COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Inferences of environmental and biotic effects on patterns of eukaryotic alpha and beta diversity for the spring systems of Ash Meadows, Nevada

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Abstract

Freshwater springs are important ecosystems. In the arid regions of North America, groundwater extraction has caused the desiccation of springs and the extinction of taxa. To better describe the biodiversity of freshwater springs in the hope of establishing a sensitive approach for monitoring the predicted change in spring systems, we used high-resolution genetic methods to estimate the alpha and beta diversity of 19 springs and two reservoirs within the Ash Meadows National Wildlife Refuge in southwestern Nevada. We discovered a large number of distinct taxa based on eukaryote ribosomal gene sequences and show water temperature, spring size, and the presence or absence of non-native predators predicts alpha diversity, and temperature predicts beta diversity. Our study highlights how DNA data support inferences of environmental factors influencing community diversity and demonstrates the method may be an important tool for monitoring ecological communities.

Keywords Alpha diversity · Beta diversity · Eukaryotes · Ecological communities · Groundwater springs

Introduction

Freshwater springs are among the most important, and most imperiled, ecological resources on Earth (Jackson et al. 2001; Dudgeon et al. 2006; Vörösmarty et al. 2010; Davis et al. 2017). There are tens of thousands of springs and seeps of varying sizes scattered across western North America (Meinzer 1923; Stevens and Meretsky 2008). Many of these isolated surface waters harbor high levels of biodiversity and endemic taxa (Soltz and Naiman 1978; Shepard 1993; Bogan et al. 2014) and have ecological and cultural importance extending far beyond their physical borders (Hunter et al. 2017). There are approximately 30,000 springs and seeps in the state of Nevada, 700 of which provide habitat for 165 of the state's 173 described endemic species (Abele 2011). Despite broad recognition of the intrinsic value of groundwater-dependent ecosystems, there

are a litany of processes threatening the integrity of regional spring systems, including water extraction, altered flow and temperature regimes due to climate change, destructive land use practices, and the spread of invasive species (Deacon and Williams 1991; Shepard 1993; Glennon 2002; Deacon et al. 2007). Of these threats, groundwater exploitation for agriculture and municipal uses is an increasingly pressing concern: groundwater extraction can cause loss of vegetation communities, land subsidence, and declines in surface water and stream flows (Zektser et al. 2005; Elmore et al. 2006).

Despite looming threats, our knowledge of freshwater spring community diversity and composition remains inadequate. Most surveys of freshwater community diversity focus on macrophytes (McCreary 1991; Lougheed et al. 2001; Hansel-Welch et al. 2003; Meerhoff et al. 2007); animals such as molluscs, insects, and vertebrates (Schlosser 1982; Agostinho and Zalewski 1995; Delong and Brusven 1998; Haag and Warren 1998; Milner et al. 2008; Vaughn et al. 2008; Hershler et al. 2014); or some combination of these taxa (Welborn et al. 1996; Friberg et al. 2009; Ruhí et al. 2014). These groups include only a small fraction of the organisms comprising freshwater ecological communities. Missing from biodiversity assessments are thousands of microbial and protist producers, consumers, and decomposers (Finlay et al. 1997; Hahn 2006). Ideally, studies of

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Communicated by Leon A. Barmuta.

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freshwater systems utilize methods enabling unbiased and more complete assessment of alpha and beta diversity when estimating the effects of physical and biotic factors on community composition and structure. With estimates of the effects of hypothesized drivers of community composition, we can begin to predict the fate of biodiversity and ecosystem function in the face of climate change, isolation and fragmentation of habitat, and the potential cascading effects of invasive species.

We studied a set of springs and seeps in Nevada's Mojave Desert within Ash Meadows National Wildlife Refuge. Our primary purpose was to predict the consequences of aquifer decline anticipated from a combination of global climate change and groundwater mining. Estimates of biodiversity and inferences of the effects of physical and biotic factors on diversity will provide a baseline for evaluating how projected environmental changes—including aquifer depletion and global warming—might influence biological diversity and inform conservation efforts. Several questions motivated the study. Are communities structured along gradients that can influence the effects of groundwater extraction and global warming on local alpha and beta diversity? Or alternatively, is each community an approximately random assemblage from a localized aquifer species pool, such that changes to, or the desiccation of, springs have little discernible effect on alpha and beta diversity? (At least until the whole system of springs is impacted.)

Our working model for the effects of groundwater extraction and increasing aridity associated with global warming is that a decline in the aquifer yields incremental desiccation of springs along an elevational gradient (Deacon et al. 2007; Unmack and Minckley 2008; Morrison et al. 2013). With an estimate of alpha diversity within springs across an elevational gradient, and the sobering recognition that spring desiccation results in extirpation of all aquatic organisms from a particular spring (e.g., Contreras-Balderas and Lozano-Vilano 1996), we can simulate spring desiccation by computationally removing individual spring diversity along the elevational gradient to estimate changes to system-wide diversity. In addition, we were interested in gaining answers to three other questions. Do larger springs harbor more alpha diversity than smaller springs as expected from island biogeographical theory? Are there demonstrable and predictable effects of water temperature and the presence or absence of introduced predatory species on alpha and beta diversity? Finally, are estimates of the composition of distinct ecological communities from DNA samples extracted from environmental samples useful for making general inferences about community structure?

Study system

Ash Meadows National Wildlife Refuge, hereafter Ash Meadows, consists of approximately 30 springs and seeps and associated wetland and riparian habitats in a relatively small area in southwestern Nevada (Fig. 1). Surface water is derived from a deep carbonate aquifer, which receives drainage from approximately 12,000 km^2 to the northeast of Ash Meadows (Winograd and Thordarson 1975). The deep aquifer is intersected by a fault at the northeast edge of the Ash Meadows system, marked by a line of Paleozoic rocky outcrops (Dudley and Larsen 1976). The fault system provides pathways for water to flow upwards to the land surface, resulting in freshwater springs. The deep aquifer is geothermally influenced: discharge localities located nearest to the fault maintain a constant temperature of ~ 34 °C, while discharge points located further away from the fault emit water with lower temperatures, reflecting cooling that occurs across subterranean distances as well as subsurface mixing with local groundwater. On the days we sampled, water temperatures among springs ranged from 16 to 34 °C.

