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Assessing Rotifer Diversity In Desert Wetlands Through Sediment Rehydration And Amplicon Sequencing

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ASSESSING ROTIFER DIVERSITY IN DESERT WETLANDS THROUGH SEDIMENT REHYDRATION AND AMPLICON SEQUENCING

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Dedication

This thesis is dedicated to my parents, Laura and Sergio Samaniego.

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by

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Abstract

Species living in temporary wetlands can be threatened due to the instability and fluctuation of rising temperatures and evaporation caused by global climate change. In addition, regions characterized by dry climates, where most temporary wetlands exist, are expected to have a stronger response to climate change than other ecosystems. While most organisms are not able to respond to rapid changes in their environments, others like freshwater invertebrates have shown great resiliency to short- and long-term ecological disturbances. Rotifers for example, inhabit temporary waters that remain desiccated for indefinite periods of time making assessment of rotifer biodiversity challenging. Fortunately, many microinvertebrates have the ability to produce resting stages that persist in surface sediments when conditions become unfavorable. Their ability to persist through resting stages is important since the structure of these communities contribute to healthy and sustainable ecosystems. Here, active rotifer communities were compared with egg banks found in surface sediments from 12 temporary desert wetlands. Dormant communities were analyzed through rehydration experiments and amplicon sequencing. It was hypothesized that more permanent ponds would have higher microinvertebrate species richness than those that are more ephemeral due to habitat stability. Rehydration of sediments from 12 sites resulted in 41 rotifer species emerging from diapause, with richness ranging from 1-16. Unique taxa not present in active species lists were found in 8 out of the 12 sites. Species richness was highest in larger, Australian sites with lowest richness occurring in small rock pools from the United States. For a subset of the sites, hatching success was compared from sediments stored up to 21 years of age in order to assess the viability of rotifer resting eggs to hatch after being stored for long periods. As expected, recovery of rotifer species from older sediments was less when compared to the recovery rates of newer sediments. One-yr old sediment collected from a Texas playa yielded the highest species richness (n=10). Amplicon sequencing of sediments showed that taxonomic diversity varied among pond types and that larger more permanent temporary ponds had higher diversity than those that were more ephemeral. 18S

sequencing resulted in 702,328 reads and 34,995 Operational Taxonomic Units (OTUs), of these, 3,610 (0.51%) reads corresponded to 55 OTUs (0.15%) that were identified as rotifers. COI sequencing resulted in 495,643 reads and 10,241 OTUs, of these, 15,687 reads (3.16%) corresponded to 149 OTUs (1.45%) that were identified as rotifers. Principal Coordinates Analysis (PCoA) plots constructed with 18S sequences showed little to no similarities among sites. Interestingly, PCoA plots based on COI sequences clustered sites by pool type. Implementing these approaches into ecological assessments of resting stages can expand our knowledge on their efficiency to fully capture species richness and how a combination of them allows detection of taxa missed by using only one method.

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Chapter 1: General Introduction

Eukaryotic microorganisms are one of the most abundant and diverse groups found in freshwater and terrestrial systems. These taxa play roles as decomposers, predators, producers, parasites, and are crucial in maintaining healthy ecosystems (Bik et al., 2012; Debroas et al., 2017; Yang et al., 2017; Banerji et al., 2018). Fluctuations of temperature, nutrient content, and biological communities in freshwater ecosystems have been largely accredited to global climate change (Tavşanoğlu et al., 2017; Darwall et al., 2018; Pinceel et al., 2018). Having an inventory of both active aquatic communities and resting stages found in sediment egg banks can increase our understanding of the overall biodiversity present in desert aquatic systems. Most studies focus on a snapshot assessment approach where active communities are only sampled once, which oftentimes is not enough to capture all the taxa present at a site. In the future, the assessment of zooplankton egg bank communities will become increasingly important with the potential of warmer temperatures and fewer rainfall events in desert regions.

Zooplankton communities, including rotifers, are typically characterized by sampling of active populations (Duggan et al., 2001; Dodson et al., 2005; Wallace et al., 2008). As mentioned above, most studies are snapshot assessments with relatively few long-term studies of community composition. Assessing active zooplankton communities through single sporadic visits will only provide a general characterization of the community structure (Stemberger et al., 2001). Analyzing both active and resting egg communities would provide a more in-depth assessment of community composition at any given site. Aquatic sediments typically contain resting stages that can be used to explore questions on ecological, community, and evolutionary ecology (Burge et al., 2018). Here, a series of rehydration experiments were conducted to assess the biodiversity of rotifer resting stages found in sediments collected from 12 wetland sites. Once recovered, rotifer communities were compared to those found during wet periods. Additionally, for a subset of the sites, hatching success was compared from sediments collected from different

time periods to see if the ability of rotifer resting eggs to hatch was affected by long periods of dormancy.

The analysis and recovery of resting egg banks through rehydration experiments can be useful to indicate the qualitative status of an ecosystem (Angeler and Garcia, 2005). Skinner et al. (2001) rehydrated sod samples from two dry lakes containing moderate to severe concentrations of salinity in the River Murray floodplain, South Australia. This was done to see how flow regulation, agricultural flow, and increased salinization would affect the emergence of taxa. Results showed that increased salinity was associated with lower species richness and suggests that propagule banks may be useful as complementary indicators of wetland health (Skinner et al., 2001). Toruan (2012) quantified the impact that increased salinity had on zooplankton communities. Sediments from three different Australian wetlands with different salinity gradients were exposed to salinity levels of 300 mg/L, 5,000 mg/L, and 15,000 mg/L for 21 days. After exposure, more taxa had emerged from less saline sediments. Also, a reduction of taxa was seen when salinity increased from 300 mg/L to 5,000 mg/L. Mabidi et al. (2018) also found that high levels of salinity reduced the amount of taxa emerging from wetland sediments with a significant decrease in emerging taxa richness and abundance seen in salinity concentrations of > 2.5 g L^{-1}. Thus, the application of resurrection ecology in wetland studies may be valuable in order to assess how changes in the environment can potentially threaten biodiversity (Carroll et al., 2014).

Rotifers were selected as model organisms in this study due to their successful strategies to persist in temporary wetlands through diapausing stages. The phylum Rotifera includes over 2,000 marine and freshwater species. The rotifer bauplan is characterized by a ciliated anterior end called the corona, a thickened body wall with variable appendages called the lorica, and a muscular pharynx called the mastax that contains chitinous jaws termed trophi (Wallace, 2002). Three groups within the phylum have aquatic representatives, the Seisonidea, Monogononta, and Bdelloidea (Segers, 2007). The endoparasitic acanthocephalans, a sister-taxon of the Bdelloidea (Mark Welch, 2000; Garcia-Varela and Nadler, 2006; Sorensen and Giribet, 2006), are also

included in the phylum Rotifera, but are not addressed in this study. Most aquatic rotifers mainly feed on algae, bacteria, and protists making them important basal consumers in food webs. Rotifers in turn are eaten by invertebrate predators and small fishes; thus transferring energy to higher trophic levels (Wallace et al., 2006).

Reproduction strategies vary among aquatic rotifer classes. The Seisonidea reproduce by ordinary meiosis. Monogononta undergo cyclical parthenogenesis (Mark Welch, 2000; Wallace, 2002). During their life cycle, amictic females produce diploid eggs that hatch into amictic females (Gilbert, 1974). Environmental signals can induce amictic females to go sexual and produce mictic females. Signals include crowding, dietary tocopherol, or changes in photoperiod length (Pourriot and Clement, 1975; Gilbert, 2004; Alekseev et al., 2007; Serra et al., 2019). Once sexual reproduction ensues, mictic females are produced. Mictic females produce haploid eggs that if fertilized, result in diploid embryos or resting eggs. Resting eggs undergo obligatory diapause, eventually hatching as amictic females. Similar to rotifer resting eggs, bdelloids have the ability to enter a state of diapause, forming resting stages called xerosomes (Wallace and Smith, 2009). Xerosomes also have the ability to withstand desiccation in sediment egg banks for extended periods of time, becoming active after rehydration (Ricci and Caprioli, 2005; Wallace and Smith, 2009). However, they are formed through a process of anhydrobiosis rather than being a product of sexual reproduction. Bdelloids reproduce entirely asexually and no males have been reported for the group (Wallace et al., 2015). In monogononts, resting stages usually persist in the top 2 cm of sediment egg banks until favorable conditions resume (Snell et al., 1983). Further, even if conditions do become favorable, rotifers have evolved risk-spreading strategies like bet-hedging to deal with their habitats. Through bet-hedging, not all diapausing eggs or xerosomes produced by a single genotype hatch at once but are spread over several growing seasons (García-Roger et al., 2014). The composition of sediment egg banks can be characterized by rehydration experiments or through environmental DNA sequencing techniques.

To further assess rotifer communities in this study, a metabarcoding approach was implemented to obtain a more comprehensive estimation of the diversity of rotifers in temporary

desert wetlands than can be provided through active collections or rehydration experiments. Using DNA metabarcoding techniques can be convenient, cost-effective, and can provide an in-depth survey of the presence or absence of communities and potentially indicate the environmental quality of a site (Keck et al., 2017). Assessing the biodiversity of all trophic levels in a community is crucial since the structure of these oftentimes overlooked rotifer communities contribute substantially to the maintenance and sustainability of healthy aquatic ecosystems.

When using a metabarcoding approach, the choice of primers important to accurately identify taxa. For example, the 18S ribosomal RNA (rRNA) gene (Blaxter et al., 1998) and the mitochondrial cytochrome c oxidase subunit I (COI) (Folmer et al., 1994) are broadly used to generate informative sequences for phylogenetic analyses of eukaryotes at the species and higher taxonomic levels. Many studies have been conducted validating the reliability of metabarcoding techniques and their ability to assess biodiversity by comparing them to traditional methods. For example, Piredda et al. (2017) compared the results obtained from a Serial Dilution Culture (SDC) method (Throndsen, 1978), which estimates the concentration of viable cells in a sample, to those given by High Throughput Sequencing (HTS) using 18S primers. They assessed the abundance and diversity of viable diatom resting stages found in surface sediment samples from a long-term ecological research station in the Gulf of Naples, Italy. Results showed that HTS-metabarcoding provided a higher number of identifiable sequences when compared to the number of taxa recorded by SDC, suggesting that HTS-metabarcoding is a more reliable approach when analyzing the diversity in resting stages of diatoms (Piredda et al., 2017). Further, Valentini et al. (2016) compared traditional surveys and historical data to an environmental DNA metabarcoding approach using fish and amphibian tissues and environmental samples to test the reliability of metabarcoding. Results showed that the detection of these taxa through metabarcoding was significantly higher or identical to the number detected using traditional surveying methods. They concluded that metabarcoding has great potential to assess and monitor aquatic diversity at various trophic levels. Recently, it has been shown that rotifer communities

can be efficiently characterized through sequencing approaches in a freshwater system (Yang et al., 2017).

