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Authors
Forrest, Matthew J
Sada, Donald W
Edwards, Matthew S
et al.

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RESEARCH ARTICLE

Oases of endemism: Regional aquifer desert springs serve as biodiversity hotspots preserving vulnerable endemic taxa in the Great Basin and Mojave Desert regions

Matthew J. Forrest,[1*] **Donald W. Sada**,[2] **Matthew S. Edwards**,[3] **Robert K. Burton**,[4] **Richard D. Norris**[1]

[1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA; [2]Division of Hydrologic Sciences, Desert Research Institute, Reno, Nevada, USA; [3]Department of Biology, San Diego State University, San Diego, California, USA; [4]Department of Applied Environmental Science, California State University-Monterey Bay, Seaside, California, USA

Abstract

Spring ecosystems in arid regions often serve as crucial biodiversity hotspots by providing some of the only reliable sources of surface water. However, anthropogenic activities and climate change have severely degraded spring ecosystems worldwide, emphasizing the need for large-scale multidisciplinary studies informing conservation efforts. Here, we analyzed characteristics of 1121 widely dispersed springs within the Great Basin and Mojave Desert regions, USA to assess the distribution of endemic animal taxa relative to hydrological, geochemical, and ecological parameters. To our knowledge this is the largest original dataset of springs that has been examined in this manner. Our results revealed that the three broad classes of springs: regional aquifer thermal springs, local aquifer cold springs, and local aquifer geothermal springs can be distinguished by their environmental and physicochemical parameters, taxa present, persistence, and natural and human perturbations. Notably, regional aquifer thermal springs support disproportionate numbers of endemic taxa, likely reflecting their stability and longevity. These patterns are particularly striking considering that these springs have been subjected to extreme anthropogenic exploitation and modifications for centuries and frequently support non-native taxa. Conversely, local aquifer cold springs, which are often highly disturbed and less persistent, support proportionately fewer endemic taxa. The high abundance of endemic taxa in regional aquifer thermal springs indicates that they represent critical biodiversity hotspots, and efforts to conserve and restore these important ecosystems should be major priorities.

Springs are biodiversity hotspots and keystone ecosystems that support a substantial number of the aquatic and riparian taxa inhabiting arid regions (Hubbs 1995; Sada and Vinyard 2002; Fensham et al. 2023). Despite their relatively small sizes, natural springs in desert regions have disproportionate influences on surrounding landscapes (Davis et al. 2017; Cartwright et al. 2020; Parker et al. 2021) and may also serve as museums of biodiversity, preserving lineages that would otherwise have gone extinct (Murphy et al. 2015). Isolation within spring refugia can also lead to diversification and the evolution of novel endemic fauna (Davis et al. 2013; Murphy et al. 2015; Campbell et al. 2022). While several studies have examined individual springs and their biota, long-term landscape-level assessments of biotic and environmental relationships of springs have rarely been investigated (Cantonati et al. 2020; Stevens et al. 2021a; Fernández-Martínez et al. 2024). Many of the world's largest aquifers are being depleted far more rapidly than they can be recharged by rain or runoff, emphasizing the need for more research and effective conservation efforts (Richey et al. 2015; Tortajada and González-Gómez 2022). Desert springs and associated groundwater dependent ecosystems are threatened by

*Correspondence: matthewforrest@gmail.com

Matthew J. Forrest and Donald W. Sada contributed equally and are the cofirst authors.

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anthropogenic disturbances including unsustainable groundwater pumping, diversions, and modifications, which have resulted in extirpations and extinctions of multiple species (Sada and Vinyard 2002; Parker et al. 2021; Sada and Thomas 2025). Because desert springs often serve as ecological refuges, conservation planning should incorporate a high level of protection for these endangered ecosystems (Stevens et al. 2021a; Fensham et al. 2023; Zhang et al. 2023).

The Great Basin and Mojave Desert regions of the western United States contain the largest areas of contiguous internally drained (endorheic) watersheds in North America. The regions are primarily characterized by mountainous and arid climates, with few widely dispersed large permanent wetland habitats. However, thousands of springs are located within the Great Basin and Mojave Desert regions, some of which are occupied by endemic organisms that are exclusively restricted to spring environments—taxa known as crenobionts, whose entire ranges may be limited to a single drainage basin, spring complex, or even a single spring (Sada and Vinyard 2002; Keleher and Sada 2012; Walters et al. 2022). For example, the regional aquifer thermal spring systems within Ash Meadows National Wildlife Refuge in the Mojave Desert contain at least 27 endemic animals and plants, which is the highest known endemicity of vertebrates, benthic macroinvertebrates, and plant taxa of any locality in the continental United States (Whiteman and Sites 2008; Fensham et al. 2023). However, more than 65% of the springs in the Great Basin and Mojave Desert regions have been severely degraded, and at least 16 endemic species, subspecies, or distinctive populations have gone extinct since the late 1880s, including 12 fishes, three mollusks, and one aquatic insect (Sada and Vinyard 2002). While there are various reasons for this, groundwater pumping and surface water flow diversions have been identified as the primary causes (Parker et al. 2021), and the documented extinctions of two species along with the extirpation of 27 populations of crenophilic taxa (spring dwelling organisms) between the late 1980s and 2013 indicate that desert springs continue to be one of the most endangered ecosystems in North America (Sada and Lutz 2016; Work 2023).