The entire system discharges approximately 40,500 L per min, or just under 21 million cubic meters per year (Walker and Eakin 1963). Individual springs vary in discharge rate from 0.1 L per second (L/s) to nearly 200 L/s (Dudley and Larsen 1976). In addition, there are two large reservoirs. Surface area of the aquatic habitats—the main pool environment—varies over six orders of magnitude. Episodic flooding occurs across the system that temporarily connects isolated springs (Fig. 1b) and provides a means for dispersal for some aquatic organisms between spring habitats distributed across Ash Meadows (Paulson and Martin 2014).

Most springs have nearly constant flow rates and temperatures over time. Additionally, the chemistry of springs varies little over time and among localities, although there are two localized exceptions in the most northern portion of Ash Meadows that differ from the other springs for nitrate, sulfate and strontium concentrations (Dudley and Larsen 1976; Thomas et al. 2013). Importantly, the springs in Ash Meadows are arrayed along an elevational gradient and there is a strong correlation between spring elevation, spring flow rate, and spring temperature; the higher elevation springs are smaller, have lower flow rates, and also tend to be warmer. The highest elevation aquatic habitat exists within a deep chasm that opens onto the aquifer surface, a geological phenomenon called Devils Hole. There are a series of small springs at the next highest elevations—termed the Warm Springs complex—that may be particularly susceptible to aquifer decline because of the very low spring flow rates. Most of the springs exist at the
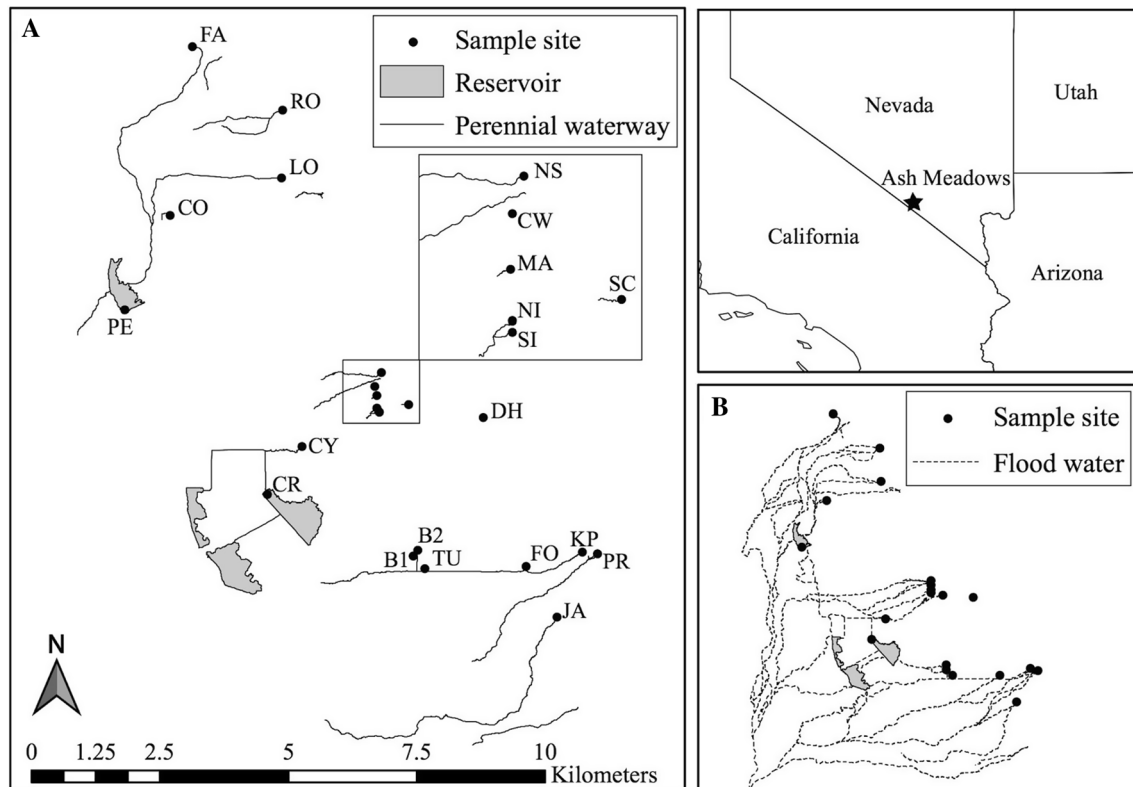
Fig. 1 Map of the study area in Ash Meadows National Wildlife Refuge, Nevada (**a**). Select abiotic and biotic characteristics of each spring are reported in Table 1. Rare flooding events can lead to increased connectivity between otherwise isolated waterways (**b**)
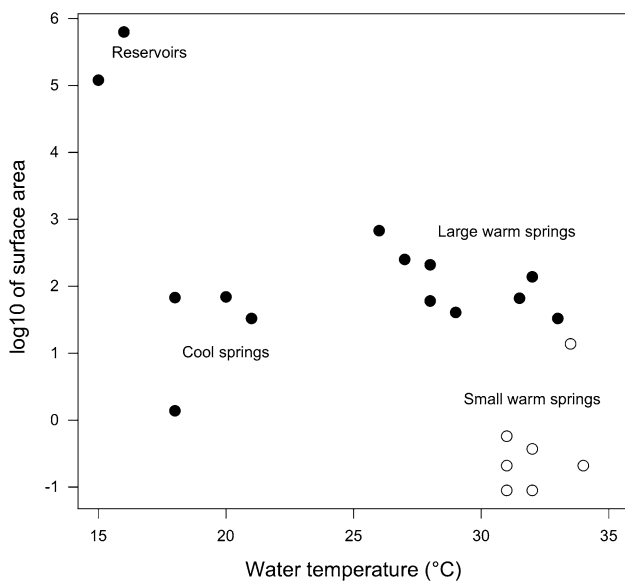
Fig. 2 Bivariate plot of the mean water temperature and log of habitat surface area for the sampled Ash Meadows spring systems. Filled and open symbols are springs with and without non-native predators, respectively

lower elevation of the refuge and vary in temperature and size. Finally, there are several reservoirs in Ash Meadows formed by earthen dams. Overall, spring size and temperature provide a useful means of describing the diversity of habitats (Fig. 2), including (1) small warm-water springs; (2) large warm-water springs; (3) large reservoirs; (4) cool-water springs; and (5) Devils Hole. Finally, the spring pools and associated outflows also vary in the presence or absence of non-native predatory species: red swamp crayfish (*Procambarus clarkii*) and large-mouth bass (*Micropterus salmoides*). While there are clear negative effects of crayfish and bass for the endemic *Cyprinodon* fishes, their impacts on the community composition are unknown.