Metabarcoding is a powerful tool but careful consideration should be taken when sequencing older samples. Carew et al. (2016) reported that archived samples older than 8 years often resulted in detection of fewer taxa and were less reliable for amplification. As part of the investigation, archived macroinvertebrate samples stored in 70% ethanol at room temperature collected over a 12-year period were sequenced to see if HTS could be used to identify them to the species level. Results confirmed the effectiveness of HTS for detecting multiple species in mixed and archived environmental samples, but amplification of material older than eight years proved to be less reliable. Further, an attempt to amplify individuals for short DNA barcodes of <300 bp showed the negative effect that long-term storage had on the quality of the DNA. Even when taking these limitations into consideration, metabarcoding provides an unparalleled ability to analyze the genomic diversity within a natural population (Ruppert et al., 2019).

The goal of this study was to fully assess rotifer biodiversity in temporary desert wetlands through comparison of active communities, species recovered through rehydration experiments, and amplicon sequencing. The Chihuahuan Desert is known for its high biodiversity in both terrestrial and aquatic systems (Dinerstein et al., 2000). In aquatic systems, endemism is pronounced in fishes (Minckley, 1978; Echelle et al., 2003; Hubbs, 2003; Hoagstrom et al., 2011) and springsnails (Hershler, 1985; Hershler et al., 2011; Hershler et al., 2014). Although many studies have characterized rotifers inhabiting springs, temporary playas, and huecos in American and Mexican regions of the Chihuahuan Desert (Wallace et al., 2005; Walsh et al., 2007, Wallace et al., 2008, Walsh et al., 2008; Walsh et al., 2014; Rivas et al., 2018), few have assessed the dormant phase of these communities. This study provides a more in-depth survey of rotifer biodiversity including sediment egg bank communities found in the southwest Texas and New Mexico region of the Chihuahuan Desert. This approach was applied in a second desert system by including samples from Australian sites to test the recovery rate of rotifer biodiversity from a geographically distinct desert system. Further, USA sites are fed exclusively by rain

events, while Australian sites are fed by water from the Chowilla floodplain. Because of their connection with the river, they are larger, more permanent, and with more heterogeneous sediment layers. In sum, these sites will provide insight into the recovery rate of rotifer biodiversity from a variety of desert systems ranging from small, ephemeral to larger more permanent systems. .

The first objective of this study was to the test the ability of rehydration experiments to recover rotifer communities and to determine similarities between rotifers recovered from active communities and sediments. It was hypothesized that more rotifer taxa would be recovered from sediments coming from larger pools and that rehydration experiments would recover a large portion of rotifer taxa found during wet periods. Additionally, sediments collected at different time periods were rehydrated to compare how the age of sediments impact rotifer recovery. We hypothesized that newer sediment would recover more rotifer taxa than older sediments. The second objective of this study was to compare environmental sequencing results to those obtained from rehydration experiments. It was hypothesized that sediment samples sequenced from larger pools would have higher rotifer diversity than those that are smaller and more ephemeral. Further, when compared to active communities and rehydration experiments, amplicon sequencing was hypothesized to provide the highest estimates of rotifer richness.

Chapter 2: Assessment of rotifer diversity in temporary desert wetlands through rehydration experiments

2.1 Introduction

Species living in temporary wetlands can be threatened due to the instability and fluctuation of rising temperatures and evaporation caused by global climate change (Pinceel et al., 2017). In addition, regions characterized by dry climates, where most temporary wetlands exist, are expected to have a stronger response to climate change than other ecosystems, a phenomenon known as "desert amplification" (Zhou, 2016). While most organisms are not able to respond to rapid changes in their environment, others like freshwater invertebrates have shown great resiliency to short- and long-term ecological disturbances (Bogan et al., 2017). Aquatic invertebrates, including rotifers, are bioindicators of wetland health due to their abundance, high species diversity, and sensitivity to environmental change (Carew et al., 2013). Many species living in temporary habitats persist as resting stages; stages that undergo suspended development when conditions become unfavorable (Datry et al., 2017).

Persisting through resting stages is beneficial because it maintains species diversity within communities and genetic diversity within populations (Garcia-Roger and Ortells, 2017). Kerfoot et al. (1999) coined the term "resurrection ecology" to describe the bringing back of ancestors or ancestral genetic material by retrieving entombed resting stages for biological tests. Recovering dormant taxa through sediment rehydration can give insights into biological species pools, how past environmental conditions impacted communities, changes in genotypes within species, and determine evolutionary responses that organisms have towards climate change (Angeler, 2007). Also, recovery of resting egg banks can be useful to indicate the qualitative status of an ecosystem by directly documenting how communities have adapted to historical environmental changes and can also allow experimental evaluation of alternative paleo-ecological and evolutionary scenarios (Kerfoot and Weider, 2004).

Temporary wetlands in arid regions typically hold a large reserve of resting stages in their sediments (Hairston and Kearns, 2002; Brock et al., 2003; Brendonck and De Meester, 2003). As drought and extreme conditions become more frequent, species that have the ability to produce stages resistant to desiccation will be able to persist while those species lacking egg banks will not (Hairston, 1996). When more favorable conditions resume, a fraction of the dormant stages become active while the remainder persist in an inactive portion, potentially serving as natural archives of community and population structure (Orsini et al., 2013). Hatching within egg banks usually occurs within hours to days following rainfall (Brendonck, 1996). Resting egg banks are not always exhausted by a single wetting event, allowing multiple generations to accumulate and develop across large temporal and spatial scales (Brock et al., 2003). Rotifers may also opt for bet-hedging strategies to avoid reproductive failure if conditions are not optimum (García-Roger et al., 2014). Bet-hedging strategies in rotifers have been proposed to have evolved in three diapause-related traits which include the timing of sex, sexual reproduction ratio, and the timing of diapausing egg hatching (García-Roger and Ortells, 2018).

Here, as categorized in Walsh et al. (2014), temporary playas were defined as being seasonally flooded pools or depressions found in the landscape of aridland systems. More ephemeral water bodies, such as rock pools, were defined as aridland basins on impervious bedrock. Both of these pool types have rare and unpredictable inundation frequencies, and generally have a hydroperiod of days, weeks, and sometimes even months. The length of time a wetland holds water is important for invertebrates especially for those that produce resting stages. If insufficient resting stages are produced within a single hydroperiod, the population of certain species may not be able to persist and form future generations. Therefore, size differences in wetland ponds may have an effect on species diversity (Oertli et al., 2002).

In temporary wetlands, habitat type and size are an important factor that can potentially affect the diversity of aquatic communities that produce resting eggs (Fontoura Freiry et al., 2016). As noted above, one of the main factors in structuring aquatic communities in pond systems is their hydroperiod (Brendonck and Williams, 2000). Serrano and Fahd (2005)

observed the influence that hydroperiod had on the species composition and richness of zooplankton in 19 temporary ponds at different spatial and temporal scales. Ponds were categorized as having long, intermediate, and short hydroperiods. Their results showed that there was a significant positive correlation between the number of zooplankton found and hydroperiod. Della Bella et al. (2005) investigated several factors, including hydroperiod, that influenced macroinvertebrate species richness in 21 temporary and permanent ponds in central Italy. They found that water permanence had a significant effect on species richness with permanent ponds having higher richness than those that were more temporary. Gleason and Rooney (2018) also looked at the effect that pond permanence had on the macroinvertebrate community structure in wetlands and found that macroinvertebrate species richness was positively associated with longer hydroperiods. These studies suggest that sediments rehydrated from larger temporary playas may have higher diversity due to their ability to support a larger number of resting stage-producing taxa than those that are more ephemeral.

This study was done to assess rotifer diversity in 12 desert wetland ponds through sediment rehydration experiments. Ponds studied varied in pool type, size, and geographic location. The first objective of this study was to test the efficiency of rehydration experiments to recover rotifer communities from these sites and to see how similar recovered communities were to active communities. It was hypothesized that more rotifer taxa would be recovered from sediments coming from larger pools and that rehydration experiments would recover a large portion of rotifer taxa also found in active communities. The second objective of this study was to compare how the age of sediments impacts rotifer recovery. It was hypothesized that more recently collected sediment would recover more rotifer taxa than sediments that had been stored for longer periods of time.

2.2 Methods

2.2.1 Active community collection

The 12 sites sampled included seven located at Hueco Tanks State Park and Historic Site (HTSP) located in El Paso Co., Texas, USA. The Hueco Mountains are made of large round boulders that oftentimes have depressions that form rock pools after rainfall events; several temporary playas are also filled. At HTSP, four playas East (E), Behind East (BE), Behind Ranch House (BRH), Laguna Prieta (LP) and three rock pools Vero (V), Stacia (S), and South Temp (ST) were sampled (Figure 1A). Two additional playas (404A (4A) and 404B (4B)) located near New Mexico State Road 404 located in Doña Ana Co., New Mexico, USA were sampled (Figure 1). Sites 404A and 404B are located on Bureau of Land Management land that is used for recreation and cattle grazing. Site 404A is located near a hiking trail that is disturbed by vehicles, cattle, and pedestrians. Site 404B is further north, is surrounded by vegetation, and is more protected from these disturbances (Figure 1A). The annual average temperature of the Chihuahuan Desert is of 18.6 ºC with a mean precipitation of 235 mm per year (Schmidt, 1986; Laity, 2008). In El Paso Co., Texas, where most of the USA sites are located, the annual average temperature is of 17.7 °C with a mean precipitation of 228 mm per year. In addition to these Chihuahuan desert sites, three waterbodies along the River Murray floodplain in Chowilla, South Australia, AUS were sampled: Lake Littra (LL), Lake Limbra (LIM), and Ryan's 2 billabong (R2) (Figure 1B). Lake Littra and Lake Limbra are temporary shallow pools fed by waters of the Chowilla floodplain, which is one of the major floodplains adjoining the River Murray in South Australia (Furst, 2013). Ryan's 2 billabong is one of three billabongs located on the grounds of the Michael Ryan Ecology Laboratory, located in the River Murray floodplain. Australian sites are larger, more permanent, and more riverine than sites located in the USA. Similar to the Chihuahuan Desert, Australian sites along the Chowilla floodplain experience an arid to semi-arid climate with an average annual rainfall of 260 mm (Furst, 2013). Table 1 summarizes the characteristics of each site.

