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Ecological divergence of a habitat constructed to harbor an endangered species

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Abstract

Ecological restoration, habitat creation, and artificial refuges are increasingly essential for conservation, particularly for species that are threatened by the loss or degradation of critical habitat. The Devils Hole pupfish (*Cyprinodon diabolis*) exists in a single small locality subject to the detrimental effects of local groundwater extraction, limited resources, and the gradual deterioration of its gene pool as a consequence of its small effective population size. Multiple attempts at establishing captive populations have been made since the 1970s, but all failed due to mechanical issues combined with lack of staffing, and in one case a hybridization event with a closely related species. In 2012, the completion of the U.S. Fish and Wildlife Ash Meadows Fish Conservation Facility included a 416 m^3 tank designed to closely emulate the Devils Hole environment. We conducted a comparative analysis of the early-stage ecological community in this newly constructed environment with that of Devils Hole. Results showed significantly different community compositions and temporal variation between the two sites. Differences between the natural and constructed communities were likely driven by environmental and successional stage differences between the natural and constructed habitats. Environmental DNA surveys revealed far greater diversity in both Devils Hole and the refuge tank than detected through traditional visual sampling methods. Our results highlight the importance of monitoring for tracking progress toward a desired ecological community outcome.

KEYWORDS

community ecology, *Cyprinodon diabolis*, Devils Hole, environmental DNA, metagenetic survey

1 | INTRODUCTION

Conservation actions aimed toward ensuring the persistence of endangered species of fish sometimes involve creating habitat, either through modification of a natural landscape or constructing an environment that mimics, to the extent possible, details of the species' natural habitat. Constructed habitat (CH) can achieve several purposes: species persistence in the event its natural habitat is destroyed, support of a refuge population that enables introduction of the species back into its native habitat following extirpation, and augmenting the productivity of populations in natural habitats (Black et al., 2016; Feuerbacher, Bonar, & Barrett, 2016; Fraser, 2008; Ruggirello, Bonar, Feuerbacher, Simons, & Powers, 2018; Snyder et al., 1996). CHs vary in the extent of control over key environmental parameters. In most cases, abiotic and biotic parameters vary depending on prevailing environmental conditions. In some cases, however, environmental and ecological variables are intensively monitored and controlled within specific ranges according to perceptions of the target habitat (Ruggirello et al., 2018; Sackett, Huerta, Kruger, Hamilton-Brehm, & Moser, 2018).

Phenotypes of fishes often differ between populations in natural and constructed or refuge habitats (Black et al., 2016; Collyer, Heilveil, & Stockwell, 2011; Jonsson & Jonsson, 2006; Lema, 2008). Because species can be defined by phenotypes, conservation actions dependent on CH are confronted with the challenge of phenotype management (Watters, Lema, & Nevitt, 2003). The crux of the issue is that characteristics of a species depend on additive genetic effects, environmental effects, and the covariance between genes and environment; change the environment, and species' characteristics change as a consequence of phenotypic plasticity and evolution (e.g., Hutchings, 2011; Lema, 2020; Olendorf et al., 2006; Scheiner & Lyman, 1989). Thus, conservation efforts that utilize CHs for the conservation of endangered species (or any species) should attempt to emulate natural habitats in an effort to manage phenotypes and direct, to the extent possible, evolution. Of course, creating a perfect analog of a natural habitat is challenging because ecological systems are complex. In addition to the numerous physical parameters that can vary, species are embedded within trophic networks and there are multiple direct and indirect biotic effects on focal species of conservation concern (Bukovinszky, van Veen, Jongema, & Dicke, 2008; Holt & Kotler, 1987).

Each species within an ecological community provides an assessment of the habitat's environmental conditions because all species exist within a multidimensional niche space (van der Putten, Macel, & Visser, 2010). One of the values of describing ecological communities is that species composition can be used as an indicator of habitat that integrates niche information across divergent lineages (Dufrêne & Legendre, 1997). Thus, characterizing compositional differences between communities can provide an assessment of habitat similarity between two or more habitats (Bista et al., 2017; Canterbury, Martin, Petit, Petit, & Bradford, 2000). Moreover, because species comprising an ecological community can have direct or indirect effects on a focal species, the extent two communities differ in composition can provide a sensitive indicator of whether ecosystem engineering through construction of habitat and control of key parameters might successfully accomplish phenotype management.

We applied community-level analyses to compare the compositions of thousands of species found in a natural aquatic habitat and an engineered analog constructed with the goal of endangered species conservation. The focal species, the Devils Hole pupfish (*Cyprinodon diabolis*), has experienced several assaults on the natural population due to human activity, and began a sustained decline in population size in 1996 (Figure 1). The species has a long history of management using CHs, although all CHs prior to the one described here had limited control over environmental parameters, and little similarity to the natural habitat; all ultimately failed (Karam, 2005; Karam, Parker, & Lyons, 2012). The native environment of *C. diabolis* is a small surface opening of a large and deep aquifer in the Mojave Desert. The pool, referred to as Devils Hole (DH), has a shallow shelf that is approximately 2.5 m by 6 m where *C. diabolis* feed and reproduce, and a deeper end that has been mapped down to 130 m. The water is geothermally heated and the temperature remains remarkably constant (≈33.5°C) over time (Hausner, Wilson, Gaines, Suárez, & Tyler, 2013).