In arid regions, spring environments represent "island" ecosystems surrounded by "seas" of deserts—often providing the sole source of water for the biota within and around them (Soltz and Naiman 1978; Work 2023). Most of the springs in the Great Basin and Mojave Desert regions are completely isolated from other aquatic systems, making them useful systems to describe relationships between biota and environments. More than 150 endemic crenobiontic (spring obligate) taxa, including fishes, mollusks, and aquatic insects, occur in persistent springs throughout the Great Basin and Mojave Desert regions (Keleher and Sada 2012; Sada and Lutz 2016; Sada and Stevens 2020). Most prior investigations of endemic crenobiontic taxa involved springsnails (*Tyronia* spp. and *Pyrgulopsis* spp.; e.g., Taylor 1985; Hershler and Liu 2008; Walters et al. 2022) and pupfish (*Cyprinodon* spp.; e.g., Hubbs and Miller 1948; Echelle 2008; Lutz et al. 2023). Pupfish are small, charismatic native fishes that have inspired desert spring conservation endeavors as well as legal challenges to water rights. In fact, the plight of the Devils Hole pupfish (*Cyprinodon diabolis*) was the motivation for a federal reserved water rights case decided by the US Supreme Court in 1976, which helped preserve its critical habitat and focused attention on the overexploitation of regional aquifer springs (Brown 2021).

Springsnails are tiny, gill-breathing gastropods that have been popular subjects of biogeographic studies due to their antiquity and close linkage with aquatic systems (Taylor 1985; Hershler et al. 2007; Walters et al. 2022). In contrast with pupfish and springsnails of the genus *Tryonia*, which are crenobionts that naturally live exclusively in regional aquifer springs (Miller 1946; Hershler and Sada 1987; Hershler 1998), *Pyrgulopsis* inhabit diverse spring-fed waters, including local cold and hot springs (Hershler et al. 2007; Hershler and Liu 2008). There are over 80 species of *Pyrgulopsis* springsnails inhabiting the Great Basin and Mojave Desert regions, and many are endemic to single springs, spring systems or drainage basins (Hershler and Sada 1987; Walters et al. 2022). While other studies have examined plants from regional aquifer springs (Love et al. 2022; Fraga et al. 2023) and recognizing that cryptic species are likely present (Witt et al. 2006; Work 2023), we focused on animal taxa. We also categorize the animals according to their "taxa" rather than "species" because many of the animals, particularly the pupfish (*Cyprinodon* spp.), have been subjected to taxonomic revisions, and several are regarded as subspecies.

We investigated relationships between environmental characteristics and the occurrences of animal taxa occupying 1121 springs within the Great Basin and Mojave Desert regions. To our knowledge, this is the largest original dataset of desert springs that has been analyzed to date. For the purposes of our study, we separated the 1121 springs into three broad categories that differ in temperature and geochemistry due to differences in residence time, depth, and locations of the aquifers. Specifically, these categories are local aquifer cold springs ($n = 1014$), local aquifer geothermal springs ($n = 62$), and regional aquifer thermal springs ($n = 45$). Local aquifer springs are generally supplied by recent precipitation and snowmelt from nearby mountain ranges, while regional aquifer thermal springs are fed by carbonate aquifers that collect and retain water precipitated over larger areas and longer time periods (Hay et al. 1986; Coolbaugh et al. 2005; Zhang 2022). We further classified the local aquifer systems into two broad categories: (1) Local aquifer geothermal springs, which are sourced locally even though they are fed by geothermal waters with year-round temperatures exceeding 25°C, and (2) Local aquifer cold springs whose waters are typically relatively unmodified from local rainfall or snow melt, with mean temperatures of $\sim 15°C$. While local aquifer springs may flow intermittently and often occur in drainages exposed to seasonal flooding and spring channel erosion, regional aquifer thermal springs generally occur in areas unaffected by natural drying and scouring floods, and have substantial and continual discharges, as well as consistent warm temperatures and water chemistries (Rose and Davisson 2003; Zdon and Love 2020; Sada and Thomas 2025).

Prior studies showed that a disproportionate number of endemic crenobiontic taxa are found in springs fed by regional aquifers (Sada and Vinyard 2002; Keleher and Sada 2012; Walters et al. 2022), and to a lesser extent local aquifer geothermal springs (Hershler et al. 2007), therefore our overarching question centered on elucidating which factors best explain these patterns. We hypothesize that the regional aquifer springs support more endemic crenobiontic taxa in the Great Basin and Mojave Desert regions due to their environmental and physicochemical aspects, such as constant warm temperatures, long-term persistence, and high discharge rates, along with consistent geochemical parameters that may exclude some taxa and influence speciation patterns and rates. We used multivariate statistical analyses to investigate how environmental variables and taxa differ between spring types and to understand which factors contribute most to these differences. These analyses will facilitate resource management by providing information to identify high conservation value springs as indicated by environmental characteristics and the presence of endemic crenobionts.

Materials and methods

Description of data

Environmental and biological data were collected between 1991 and 2008 at each of the 1121 spring sites (Fig. 1) by Sada and his colleagues (W. Pratt, R. Hershler, G. Vinyard, C. Rosamond, and A. Schwaneflugel). Water chemistry information (pH, conductivity, temperature) was collected only at the eucrenal (spring source) environments because these parameters often change along the spring flowpath, while springbrook width, water depth, vegetative cover and substrate composition were collected within spring channels. When data were collected multiple times at the same spring, we used the data from the most recent sampling endeavors. Therefore, data from each spring site was represented by one collection date. Regional springs discharging from the carbonate-rock aquifer regional flow systems within the Great Basin and Mojave Desert regions have substantial and continual discharges, as well as consistent warm temperatures, water chemistry, and isotopic signatures (Thomas et al. 1996; Rose and Davisson 2003; Zdon and Love 2020); therefore, a single sampling campaign can adequately capture their characteristics (Hershey et al. 2010). The data comprised five categories, including (1) survey date and spring location, (2) water chemistry parameters (i.e., temperature, pH, and conductivity), (3) physical characteristics of the aquatic and riparian environment (i.e., springbrook width, water depth, vegetative cover, and substrate composition), (4) natural and anthropogenic factors that may be stressing the aquatic and riparian systems (i.e., the presence of diversions, cattle, feral horses, and burros [hereafter referred to as "equine"], and modifications to the hydrology for recreational activities like bathtubs or constructed pools), and (5) the presence of crenophilic, endemic crenobiontic, springsnails, native fish, and non-native taxa (Fig. 2a, b). For more details about spring parameters see Sada and Lutz (2016).