Active communities from the desert southwest USA were collected using a 64 μm plankton net during the past 20 years (HTSP and NM) and examined as part of other studies conducted by Walsh (personal communication; see Table 1 for sampling effort). Species list from plankton samples were available in published studies for Australian sites (Lake Limbra, Lake Littra; Furst, 2013) while Ryan's 2 billabong species lists were provided by Russ Shiel (pers. comm.). Sampling effort of active communities varied greatly among sites (Table 1). Examples of pool types sampled during dry and wet periods are shown in Figure 2.

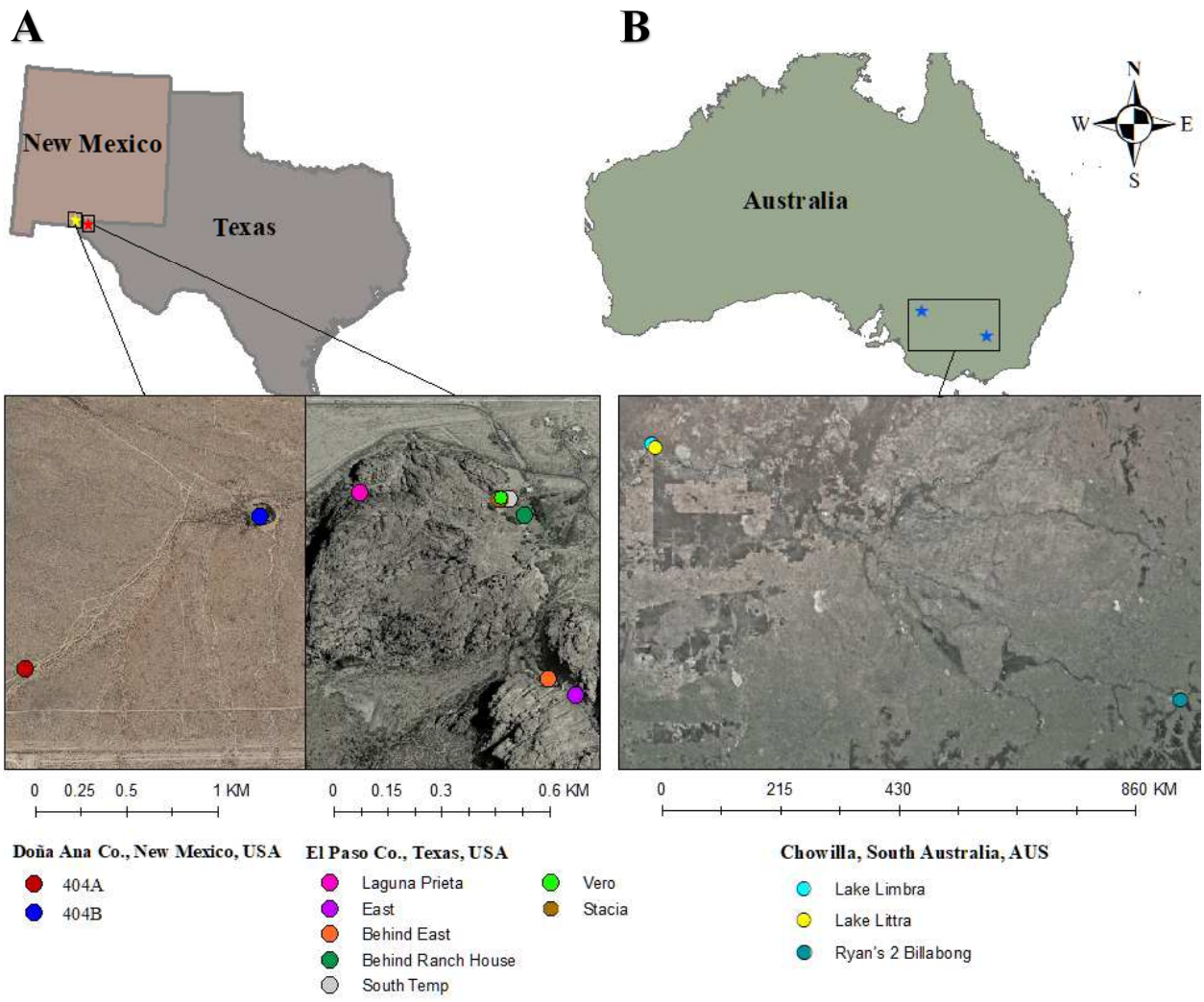

Figure 1 Map of the 12 sites sampled for active communities, sediments used for rehydration experiments, and amplicon sequencing. Panel A from left to right: Map of Texas and New Mexico is provided to show geographic proximity of the US sites; below are sites near New Mexico State Road 404 located in Doña Ana Co., New Mexico, USA and HTSP sites located in El Paso Co., Texas, USA; Panel B: Map of Australia is provided to show geographic proximity of the Australian site; below are lake sites located along the River Murray floodplain in Chowilla, South Australia, and the billabong site located on the grounds of the Michael Ryan Ecology Laboratory.

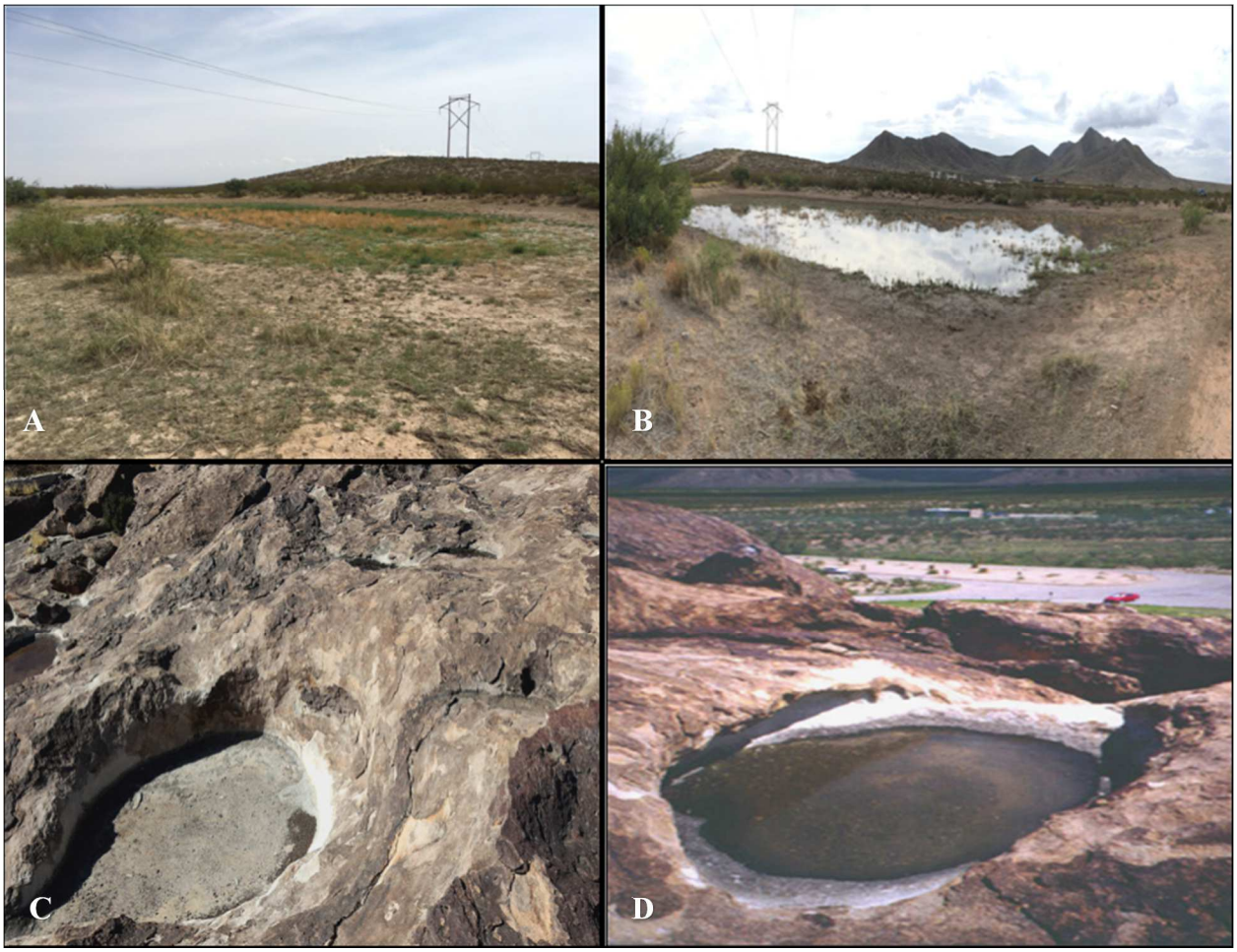

Figure 2 Examples of pool types included in this study before and after the monsoon season. Panel A = dry temporary playa (404A); Panel B = filled temporary playa (404A); Panel C = dry rock pool (Stacia); Panel D = filled rock pool (Stacia).

Table 1 Site characterization of 12 desert wetlands including species richness of active rotifer communities is provided for comparison between habitat types (temporary playas versus rock pools). Sampling intensities for active species lists are indicated by asterisks (* = low, ** = moderate, *** = high).