In 2010, the U.S. Fish and Wildlife Service initiated construction of the Ash Meadows Fish Conservation Facility (FCF) in response to low counts of pupfish in

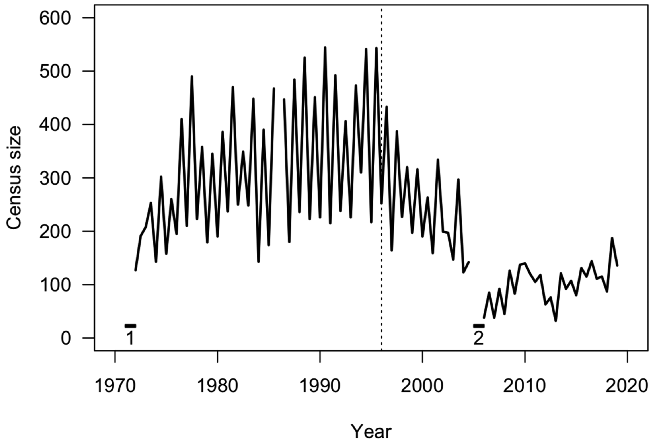

FIGURE 1 Estimated census population size of the Devils Hole pupfish in Devils Hole. Census size is based on counts in the spring and fall of each year. The short horizontal lines labeled 1 and 2 represent two (of the three) significant assaults on the population due to humans: 1 = groundwater pumping that reduced the available habitat by ≈85% and 2 = the loss of approximately 80 individuals (∼37% of the population) due to inadvertent deaths from human error. The third assault happened in 1930 when large numbers of individuals were collected by biologists interested in describing the morphological uniqueness of the species (Wales, 1930). The vertical dotted line shows the beginning of the most recent long-term decline, which began in 1996

DH. The FCF was completed in 2012 and became operational in 2013. This facility represented the most significant attempt at sustaining an analog of the native DH habitat (USFWS, 2016). Design of the facility took into account some of the sources of error that led to the failures of previous refuge attempts (Karam et al., 2012). A 416 m^3 pool was designed to mimic the cavernous setting and geologic contours of DH, with a shallow shelf and a 6.7 m deep end (Figure 2). To replicate the sunlight regime of DH, the tank was oriented along the same directional axis and exists within a structure that shades the pool on the southeast and northwest, with windows on the southwest end, and the ceiling of the facility has adjustable louvers to control the amount of light entering the facility from above (Figure 3). The shallow shelf was modeled off the topography of the shelf in DH, and maintains a water level characteristic of DH prior to local groundwater pumping that permanently altered the water level (Andersen & Deacon, 2001). Water comes from the same aquifer source that feeds DH and is conditioned prior to entering the tank to closely mimic the water parameters of DH. Despite structural similarities and the same water source, there were habitat parameter differences between DH and the CH at the time of this study, including, but not limited to, higher average water temperature in DH than the CH, lower pH in DH than the CH, lower dissolved oxygen in DH than the CH, lower dissolved organic carbon in DH than the CH, and higher arsenic in DH than the CH (Sackett et al., 2018). The CH monitoring system is equipped with alarms and backup measures in case of mechanical failures. There are also anti-contamination protocols to prevent invasion by non-DH taxa and pathogens.

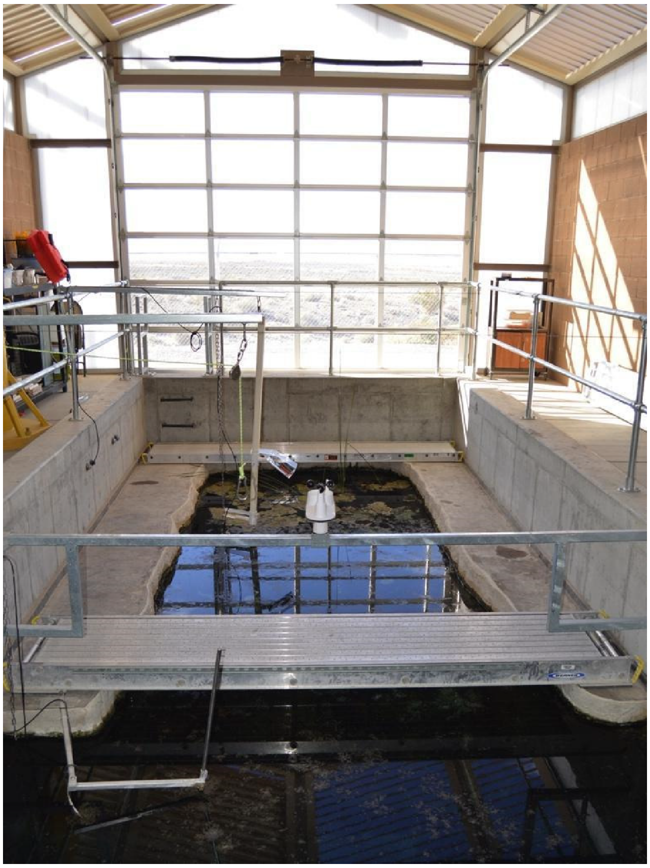

FIGURE 3 The constructed habitat is shaded on the southeast and northwest sides and has a wall of windows to the southwest to mimic the aspect and exposure of Devils Hole. An adjustable louvered ceiling was designed to further replicate the insolation regime of Devils Hole

FIGURE 2 Devils Hole (left) and the constructed habitat at the Ash Meadows Fish Conservation Facility (right), in June 2015

The FCF has the capacity for isolation and propagation of invertebrates and algae for assembly and management of the ecological community. To establish the CH community, algae and invertebrates were collected from DH, sorted, isolated, propagated, and introduced into the CH beginning on June 28, 2013. The majority of introductions prior to this study happened between June 2013 and February 2014 (Table 1). In addition, 29 *C. diabolis* were introduced in May 2014; this date was earlier than originally planned due to extremely low population counts in DH (O. Feuerbacher, personal communication, September 23, 2020). At the time of this study, the native Hydrobiidae snail *Tryonia variegata*, an important component of the DH ecosystem that comprises approximately 80% of invertebrate mass (Wilson & Blinn, 2007), was intentionally excluded from the CH over concerns that the species might clog the filtration system, and the possibility that the mollusk harbored a fish parasite. However, they were introduced into the system after this study was completed (O. Feuerbacher, personal communication, September 23, 2020).