Springbrook length, estimated in meters, was measured by pacing distance following calibration for the length of stride. Water temperature and conductivity were measured as close to the spring source as possible using a YSI Model 30 m, and pH was measured using an Oakton pHTestr2 meter that was calibrated daily. Emergent cover was recorded by qualitatively estimating to the nearest 10% the amount of riparian and instream vegetation, debris, or other material arising within the wetted perimeter and shading the substrate of the spring and its spring brook and totals were calculated as the mean estimated emergent cover across five transects that were placed at 5-m intervals along the length of the springbrook channel. Vegetative bank cover was qualitatively estimated to the nearest 10% as the amount of live vegetation that covers spring brook banks within the riparian zone and totals were calculated as the grand mean from the two banks at the end of transects. Substrate composition was qualitatively estimated ($\sim$ 10% accuracy for each category) using a Wentworth particle scale analysis, which describes the substrate by the proportional composition of materials, where materials are classified as: fines (< 1 mm), sand (1–5 mm), gravel (> 5–80 mm), cobble (> 80–300 mm), boulder (> 300 mm), or bedrock. Size is defined as the minimum particle size of substrate as measured on a two-dimensional axis, as would pass through a substrate sieve. Benthic macroinvertebrate samples were collected using a 500 μm net and combining five samples collected within 120 cm^2 quadrats placed within the upper 25 m of spring brooks. Quadrats were placed along five transects at 5-m intervals and oriented perpendicular to the thalweg. Quadrats were sequentially placed along transects at spring brook center, right bank, center, left bank, and center. The presence of certain animals including amphibians, finger clams, amphipods, pulmonate gastropods, flatworms, fishes, ostracods, and non-native species was also recorded. Qualitative estimates of disturbances from diversion, horses and burros, cattle, and human recreation were each categorized on a scale from 1 to 4, where 1 was the least disturbed and 4 was the most disturbed.

The environmental data used in the multivariate analyses include spring temperature, conductivity, pH, percent emergent cover, percent bank cover, percent silt, sand, gravel, and cobbles, and springbrook width and water depth (Table 1). Biological parameters used in analyses included the presence of crenophilic taxa, endemic taxa, non-native taxa, springsnails, native fish taxa, and disturbance factors (diversion, equine, cattle, recreation) (Table 2). Counts of individual taxa were not collected—only their presence was recorded. In

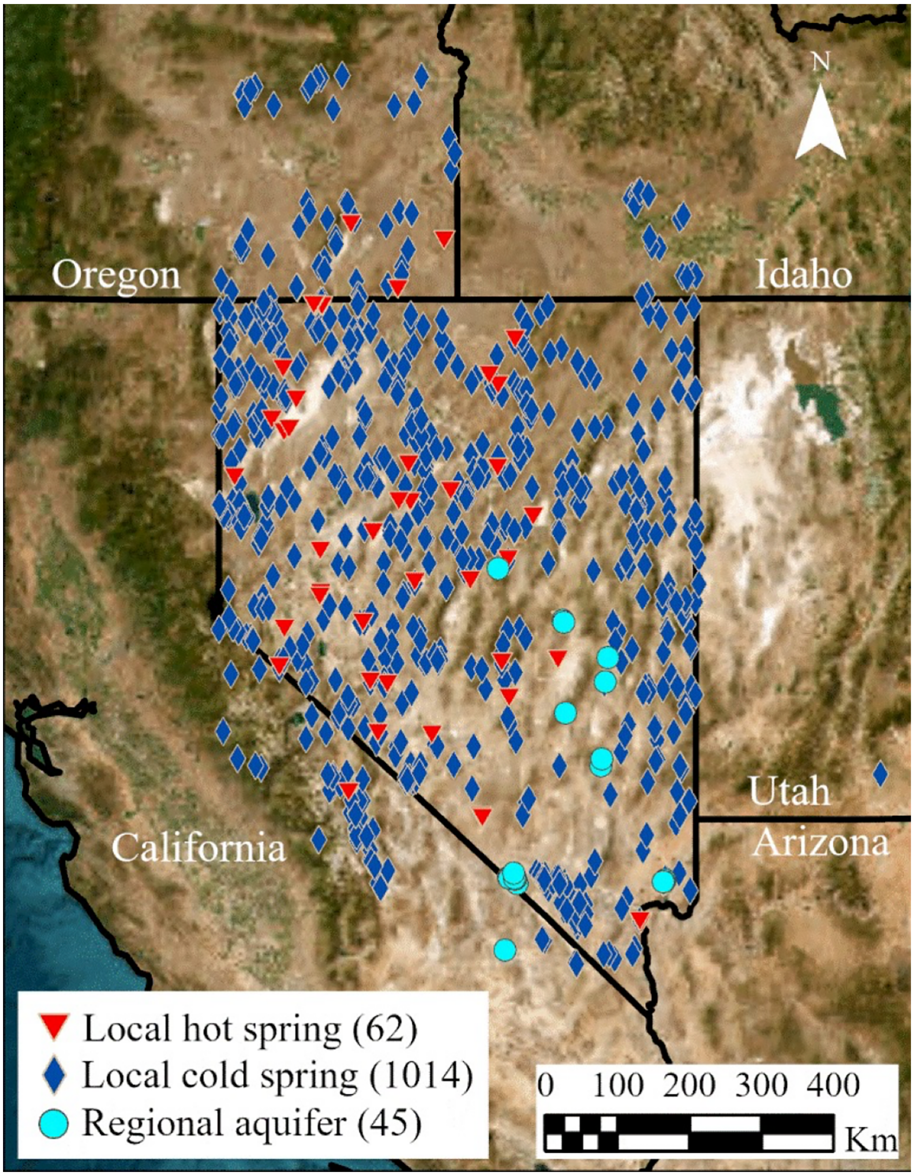
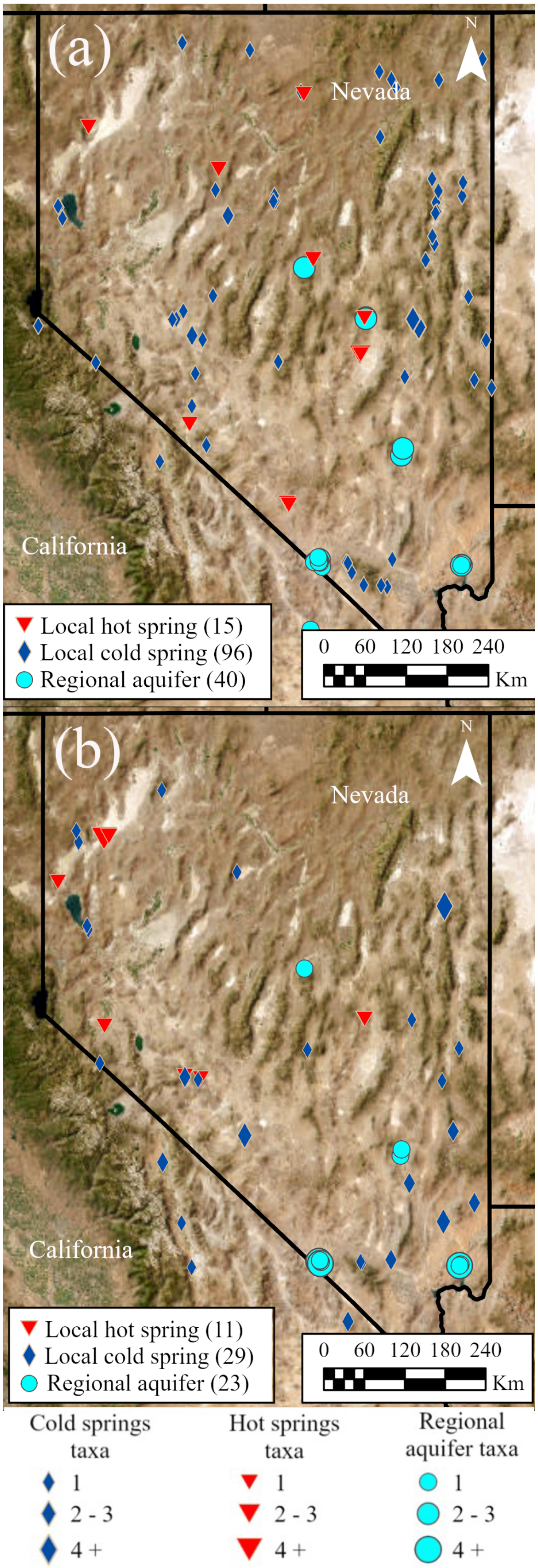