Site	Geographic Location	GPS Coordinates Latitude	GPS Coordinates Longitude	Pool Type	Surface Area (m^2)	Maximum Depth (m)	Sediment Collection Date	Species Richness
Lake Littra*	Australia	-33.95083333	141.0305556	Temporary Playa	868,155	1.00	2012	45
Lake Limbra*	Australia	-33.92806666	140.9531111	Temporary Playa	1,200,000	2.00	2012	49
Ryan's 2 Billabong***	Australia	-36.11072222	146.9666444	Temporary Playa	61,026	2.50	2017	83
404A**	USA	32.01258444	-106.5234270	Temporary Playa	1,648	0.30	2016	16
404B**	USA	32.01832555	-106.5171590	Temporary Playa	2,755	1.00	2016	18
East***	USA	31.91861111	-106.0402778	Temporary Playa	3,432	1.26	2018	51
Behind East***	USA	31.91916667	-106.0408333	Temporary Playa	1,644	2.00	2018	26
Behind Ranch House***	USA	31.92388889	-106.0416667	Temporary Playa	1,835	1.30	2018	36
Laguna Prieta***	USA	31.9246288	-106.0466750	Temporary Playa	4,306	2.00	2018	39
Vero***	USA	31.92444444	-106.0425000	Rock Pool	5.8	0.08	2018	3
Stacia***	USA	31.92458333	-106.0425556	Rock Pool	3.7	0.20	2003, 2008, 2016	4
South Temp***	USA	31.92444444	-106.0422222	Rock Pool	10.8	0.22	2018	21

2.2.2 Sediment collection

Composite sediment samples from the USA were collected from nine sites in 2016 and 2018, with one exception. Sediments from one rock pool (Stacia (S)) for one replicate consisted of a mixture of sediments collected from 2003, 2008, and 2016 due to limited availability of sediments from more recent collections. Sediments were stored in cool, dark conditions until used. In general, samples were taken from upper playa and rock pool surfaces (~2.5 cm) with a small hand trowel from several locations within a dry basin and then combined. Dried sediments from Australian sites Lake Littra and Lake Limbra were collected in 2012 and Ryan's 2 Billabong was collected in 2017. Drs. Russ Shiel, John Gilbert, and Daryl Nielsen provided dried sediments from the Australian sites.

2.2.3 Rehydration experiments

2.3.2A. Rehydration of sediments from 12 desert sites

Dried surface sediments (3 g) from all sites were placed in small plastic containers (740 mL capacity) and were rehydrated in 400 mL of EPA artificial freshwater (pH 7.5; Weber, 1993) and incubated at 25 °C with a light-dark cycle of 18 L:6 D to initiate emergence of rotifers from diapause. Rehydrated sediments were observed periodically for one month, held for one week, then re-observed a final time and were discarded if no new taxa emerged. All hatching experiments were independently replicated four times. Rotifers were identified to species level using keys by Koste (1978) and in the Guides to the Identification of the Microinvertebrates of the Continental Waters of the World (Edmondson, 1949, 1959; Berzins, 1951; Donner, 1965; Ruttner-Kolisko, 1974; Koste, 1978; Stemberger, 1979; Koste and Shiel, 1986; Nogrady et al., 1993; Nogrady et al., 1995; Segers, 1995; De Smet, 1996; De Smet and Pourriot, 1997; Ricci and Melone, 2000; Nogrady and Segers, 2002).

2.3.2B Hatching success experiment

For a subset of the sites, hatching success was compared from sediments of different ages to determine if the age of sediments had an impact on rotifer recovery. Sites were chosen based on the availability of sediments stored in the Walsh laboratory. Using the same methods as above, sediments were rehydrated from two playas, Behind East (BE) and Lake Littra (LL), and a rock pool, Vero (V) (Table 2). Further, a hatching phenology of rotifers recovered from sediments of different ages was included. For the hatching phenology, unique colors were assigned to rotifer species as they hatched. Presence of color indicates hatching of a rotifer species on that specific day.

Table 2 Rehydration experiments comparing hatching success from sediments of different ages. Site name, date when sediment was collected, and species richness of rotifers found during wet periods and after rehydration is provided. See Table 1 for site characteristics.

Site	Sediment Collection Date	Species Richness of Active Community	Species Richness of Recovered community
Behind East	04.16.1998	26	8
Behind East	06.03.1999	26	4
Behind East	09.12.2014	26	6
Behind East	01.13.2018	26	10
Vero	06.20.2003	3	1
Vero	01.13.2018	3	1
Lake Littra	2012	45	14

2.2.4 Diversity in active populations and sediments

Presence-absence data from active and recovered species lists was used to calculate the Sorensen index of dissimilarity: $D_s = b+c/2a+b+c$, where a = number of species present in active and recovered lists; b = number of species absent in active lists and present in recovered lists; c = number of species present in active lists and absent in recovered lists (Sorensen, 1948). Active lists were compared with each other in order to assess how similar species composition was between localities. Species lists of rotifers recovered from dormant populations through rehydration experiments versus those found in existing active species lists were also compared. Sorenson dissimilarity index was calculated using the vegan package in R version 3.4.0 (R Core Team 2013).

2.3 Results

2.3.1 Active population species richness

A total of 165 rotifer taxa were found in plankton collections from the 12 sites (Table 3). Species richness was greatest in Australian sites, with 45 taxa in Lake Littra (LL), 49 in Lake Limbra (LIM), and 83 in Ryan's 2 billabong (R2). At HTSP, richness was highest in East (E) with 51 taxa, followed by Laguna Prieta (LP) with 39, and Behind Ranch House (BRH) with 36. Rock pools had the lowest richness with 21 taxa in the largest pool South Temp (ST) and the smaller pools had 3 (Vero (V)) or 4 (Stacia (S)) species.

Table 3 Presence/absence of rotifer taxa found in active and recovered species lists. Key to abbreviations: A = active rotifer populations; R = recovered rotifer populations; R* = unique recovered rotifer species not found in active populations; A/R = both active and recovered populations; E = East, HTSP, USA; BE = Behind East, HTSP, USA; BRH = Behind Ranch House, HTSP, USA; LP = Laguna Prieta, HTSP, USA; ST = South Temp, HTSP, USA; V = Vero, HTSP, USA; S = Stacia, HTSP, USA; LL = Lake Littra, Australia; R2 = Ryan's 2 Billabong, Australia; LIM = Lake Limbra, Australia; 4A = Playa 404A, New Mexico, USA; 4B = Playa 404B, New Mexico, USA. Active species lists for LL and LIM are from Furst (2013). Active species list for R2 was provided by Russ Shiel (pers. com.).

Authority name	E	BE	BRH	LP	ST	V	S	LL	R2	LIM	4A	4B
Adineta vaga (Davis, 1873)			A									
Anuraeopsis fissa (Gosse, 1851)	A			A	A				A			
Anuraeopsis sp.								A		A		
Ascomorpha saltans Bartsch, 1870									A			
Asplanchna asymmetrica Shiel and Koste, 1985								A	A	A		
Asplanchna brightwellii Gosse, 1850								A/R	A/R	R*		
Asplanchna girodi Guerne, 1888	A											
Asplanchna intermedia Hudson, 1886				A/R								
Asplanchna priodonta Gosse, 1850								A		A		
Asplanchna sieboldii (Leydig, 1854)	A			A				A	A	A		
Asplanchna sp.			A/R									A
Asplanchnopus hyalinus Harring, 1913	A	A										
Asplanchnopus multiceps (Schrank, 1793)		R*		A					A			
Asplanchnopus sp.	A											
Bdelloid spp.		R*						R*		R*		A
Brachionus angularis Gosse, 1851	A	A	A/R	A				A/R	A/R	A		
Brachionus bidentatus f. *testudinarius* Jakubski, 1912										A		
Brachionus budapestinensis Daday, 1885								A	A	A		
Brachionus calyciflorus Pallas, 1776	A	A	R*	A	A			A/R	A/R	A/R		A/R

Brachionus calyciflorus f. *anuraeiformis* Brehm, 1909								A			
Brachionus calyciflorus “round”							R*	R*			
Brachionus calyciflorus “spines”											A/R
Brachionus calyciflorus var. *amphiceros* Ehrenberg, 1838									A		
Brachionus caudatus Barrois and Daday, 1894							A		A		
Brachionus dichotomus Shephard, 1911								A	A		
Brachionus diversicornis (Daday, 1883)							A	A	A		
Brachionus falcatus Zacharias, 1898							A		A		
Brachionus lyratus Shephard, 1911							A	A	A		
Brachionus nilsoni Ahlstrom, 1940							A		A		
Brachionus novaezealandiae Morris, 1913							A	A			
Brachionus plicatilis Müller, 1786							R*	R*			A/R
Brachionus quadridentatus Kertész, 1894	A	A	A/R	A	A		A	A	A	A/R	A/R
Brachionus quadridentatus f. *brevispinus* Ehrenberg, 1832								A			
Brachionus quadridentatus f. *cluniorbicularis* Skorik, 1894							A		A		
Brachionus sp.							R*		A		
Brachionus urceolaris Müller, 1773	A		A				A		A		
Brachionus variabilis Hempel, 1896	A										
Cephalodella catellina Müller, 1786	A	A/R	A/R	A/R	A/R						A/R
Cephalodella forficula (Ehrenberg, 1838)	A							A			
Cephalodella gibba (Ehrenberg, 1830)	A/R	R*			R*		A/R		A/R	A/R	A/R
Cephalodella gracilis (Ehrenberg, 1830)	A									A/R	
Cephalodella megalocephala (Glascott, 1893)										A/R	
Cephalodella plicata Myers, 1924					A						
Cephalodella sterea (Gosse, 1887)	A	A		A				A			
Cephalodella tenuiseta (Burn, 1890)								A			
Cephalodella tinca Wulfert, 1937								A			
Cephalodella uncinata (Müller, 1773)				A							
Cephalodella ventripes (Dixon-Nuttall, 1901)		A	A	A	A						
Collotheca coronetta (Cubitt, 1869)	A										
Collotheca ornata (Ehrenberg, 1830)		A	R*								
Collotheca sp.			A	A							
Colurella colurus (Ehrenberg, 1830)			A								
Colurella obtusa (Gosse, 1886)	A	A	A	A	A			A			
Colurella uncinata (Müller, 1773)			A					A			