Methods

We sampled 21 sites across Ash Meadows that varied in water temperature, size, elevation, and presence or absence of red swamp crayfish and largemouth bass (Table 1). Samples were collected from algal mats where present, benthic sediments, and the water column at each site in an effort to estimate community composition for the whole ecosystem in each spring. Sampling sites for the larger springs were located at the springhead, which forms a distinct

Table 1 Characteristics of springs sampled in Ash Meadows National Wildlife Refuge, Nevada

Spring name	#	Latitude	Longitude	NNP	°C	Elevation	Log10 (size)
Bradford 1	1	36.401166	− 116.3028057	Crayfish	18	683.56	1.83
Bradford 2	2	36.402167	− 116.3024522	Crayfish	20	683.22	1.84
Cold	3	36.460790	− 116.3459333	Crayfish	18	681.79	0.14
Cottonwood	4	36.431621	− 116.3097668	None	32	705.36	− 1.05
Crystal	5	36.420127	− 116.3233199	Crayfish	32	670.61	2.14
Crystal Reservoir	6	36.412053	− 116.3284355	Bass	16	664.98	5.80
Devils Hole	7	36.425342	− 116.2914326	None	33.5	742.47	1.14
Fairbanks	8	36.490436	− 116.3421221	Crayfish	28	692.10	2.32
Forest	9	36.399170	− 116.2836293	Crayfish	26	695.78	2.83
Jackrabbit	10	36.390043	− 116.2784375	Crayfish	28	694.33	1.78
Kings Pool	11	36.401535	− 116.2738525	Crayfish	31.5	704.30	1.82
Longstreet	12	36.467514	− 116.3264313	Crayfish	27	703.31	2.40
Marsh	13	36.429059	− 116.3100024	None	31	700.63	− 0.24
North Indian	14	36.426914	− 116.3098409	None	31	696.62	− 0.68
North Scruggs	15	36.433120	− 116.3091260	None	34	708.00	− 0.68
Peterson Reservoir	16	36.444840	− 116.3539505	Crayfish	15	661.58	5.08
Point of Rocks	17	36.401248	− 116.2715388	Crayfish	33	707.17	1.52
Rogers	18	36.479191	− 116.3262426	Crayfish	29	694.63	1.61
School	19	36.427719	− 116.3042623	None	32	713.45	− 0.43
South Indian	20	36.426480	− 116.3099131	None	31	695.77	− 1.05
Tubbs	21	36.399084	− 116.3011726	Crayfish	21	685.94	1.52

NNP non-native predators, *°C* water temperature at time of collection, *Log10 (size)* log10 of spring area

pool of water in those higher-flow systems; for the lower-flow springs (North Indian, South Indian, School, North Scruggs, Marsh, and Cottonwood) sample sites were located at the springhead and four downstream sites approximately ten meters apart due to the habitat heterogeneity that occurs along these more linear systems. Because the goal was to estimate diversity for whole springs, not habitats within springs, care was taken to remove visible traces of contamination from tools between but not within sites, including a final rinse with ethanol. A large-bore pipette was used to collect approximately 300 mL of water, sediment, and algal material (if present) from three locations within each habitat type, which were then pooled by habitat type within each spring and stored in sterile WhirlPak bags at − 20 °C. All sites were sampled in November 2013, except for Devils Hole which was sampled in December 2014 due to permit issues.

DNA was extracted using MO BIO PowerWater DNA isolation kits (MO BIO Laboratories, Carlsbad, CA), per the manufacturer's protocol with two modifications. First, water samples were centrifuged at 4000*g* for 8 min, and the pellet was used for extraction. For algal and sediment samples, two 300-μL sweeps were pulled from the sample for extraction (i.e., the sample was stirred with the pipette tip while drawing up material); the same total volume was pulled from water samples, including the pellet. Second, samples were heated at 65 °C for 10 min after addition of the PW1

reagent. All samples were extracted in triplicate to ensure that maximum taxonomic scope was encompassed per habitat type, per site, in order to thoroughly explore the diversity present in comparative analyses. Devils Hole samples were not extracted in triplicate, but increased sampling at that site yielded the same number of total data points for the site (three each of water, algal mats, and sediments).

A short, variable region of the 18S rDNA gene region was amplified and sequenced to broadly target eukaryotic taxa. This is a commonly used gene region for eukaryotic DNA studies, as it is conserved enough to capture diversity across the eukaryotic tree of life, but this level of conservation results in taxonomic resolution that is reliable only to the family level. DNA amplification followed the Earth Microbiome Project protocol for 18S Illumina library preparation (http://www.earthmicrobiome.org/emp-standard-protocols/18s/), with the exception of using 2 μL template DNA per reaction volume. The forward Illumina Euk 1391f and reverse Illumina EukBr primer set was used to amplify and barcode approximately 200 base pairs of the hypervariable V9 region of the 18S rDNA locus (Amaral-Zettler et al. 2009). PCR was performed in triplicate, and triplicate reactions were pooled per sample. Pooled amplicons were quantified to normalize pooling per plate, and pooled plate amplicons were quantified to normalize further pooling into a single library. The library was sequenced on an Illumina MiSeq platform with a V2 300 cycle kit (Illumina,

San Diego, CA), at the University of Colorado BioFrontiers Institute Next-Gen Sequencing core facility. Raw MiSeq reads and the mapping file associated with the samples used for this manuscript are archived at figshare.com under https ://doi.org/10.6084/m9.figshare.7789133; additional samples from the same study area which were not used for this manuscript can be found in the same fileset, as well as under https ://doi.org/10.6084/m9.figshare.7786547.

