.3	80.0	69.1 (6.9)	31.3	31.3	58.1	59.3	17.5	39.5 (8.2)	84.3
Percent Sprawlers*	5.7	12.9	5.7	0.0	0.0	8.1	5.4 (2.0)	42.7	42.1	27.3	15.3	37.5	33.0 (5.2)	0.0
Percent Climbers*	0.0	5.7	0.0	0.0	0.0	0.0	1.1 (0.9)	11.5	16.4	10.0	18.4	15.3	14.3 (2.6)	0.0
Percent Burrowers	0.0	8.1	0.0	0.0	0.0	0.0	1.6 (1.4)	0.0	22.8	0.0	8.1	26.6	11.5 (5.6)	0.0
<u>HBI related Metrics</u>														
Community tolerance value	5.8	5.9	4.9	4.5	4.2	4.9	4.9 (0.3)	4.8	4.9	4.4	4.2	4.9	4.6 (0.1)	4.2
Percent intolerant EPT*	0.0	5.7	0.0	0.0	0.0	0.0	1.1 (0.9)	25.8	14.2	27.3	25.1	37.5	26.0 (3.7)	0.0
Percent tolerant EPT	0.0	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)	0.0	0.0	0.0	0.0	0.0	0.0 (0.0)	0.0
Percent Tolerant taxa	26.6	27.3	5.7	0.0	22.0	18.4	14.7 (4.6)	33.8	17.5	32.6	15.3	30.0	25.8 (3.9)	8.1
Percent Intolerant taxa*	18.4	23.6	0.0	0.0	0.0	18.4	8.4 (4.6)	26.6	11.5	41.0	36.3	30.0	29.1 (5.0)	0.0

4.8 Supplementary Information:

4.8.1 Planktonic versus benthic microbes

Our findings are in line with the results of the previous studies (Griebler et al. 2002, Flynn et al. 2013, Ayayee et al. 2018), which revealed that the structure of planktonic (free-floating) communities is different from benthic (attached to sediment) communities. We detected a stronger relationship between BMI and benthic microbial communities than planktonic communities, based on the comparison of the macroinvertebrates and microbial communities in NMDS plots (Figures 5 and S1). The NMDS plot of planktonic communities is color coded to show the three clusters observed in the HCA plot of geochemical classification (Figure S3). The ordination pattern of benthic microbial communities followed the same pattern as the BMI ordination pattern (ANOSIM: global R = 0.58, P-value = 0.002), whereas planktonic communities did not demonstrate a similar pattern as BMI communities (ANOSIM: global R = 0.2, P-value = 3.5). This might be because planktonic samples represent momentary populations that were present at the time of sampling, whereas benthic microbial communities provide information about the established and colonized microorganisms (Rizoulis et al. 2013).

4.8.2 Similarity percentage (SIMPER)

Similarity percentage (SIMPER) was calculated using a Bray-Curtis matrix to detect shared organisms that were primarily responsible for the observed dissimilarity between the groups identified in the NMDS plot. Primer® v6 was used to calculate SIMPER (Clarke and Gorley 2006). The SIMPER analysis revealed that *Pyrgulopsis* (class: Gastropoda, Tolerance Value [TV]: 5), *Argia* (order: Odonata, TV: 7), and *Hyalella* (order: Amphipoda, TV: 8) were the BMI taxa shared among springs in cluster 1 (explaining the 73 percent of the similarity in Group 1). However, *Lepidostoma* (order: Trichoptera, TV: 1), Malenka (Order: Plecoptera, TV: 2), and *Hesperoperla* (order: Plecoptera, TV: 2) were the most abundant shared BMI taxa among springs in cluster 2 (explaining 53 percent of the similarity in Group 2). The structure of the BMI community within Warm Spring A was only comprised of four taxa. Genus Microcylloepus was dominant in this spring (consisting of 81percent of the sample population) and was only present in Warm Spring.