Colurella uncinata bicuspidata (Ehrenberg, 1830)									A			
Conochilus coenobasis (Skorikov, 1914)								A				
Conochilus dossuarius Hudson, 1885							A	A	A			
Conochilus natans (Seligo, 1900)								A				
Conochilus unicornis Rousselet, 1892	A											
Cupelopagis vorax (Leidy, 1857)								A				
Cyrtonia tuba (Ehrenberg, 1834)								A				
Dicranophoridae species unidentified							A		A			
Dicranophoroides caudatus (Ehrenberg, 1834)								A				
Dicranophorus epicharis Harring and Myers, 1928	A											
Eosphora/*Eothinia* sp.	A			A						A/R	A	
Epiphanes brachionus (Ehrenberg, 1837)		A	A/R	A/R			R*	A		A/R	A/R	
Epiphanes chihuahuaensis Schröder and Walsh, 2007	A	R*	A	A	A							
Epiphanes daphnicola (Thompson, 1892)								A				
Epiphanes macroura (Barrois and Daday, 1894)						A						
Euchlanis dilatata Ehrenberg, 1830	A/R	A/R	A/R	A/R			R*	A		A/R	A/R	
Euchlanis dilatata f. *larga* (Kutikova, 1959)								A				
Euchlanis triquetra Ehrenberg, 1838	A			A								
Filinia australiensis (Koste, 1980)							A	A	A			
Filinia cornuta (Weisse, 1848)	A									A/R	A/R	
Filinia grandis Koste and Shiel, 1980							A		A			
Filinia limnetica (Zacharias, 1893)								A				
Filinia longiseta (Ehrenberg, 1834)	A		R*				A/R	A	A/R			
Filinia opoliensis (Zacharias, 1898)							A		A			
Filinia passa (Müller, 1786)							A	A	A			
Filinia pejleri Hutchinson, 1964							A	A	A			
Filinia saltator (Gosse, 1886)								A				
Floscularia ringens (Linnæus, 1758)								A				
Floscularia sp.	A											
Gastropus stylifer Imhof, 1891								A				
Hexarthra sp1.				R*		A	A/R					
Hexarthra intermedia (Wiszniewski, 1929)							A/R		A/R			
Hexarthra sp2.					A							
Horaella brehmi Donner, 1949							A	A				
Itura aurita (Ehrenberg, 1830)								A				
Keratella americana Carlin, 1943				A								
Keratella australis Bērziņš, 1963							A	A				

Keratella cochlearis (Gosse, 1851)							A	A	A		
Keratella procurva (Thorpe, 1891)							A	A	A		
Keratella procurva robusta Koste and Shiel, 1980								A			
Keratella slacki Bērziņš, 1963							A	A			
Keratella tropica (Apstein, 1907)	A		A	A			A	A/R	A		
Lacinularia flosculosa (Müller, 1773)	A		A	A/R			R*	R*			
Lecane agilis (Bryce, 1892)								A			
Lecane bulla (Gosse, 1851)	A/R	A/R	A/R	A/R	R*		R*	A	A/R	A/R	A/R
Lecane closterocerca (Schmarda, 1859)	A	A	A	A	A			A	A		
Lecane elsa Hauer, 1931	A		A								
Lecane flexilis (Gosse, 1886)									A		
Lecane hamata (Stokes, 1896)	A	A		A	A			A	A		
Lecane inermis (Bryce, 1892)			A					A			
Lecane luna (Müller, 1776)	A	A/R	A/R	A/R	R*		R*	A	R*	A/R	A/R
Lecane papuana (Murray, 1913)							A		A		
Lecane pyriformis (Daday, 1905)								A			
Lecane quadridentata (Ehrenberg, 1830)	A/R	A/R	A/R	A/R	A/R					A/R	A/R
Lecane ungulata (Mola, 1913)								A			
Lepadella acuminata (Ehrenberg, 1834)								A			
Lepadella biloba Hauer, 1958								A			
Lepadella colurella[1]									A		
Lepadella patella (Müller, 1773)	A	A	A	A	A			A	A		
Lepadella rhomboides (Gosse, 1886)	A		A/R	A	A		A/R		A		
Lepadella triptera (Ehrenberg, 1830)	A	A	A	A	A			A			
Limnias ceratophylli Schrank, 1803								A			
Limnias melicerta Weisse, 1848	A										
Lindia torulosa Dujardin, 1841			A								
Macrochaetus collinsii (Gosse, 1867)					R*		R*				
Mytilina mucronata (Müller, 1773)	A										
Mytilina ventralis (Ehrenberg, 1830)								A			
Notommata copeus Ehrenberg, 1834				A							
Notommata glyphura Wulfert, 1935	A	A									
Notommata sp.			A							A/R	
Philodina acuticornis Murray, 1902	A/R		A/R	A/R							
Plationus patulus (Müller, 1786)			A					A			
Platyias quadricornis (Ehrenberg, 1832)	A/R	A/R	A	A/R	A		A	A			
Pleuretra lineata Donner, 1962						A/R	A/R			A/R	

Polyarthra dolichoptera Idelson, 1925							A		A		
Polyarthra longiremis Carlin, 1943								A			
Polyarthra sp.	A	A	A/R	A				R*		A/R	A/R
Polyarthra vulgaris Carlin, 1943								A/R			
Pompholyx complanata Gosse, 1851								A			
Proales sp.	A		A	A	A	A					
Proalides tentaculatus Beauchamp, 1907								A			
Ptygura crystallina (Ehrenberg, 1834)								A			
Ptygura intermedia (Davis, 1867)								A			
Ptygura longicornis (Davis, 1867)								A			
Rhinoglena frontalis Ehrenberg, 1853	A			A							
Rhinoglena ovigera Segers and Walsh, 2017										A/R	A/R
Rhinoglena texana Segers and Walsh, 2017		A	A								
Rotaria neptunia (Ehrenberg, 1830)							A		A		
Rotaria tardigrada (Ehrenberg, 1830)								A			
Rotatoria sp.	A		A	A							
Sinantherina socialis (Linnaeus, 1758)								A			
Squatinella lamellaris (Müller, 1786)				A							
Squatinella lamellaris f. *mutica* (Ehrenberg, 1832)								A			
Squatinella rostrum (Schmarda, 1846)	A	A	A		A						
Synchaeta oblonga (Ehrenberg, 1832)							A		A		
Synchaeta pectinata (Ehrenberg, 1832)							A	A	A		
Synchaeta species unidentified							A		A		
Testudinella patina (Hermann, 1783)								A	A		
Trichocerca brachyura (Gosse, 1851)								A			
Trichocerca collaris (Rousselet, 1896)	A										
Trichocerca myersi (Hauer, 1931)								A			
Trichocerca porcellus (Gosse, 1851)								A			
Trichocerca pusilla (Jennings, 1903)							A	A	A		
Trichocerca rattus (Müller, 1776)	A/R	A		A/R	A/R			A/R			A/R
Trichocerca similis (Wierzejski, 1893)								A			
Trichocerca similis grandis Hauer, 1965							A		A		
Trichocerca sp.			A	A	A	A				A/R	
Trichocerca tenuior (Gosse, 1886)					A						
Trichocerca brachyura (Gosse, 1851)	A	A									
Trichocerca vernalis (Hauer, 1936)	A	A									
Trichotria tetractis similis (Stenroos, 1898)							A	A			

2.3.2 Rehydration experiments

2.3.2A. Sediment rehydration and comparison between active communities

Rehydration of surface sediments from the 12 sites resulted in 41 rotifer species, richness ranged from 1-16 (Table 3). The highest species richness occurred in Lake Littra (LL) and the lowest occurred in the rock pool Vero (V). Unique taxa not present in active species lists were found in 8 out of the 12 sites (Behind East (BE), Behind Ranch House (BRH), Laguna Prieta (LP), South Temp (ST), Stacia (S), Lake Littra (LL), Lake Limbra (LIM) and Ryan's 2 billabong (R2)) (Table 3). Other invertebrate taxa emerged from diapause but were not analyzed.

Playa sites at HTSP (E, BE, BRH, LP) had a total of 70 rotifer species in active populations with 24 of those rotifer species being recovered in rehydration experiments. In rock pools (ST, Vero, S), 24 rotifer species were found in active populations and 10 emerged during rehydration experiments. Most rotifers found in active rock pool populations (87.5%) and recovered after rehydration experiments (70%) were found in South Temp (ST), which is the largest of the rock pools and has the deepest sediment layer. Furthermore, rehydration experiments using sediments from HTSP sites yielded 1-4 species that were not found in active populations that had been sampled many times for the past 20 years. Additionally, several additional taxa found in active and recovered species lists were also recovered by environmental sequencing. These findings indicate that resting stages are present but do not hatch under experimental conditions, or that individuals hatch but can not successfully colonize playas and rock pools found at HTSP. New Mexican sites 404A and 404B had a total of 23 rotifers in active communities with 21 of those rotifer species being recovered in rehydration experiments. Australian sites (Lake Littra, Lake Limbra, and Ryan's 2) had a combined total of 109 species in active populations with 20 of those species being recovered in rehydration experiments. Again, other invertebrate taxa emerged from diapause but were not analyzed.

Active species lists were compared among sites to determine dissimilarities between rotifer communities by locality and pool types (Table 4). As expected, low dissimilarities (<0.50) were found between sites that were located in close geographic proximity. Active communities

from larger playas found at HTSP (E, BE, BRH, LP) were more similar to each other with dissimilarities ranging from 0.36-0.47. Interestingly, rock pool South Temp (ST), which is the largest of all 3 rock pools, showed similarities with larger playas Behind East (BE) (0.45) and Laguna Prieta (LP) (0.43). Active communities from rock pools Vero (V) and Stacia (S) shared dissimilarities of 0.43. New Mexican sites 404A (4A) and 404B (4B) had high dissimilarities between the other 10 sites with values ranging from 0.55-0.93. Highest similarities were found in the New Mexican county sites 404A (4A) and 404B (4B), with a dissimilarity value of 0.35. For Australian sites, Lake Littra (LL) and Lake Limbra (LIM) were highly similar to each other with low Sorenson values of 0.21. Ryan's 2 billabong (R2) had high Sorenson values (>0.50) when compared to all sites. Lake Littra (LL) was most different from R2 with a Sorenson value of 0.61.