Fig. 1. Map of all 1121 springs from the Great Basin and Mojave Desert regions analyzed in this study showing locations of regional aquifer thermal springs (represented by light blue circles), local aquifer geothermal (red upside-down triangles), and cold springs (blue diamonds).

this study, the biological parameters were analyzed as independent variables that could be used to characterize spring types, not dependent variables as in some other studies of springs. Endemic crenobionts were determined and classified from the literature, and non-native taxa (taxa that do not occur naturally in the study area) were recorded during field surveys. The non-native taxa were neither crenobiontic nor exclusively crenophilic, and the most common non-native taxa were bullfrogs (*Lithobates catesbeianus*), western mosquito fish (*Gambusia affinis*), sailfin mollies (*Poecilla latipinna*), and other aquarium fishes, redrim melania snails (*Melanoides tuberculata*), and red swamp crayfish (*Procambarus clarki*) (Sada and Vinyard 2002; Hershler 1998). Springs were classified as described above and labeled as either local aquifer cold springs (hereafter referred to as "local cold"), local aquifer geothermal springs ("local hot"), or regional aquifer thermal springs ('regional aquifer') for comparisons using multivariate analyses. These assignments were made based on extensive research and knowledge by Donald W. Sada prior to statistical analyses to avoid bias in the analyses. While other authors have further divided springs into more specific categories based on geomorphic, hydrogeochemical, and ecological criteria (Springer et al. 2008; Springer and Stevens 2009; Stevens et al. 2021b), we elected to use a coarser approach to provide more details regarding the environmental and physicochemical aspects that contribute most significantly to the



presence of endemic crenobiontic taxa. We also used Hierarchical Cluster Analyses to support our groupings according to these classifications (Figs. S1, S2A–C). Spring complexes, here defined as multiple springs within a single relatively small geomorphological setting, were analyzed by selecting the most representative spring to prevent biases due to pseudoreplication and spatial autocorrelation.

Multivariate statistical analyses

We analyzed a total of 1121 springs, of which 1014 were local aquifer cold springs, 62 were local aquifer geothermal springs, and 45 were regional aquifer thermal springs (Fig. 1). We analyzed the dataset for three water chemistry parameters (conductivity, pH, temperature), eight physical characteristics (depth, width, emergent cover, silt, sand, cobble, gravel, bank cover), four disturbance factors (diversion, equine, cattle, recreation), and five taxonomic groups (endemics, crenophiles, springsnails, non-native taxa, native fishes). Data were analyzed three ways: (1) using both the environmental and biological data, including locations; (2) using only the environmental data; (3) using only the biological and anthropological disturbance data. We used these three different methods because our dataset is very lengthy and complex and because we did not collect all data at the same times and places. Therefore, we felt that these separate analyses would independently and together corroborate our groupings, and more importantly, help elucidate which factors contribute most significantly to our hypothesis that the regional aquifer springs support a disproportionate number of the endemic crenobiontic taxa in the Great Basin and Mojave Desert regions due to their unusual environmental and physicochemical parameters.