4.8.3 Supplementary Figures and Tables

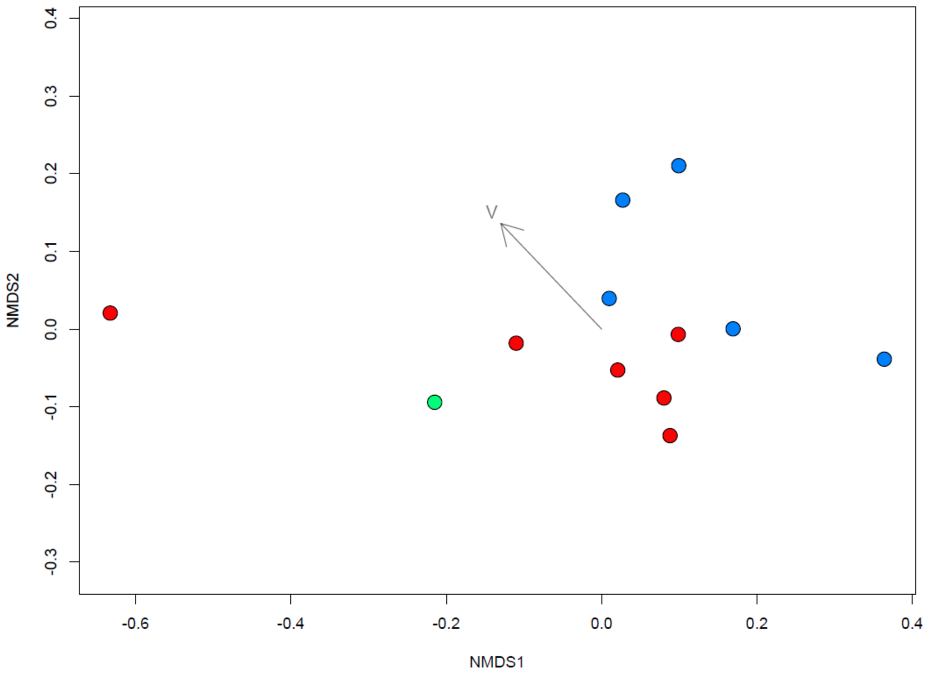

Figure 4.S1. NMDS plot based on planktonic ASVs. ANOSIM Global R =0.28, and the significance level of the ample statistic = 0.035. ANOSIM Pairwise R-value between Groups 1 and 2 =0.2, and p-value =0.38.

Table 4.S1. Observed counts, Shannon diversity index, and Gini-Simpson index for microbial ASVs (F: filter sample, S: average of sediment samples).

		Amplicon Sequence Variants		
Spring Name		Observed	Shannon	Simpson
Birchim Spring B	S	852	5.97	0.993
	F	1040	6.36	0.997
Boron Spring A	S	795	5.80	0.984
	F	953	5.82	0.993
Elderberry Canyon	S	588	5.72	0.994
	F	1185	7.00	0.997
Grover Anton Spring	S	854	6.16	0.996
	F	809	6.00	0.996
Hogback Creek A	S	862	6.22	0.997
	F	1110	6.44	0.997
Lubken Canyon Spring 2	S	714	6.03	0.995
	F	869	6.01	0.994

		Amplicon Sequence Variants		
Spring Name		Observed	Shannon	Simpson
North Harry Birch Spring	S	735	6.03	0.996
	F	1297	6.31	0.993
Reinhackle Spring	S	553	5.10	0.947
	F	1039	6.00	0.997
Red Mountain Spring	S	271	4.17	0.952
	F	150	4.82	0.991
South Harry Birch Spring	S	840	6.15	0.996
	F	1124	6.35	0.993
Well Meadows B	S	677	5.82	0.993
	F	1649	6.96	0.998
Warm Spring A	S	479	4.02	0.875
	F	1322	6.65	0.997

Table 4.S2. Richness, total number of individuals, Shannon diversity index, and Simpson index for BMIs.

	Benthic Macroinvertebrates			
Spring Name	Richness	Counts	Shannon	Simpson
Birchim Spring B	10	>300	0.7	0.3
Boron Spring A	10	>300	0.5	0.2
Elderberry Canyon	14	83	1.1	0.5
Grover Anton Spring	16	54	1.9	0.8
Hogback Creek A	10	>300	0.9	0.5
Lubken Canyon Spring 2	19	>300	0.4	0.1
North Harry Birch Spring	16	26	2.5	0.9
Reinhackle Spring	8	94	1.4	0.7
Red Mountain Spring	7	223	0.5	0.2
South Harry Birch Spring	23	168	2.0	0.8
Well Meadows B	7	29	1.4	0.7
Warm Spring A	4	>300	0.0	0.0

Table 4.S3. General chemistry data and geochemical groupings.