Our study focused on comparing the two habitats supporting populations of *C. diabolis*, and specifically evaluated the extent to which the CH was an ecological analog of the DH habitat for the time period from December 2014 to November 2015. We tested two alternative hypotheses: (a) the two habitats exhibited ecological equivalence and (b) the two habitats were ecologically divergent. We focused on four testable categories of predictions (Figure 4): (a) the overall similarity of ecological community composition between the two habitats, (b) the extent that seasonal change in community composition followed similar trajectories, (c) whether the rank-order abundances of the most abundant taxa were more correlated between the natural and CHs than between a distribution of correlations estimated for a sample of similar environments across the Ash Meadows aquifer system shared by DH and the CH, and (d) whether samples of the ecological communities from different environments clearly separate using hierarchical clustering analysis.

2 | METHODS

We estimated ecological community composition using a meta-genetic approach (see Paulson & Martin, 2019 for detailed methods). Sampling was conducted in DH and the CH approximately bimonthly—December, February, April, June, August, October, and November—from 2014 to 2015. A second set of samples was collected in October after an extreme flooding event on 18 October resulted in an influx of allochthonous material and water into both DH and, to a lesser degree, the CH environment. Parallel sampling was conducted in nearby School Spring in Ash Meadows National Wildlife Refuge for use as comparative "outgroup" data. School Spring is fed by the same aquifer source as DH, is located less than 1 km away, and is occupied by the Warm Springs pupfish (*Cyprinodon nevadensis pectoralis*)—a close relative of *C. diabolis*. We surveyed three habitats within DH and the CH in an effort to assess the whole community: algal mats, the water column, and the benthic sediments. From each of these habitats, we collected a ∼300 ml sample using a large-bore pipette at five dispersed locations across the shallow shelf, yielding 5 samples each from the algal mats, water column, and sediments, for a total of 15 samples per sampling episode, per site. In addition, one sample was also collected directly from the well that provides water to the CH to assess possible contributions of DNA to the CH environment from organisms occurring in the groundwater (Bradford, Adams, Humphreys, Austin, & Cooper, 2010; Hahn & Matzke, 2005). Although the filtration system prevents seeding of organisms from the groundwater system into the CH, detection of DNA in the CH environment may influence the comparison between the ecological communities of DH and the CH.

All samples were frozen until DNA extractions were performed. DNA was extracted from 600 μl of a representative aliquot of each sample. Water samples were centrifuged for 8 min at 4,000*g* prior to extraction. MO BIO PowerWater DNA extraction kits were used (MO BIO Laboratories, Carlsbad, CA), following the manufacturer's protocol, including the additional cell lysis step (10 min at 65°C with PW1, prior to bead-beating).

We targeted all eukaryotic taxa using a metagenetic, environmental DNA approach. Amplification, sequencing, and sequence processing of the V9 region of the 18S rDNA gene followed methods described by Paulson and Martin (2019). OTU table and mapping file are available at https://doi.org/10.6084/m9.figshare.12950021. All analyses were conducted in R (R Core Team, 2018).

For evaluating differences between the two environments, all samples were rarified using a common sample size of 2,312 sequences prior to analysis. We estimated the effective number of species (Chao, Chiu, & Jost, 2014) for each sample episode in both environments and plotted the data to evaluate whether there were differences in species richness over time. We used nonmetric multidimensional scaling (NMDS) for visualizing the similarity of all samples from DH and the CH. The variation in community composition was statistically evaluated using the pairwise PERMANOVA (adonis) function in mctoolsr based on Bray Curtis distances from square-root transformed abundance data (Leff, 2015). If the CH effectively emulated the environment of DH at

TABLE 1 Dates and approximate abundances of all taxa intentionally introduced into the refuge tank. *Spirogyra* and cyanobacteria are listed by weight (g)

Inoculation date	Rotifers	Paramecia	Other ciliates	Nematodes	Copepods	Ostracods	Amphipods	Oligochaetes	*Spirogyra sp.* (g)	*Oscillatoria sp.* (g)
June 28, 2013	63,000	780,000	30,000		199				25	
July 12, 2013	20,000	100,000	10,000		100				25	
August 12, 2013							400			
August 16, 2013	28,000	41,250			2,500		175		25	
August 30, 2013	10,000	30,000	15,000		500		200			
September 18, 2013	570							570	23	
September 20, 2013	40,000	70,000	20,000		100		40			
November 19, 2013							106			
November 30, 2013					500		200			
December 5, 2013	22,200	6,000	3,000	9,000					60	20
January 7, 2014	60,000	600	114,000							12
February 25, 2014	56,750	50,250	35,000	19,500				126,000		
March 6, 2014	4,500								10	
March 21, 2014									5	
October 15, 2014						150				
February 18, 2015						3,504		2,280		
February 23, 2015						2,952		2,496		
March 16, 2015						2,652		2,142		
June 29, 2015					54	6,195				
November 4, 2015	10,000		25,000	3,000		6,500				
Totals	315,020	1,078,100	252,000	31,500	3,953	21,953	1,121	133,488	173	32