Prior to analyses, the biological data (numbers of crenophilic taxa, endemic taxa, non-native taxa, springsnails, and native fish taxa found in each spring) were square root transformed to ensure bivariate normality as recommended by Clarke and Gorley (2006) and the data were normalized to place the different variables in similar scales. The environmental data were not transformed but rather normalized to place

Fig. 2. (**a**) Map of springs from the Great Basin and Mojave Desert regions analyzed in this study showing locations of endemic taxa inhabiting regional aquifer thermal springs (represented by light blue circles), local aquifer geothermal (red upside-down triangles), and cold springs (blue diamonds). Sizes of the symbols reflect the number of taxa present in each spring or spring complex (see legend). Numbers in parentheses in legend show the number of springs with endemic taxa in each spring type. (**b**) Map of springs from the Great Basin and Mojave Desert regions analyzed in this study showing locations of non-native taxa inhabiting regional aquifer thermal springs (represented by light blue circles), local aquifer geothermal (red upside-down triangles), and cold springs (blue diamonds). Sizes of the symbols reflect the number of taxa present in each spring or spring complex (see legend). Numbers in parentheses in legend show the number of springs with non-native taxa in each spring type.

Table 1. Average (untransformed) values of environmental parameters from regional aquifer springs (regional aquifer; $n = 45$), local aquifer geothermal (local hot; $n = 62$), and cold springs (local cold; $n = 1014$). Standard deviations in parentheses.

Aquifer type	Depth (cm)	Width (cm)	Temperature (°C)	Conductivity (μS cm^{-1})	pH (pH units)	Emergent cover (%)	Bank cover (%)	Silt (%)	Sand (%)	Gravel (%)	Cobble (%)
Regional	23 (38)	286 (493)	26.4 (6.8)	1226.5 (712.3)	7.55 (0.2)	52 (37)	85 (25)	67 (37)	12 (19)	7 (18)	9 (25)
Local hot	24 (44)	475 (1230)	34.1 (11.1)	1254.4 (1203.1)	7.85 (0.7)	31 (36)	65 (37)	58 (43)	21 (29)	15 (25)	4 (11)
Local cold	10 (29)	352 (1049)	15.2 (4.1)	512.2 (720.1)	7.78 (0.5)	55 (38)	67 (35)	50 (42)	19 (25)	21 (28)	9 (19)

them in a similar scale. These data were then used to generate a Euclidean distance-based resemblance matrix between site pairs for subsequent multivariate statistical analyses. We performed Hierarchical Cluster Analysis using only temperature, pH, and conductivity to confirm our classifications of the individual springs as either local aquifer cold springs, local aquifer geothermal springs, or regional aquifer thermal springs (Figs. S1, S2A–C). We then used a combination of PERMANOVA, distance-based linear models (DistLM), principal components analysis (PCA), and similarity percentage analyses (SIMPER) to characterize biological and physical differences between local cold springs, local hot springs, and regional aquifer springs. All multivariate statistical analyses were done using PRIMER (v. 6.0) software (Clarke and Gorley 2006) except for the PCA analyses, which were done using R.

Results

Hierarchical cluster analysis of all springs using only temperature, pH, and conductivity supported our groupings of the individual springs as local cold springs, local hot springs, or regional aquifer springs, with each group generally forming clusters together with only minor overlap between local hot and regional aquifer springs (Fig. S1). Indeed, with few exceptions, the springs grouped together according to their assigned categories. However, given there were more than 1100 springs in the cluster analysis, which obscured smaller differences among similar spring types when all were examined together in a single dendrogram (Fig. S1), we further examined three regions of the cluster analysis that roughly identified the three spring types to elucidate more detailed groupings of the spring types (Fig. S2A–C). These analyses supported our assignments of individual springs to the three spring types.

When all environmental and biological data were considered, local cold springs, local hot springs, and regional aquifer springs differed significantly from one another (PERMANOVA: pseudo-$F_{2,1118} = 141.5$, $p(\text{perm}) = 0.001$), with differences among spring types explaining 59% of the overall variation among all springs, and differences among springs within each type explaining 41% of the overall variation (Table 3). The DistLM identified a best model that included 11 of the 15 environmental variables that significantly ($p < 0.05$) influenced the biological differences among spring types. These were: springbrook depth and width, temperature, conductivity, percent emergent cover, bank cover, the percentage of silt and sand, diversion, equine, and cattle (see Table S1 for a complete listing of environmental variables).

Regional aquifer springs generally formed a group distinct from both the local aquifer cold springs and local aquifer geothermal springs when the environmental and biological data from all three spring types were analyzed together using PCA (Fig. 3a). In contrast, there was a great deal of overlap between the local cold and local hot springs even though some of the local hot springs grouped with the regional aquifer springs

Table 2. Average untransformed values of biological and anthropogenic disturbance parameters (on a scale from 1 to 4) from regional aquifer springs (regional; $n = 45$), local aquifer geothermal (local hot; $n = 62$), and cold springs (local cold; $n = 1014$).

Aquifer type	Endemics (# taxa)	Crenophiles (# taxa)	Springsnails (# taxa)	Non-native (# taxa)	Native fish (# taxa)	Diversion (1–4)	Equine (1–4)	Recreation (1–4)	Cattle (1–4)
Regional	2.64	2.98	1.73	1.27	1.13	1.78	1.02	1.16	1.16
Local hot	0.34	0.47	0.35	0.24	0.24	1.87	1.1	1.82	2.26
Local cold	0.11	0.42	0.41	0.04	0.05	1.8	1.19	1.27	2.47

(Fig. 3a), which was also observed in the cluster analyses (Figs. S1, S2A–C), and appeared to be due to the similarities in the environmental data and the presence of endemic taxa in some local hot springs. However, when only the biological data were considered, which included agents of disturbance such as diversion, cattle, equine, and recreation, PCA showed that each spring type formed fairly distinct groups (Fig. 3b). The local cold springs and local hot springs were more scattered but often grouped together, while the regional aquifer springs mostly grouped separately, although certain local hot springs grouped with them. SIMPER analyses also indicated that regional aquifer springs differ from both the local hot and local cold springs primarily due to biological characteristics, with the regional aquifer springs having greater numbers of individuals from various taxonomic groups (Table S2A–C).