	Spring ID	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	K^{+} (mg/l)	Na^{+} (mg/l)	Sr^{2+} (mg/l)	Cl^{-} (mg/l)	NO$_3^{-}$ (mg/l)	NO$_2^{-}$ (mg/l)	PO$_4^{3-}$ (mg/l)	SO$_4^{2-}$ (mg/l)	CaCO$_3$ (mg/l)	HCO$_3^{-}$ (mg/l)	Balance (%)	Si as SIO2 (mg/l)	DO (mg/l)	DO (%)
Group 1	IES054	19.7	3.5	2.82	17.9	0.284	5.48	0.16	ND	ND	12.8	75	91	4.9	35.4	7.9	83
	IES024	36	8.15	2.22	24	0.411	5.52	2.71	ND	ND	9.33	162	198	-1.06	50.6	6.8	72.9
	IES027	33.9	4.88	2.58	28.7	0.174	13.6	2.01	ND	ND	32.4	126	154	-2.93	18.3	6.3	63
	IES029	14.9	3.76	2.15	9.77	0.098	1.9	0.74	ND	ND	9.51	66	81	-2.08	31.6	5.8	58.8
	IES026	27.4	5.65	4.07	39.7	0.195	16.9	0.52	ND	ND	22.8	134	164	-0.24	23.6	6.7	69.8
	IES034	20.7	4.48	4.13	44.8	0.108	19.4	2.35	ND	ND	13.6	128	156	-0.17	51	6.6	70
	Average	25.43	5.07	3.00	27.48	0.21	10.47	1.42	ND	ND	16.74	115.17	140.67	-0.26	35.08	6.68	69.58
	(±SE)	3.43	0.69	0.36	5.38	0.05	2.91	0.44	ND	ND	3.72	15.12	18.50	1.12	5.54	0.28	3.42
Group 2	IES038	10.3	0.62	1.3	3.74	0.031	0.28	0.76	ND	ND	4.1	34	41	-2.75	13.8	7.6	69.6
	IES028	44.9	3.38	2.79	16.4	0.188	13.7	0.84	ND	ND	23.8	121	147	-0.45	25.6	7.2	72.3
	IES033	14.6	0.38	1.27	6.19	0.053	0.68	1.34	ND	ND	14.6	34	42	0.55	15.7	8.7	81.8
	IES042	29.7	1.71	1.79	10.1	0.129	1.87	0.54	ND	ND	15.2	87	106	-0.99	18	5.4	53
	IES043	34.7	2.43	1.96	12.4	0.164	2.43	0.11	ND	ND	12.5	104	127	1.68	22.3	2.9	29.5
	Average	26.84	1.70	1.82	9.77	0.11	3.79	0.72	ND	ND	14.04	76.00	92.60	-0.39	19.08	6.36	61.24
	(±SE)	6.40	0.56	0.28	2.24	0.03	2.51	0.20	ND	ND	3.15	17.97	21.85	0.75	2.16	1.02	9.19
Group 3	IES031	46.50	20.70	3.31	28.80	0.49	11.70	0.95	ND	ND	65.00	183.00	224.00	-0.29	24.10	4.90	64.10

- ND: Not Detected.

4.9 Reference

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Chapter 5
Summary and Recommendation

This dissertation is a product of four years of collaboration between several universities (the University of Nevada Reno, the Purdue University, the University of the Pacific, the University of Nevada Las Vegas, and the New Mexico Institute of Mining and Technology), Desert Research Institute, National Science Foundation, and permitting agencies in an Integrated Earth System project. Specifically, this dissertation goal was characterizing modern spring systems and examining the relationship between ecology, hydrology, and geology that will be used as an input for the local-scale snapshot model. Snapshot models are local fine resolution hydrologic models to understand spring evolution during glacial/interglacial periods.

5.1 Summary

In chapter 2, the temporal variation of BMI communities was presented by comparing the structure of BMI communities within 12 undisturbed springs in Owens Valley, California. Environmental metrics and BMI samples were collected near the source of undisturbed springs during the summers of 1999 and 2000 and were compared with similar samples collected in 2017. Within these 17 years, three prolonged droughts occurred, followed by extreme wet years in between. The longest drought occurred between 2012 and 2016, which was distinguished as the driest period on record. This chapter gives us an insight into the role of aridland springs as refugial aquatic habitats protecting the benthic communities, especially crenobiontics, during frequent droughts and scouring floods in arid regions.