MiSeq reads were processed using a combination of USE-ARCH 8 (Edgar 2010) and QIIME 1.9 (Caporaso et al. 2010) scripts. First, adaptors were trimmed, reads were de-multiplexed, and paired reads were merged. Quality filtering was conducted with a maximum e rate of 0.005, merged reads were de-replicated, and singletons were removed. Next, a de novo database was assembled at 97% clustering similarity using the USEARCH algorithm. The database was then filtered using the SILVA 127 reference set (Quast et al. 2013) so that only sequences with at least 75% similarity to those found in the reference set were retained. Last, the demultiplexed merged reads (including singletons) were mapped to the filtered de novo database with a 97% similarity cutoff to assemble the final database. Taxonomy assignment was performed with the RDP classifier (Wang et al. 2007). An OTU table was constructed from this database for downstream analyses and is archived at figshare.com under https://doi.org/10.6084/m9.figshare.7789133.

Characterization of habitats

Temperature data were collected by Ash Meadows personnel. All spatial, geographic, and elevation data were collected in ArcGIS 3.1 (ESRI, Redlands, CA). Elevation data for springheads were obtained from a 10-m digital elevation model. Spring size was estimated by surface area at the springhead measured from aerial imagery. In addition, we recorded the presence or absence of non-native predators (NNP) for each locality.

Alpha diversity

Alpha diversity was estimated for each spring after rarefaction to 25,203 sequences (merged reads) per site. Alpha diversity was estimated by the number of distinct OTUs as a species richness estimate. For the Warm Springs complex, we calculated the mean diversity across the five separate sample sites along each stream to represent per-spring diversity. We explored whether there were detectable associations between OTU richness with various combinations of water temperature, spring size, elevation, and presence or absence of non-native predators using linear models. All possible additive models were compared using AICc (Burnham and Anderson 2004) implemented in the 'MuMin' R package (Barto 2008). We focused on using a Gaussian linear model

for estimating parameters and making predictions from the best model defined by AICc. Conformity of the data with the assumptions of a Gaussian model was evaluated using Q–Q plots.

Beta diversity

For characterizing beta diversity, we used Bray–Curtis community dissimilarities calculated from square-root transformed abundances and transformed the distance values using non-metric multidimensional scaling (NMDS) plots. We evaluated the effect of temperature, habitat area, elevation, and presence of non-native predators in two ways. First, we used the BIOENV function in 'vegan' (Clarke and Ainsworth 1993; Oksanen et al. 2011) and partial Mantel tests to estimate the independent contributions of environmental distance and geographic distance to Bray–Curtis distances. Environmental distances between communities were obtained from the BIOENV results. For geographic distance, we calculated Euclidean distances between sample sites. Second, we visualized the data as a bivariate plot of NMDS 1 and NMDS 2 for each of the predictive environmental parameters.

Metacommunity structure

We used a hierarchical metacommunity analysis approach to discern community structure along the elevational gradient implemented using the R package 'metacom' (Dallas 2014). We also repeated the analysis for ordinations of temperature and spring size. Taxon occurrence data for each spring were coded as presence or absence for all taxa observed using an abundance threshold of > 100 sequences (merged reads) per taxon per site. We calculated three statistics for inferring metacommunity structure: coherence, turnover, and boundary clumping (Leibold and Mikkelson 2002). These three statistics distinguish among different categories of metacommunity structure (see Leibold and Mikkelson 2002; Mihaljevic et al. 2015).

Predictive effects of aquifer decline on alpha diversity

We evaluated the potential effects of aquifer decline on local biodiversity by counting the number of OTUs across all springs after stepwise removal of each locality beginning with the highest elevation spring. To evaluate whether the predicted decline in diversity based on the observed elevational distribution of taxa differed from a null model, we randomly assigned OTUs across springs, keeping the number of OTUs per spring constant, and repeated the stepwise removal analysis 1000 times. From the simulated decay curves for the randomized occurrence data, we extracted

the 2.5% and 97.5% quantiles and compared the observed decline in richness to the quantile range for the randomized model. All analyses were performed in R (R Core Team 2018).

Results

Environmental variation among springs

We utilized four environmental parameters across 21 aquatic environments: temperature (°C), the log of spring area (Area), presence or absence of non-native predators (NNP), and elevation (Elev) (Table 1). All environmental parameters were highly correlated: the absolute magnitude of all pairwise Pearson coefficients was > 0.58 and all pairwise p values were < 0.005.

Taxonomic diversity

We estimated the taxonomic composition across all communities for the data set rarified to 46,008 sequences (merged reads) per spring. From the $21*46,008 = 966,168$ sequences, we discovered 6,769 distinct operational taxonomic units (OTUs) using the criterion of 3% ribosomal DNA sequence

difference from other sequences. We detected an immense taxonomic diversity of organisms (Fig. 3). The most common higher-order taxonomic super group was the diverse, monophyletic assemblage of eukaryotes known as SAR: including stramenopiles, alveolates, and rhizaria. Within this group we discovered 406 different diatoms. The second most abundant group was the opisthokonts, including fungi and metazoans. Within the fungi, the most species-rich group comprised sequences that did not match an identified OTU and were therefore classified as unidentified. A large number of different ascomycetes (275), chytrids (130), and basidiomycetes (110) were detected. Finally, we detected a variety of different animals. Arthropods were the most common major animal group within which we discovered 86 different crustacea, 61 different insects, and 27 chelicerates (e.g., water mites and spiders). Most of the crustacea were copepods (~60%) and ostracods (~20%). We detected Nematoda, Platyhelminthes, Annelida, Rotifera, and miscellaneous protist metazoans. Additionally, we detected six different fish OTUs, five different molluscs, four mammals, one bird and one crayfish (also counted as a crustacean). Two of the mammal OTUs were identified as human, most likely a contaminant from sampling. The final major group we focused on was the archaeplastids, which comprised 300 different algae and plants. Overall, our results underscore the