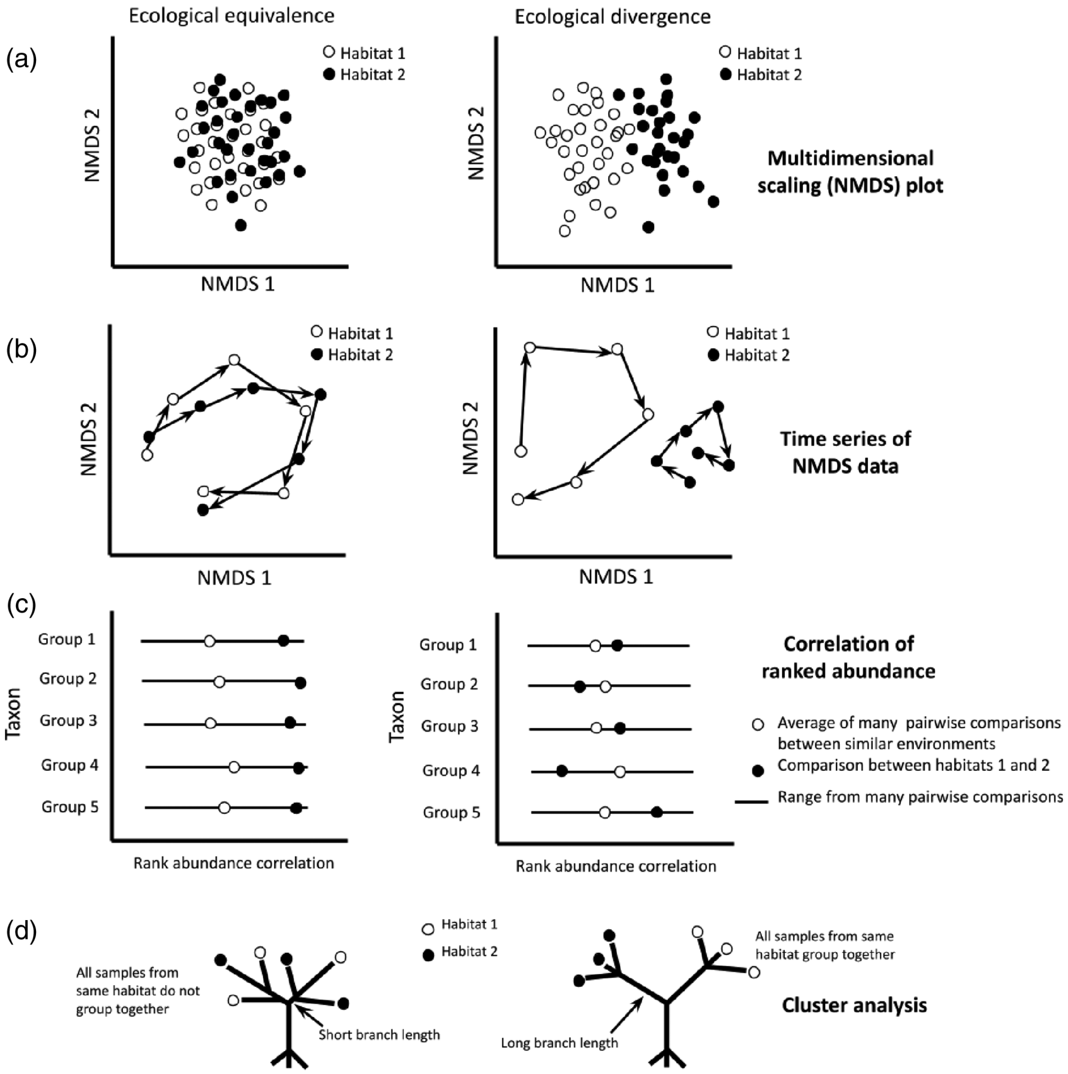

FIGURE 4 Predictions based on hypotheses of ecological equivalence (left column) and ecological divergence (right column) for four measures of community composition. (a) Each point is a single sample from a community and the x,y coordinate of each point is determined by community composition. Points closer together have more similar communities than points further away. NMDS is nonmetric multidimensional scaling. (b) Data for multiple samples for each habitat are combined into a single point and each point is a different date. The lines connect consecutive sampling dates and the data show the community changes over time. (c) Pairwise correlation coefficients for rank-order abundances between pairs of environments. The horizontal line is the range of values obtained from all pairwise comparisons for 21 estimates of community composition published by Paulson and Martin (2019). The open circle is the average correlation and the black dot is the correlation for the comparison between Devils Hole (DH) and the constructed habitat (CH). DH and the CH would be ecologically equivalent if the observed pairwise correlation was more similar than the average correlation based on a large number of pairwise correlations between similar habitats (left graph). Ecological divergence is supported if the observed correlation between DH and the CH is similar to, or less than, the pairwise differences between similar habitats (right graph). (d) Cluster analysis of community composition

the time of this study, we expected overlap in the distribution of points in the two-dimensional community space defined by the NMDS axes (Figure 4a). Additionally, if the CH effectively emulated the environment of DH, we expected similar patterns for the time series of NMDS values for each habitat (Figure 4b). To visualize the time series data, sequences were summed per sampling episode, and then rarefied to 41,122 sequences. Taxa driving differences between the two sites were identified using Kruskal–Wallis tests in mctoolsr (Leff, 2015), with rare taxa (<0.05 relative abundance) filtered out.