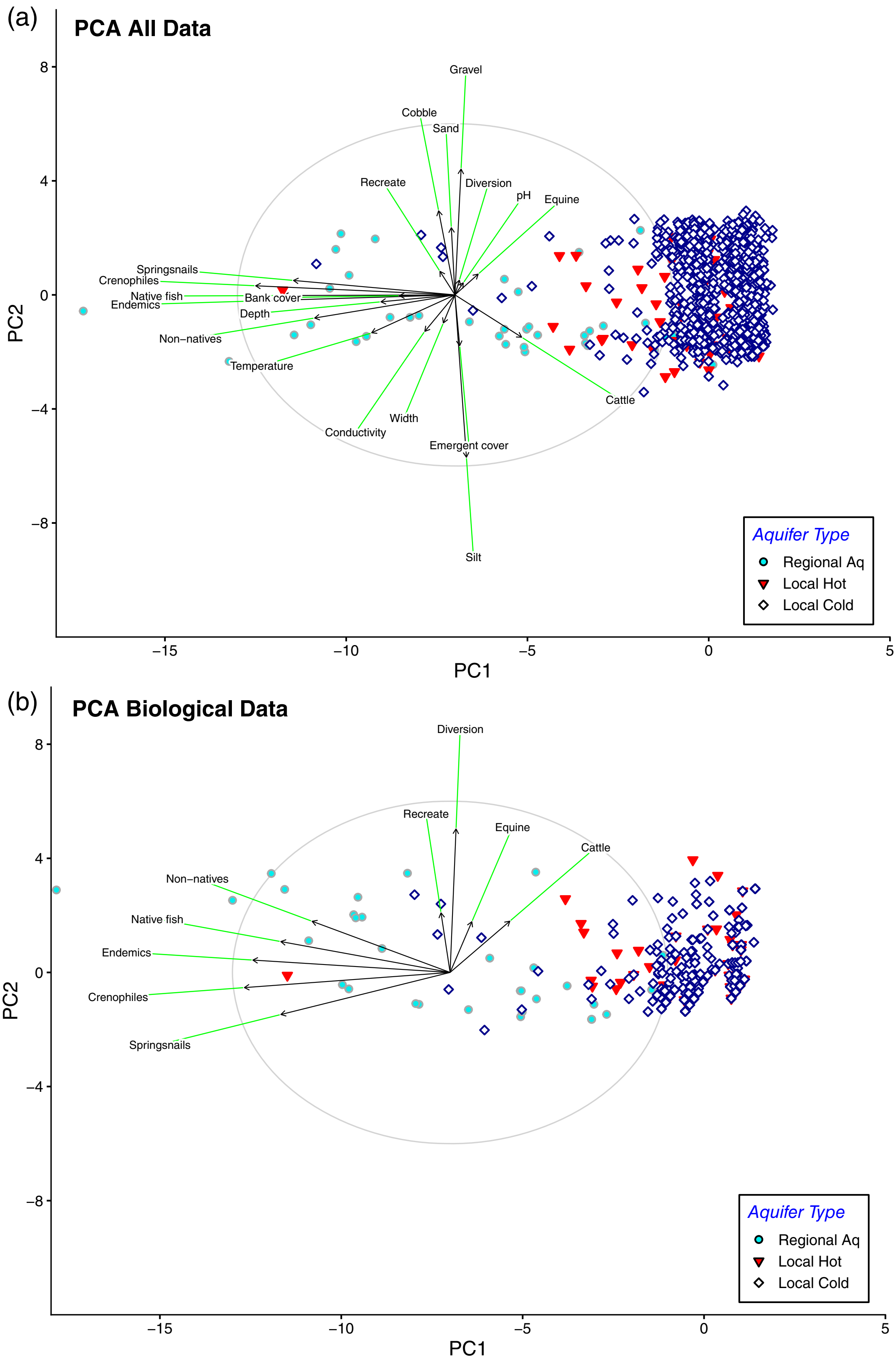
Taken together, our analyses revealed that the individual spring types largely grouped together according to their predetermined assignments due to similarities in their biological and environmental characteristics, and that the three spring types differed significantly from each other. Despite some overlap in environmental parameters with local hot springs (i.e., greater depths and higher temperatures and conductivity), regional aquifer springs differed from both the local hot and cold springs due primarily to biological factors, with the regional aquifer springs harboring proportionally more endemic, crenophilic, native fish, and springsnail taxa than the other two spring types (Fig. 4). Conversely, the local hot and local cold springs differed from each other primarily due to environmental characteristics, with the local hot springs being deeper and wider, more likely to be diverted and disturbed by recreational use, having higher temperatures, pH, and conductivity, but also having lower bank and emergent coverage. For more details regarding the PCA analyses including eigenvalues see Supporting Information Section and Tables S3 and S4A, B. For more details regarding SIMPER analyses see Supporting Information Section and Table S2A–C.

Table 3. Results of one-way PERMANOVA testing differences in biological and environmental characteristics among springs that were characterized as either cold aquifer springs, geothermal aquifer springs, or regional aquifer springs. The final column (w^2) indicates the percent of the total variation among all springs that was accounted for by differences among aquifer types and among springs of each type (i.e., residual) based on variance components analyses (28).