Fig. 3 Summary of some of the distinct taxa discovered across all springs within Ash Meadows. The number of sampled sequences was 966,168
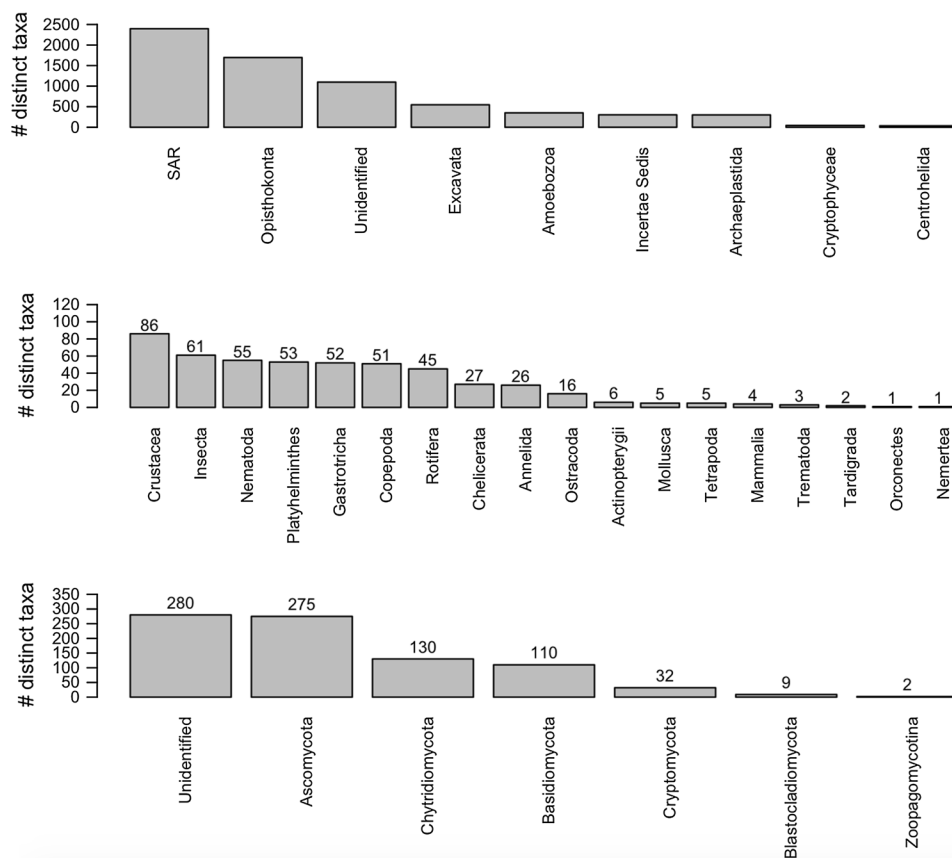
power of DNA surveying for detecting a diverse assemblage of taxa.

Alpha diversity

For comparing alpha diversity across sites, we rarified the number of sequences (merged reads) per site to 25,203. The Warm Springs complex springs were sampled at five different sites along each spring outflow (due to the absence of large pools at the springheads); for these springs we calculated the mean number of OTUs across sites. Estimates of OTU richness within sites ranged from 388 in Devils Hole to 1248 in Bradford 2. The mean number of distinct OTUs per site was 674. The rank abundance distributions of OTUs across sites exhibited a typical log-normal pattern with a large number of rare sequences and exponentially fewer higher-abundance OTUs. Most OTUs were detected from only a single spring and there were exponentially fewer OTUs detected from an increasing number of sites. Fifteen different predictive models were evaluated for estimating the effects of water temperature, habitat size, elevation, and the presence or absence of non-native predators on alpha diversity (OTU richness). For some of the models, Q–Q plots revealed limited but recognizable hyper-dispersion of the residuals, suggesting AICc may have favored a more

parameter-rich model. Furthermore, estimated *p* values may be inaccurate. Figure 4 shows all four univariate plots. The best model included temperature, area and NNP as predictors (Table 2). In both the best and second-best models, the estimated effects of temperature and area were negative and the effect of NNP was positive (Table 3). We used the residuals from the best model to visualize the springs harboring more or fewer species than expected; the most notable result was greater than expected diversity in Bradford 2 and Jackrabbit (Fig. 5).

Beta diversity

We constructed a visualization of beta diversity using two non-metric multidimensional scaling axes for all springs based on pooled samples from each spring (Fig. 6). Environmental variables were correlated with Bray–Curtis distances between sites, with temperature having the largest effect (Table 4). Mantel correlation coefficients from partial Mantel tests conditioned on geographic distance were all statistically distinguishable from the expectations of sampling error for all four models (Table 4). Overall, multidimensional scaling scores of Bray–Curtis distances for whole community data revealed a strong dependence of community

Fig. 4 Bivariate visualizations of alpha diversity (estimated as number of distinct OTUs) and four predictive variables: temperature, spring area, spring elevation, and presence or absence of non-native predators. Numbers in the open circles identify each spring (see Table 1). For a summary of alternative predictive models, see Table 2
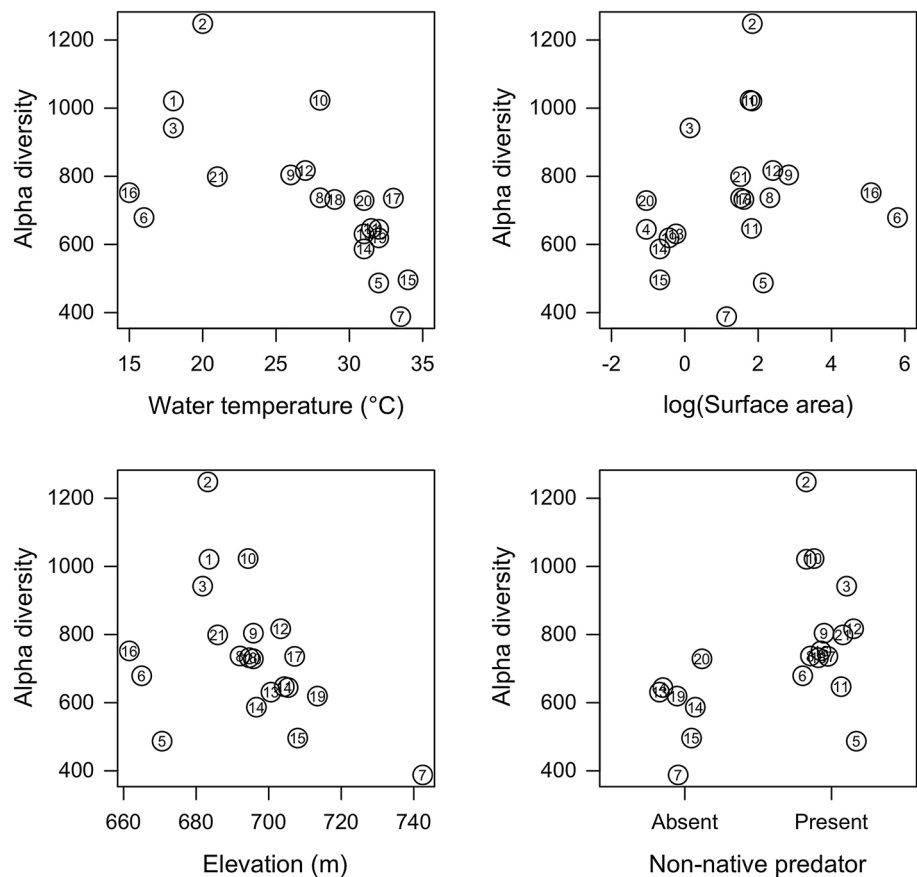
Table 2 Results from AICc analysis of 15 different models for predicting variation in OTU richness among springs (ALPHA)