Table 4 Sorenson matrix of active rotifer communities from 12 desert aquatic sites**.** Dissimilarities were determined between sites using rotifer community composition found during filled periods. Values closer to zero indicate higher similarities between sites.

Site	East	Behind East	Behind Ranch House	Laguna Prieta	South Temp	Vero	Stacia	Lake Littra	Ryan's 2	Lake Limbra	404A	404B
East	0.00	-	-	-	-	-	-	-	-	-	-	-
Behind East	0.43	0.00	-	-	-	-	-	-	-	-	-	-
Behind Ranch House	0.47	0.45	0.00	-	-	-	-	-	-	-	-	-
Laguna Prieta	0.36	0.42	0.36	0.00	-	-	-	-	-	-	-	-
South Temp	0.56	0.45	0.51	0.43	0.00	-	-	-	-	-	-	-
Vero	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-	-	-
Stacia	0.96	1.00	0.90	0.91	0.84	0.43	0.00	-	-	-	-	-
Lake Littra	0.79	0.89	0.85	0.83	0.88	1.00	1.00	0.00	-	-	-	-
Ryan’s 2	0.72	0.72	0.75	0.69	0.81	1.00	1.00	0.61	0.00	-	-	-
Lake Limbra	0.74	0.81	0.81	0.77	0.83	1.00	1.00	0.21	0.64	0.00	-	-
404A	0.70	0.67	0.65	0.67	0.84	0.89	0.80	0.93	0.90	0.91	0.00	-
404B	0.65	0.55	0.67	0.61	0.74	1.00	1.00	0.90	0.86	0.88	0.35	0.00

When comparing recovered rotifer species lists to those found in active species lists, those from larger playas Laguna Prieta (LP), East (E), Behind East (BE), Behind Ranch House (BRH), and rock pool South Temp (ST) had high dissimilarity values ranging from 0.53 - 0.87 (Figure 3). Similarities were found between rock pools Vero (V; 0.50) and Stacia (S; 0.43) with a low Sorenson dissimilarity index of < 0.50 when compared to recovered communities. Recovered communities from New Mexican sites were highly similar to those found in active species lists. Site 404A had a dissimilarity index of 0 meaning that recovered communities were identical to active species lists. Similarly, site 404B had a high similarity when compared to active communities with a dissimilarity value of 0.09. This might have been because active communities and sediment samples collected from New Mexican sites 404A and 404B were sampled with a lower intensity than sites from HTSP that were sampled many times over the years by Dr. Walsh's laboratory. Rotifer communities recovered from Australian sites (Lake Littra, Lake Limbra, Ryan's 2) were highly dissimilar to active species lists with values ranging from 0.74 - 0.87.

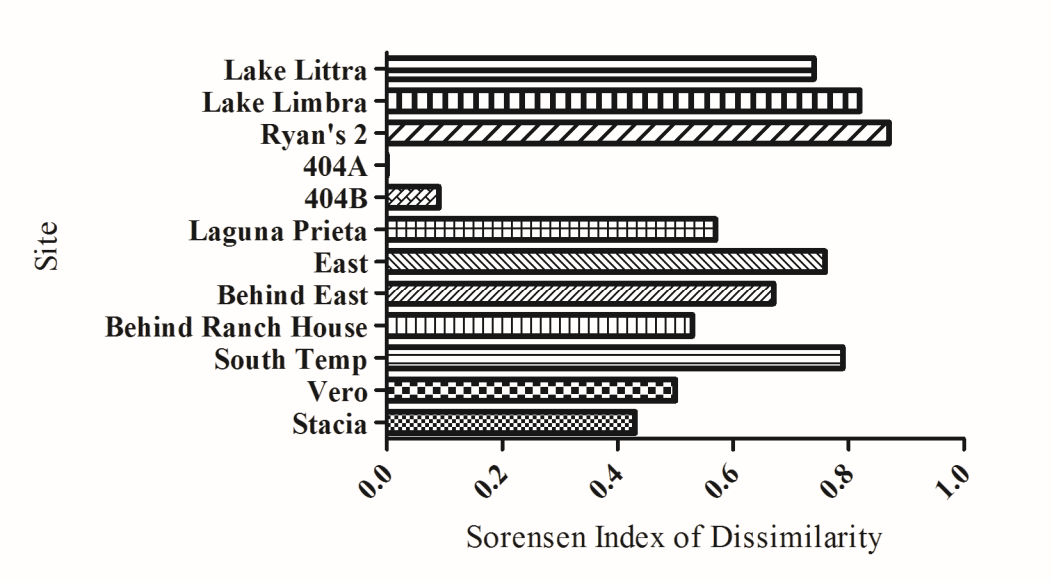

Figure 3 Dissimilarities between rotifer species richness at 12 sites when comparing communities recovered from sediments (excludes communities recovered from hatching success experiment) to active communities. Values closest to zero suggest higher similarities between active versus recovered communities.

2.3.2B Hatching success experiment

A subset of rotifers found in active species lists hatched from desiccated sediments as well as other taxa not analyzed here (i.e., fairy shrimp, gastrotrichs, nematodes) (Walsh et al., 2014). Most rotifers hatched within the first two weeks after rehydration (Figure 4). Larger playas had higher richness with 4-12 rotifer species emerging per site. In rock pool Vero (V), one out of the three species found during wet periods was recovered after experiments. Rehydrated sediments from Behind East (BE) collected in 1998 (21 years old) yielded eight rotifer species representing 32% of the species in active populations (Table 2). Comparatively sediments collected in 1999 from BE (20 years old), four rotifers hatched representing 16% of the active species. Additionally, more recent sediments from Behind East (BE) collected in 2014 (5 years old) yielded 6 rotifer species 24% of the species found during filled periods. Newer sediments from Behind East collected in 2018 (1-year-old) yielded 10 rotifer species representing 40% of active communities. In rock pool Vero (V), sediments from 2003 (16 years old) yielded one rotifer representing 33%, which is the majority of the active population. Similarly, sediment collected in 2018 from rock pool (V) also yielded only one rotifer. Australian sediment from Lake Littra collected in 2012 (7 years old), was also rehydrated and analyzed. Fourteen out of 44 (32%) rotifer species found in the lake's active community were recovered from sediment. In general, sediment that had been stored for less amount of time had more rotifer richness than those that had been stored for long periods. Shortly after week 2, rotifer emergence from newer sediment ceased, while emergence from older sediment continued throughout week 4.

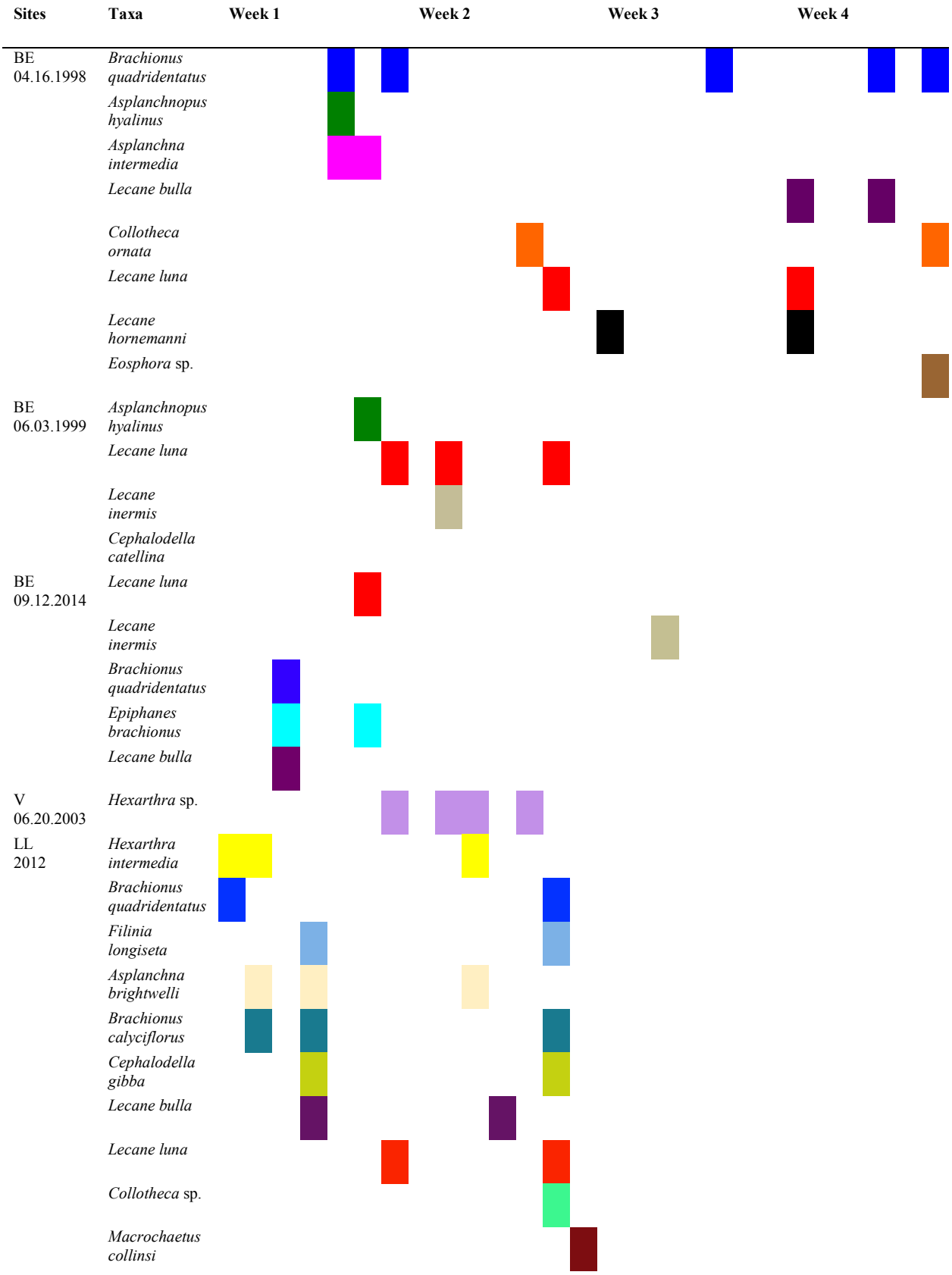
Sites
Taxa
Week 1
Week 2
Week 3
Week 4
BE 04.16.1998
Brachionus quadridentatus
Asplanchnopus hyalinus
Asplanchna intermedia
Lecane bulla
Collotheca ornata
Lecane luna
Lecane hornemanni
Eosphora sp.
BE 06.03.1999
Asplanchnopus hyalinus
Lecane luna
Lecane inermis
Cephalodella catellina
BE 09.12.2014
Lecane luna
Lecane inermis
Brachionus quadridentatus
Epiphanes brachionus
Lecane bulla
V 06.20.2003
Hexarthra sp.
LL 2012
Hexarthra intermedia
Brachionus quadridentatus
Filinia longiseta
Asplanchna brightwelli
Brachionus calyciflorus
Cephalodella gibba
Lecane bulla
Lecane luna
Collotheca sp.
Macrochaetus collinsi

Figure 4 Hatching phenology of rotifers recovered from sediments of different ages monitored for 4 weeks. Unique colors were assigned to rotifer species as they hatched. Presence of color indicates hatching of a rotifer species on that specific day.