We also constructed rank-order abundances for the 10 most abundant taxa across 12 divergent taxonomic groups based on counts of sequences. For each group, we included the taxa based on the rank of the sums of the number of sequences from the two environments. Nonparametric Spearman correlations were calculated. For comparative purposes, we performed the same analysis for all pairwise rank-order correlations between 21 spring systems in the Ash Meadows system using the data from Paulson and Martin (2019) and compared the values for the DH and CH comparison with the means and ranges from all pairwise comparisons. Because the samples from the 21 springs were sampled in November 2013 (Paulson & Martin, 2019), we also repeated this analysis using only the data from December 2014 and November 2015. If the CH effectively emulated the environment of DH, we expected the rank-order correlation between DH and the CH to be larger than the average for the 21 pairwise comparisons (Figure 4c).

Finally, we performed a hierarchical clustering analysis on Bray-Curtis distances for each group of sequences by month from the two focal environments (DH and the CH) as well as a nearby "outgroup" spring (School Spring), using the Ward D2 method (Murtagh & Legendre, 2014) in the hclust() R base function. If the CH effectively emulated the environment of DH, we expected the cluster analysis to show more similarity (shorter branch lengths) between the communities in the two focal environments than the community in the outgroup environment (Figure 4d).

3 | RESULTS

Between December 2014 and November 2015, 15 environmental samples from each of the two habitats were obtained approximately every 2 months. During this time, there were two extraordinary precipitation events with noticeably different visible effects on the two systems. Both events happened during the month of October. The first event, on October 4 and 5, 2015, resulted in a small stream of water that ran into the southwest end of DH and formed a small waterfall off the southern cliff walls. At the CH, the rain washed over the louvered ceiling into the refuge tank. The second flooding event on October 18 was severe. Water flooded into DH in a torrent and deposited a mound of rocky debris on the shallow shelf that rose above the surface of the water. This event caused a moderate to severe disturbance to the DH ecosystem and reduced algal cover on the shallow shelf. The severe flooding event that happened in DH did not have an equivalent, severe impact on the CH, although flooding did happen at the FCF. An additional set of seven samples were collected from both systems approximately 10 days after this second flooding event.

Between December 2014 and November 2015, 4,605 different OTUs were detected in DH and 2,836 OTUs were detected in the CH. Of these, 1,891 OTUs were found in both systems and 2,714 and 945 OTUs were unique to DH and the CH, respectively. A total of 85 OTUs were detected in the CH well water sample; 69 of those OTUs were found in both DH and the CH, 7 were shared between the well water and the CH, 2 were shared between the well water and DH, and 7 were found only in the well water sample. Because the majority of OTUs detected in the well water were found in both the CH and DH, we did not remove those OTUs from further analyses. The effective number of species was more variable and higher in DH than in the CH; in particular, there was a marked difference beginning with the October samples that persisted through the end of the study (Figure 5). It seems inescapable that the difference in alpha diversity beginning in October reflects, to some degree, a differential effect of flooding from extreme precipitation events.

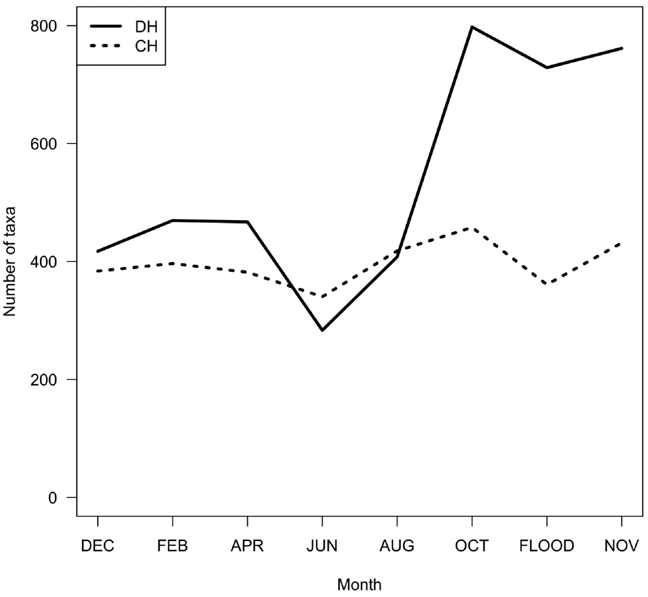

FIGURE 5 Effective number of species between December 2014 and November 2015, including additional samples collected after a major October flooding event (labeled FLOOD)

Community compositions were detectably different between DH and the CH ($p = .001$, $R^2 = .13$, Figure 6). A plot of the community compositions in DH and the CH over time revealed directionally different and non-overlapping trajectories: particularly evident was the large variation in community composition over time in DH relative to the CH (Figure 7). The top taxa driving differences between the two sites were Mollusca (0.16 relative abundance in DH, 0.0005 in the CH; FDR corrected $p = .02$); Embryophyta (0.07 relative abundance in the CH, 0.02 in DH; FDR corrected $p = .03$); and Nematoda (0.09 relative abundance in the CH, 0.02 in DH; FDR corrected $p = .05$).