In chapter 3, we examined the association between the structure of BMI communities (ecology) and the geochemical fingerprint of the spring waters (hydrogeology). Meyers (2020) showed that the geochemistry of spring waters in Owens Valley is a function of the interactions between groundwater and geology of the contributing area. Our understanding of the relationship between hydrogeological parameters and the ecology of spring systems is in its early development stages. Spring systems are unique freshwater habitats because of their environmental stability, small size, and lack of large predators. Twelve mountain front springs along the eastern Sierra Nevada frontal fault zone were sampled for environmental characteristics and BMI taxa in 2017. Relationships between spring geochemistry, physical environment, and structural features of BMI communities were studied using nonmetric multidimensional scaling and nonparametric analyses. This chapter revealed an association between weathering-derived geochemical signals of spring waters and the spatial distribution of BMI communities in persistent aridland springs.

In chapter 4, we compared the ecological patterns of BMI and microbial communities found in aridland springs in Owens Valley, California, to examine the relationship between these biological organization. Also, we examined the potential drivers of ecological processes that structure BMI and microbial communities these spring systems. Eleven mountain front springs, as well as one regional spring, were sampled for characterizing their BMI, microbial, and water geochemistry compositions. A free ordination tool, nonmetric multidimensional scaling, was applied on both biological datasets to compare their community assembly. Mantel test and co-occurrence heatmap were also used to calculate and visualize the correlation between BMI and microbial communities. As far as we know, there are no studies that have been conducted directly comparing the community structures and ecological patterns of BMI and microbial communities in freshwater ecosystems. In this chapter, we found similar ordination patterns and a significant relationship between BMI and microbial communities in undisturbed aridland springs. Our findings highlight the geochemistry of spring water as a primary factor in shaping both BMI and microbial communities.

5.2 Recommendations

The spatial distribution of BMI assemblages along the spring run- All questions in this dissertation were answered based on our source samples of BMIs. We collected the BMI samples at three locations along the channel in longer springbrooks (>150 m) and two locations in shorter springbrooks. All samples included source samples (within 15 m of the source), followed by the middle of the springbrook (in brooks < 150 m), then at 150 m from the source where three samples were collected. One can use this data and examine the spatial distribution of BMI assemblages along the spring run. McCabe (1998) reviewed environmental changes occurring along the spring channel from the source to the terminus to examine the possible response of BMIs to environmental changes along the spring channel. Previous studies showed that some species (i.e., crenobiontic taxa) tend to live near the source, and some can only be found downstream. Our findings in chapter 2 (based on source samples) suggest that either: (1) BMI communities are minimally affected by environmental condition and variation; (2) communities were disturbed by drought event(s), but conditions during wet years rapidly recovered the communities to their predisturbance condition; or (3) environmental variation over this period was insufficient to influence the structure of BMI communities. All scenarios highlight the role of a spring source as climatic refugia and its importance in conserving endemic and endangered flora and fauna in the region.

Although we showed that the environmental stability of spring systems near their sources protects BMI communities over climatic disturbances, the response of BMI organisms in the lower section of the spring run to these disturbances are more puzzling and unknown. Understanding changes in the communities and biodiversity of BMIs along the spring channel become a concerning issue as population growth and climate change causes higher pumping and lowering the spring discharge in arid regions. As a consequence, BMIs living in the spring channel is facing frequent and severer stress that BMIs near spring sources. Thus this recommended study is vital to identify springs that require extraordinary protection measurements and springs that are resilient and can support their biota for a longer time. Results coming out of this potential research provide an informative tool for the conservation and management of spring ecosystems in arid regions.

Expand the hydrogeology and Aquatic Ecology Relationship- In chapter 3, we found that weathering-derived geochemical signals of spring waters explain the spatial distribution of BMI communities in undisturbed springs within a valley. The geologic heterogeneity of the eastern Sierra Nevada in Owens Valley provides a unique case study to identify the factors explaining the structure of BMI communities in a valley-scale study. We collected BMI samples from different areas of interest (Owens Valley, Death Valley, Ash meadow, and Spring Mountain). Our dataset from springs in the southern Great Basin provides the opportunity to study the significance of the relationship between hydrogeology and ecology among springs in different valleys with distinct topography and geology. Springs were selected throughout the southern Great Basin to cover a broad gradient of residence times (e.g., modern, a mixture of modern and old, and very old groundwater), landscape associations (mountain, local, and regional aquifers), topography (high elevation White Mountain to the lowest elevations in Death Valley), hydrogeological units (granitic, volcanic, and carbonate units), and BMI taxa (crenobiontics, intolerant taxa, and tolerant taxa). All springs are permanent springs with relatively stable conditions. The majority of springs in the area are rheocrene (flowing into a channel), and several springs, especially in Ash Meadow, emerged in large pools before flowing into a channel (limnocrene).