Source	df	SS	MS	Pseudo-*F*	*p*(perm)	w^2
Aquifer type	2	224	112	142	0.001	0.59
Residual	1118	886	0.79			0.41

Discussion

Our multivariate statistical analyses revealed that the main differences between regional aquifer springs and local cold and hot springs within the Great Basin and Mojave Desert regions are primarily related to biological factors, while the local hot and cold springs differed from each other mainly due to environmental characteristics (Fig. 4). These results revealed that despite some obvious differences in environmental characteristics (e.g., water temperatures and conductivity), the main parameters that describe most of the variation between these spring systems are primarily biological, such as the number of non-native taxa, endemic taxa, and disturbance by cattle, burros, and feral horses. Spring depth and width are also important factors; deep, wide springs reflect constant high discharge flows and may contribute to the persistence of crenobionts due to their greater size, habitat availability and complexity (Morrison et al. 2013). Springs with higher discharges also generally support more diverse benthic fauna and include taxa absent from springs with smaller discharges (Lindegaard et al. 1998; Dumnicka et al. 2013; Work 2023). We also found that most of the springs from our study have been diverted and disturbed by humans. Regional aquifer springs and local hot springs are more likely to be affected by recreational usage, as people throughout history have exploited hot and warm springs for bathing and therapeutic purposes (Waring and Blankenship 1965; Sada and Thomas 2025), whereas local cold springs are more likely to be used to provide drinking water for cattle and horses (Sada and Lutz 2016). The Great Basin and Mojave Desert regions are extremely arid, and virtually every spring in the region has a long history of anthropogenic exploitation and disturbance (Brues 1928; Sada and Vinyard 2002; Deacon et al. 2007) such as flow and pathway modifications, the introduction of non-native taxa and the extraction of groundwater (Boulton et al. 2014; Work 2023; Beasley-Hall et al. 2024).



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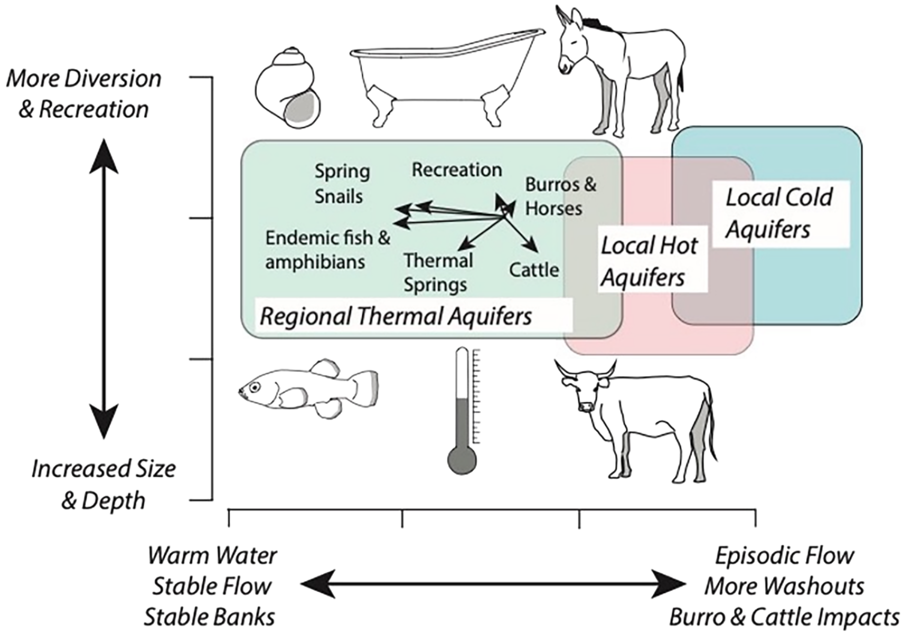

Fig. 4. Some of the main variables in the classification and biological components of desert springs. Arrows in the middle of the diagram show the axes of the principal components analysis (PCA) shown in Fig. 3a. The PCAs showed that regional aquifer springs, local aquifer hot springs, and local aquifer cold springs differ from each other due to biological, disturbance, and environmental factors. The regional aquifer springs differed from the local hot and cold springs due primarily to biological factors—because they harbor more endemic, crenophilic, native fish and springsnail taxa than the other two spring types. The local hot and cold springs differ from each other primarily due to environmental characteristics such as conductivity, temperature, size, and depth.

Regional aquifer springs not only support proportionately more of the endemic aquatic taxa within the Great Basin and Mojave Desert regions, but also a disproportionate number of taxa that are non-native to the study area (Fig. 2a, b). The reasons for these patterns are likely similar: regional aquifer springs serve as refuges for non-native taxa as well as endemic crenobionts. Greater water depths along with longer springbrooks within regional aquifer springs also provide enhanced niche space for both endemic and invasive taxa (Fleishman et al. 2006). The longevity and persistence of regional aquifer springs may also help account for the presence of both endemic and non-native taxa within them because they can survive even if they are cut off from further immigration. Regional aquifer springs can flow continuously for hundreds of thousands to millions of years (Brock 1967; Hay et al. 1986; Hershler et al. 2007), and they provide stable environments that do not freeze nor dry up (Brock 1967; Brown and Feldmeth 1971). The most common non-native taxa documented in the springs considered from this study were bullfrogs (*Lithobates catesbeianus*), western mosquito fish (*Gambusia affinis*), sailfin mollies (*Poecilla latipinna*), and other aquarium fishes, redrim melania snails (*Melanoides tuberculata*), and red swamp crayfish (*Procambarus clarki*) (Hubbs et al. 1974; Hershler and Sada 1987; Sada and Vinyard 2002). Many of these non-native taxa were introduced intentionally, and once established, they typically become invasive, often causing native populations to be locally extirpated or become extinct (Sada and Vinyard 2002). It is difficult to eradicate non-native taxa without also negatively impacting local native taxa (Peck 2020); therefore, it may be preferable to manage the systems by actively enhancing critical habitat for endemic crenobionts and other native taxa rather than attempting to completely remove non-native taxa (Simberloff 2002).