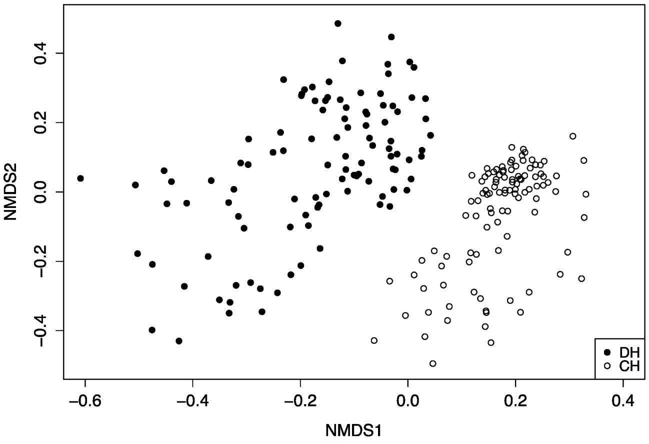

FIGURE 6 Nonmetric multidimensional scaling (NMDS) ordination of all samples collected between December 2014 and November 2015, excluding those collected after the major flood. Sequence data were rarified to a depth of 2,312. The constructed habitat (CH) and Devils Hole (DH) had significantly different community compositions ($p = .001$, $R^2 = .13$)

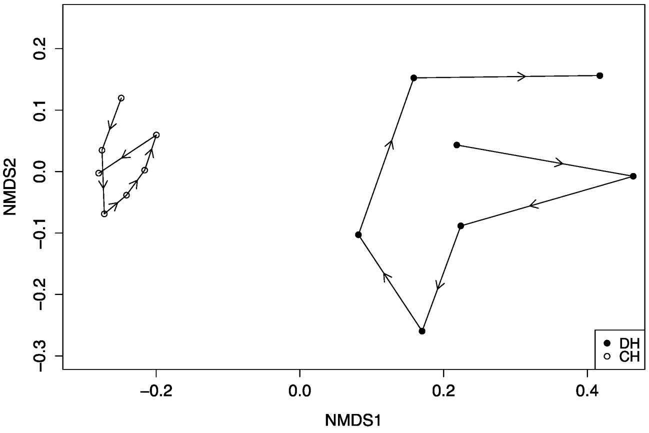

FIGURE 7 Nonmetric multidimensional scaling (NMDS) ordination of whole community estimates per site, per sample date, from Bray Curtis dissimilarities. Sequence data were rarefied to a depth of 41,122. Arrows between points show the sequential trajectories from December 2014 to November 2015 (not including samples collected after the major flood). The two sites changed along differing trajectories across the chronosequence, and the Devils Hole community showed wider variation over the dates sampled

Rank-order correlations of the 10 most abundant taxa for 12 divergent taxonomic groups revealed 9 of the correlations were larger for the comparison between DH and CH than for comparisons across 21 different springs in Ash Meadows ($p = .097$), although the Spearman coefficients were, for all taxonomic groups, less than the maximum values for the comparison springs' correlations (Figure 8).

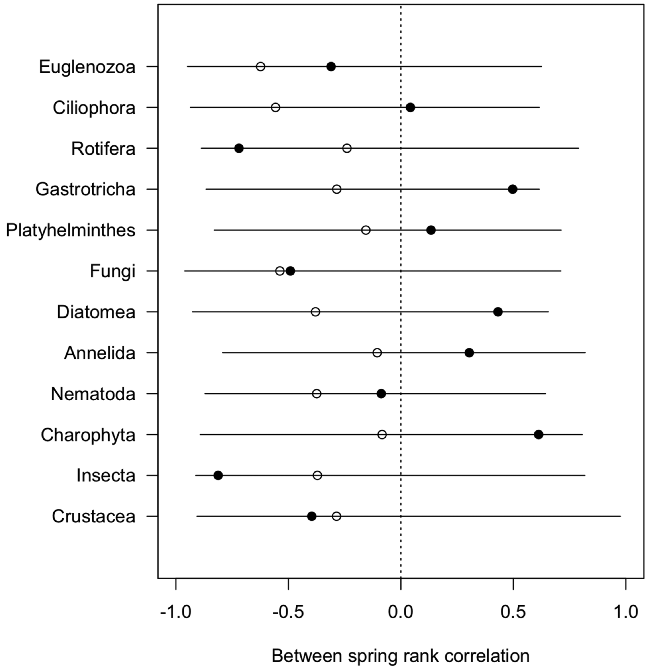

FIGURE 8 Nonparametric correlation coefficients of the rank-order abundance for pairs of communities based on the 10 most abundant taxa across 12 divergent groups. Filled circles are the correlations from the comparison of Devils Hole (DH) and the constructed habitat (CH) (without the flood samples). Open circles are the average correlations based on pairwise comparison of 21 spring systems across Ash Meadows (from Paulson & Martin, 2019). Horizontal segments are the range of correlation values from pairwise comparisons of 21 spring systems. Of the 12 groups, 9 of the correlations were greater for the DH and CH comparison than the mean across the 21 spring systems ($p = .096$)

Hierarchical clustering analysis of communities by sample date revealed clear evidence of separation between DH and the CH, although the two communities were more similar to each other than either was to the outgroup spring (Figure 9).

4 | DISCUSSION

Analysis of a meta-genetic survey of community composition for the DH and CH environments over a year revealed differences in the communities. The data were sufficient to refute the ecological equivalence hypothesis for all testable predictions. Ecological community differentiation suggests the CH did not completely mimic DH for the duration of this study.