Model	df	AICc	ΔAICc	Relative likelihood	Weights
ALPHA ~ Temp + Area + NNP	5	272.5	0	1	0.705
ALPHA ~ Temp + Area + Elev + NNP	6	275.63	3.13	0.209	0.147
ALPHA ~ Elev	3	278.21	5.71	0.058	0.041
ALPHA ~ Area + NNP	4	279.12	6.62	0.037	0.026
ALPHA ~ Temp + Area	4	279.32	6.83	0.033	0.023
ALPHA ~ Temp + Elev + NNP	3	280.31	7.81	0.02	0.014
ALPHA ~ Elev + NNP	4	280.47	7.97	0.019	0.013
ALPHA ~ Temp + Elev	4	281.16	8.66	0.013	0.009
ALPHA ~ NNP	5	281.88	9.38	0.009	0.006
ALPHA ~ Area + Elev + NNP	5	282.71	10.22	0.006	0.004
ALPHA ~ Temp + Area + Elev	5	282.81	10.31	0.006	0.004
ALPHA ~ Temp + NNP	4	283.07	10.57	0.005	0.004
ALPHA ~ Temp	3	284.36	11.86	0.003	0.002
ALPHA ~ Area	3	287.35	14.85	0.001	0.000
ALPHA ~ Area + Elev	4	287.42	14.92	0.001	0.000

The model in bold is the best model

Table 3 Summary of estimated effects for the two best alpha diversity models based on AICc (see Table 2)

Predictive variable	Effect	SE	T
Best model			
Temperature	− 21.0	5.98	− 3.52
Area	− 79.4	24.48	− 3.24
NPP	290.3	88.33	3.29
Second best model			
Temperature	− 24.4	7.32	− 3.34
Area	− 78.5	24.74	− 3.17
NPP	305.7	91.14	3.35
Elevation	2.0	2.48	0.82

Fig. 5 Residuals from the best model estimating the effects of predictive variables on alpha diversity. Vertical dashed lines are ± 2 standard deviations from predicted values
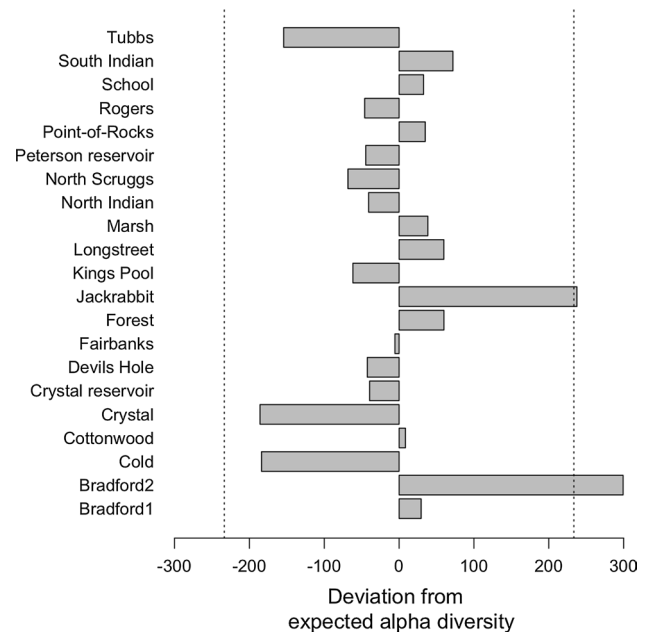
similarity on the measured environmental variables, especially temperature.

Metacommunity structure

There was no evidence of interpretable metacommunity structure for any of the three ordinations of the data because the inference of structure was described as quasi-nested (see Leibold and Mikkelson 2002).

Predicting effects of aquifer decline on alpha diversity

Our inference of the predicted effect of groundwater extraction on alpha diversity involved stepwise removal of springs from the local pool of species along the elevational gradient, recording the total number of species across all remaining springs, and comparing the results to the expectations for a random distribution of taxa across sites. The observed decline in system diversity fell within the expectation of the null. The lack of an effect of stepwise desiccation and extirpation of taxa reflects the lack of any statistically detectable effect of elevation on diversity (e.g., Table 2).