2.4 Discussion

Highest species richness was found in larger temporary playas in rehydration experiments. Lowest richness was found in smaller more ephemeral rock pools at HTSP. Larger wetland playas at HTSP had over twice the species richness of rock pools. When active species lists from all 12 sites were compared to determine dissimilarities between locality and pool type, low dissimilarities were found between sites that were located in close geographic proximity. Further, when species recovered after rehydration experiments were compared to species found in active communities, dissimilarity results varied for all 12 sites. Larger playas (LP, E, BE, BRH, LL, LIM, R2) and large rock pool ST were highly dissimilar to active communities while Rock pools (V) and (S) and New Mexican playas (4A) and (4B) were more similar.

The inability to adapt to on-going fluctuations in temperature due to global climate change is an issue for most species living in extreme arid environments (Wiens, 2016). Further, knowledge of the distribution of many invertebrate groups is limited; this is especially true for zooplankton including rotifers (Walsh et al., 2009). Through resting stages, rotifers have the ability to persist even the most unfavorable conditions (Gilbert, 1974, Pourriot and Snell, 1983). The ability of rotifer resting stages to withstand long periods of desiccation has been well documented (Battauz et al., 2014; Walsh et. al., 2017) but it is still unclear how long periods of desiccation may affect their hatching success. Depending on the pool type, sediments from these systems may contain large reserves of rotifer resting stages that are essential for their re-establishment of communities when conditions become more favorable (Nielsen et al., 2000; Jenkins and Boulton, 2003; Gaikwad et al., 2008). Therefore, ecologists have long recognized sediments as a viable source to explore questions regarding community ecology (Brendonck and De Meester, 2003; Burge et al., 2018).

Implementing resurrection ecology approaches can offer an insight into the impact that changes in the environment have on the evolution of species (De Meester et al., 2019). For example, Hairston et al. (1999) compared the mean resistance of *Daphnia* to dietary cyanobacteria. *Daphnia* were resurrected from dormant eggs found in sediments ranging from

1969-71 and 1962-64 (before and just after the appearance of cyanobacteria), 1978-80 (when peak eutrophication had occurred), and 1995-97 and 1992-94 (when eutrophication had passed). Results showed that when exposed to a diet containing toxic cyanobacteria, younger genotypes of *Daphnia* (resurrected from younger sediments) exhibited a higher growth-rate than those hatched from older sediments. Geerts et al. (2015) conducted an experiment where resurrection ecology was used to analyze the evolutionary changes in Critical Thermal Maximum (the tolerance an organism has to extreme temperatures) for *Daphnia magna* (Straus, 1820). They compared resurrected clonal populations of *D. magna* from 1955-1965 to more recent populations dating to 1995-2005. Results showed that resurrected *D. magna* from more recent populations evolved higher heat tolerances than those resurrected from historic sediment. On a follow up study using the same population as Geerts et al. (2015), Jansen et al. (2017) assessed plastic and genetic responses to extreme temperature events and warming. They identified five genes potentially linked to temperature adaptation from the resurrected *D. magna* populations. Yousey et al. (2018) investigated how thermal tolerance had changed in a population of *Daphnia pulicaria* over time by implementing resurrection ecology techniques. Overall, younger genotypes recovered from sediment cores exhibited higher thermal tolerance than older genotypes. Studies that investigate rapid evolutionary responses to environmental change aid in the prediction of future occurrences of species with climatic fluctuations in temperature and suggest that this may also reduce colonization success of immigrant species (Geerts et al., 2015). Recovering communities from different time periods can provide insights into how evolution and changes in gene composition aid species to respond to environmental change in aridland systems.

Resting eggs can remain dormant over many years and can be useful in resurrection ecology experiments to answer questions in evolution ecology in rotifers (Cáceres and Hairston 1998; Orsini et al., 2013; Alekseev et al., 2007). Studies have looked at the recovery of dormant rotifers from sediments, but only one has focused on the sites covered here (Walsh et al., 2014). In this investigation, rotifer recovery varied depending on the region and pool type. For example, sediment from sites located in the Chihuahuan desert yielded a total of 35 rotifer species from

temporary playas and rock pools combined. Comparatively, Walsh et al. (2014) found that 11 temporary Chihuahuan Desert habitats, some of which were included in this study, yielded 32 rotifer species. Rehydration of Australian sediments from temporary playas Lake Littra, Lake Limbra, and Ryan's 2 billabong recovered 20 rotifer species. Similarly, rehydration experiments by Furst (2013) using sediment cores from Australian sites Lake Littra and Lake Limbra, recovered 11% of the active rotifer community following rehydration for Lake Littra and 22% for Lake Limbra. The experiment conducted here recovered 36% of the active rotifer communities found in Lake Littra and 16% from Lake Limbra. In billabongs, Tan and Shiel (1993) investigated pre- and post-flooding of Ryan's 1 billabong, found in close proximity to Ryan's 2, and recorded a total of 63 rotifer species. Here, a total of only 10 rotifer species were recovered from Ryan's 2 billabong. In general, results found in this study were similar to those found in the past, therefore supporting the effectiveness of the methods applied for assessing rotifer biodiversity.

Several limitations were associated with this study. One limitation was the amount of sediment used in rehydration experiments. Using the protocol in this study, only 10 rotifer species were recovered from Australian billabong Ryan's 2, which was expected to have more rotifer species emerge from diapause due to its larger size. The discrepancy in these results might be related to the low densities of resting eggs found in sediments of Ryan's 2 billabong as compared to Ryan's 1 and Ryan's 3 billabongs (Shiel et al., 2001). Another factor that might have been the amount of sediment rehydrated or the conditions used in the experiment. A summary of studies that have focused on the recovery of rotifer resting stages from various pool types using an array of methods is presented in Table 5. As can be seen, sampling method, amount of sediment rehydrated, light-dark cycle, and overall mesocosm design typically had an effect on the number of taxa recovered (Table 5). Results from the study presented here are comparable to those reported previously (Table 5), where rotifer species recovered from sediments ranged from 7 to 54 individuals. Most past experiments used substantially more sediment than was used here. Sediment rehydrated ranged from 20 to 5,000 g. Only 3 g of

sediment were used for rehydration experiments in this study. The reason behind using such small quantities is that most rock pools have a very shallow sediment layer. Collecting large quantities of sediment from rock pools would have been in some cases impossible and might have potentially exhausted the entire sediment egg bank. However, the use of more sediment in these experiments would have likely increased representation of resting stages in the sample and thereby increase the number of taxa recovered after rehydration.

The effect of pond permanence is a well-known factor that determines community composition (Wellborn et al., 1996). Here, sediments from larger pools yielded more rotifer species than those that are smaller and more ephemeral. Larger pools likely have longer hydroperiods and support more active populations which is usually positively correlated with the density of resting stages (Schröder and Gilbert, 2004; Vanschoenwinkel et al., 2010; Walsh et al., 2014). Comparatively, smaller more ephemeral rock pools are usually very short-lived, usually have few macrophytes if any (Barrett et al., 2016), and likely have lower carrying capacities (Brendonck et al., 1998; Brendonck and Riddoch, 2001; Wallace et al., 2005; Walsh et al., 2014). Another limitation of this study was the size of the mesocosms. The mesocosms used here were much smaller than some used in the select studies presented in Table 5. Implementing larger mesocosms might mimic more permanent hydroperiods and possibly provide cues that initiate the remaining resting stages to hatch. For example, Eskinazi-Sant'Anna and Pace (2018) investigated how viability and diversity of zooplankton resting stages in sediments were affected when exposed to varying hydroperiods. Their results showed that taxa recovered from sediments belonging to more predictable wetting periods had approximately twice the abundance and diversity. Sediments from more predictable wetting periods also had better preserved resting egg banks than lakes with more unpredictable wetting periods. Hatching may vary among species; therefore, implementing other hatching conditions may help understand which environmental cues aid in fully recovering rotifer species composition.

Table 5 Summary of sampling methods and results from selected studies assessing the recovery of zooplankton communities through sediment rehydration. Species recovered includes only rotifers.