Springs in the study area arise from different hydrogeologic units. These units consist of plutonic rock (Sierra Nevada), carbonate rock (Spring Mountains and Ash Meadow), and alluvial-filled basins (Death Valley and Owens Valley) (Burchfiel et al. 1974, Belcher and Sweetkind 2004, Lutz et al. 2017). Forty-six springs were initially chosen for isotope hydrology and geochemistry analysis. Twelve springs were later added to improve the resolution of the geochemistry and BMI data in the study area. Approximately 35 references (undisturbed and naturalized) springs were selected for ecological analysis. We selected 18 springs along the Owens Valley, nine springs in Death Valley and Ash Meadows, seven springs in the Spring Mountains, and one spring in the White Mountains.

One can test this relationship among springs with distinct aquifer types and landscape associations (e.g., Sada and Thomas (in press)). Mountain, local, and regional aquifers are the main types of supporting aquifer provenances in the central-southern Great

Basin (Mifflin, 1968; Thomas et al., 1996). Mountain aquifer system supports springs with a short groundwater residence time that discharge within the high elevation of mountains. Most of Owens Valley, White Mountains, and Spring Mountains springs are mountain springs. Mountain springs are cooler (<15°C) and more dilute (EC< 200 uS/cm) (Sada and Thomas in press). The majority of local springs discharge on the alluvial fan (bajadas) and valley floors. Groundwater residence time in a local aquifer is generally longer than a mountain aquifer. Bajada springs are typically cold (10 °C < to <50 °C), and their geochemistry is similar to mountain springs (EC< 500 uS/cm). However, valley springs are mostly a mixture of different flowpaths with a wide range of chemical characteristics; they are typically warmer and contain higher chemical concentration than mountain and bajada springs. Regional springs also occur on valley floors, and most of them are permanent aquatic habitat because of their deep groundwater circulation and constant discharge. The regional springs have warm (25°C to 40°C) water with stable electrical conductance (EC = 500 to 1000 uS/cm). Springs at Death Valley and Ash Meadows are mainly valley or regional springs. We did not collect any playa and geothermal springs because of their harsh environment and disturbed condition. The hydrological and ecological characteristics of these springs were mainly outlier in previous studies.

One can also test the hydrogeology-ecology association among springs with distinct groundwater residence time by evaluating the response of BMI organisms to “young” versus “old” groundwater residence times. Pinder and Jones (1969) suggested that deeper flowpaths have a longer groundwater residence time with higher ion concentration and warmer water (geothermal heating) at the spring emergence, while local groundwater flowpaths have shorter mean groundwater residence time with lower solute loads.

Furthermore, shallow flowpaths are more sensitive to climate change and will dry earlier relative to springs with a longer residence time (Rademacher et al. 2001, Frisbee et al. 2013). The potential hypothesis would be that intolerant taxa tend to live in springs with short groundwater residence time and cold temperature. In contrast, tolerant taxa would be dominant in springs with longer groundwater residence time (which has higher temperatures and electrical conductance). Ultimately, developing the field of ecohydrogeology enhances the efficiency of the management and monitoring of groundwater-dependent ecosystems.

Providing input for the landscape evolution model- Geologists at New Mexico Institute of Mining and Technology will combine existing data from tectonic development, paleo-hydrological evolution, and climate fluctuations to construct a 3-D model of landscape evolution over the past 14 Ma in the southern Great Basin. The model will quantify the hydrologic fragmentation of the originally lentic and lotic ecosystems into isolated springs. This model will provide a better understanding of interbasin hydrological connections that permitted minimally-vagile aquatic species (benthic macroinvertebrates and fishes) to colonize the southern Great Basin. The outcome of this dissertation will be used as an input to validate the model.

Examining the underlying processes responsible for the similar ordination patterns among BMI and microbial communities- In chapter 4, we compared the ordination pattern of microbial communities with the well-studied BMI community compositions in a freshwater ecosystem. Our results show a similar ordination trend and significant relationship among BMI and microbial communities in undisturbed aridland springs. However, further experimental and field studies on these distinct biological organizations are necessary to improve our understanding of microbial and BMI symbioses, as well as their survival strategies and physiological adaptations. This provides insight into the underlying processes shaping these communities.

Furthermore, since macroinvertebrates are well known in examining the ecological health of ecosystems, the similar spatial distribution patterns and significant co-occurrence relationships of microbial and BMI communities, may suggest that microbial communities could serve as an alternative bioindicator for evaluating the environmental conditions of spring systems. More into depth research on microbial response to different environmental conditions can help us to create bio-assessment metrics based on microbes.

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