Ecological divergence may be explained by the combined effects of differences in (a) key physical parameters, including water temperature, dissolved oxygen concentrations, and solar radiation; (b) community assembly as a consequence of introduction and

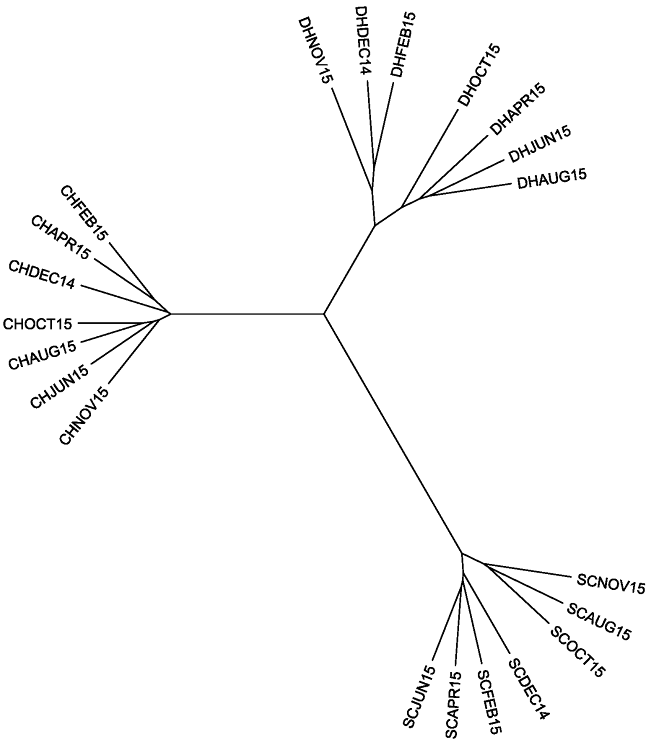

FIGURE 9 Hierarchical clustering of whole community estimates per sampling episode, including samples from a nearby "outgroup" spring called School Spring (SC). Branch lengths were calculated from Bray Curtis dissimilarities. The constructed habitat (CH) communities were more similar to those found in Devils Hole (DH) than School Spring; however, the CH and DH communities clustered separately from each other

establishment of taxa from DH into the CH; and (c) stochastic factors, including two exceptional rainfall events that caused differential deposition of allochthonous materials into the two environments. The DH and CH environments differed in deliberate ways for three physical parameters, and unintentionally for a fourth. The CH was maintained at lower water temperature, higher dissolved oxygen, and higher water level over the shelf than typical for DH. These differences from DH were intentionally implemented because they were perceived to improve conditions for *C. diabolis* pupfish. In addition to these three factors, the amount and timing of incident solar radiation also differed between the two environments as a consequence of structural design of the CH. DH is 15 m below ground level and receives limited to no direct sunlight throughout the year (Wilson & Blinn, 2007); by contrast, the CH is situated above ground, and received direct sunlight throughout the year at the time of this study. For example, direct solar radiation reached the surface of the CH pool for 3.4 hr on December 22, 2014 (USFWS, unpublished data), the day after the shortest day of the year; by contrast, sunlight did not reach the water in DH on any day in December (Wilson & Blinn, 2007).

In addition to these quantifiable differences, the two systems also differed in overall complexity. DH is connected to a deep aquifer with geologic and hydrologic complexity and is located at the bottom of a rocky crevasse with walls that have been shaped over geologic time. It is open to the surrounding landscape, and flooding events can result in streams and cascades of water that collect debris along the surrounding land surface and crevasse walls and pour into DH. Owls and bats roost in the cavern at the north end of DH, and vertebrate fecal pellets have been documented as an important source of nitrogen in the system (Wilson & Blinn, 2007). In contrast, the CH is enclosed in a manmade structure on top of the land surface. The louvered ceiling covering the CH was frequently closed at the time of this study to protect the system from high-intensity direct sunlight during the summer as well as extreme weather events (such as high precipitation and winds). DH is also unique in that it experiences seiches, sometimes multiple times per year, from earthquakes across the globe. These events are known to clear much of the algae and biological materials from the shelf and cause significant disturbance (Chaudoin, Feuerbacher, Bonar, & Barrett, 2015; Weingarten & Ge, 2014). These differences in environmental conditions may have imposed selective filters on possible colonizing organisms, potentially driving differences in community composition.

The differences in solar input likely have consequences for patterns of primary production throughout the year (Diehl, 2002; Tirok & Gaedke, 2007), which may affect consumer population dynamics. Additionally, DH is highly oligotrophic (Hausner, Wilson, Gaines, & Tyler, 2012) and nutrient cycling by both macroinvertebrates and fish may provide an important source of nitrogen. Snails and benthic insects may contribute as much as 15–70% to algal nitrogen demand in oligotrophic desert systems (Grimm, 1988); and fish excretion has been found to account for >75% of ecosystem demand for dissolved inorganic nitrogen in certain environments (McIntyre et al., 2008). Snails were intentionally excluded from the CH during the time period when our research was conducted, and the first inoculation of fish into the refuge tank consisted of 29 captive raised individuals on May 16, 2014 (compared to DH in which census size was approximately 100). Thus, there were likely biologically significant differences in nutrient cycling between the two systems mediated by the different abundance of fish and snails. Additionally, allochthonous carbon is also an important nutrient source for DH (Wilson & Blinn, 2007); the landscape position and relative containment of the CH likely result in much less allochthonous carbon input.