Fig. 6 Plot of the first two axes from non-metric multidimensional scaling of Bray–Curtis distances between sample localities with points labeled relative to the four predictive variables. For each community, the number of sampled sequences was rarified to ≈ 25,000
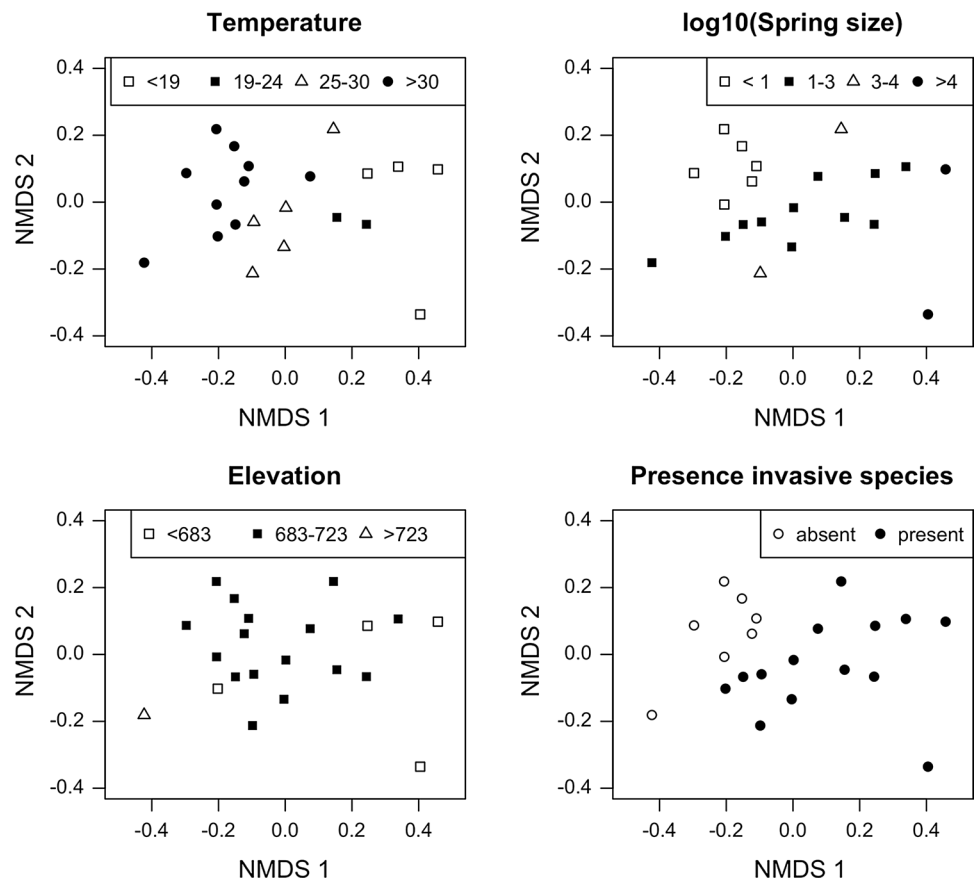
Table 4 BIOENV results from correlation analysis between Bray–Curtis distances and environmental distances

Environment variables	Pearson's r	Partial Mantel r	P
Temp	0.7017	0.6699	0.001
Temp + Elev	0.7128	0.6882	0.001
Temp + Elev + log (Area)	0.7486	0.7308	0.001
Temp + Elev + log (Area) + NNP	0.6903	0.7229	0.001

Partial Mantel correlations were calculated controlling for geographic distance

Discussion

Ash Meadows National Wildlife Refuge includes a remarkable and environmentally variable set of springs scattered across approximately 150 km^2 of the Mojave Desert near Death Valley. Metacommunity analysis using DNA based on eukaryotic 18S ribosomal gene sequences revealed a high diversity of OTUs across the three major habitat types in this system. Our study adds to a growing number of descriptions of freshwater community diversity using DNA barcoding approaches (e.g., Nolte et al. 2010; Bråte et al. 2010; Monchy et al. 2011; Charvet et al. 2012;

Bradford et al. 2013; Mangot et al. 2013; Stoeck et al. 2014; Debroas et al. 2015, 2017). These data can serve as an important baseline assessment of ecological community diversity useful for future monitoring.

Taxonomic diversity

The value of DNA analysis was evident when we compared the results of this study with previous taxonomic surveys of regional freshwater springs. For example, an in-depth study of the algae of Devils Hole (Shepard et al. 2000) discovered 84 "terminal identifications" across 44 genera. Using the 97% similarity criterion for delineating distinct lineages, we discovered 24 different diatoms and an additional 13 lineages of Charophyta, Chlorophyta, and Rhodophyceae from Devils Hole. When we expanded the scope to include all 21 sampled springs, we discovered 406 different diatoms and 300 distinct algal and plant taxa. The number of taxa would likely be much higher if we conducted more exhaustive sampling over time, and the data suggest DNA surveys will likely yield more exhaustive estimates of the alpha diversity of springs than morphology-based assessments. We also compared our data from insects with a previous morphology-based study of Odonata, a single order of insects (damselflies and dragonflies) (Stevens and

Bailowitz 2008). Stevens and Bailowitz discovered 32 species based on bimonthly sampling from 12 springs over the course of 10 months. Our DNA survey discovered 61 distinct sequences across multiple orders of insects, but only five were identified as Odonata.

Prior to this study there were no published estimates of fungal diversity from Ash Meadows. We discovered 838 distinguishable lineages, including a large diversity of the ascomycetes, basidiomycetes, and chytrids, as well as a large fraction of unidentifiable fungi. Other DNA-based surveys have revealed many environments harbor a tremendous diversity of fungi (Freeman et al. 2009; Sohlberg et al. 2015; Röhl et al. 2017). These results underscore the ubiquity of fungi across diverse ecosystems.

Inferred effect of water temperature

Temperature was inferred to have the strongest association with alpha and beta diversity. Our discovery of a negative effect of temperature on alpha diversity may be due to a variety of different factors. First, DNA is less stable in warmer environments which may result in greater decay rates of DNA than in cooler water (Strickler et al. 2015). Second, lower diversity in warmer springs may reflect environmental selection that effectively eliminates some taxa. Similarly, the inferred temperature effect on beta diversity suggests temperature may cause differential survival of species among the different communities. Before estimates of community composition are generally predictive of biological processes structuring communities, it will be important to estimate whether there is a detectable and predictable bias associated with DNA decay rates with temperature.