Our analyses of Great Basin and Mojave Desert springs show that most of them have been impacted by a variety of physical and biological disturbances ranging from washouts from flood events and diversions by people to disruption by cattle, horses, and burros including trampling, hoof damage to spring sources, and increased nutrient loads from excrement (Beasley-Hall et al. 2024). Invasions of springs by

Fig. 3. (**a**) Euclidean distance-based principle components analysis (PCA) plot of biological and environmental data from regional aquifer thermal springs (represented by light blue circles), local aquifer geothermal (red upside-down triangles), and cold springs (open blue diamonds). (**b**) Bray Curtis similarity-based PCA plot of only biological data from regional aquifer thermal springs (represented by light blue circles), local aquifer geothermal (red upside-down triangles), and cold springs (open blue diamonds).

non-native taxa are often facilitated and accelerated by anthropogenic disturbances (Hubbs et al. 1974; Sada and Vinyard 2002; Fleishman et al. 2006). Groundwater pumping threatens the flows and biota of springs in Ash Meadows (Deacon et al. 2007), where most of the springs have already faced perturbations due to mining, agriculture, and municipal development (Hershler and Sada 1987). Subsequently, the introduction and persistence of non-native taxa have caused further alteration of spring ecosystems (Hubbs et al. 1974; Hershler and Sada 1987; Sada and Vinyard 2002), resulting in population declines of virtually all of the endemic taxa within Ash Meadows (Hershler and Sada 1987). It is also likely that endemic species were lost before ever being described (Scoppettone 2013), as local extirpations and extinctions of at least 11 crenobiontic taxa have been documented in regional aquifer springs within the Great Basin and Mojave Desert regions during the past century (Sada and Vinyard 2002; Sada and Lutz 2016; Parker et al. 2021).

Some of the best examples of the roles of desert springs as refugia for endemic crenobiontic taxa are the prior investigations of springsnails (*Tyronia* spp. and *Pyrgulopsis* spp.) and pupfish (*Cyprinodon* spp.). These two taxonomic groups also represented most of the endemic crenobiontic species from our dataset. Springsnails and pupfish are generally considered poor dispersers only capable of spreading within their local habitats in the absence of waterway connections (Taylor 1985; Knott et al. 2018). Hydrologic barriers produced by faulting and volcanism fragmented the fluvial network forming endorheic basins within the Great Basin and Mojave Desert regions, while drier climactic conditions isolated previously interbreeding springsnail and pupfish populations, leading to the evolution of endemic species in the regions' modern springs (Hubbs et al. 1974; Echelle 2008; Lutz et al. 2023). The deep divergences in pupfish and springsnail taxa also suggest that regional aquifer springs enhance diversity by providing isolated habitats and long-term stability, thereby furthering the persistence of endemics. While temperature and oxygen levels may vary among different springs, they are relatively constant within individual springs, which may explain the divergences between populations of pupfish as a result of adaptation, in addition to genetic drift from isolation (Campbell et al. 2022). Elevated rates of speciation may also occur within thermal springs, because higher temperatures can accelerate mutation rates (Berger et al. 2021; Waldvogel and Pfenninger 2021) and increase the number of generations produced each year (Miller 1950; Martin and Höhna 2018).

The desert springs of the Great Basin and Mojave Desert regions in western North America provide useful systems to study biogeographic and evolutionary patterns as well as relationships between their environmental and biological characteristics. The regional aquifer springs were often historically interconnected through pluvial lakes during the Pleistocene, enabling the spread of biota to neighboring springs within a larger lake or drainage system. As the pluvial lake systems evaporated when climatic conditions became progressively drier, endemic taxa became stranded in spring ecosystems (Sada and Thomas 2025). While many springs may have initially preserved spring-associated taxa, once connectivity was lost with other water bodies the regional aquifer systems were more likely to retain their endemic taxa than local aquifer springs. Unfortunately, Great Basin and Mojave Desert springs continue to be threatened by anthropogenic impacts including groundwater depletion and pollution, alteration of flow regimes, trampling by ungulates, and the introduction of non-native taxa (Sada and Lutz 2016; Beasley-Hall et al. 2024; Sada and Thomas 2025).

The disproportionate number of endemic taxa inhabiting regional aquifer thermal springs and local aquifer geothermal springs within the Great Basin and Mojave Desert regions indicates that these habitats represent critical biodiversity hotspots, and efforts to conserve and restore these ecosystems should be a major priority to ensure that the valuable information that these relict taxa can provide is not lost forever. Regional aquifer springs (as well as cold and geothermal springs) supporting endemic species are present throughout the world. In the Great Artesian Basin Region of Australia, regional aquifer springs contain at least 96 endemic species and subspecies of fishes, mollusks, crustaceans, and plants (Beasley-Hall et al. 2024), and regional aquifer springs in Cuatro Ciénegas, México, support at least 34 endemic species (Fensham et al. 2023). Recent studies from the Mediterranean show similar patterns, with higher rates of endemicity in long-lived perennial springs fed by large karst aquifers (Fernández-Martínez et al. 2024). Therefore, we believe that our findings and recommendations informing conservation efforts are broadly applicable to arid and semi-arid regions throughout the world.

Author Contributions

Donald W. Sada collected and provided data used in this manuscript. Matthew S. Edwards, Robert K. Burton, and Richard D. Norris analyzed the data, helped write the manuscript, and designed the figures.

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Conflicts of Interest

None declared.

Data Availability Statement

The data we used for our analyses are available on the Zenodo Data Repository using the following

DOI: https://doi.org/10.5281/zenodo.19686943. We do not include the GPS locations for the springs out of caution because many of the endemic species included are endangered or vulnerable.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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