In addition to environmental differences between the two systems, community composition may have differed in the CH due to protocols for introducing organisms from DH to the CH. Although many taxa were targeted for propagation and introduction at the time of this study (Table 1), those taxa were a small fraction of the diversity detected in DH (we discovered 4,605 OTUs). In a visual survey of DH algae, Shepard, Blinn, Hoffman, and Kantz (2000) described 80 taxa; two primary taxa were seeded into the CH: *Oscillatoria sp*. as part of an initial inoculation of biological materials, and *Spirogyra sp*. which was selectively propagated and seeded on an ongoing basis (Table 1). Moreover, traditional visual methods used to survey biodiversity in DH revealed 15 macroinvertebrate species (Herbst & Blinn, 2003; Wilson & Blinn, 2007); the number of identified taxa introduced at the time of this study was 8 (Table 1). Replicating the diversity of DH in the CH could be attained through more liberal establishment protocols and expansion of targeted taxa beyond those that can be visually identified. While less stringent protocols may increase the probability of introduction of pathogens and parasites from the DH system into the CH, research has shown that the latter can be an important prey item in freshwater food webs (Lafferty, Dobson, & Kuris, 2006; Thieltges et al., 2013) and may comprise important components of the trophic network in which *C. diabolis* pupfish are embedded.

Finally, we were able to observe the effects of two heavy precipitation events—one moderate and one severe—on the natural and CHs. The first event, on October 4 and 5, 2015, resulted in a small stream of water that ran into the southwest end of DH, and light cascades off the southern cliff walls. At the FCF, the rain washed over the louvered ceiling into the CH. This first moderate flooding event in October corresponded to an observed increase in alpha diversity in both systems, with a substantially greater increase observed in DH. The second flooding event on October 18 was severe. Water flooded into DH in a torrent, depositing a mound of rocky debris on the shallow shelf that rose above the surface of the water. This event imposed a moderate to severe disturbance on the DH ecosystem, resulting in reduced algal cover on the shallow shelf and sediment deposition. In contrast, this second, severe flooding event did not have an equivalent, severe impact on the CH. The different effects of these disturbance events on the two systems may have provided an additional source of variability between the natural and constructed environments, which could have led to differences in community composition, either due to the physical effects of the disturbance or as a consequence of differential colonization of organisms into the two systems. Our results show an increase in community differentiation after the floods: higher alpha diversity in DH (Figure 5), and increased distance in NMDS ordination space between DH and CH (Figure 7).

Overall, community divergence between the natural and CHs may reflect differences in key abiotic and biotic parameters, and stochastic effects. In addition to disturbance from flooding, other stochastic effects might include ecological drift (Gilbert & Levine, 2017; Hubbell, 2001) and priority effects (Fukami, 2015).

In conclusion, comparison of the natural habitat of *C. diabolis* with a constructed environment revealed differences in the ecological communities that can be explained, in part, by differences in key environmental parameters, restrictions on community assembly, and the role of stochastic factors. These differences may have been substantial enough to drive the *C. diabolis* population in the CH along a divergent evolutionary trajectory; however, the genetic composition of that population has been managed since its introduction in an effort to maintain its similarity to the wild population, through continual introduction of new individuals grown from eggs harvested from DH. Publication of comparative genetic analyses of the two populations are pending, however preliminary results show they are similar (O. Feuerbacher, personal communication, September 23, 2020). Since the time of this study, changes have been implemented at the FCF that would have been recommended by the authors based on our results: environmental parameters have been adjusted in the CH to increase similarity to DH abiotic conditions (O. Feuerbacher, personal communication, September 23, 2020), and additional organisms—including snails—have been targeted for seeding (O. Feuerbacher, personal communication, September 23, 2020). Successional development of the ecological community over time may have increased the similarity of the CH to DH; however, additional research is required to assess the effects of these management changes.

Although there were marked differences in the ecological communities found in DH and the CH at the time of this study, the efforts of the FCF to harbor a reproductive population of *C. diabolis* have been successful (O. Feuerbacher, personal communication, March 22, 2021). It has been hypothesized that difficulties in breeding captive *C. diabolis* prior to the CH may have been due to the absence of specific environmental variables (O. Feuerbacher, personal communication, March 22, 2021); accordingly, the successful reproduction that has occurred in the CH may indicate sufficient environmental similarity. In addition, all families of taxa known to be important food items for *C. diabolis* (Minckley & Deacon, 1975) were detected in the CH (see OTU table at https://doi.org/10.6084/m9.figshare.12950021), with the exception of the intentionally excluded *Tryonia* snails that have since been established. Overall community similarity of the CH to DH is likely important for the evolutionary trajectory of the CH *C. diabolis* population (e.g., Hutchings, 2011; Lema, 2020; Olendorf et al., 2006; Scheiner & Lyman, 1989), however short-term conservation goals are currently being met by the facility.

Without monitoring, ecological community differentiation between DH and the CH may not have been detected. As is also the case for ecological restoration projects, monitoring of community change over time provides quantitative feedback about progress toward ecological goals, as seeding of targeted taxa does not always yield the expected community outcome (Suding, Gross, & Houseman, 2004). The methods described here facilitated rapid, broad surveys of thousands of species, providing a time- and cost-effective tool for informative and quantitative ecological monitoring.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Elizabeth L. Paulson and **Andrew P. Martin**: Designed the study, analyzed data, and wrote the manuscript. **Elizabeth L. Paulson**: Conducted fieldwork. **Ambre Chaudoin**: Contributed to the manuscript and provided the information in Table 1.

DATA AVAILABILITY STATEMENT

All data used for this study are publicly available at https://doi.org/10.6084/m9.figshare.12950021.

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