Our results were similar to other DNA surveys. For instance, Torstensson et al. (2015) discovered a negative association between alpha diversity and temperature for sea ice communities, and Sharp et al. (2014) discovered bacteria and Archaea diversity is predicted by a Gaussian model with maximum diversity near 24 °C. If the effect is due to taxon selection, dependence of alpha diversity on environmental temperature suggests the community composition of springs will change with the predicted increase in temperature and declines in precipitation in the Desert Southwest (Seager et al. 2007; Cayan et al. 2010). Finally, different groundwater sources may have correspondingly different biotic communities (Korbel et al. 2013); consequently, the effect of temperature may indicate mixing of different communities below ground. In this case, one set of taxa may occur in the warm, deep regional aquifer and the other inhabits the shallow, local aquifer. The higher diversity of cooler water springs may be due to mixing. The statistical association between temperature and beta diversity, coupled with the lack of an apparent effect of geography, suggests community composition may reflect environmental filtering over ecological time

frames (Baas Becking 1934). Overall, temperature appears to be the main axis of environmental filtering, although there may be other important axes structuring variation we did not measure.

Inferred effect of spring size

In contrast to many studies (e.g., Green and Bohannan 2006), we found spring size and alpha diversity were negatively correlated. One prominent feature of Ash Meadows springs that may partially explain the lack of a detectable association between habitat area and diversity was the two largest aquatic habitats were constructed reservoirs with very different geomorphological characteristics than the springs. Additionally, a general positive association between diversity and geographic area may be confounded by the strong effect of temperature, especially since, as we noted, temperature reflects the mixing of different water sources.

Inferred effect of the presence or absence of non-native predators

We did detect an effect of non-native predators (NNP) on alpha diversity, and there appears to be an association between beta diversity and NNP. Interestingly, the inferred effect of NNP on alpha diversity was positive. The cause of the effect is unknown but may be due to the introduction of protists associated with the non-native predators (Bangyeek-hun et al. 2001; Lafferty et al. 2006). However, until we can associate specific taxa detected using 18S barcodes with the invasive crayfish, fish, and amphibian species, the cause of the inferred effect remains unknown.

No detectable effect of elevation

Multiple case studies exist demonstrating the dramatic effects of groundwater pumping on biodiversity. When groundwater pumping reduces the aquifer, there is a decline and eventually a complete loss of biodiversity in freshwater spring systems (Deacon et al. 2007). The effects of groundwater pumping are not limited to the aquatic organisms: aquifer decline challenges the existence of many terrestrial plants because of their dependence on the proximity of the aquifer to their roots.

One of our motivations for pursuing this survey was to document spring diversity and then ask whether a decline in the aquifer volume from the combined influence of water mining and climate change will cause potentially irreversible extirpations of ecological communities that may be restricted to particular springs. In this case, spring elevation provides a proxy for the sensitivity of a spring to aquifer decline. Higher elevation springs with lower flow rates are more likely to desiccate than lower elevation springs

with higher flow rates. Our null model assumed each spring was phylogenetically or taxonomically redundant with other springs because they support, more or less, a random sample of the regional species pool. We simulated the loss of diversity for both models by stepwise removal of springs along an elevational gradient. The decline of biodiversity after the removal of most springs was no different from the expectation of the null model. These results suggest individual springs do not harbor unique assemblages of lineages, and the loss of the low-flow, relatively high-elevation springs—the Warm Springs complex—will likely have a relatively negligible effect on the overall eukaryote community because many of the lineages exist elsewhere in the Ash Meadows system. This result was also evident from the metacommunity analysis in which we failed to find a signal of community structure for ordinations of springs along the elevational gradient. We stress, however, that our inferences are based on a broad-scale survey of the entire eukaryote community and they do not apply to specific taxa. For example, there are some species endemic to particular springs (e.g., spring snails, Hershler et al. 2013) and the desiccation of particular springs will likely result in extinction.

Caveats

There are a number of limitations of the study. The first is that our sampling was limited to one point in time. All communities, and especially microbial communities, vary across time. However, our purpose was not an exhaustive characterization of the community composition of Ash Meadows springs, but rather an assessment of whether DNA can provide useful information about alpha and beta diversity. Our results suggest metacommunity analysis using DNA can be an efficient means of characterizing communities and our results suggests a temporal analysis of springs will likely yield relevant information for gauging the potentially deleterious effects of groundwater mining and climate change on the ecological integrity of groundwater-supported aquatic communities, especially in arid western North America.

Another limitation of our study was that we have not established an unambiguous link between 18S sequence barcodes and particular species; therefore, we did not assign generic or species names to the particular taxa identified using DNA. Nonetheless, it would be extremely useful to connect repeatedly sampled and relatively abundant DNA barcodes with phenotypes and species. This is a time-intensive process.

Our 18S ribosome gene-based survey likely underestimated diversity. While our survey discovered a large number of different OTUs, the use of the conserved 18S ribosomal gene yields an almost order of magnitude less diversity than other barcode markers like cytochrome oxidase 1 (Tang et al. 2012). Comparison between morphological and molecular

surveys, and between surveys using different barcode sequences, underscores different methods of documenting diversity will yield different results. If the goal is to monitor particular taxa that may be of high apparent cultural value, like endangered fish and snails, then DNA surveys of whole community diversity using the highly conserved ribosomal 18S gene may yield largely uninformative data in part because the resolution of a small segment of a highly conserved gene provides relatively poor taxonomic resolution. (We did, however, detect six different fish and five different molluscs.) If, however, the goal is to rapidly and efficiently estimate the composition of ecological communities for the purpose of monitoring alpha and beta diversity as indicators of ecological change, then DNA surveys using 18S should prove extremely useful (Carignan and Villard 2002; Walther et al. 2002; Röhl et al. 2017). The utility of DNA for monitoring freshwater ecological communities depends on a commitment to long-term and regular sampling so the range of variation is sufficiently well documented to detect anomalous patterns that may be indicative of conditions requiring management actions.

Finally, the inferred effects of temperature, spring size, elevation and the presence or absence of non-native predators remain hypotheses rather than general claims. Ideally, this study is repeated, perhaps using a different barcode and at different times of year, to assess whether the patterns observed for the 18S gene from samples obtained in late fall are generally true.

Author contribution statement ELP and APM conceived of and designed the study. ELP performed the fieldwork and lab work. ELP and APM conducted analyses and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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