Author(s)	Pool Type	Sediment Sampling Method	Amount of Sediment	Mesocosm Design	Media and Amount Used For Rehydration	Time Observed	Sites (#) and Species Recovered (#)
May, 1986	Large lake	Sediment core	Top 5 cm of ~10 cm core then mixed	250 mL Erlenmyer flask incubated at 5, 10, or 15°C L:D cycle of 12:12h	20 mL aliquots of mud suspension were diluted with filtered lake water to give final volume of 200 mL in mesocosm	~12 weeks	1 site: 15 species
May, 1987	Large lake	Sediment core	Top 5 cm of mud core was mixed; 20 mL subsample was used for experiment	250 mL Erlenmeyer flask incubated at 5, 10, or 15°C L:D cycle of 12:12h	20 mL of mud was diluted with filtered lake water to give final volume of 200 mL in mesocosm	Until no new taxa emerged after 3 consecutive observations	6 sites: 13 species
Hairston et al., 2000	Large lake	Sediment emergence traps	Placed trap into 10 cm of actual lake mud	Outdoor in lake	10 L of lake water	113 days	1 site: 7 species
Langley et al., 2001	Large billabongs and ephemeral pond	By hand to a depth of 10cm	4-5,000 g	1.6 m X 1.2 m mesocosm outdoors	20 cm of rainwater filtered with 10 µm mesh	35 days	3 sites: 54 species
Duggan et al., 2002	Large lake	155 X 160 mm Ekman grab depths of 12m and 25m	30 mL (sediment was measured using syringe) taken from top 5 cm of sample	250 mL Erlenmyer flask Incubated L:D cycle of 12:12h in water bath @10, 15, 20°C	Surface water collected from lakes filled Erlenmeyer flask until final volume of 250 mL (with 30 mL of sediment in flask)	82 days	2 sites: 38 species

Albritton and White, 2004	Different sites along floodplain	15 X 15 cm plexiglass pushed into sediment 2 cm deep; 15 X 15 Ekman grab; sediment core with 4 cm diameter core	200 g removed from surface samples; for cores, depth intervals of 4-10 cm and 14-20 cm were separated, 75 g were removed, then used	1 L container; 120 mL plastic containers incubated at 25°C with L:D cycle of 12:12h	500 mL lake water filtered through a 0.4 µm filter	~130 days	8 sites: 7 species
Gaikwad et al., 2008	Permanent and ephemeral ponds	10 X 10 X 10 cm quadrate	100 g	250 mL beaker incubated at ~25°C	Autoclaved tap water	12 days	4 sites: 7 species
García-Roger et al., 2008	Small ponds and large lake	Van Veen grab	25 g wet weight	250 mL Erlenmeyer flask incubated at 20°C with a L:D cycle of 12:12h	~200 mL filtered water from each site	30 days	4 sites: 18 species
Segers and Shiel, 2008	Large billabongs	Sediment core	"Small fractions" of the sediment core were added	2 L containers placed in front of a window under fluctuating temperature and light conditions	1 L distilled water	~ 6 weeks	2 sites: 48 species
Battauz et al., 2014	Permanent floodplain	By hand to a depth of <10 cm	20 g	Plastic trays with a 165 cm^2 surface Incubated at 25°C with a L:D cycle of 16:8h	2-3 cm of lake water filtered through 20 um mesh and brought to a boil	90 days	3 sites: 27 species
Walsh et al., 2014	Temporary playas and rock pools	Surface sediments					11 sites: 32 species

Sorensen index of dissimilarity was further implemented to compare recovered rotifer communities to those found in active species lists. Similarities found between lists were largely affected by the number of species present in active communities. High dissimilarities were found in most larger playas while smaller rock pools containing 1-3 rotifers in active species lists, had highest similarities to those recovered after rehydration (Figure 3). In comparison, Australian site Ryan's 2, which has the largest active species list (83 rotifers), had highest dissimilarities (Figure 3). The amount of rotifer species recovered from sediments varied between sites. This might be due to sediments used for rehydration experiments varying in age. Sediments that have been stored for longer periods may have caused variation in hatching rates because resting stages from older sediments might not be as viable as sediments that were taken from more recently created egg banks (Boulton and Lloyd, 1992; Schröder, 2005; Radzikowski, 2013). Also, sediments had different characteristics, amounts of organic matter, and textures that could have potentially had an impact on the viability of resting stages to hatch.

Little difference was seen in hatching success when comparing the number of rotifers recovered from older sediments to those yielded from younger sediments in hatching success experiments. Overall, younger sediments yielded more rotifers than older samples. Interestingly, in older sediments from BE (1998 (21 yrs old) and 1999 (20 yrs old)), emergence of rotifers continued throughout the entirety of the one-month long experiment, while emergence of rotifers from newer sediment ceased shortly after week two (Figure 4). As mentioned above, rotifers have evolved risk-spreading strategies (i.e., production of resting stages and bet-hedging) to deal with their ever-changing habitats. Through bet-hedging strategies, not all resting eggs produced by a single genotype hatch at once but are spread over several growing seasons (García-Roger et al., 2014). Since sediments had been stored for long periods of time, this rewetting event might have been a cue as the last opportunity to hatch, possibly being the reason for continued hatching throughout the entire experiment.

Also, sampling intensities of active lists varied between sites possibly underestimating the actual rotifer diversity if sites weren't sampled with enough intensity (Walsh et al., 2007).

Sites at HTSP have been sampled many times over the past 20 years yielding thorough and extensive active species lists. New Mexican sites 404A and 404B were also sampled for the past 20 years, but more sporadically than the sampling effort at HTSP. Therefore, active species lists for New Mexican sites might were not as extensive and representative as active lists from HTSP. Sampling efforts for Australian sites Lake Littra and Lake Limbra were sampled with low intensities for 11 and 6 months, respectively (Furst 2013). Similar to sites at HTSP, the Ryan's billabongs have been sampled and studied for more than 20 years (Langley et al., 2001). Factors such as sediment age and sampling intensities likely affected the number of recovered species and thus the similarities between active and recovered rotifer communities. Implementing resurrection ecology experiments using resting eggs found in sediments can help us better understand how present communities have evolved genetically to persist in an environment where on-going climate change is an issue (Weider et al., 2018).

Sediment rehydrations were an effective way of recovering resting communities here and in other studies (Table 5). Recovery of unique species (those not present in active lists) was also found through rehydration experiments in 8 out of the 12 sites investigated herein (Table 3). These results expand our knowledge of the species present in these sites and confirm that rehydration experiments are a good method to recover and assess rotifer diversity. This study provides an insight into the ability to recover rotifer diversity in sediments from various pool types. The results supported the hypothesis that more rotifer taxa would be recovered from sediments coming from larger pools and are inline with others that have found that habitat size has an influence on resting stage densities (Oertli et al., 2002; Altermatt and Ebert, 2008; Ripley et al., 2009; Zokan and Drake, 2015). Also, rehydration experiments were able to recover a large portion of rotifers found in active communities from some sites. Despite its limitations, the methodology of recovering rotifer communities through sediment rehydration continues to be a viable pathway of exploring questions regarding community ecology in rotifers.

Chapter 3: Amplicon sequencing of sediments

3.1 Introduction

Monitoring species composition has traditionally relied on visual surveys that depend heavily on morphological identification that requires taxonomic expertise to correctly identify species (Thomsen and Willerslev, 2015). It is often very difficult to identify planktonic organisms under light microscopy; this process is further complicated by the possibility of cryptic life history stages (Briski et al., 2011; Darling and Mahon, 2011; Uusitalo et al., 2013) and cryptic species complexes (Mills et al., 2017; Kordbacheh et al., 2018). Amplicon sequencing can be applied to identify these taxa by analyzing DNA found in environmental samples such as water and sediments, without the need of morphological identification (Taberlet and Coissac, 2012; Rees et al., 2014; Bista et al., 2018). This approach can be especially helpful to reduce errors when identifying species that share similar characteristics (Thomsen and Willerslev, 2015). Implementing a sequencing approach can aid in capturing both past and present biodiversity in temporary habitats, without the need of active organisms (Bohmann et al., 2014).

Primer choice is important to consider when assessing the biodiversity of an ecosystem (Hong et al., 2009; Hadziavdic et al., 2014). In this study, the 18S ribosomal RNA (rRNA) gene (Blaxter et al., 1998) and the mitochondrial cytochrome c oxidase subunit I (COI) (Folmer et al., 1994) were selected because of their ability to generate informative sequences for the analyses of eukaryotes at the species and higher taxonomic levels. For example, the 18S gene is widely used and is a popular choice in detecting the biodiversity of eukaryotic microscopic organisms but is often considered ineffective for species level identification (Tang et al., 2012; Lindeque et al., 2013; Lie et al., 2014; Wang et al., 2014). Comparatively, the COI gene has been widely used in diversity assessments due to its high resolution when identifying taxa at the species level (Ogedengbe et al., 2011; Tang et al., 2012; Wu et al., 2015). Assessing rotifer diversity with

these two primers should provide a broad taxonomic coverage of species and resting stages found in sediment (Drummond et al., 2015). Additionally, taxonomic identification of DNA sequences largely consists in comparing these sequences to DNA reference databases containing information on specific taxonomic groups (Taberlet et al., 2018). Here, the SILVA 18S (Quast et al., 2013) and the BOLD COI (Ratnasingham and Hebert, 2007) reference datasets were used due to their extensive availability of eukaryotic and animal specific sequences. A specially curated reference dataset of rotifer specific sequences obtained from the Walsh laboratory, Dr. Diego Fontaneto (downloaded from GenBank), and rehydration experiments were also included to complement existing datasets.

Sequencing has shown great promise when assessing species diversity in aquatic systems due to its efficiency, rapid processing speed of samples, and ample taxonomic coverage (Hajibabaei et al., 2011; Bik et al., 2012; Hirai et al., 2015). For example, Machler et al. (2015) compared the performance of eDNA sequencing to conventional kicknet-sampling methods to assess macroinvertebrate biodiversity. They found that results from morphological assessment of kicknet-samples and sequencing methods were very similar and had very high consistency rates. Hirai et al. (2017) assessed zooplankton communities in a marine ecosystem by implementing a sequencing approach using the 18S gene. Further, sequencing results were compared to morphological identification data of plankton net samples. The sequencing approach detected 561 molecular taxonomic units compared to 201 taxonomic groups detected by morphological analysis. Sequencing of sediments can also prove to be beneficial when assessing resting stage biodiversity in aquatic systems. Trottet et al. (2018) used a molecular approach to assess the biodiversity of planktonic organisms that are capable of producing resting stages in Singapore waters. After comparing their results with existing records on the density of resting stage producing species, they found that at least 95 genera were capable of producing resting stages.

Pond permanence determines abiotic and biotic interactions that may influence species richness and community structure (Ward and Blaustein, 1994; Skelly, 1996; Schneider and Frost, 1996). Applying a sequencing approach to the assessment of zooplankton communities in

aquatic habitats might be useful to observe the influence that pool types have on species richness (Araújo et al., 2013). Anusa et al. (2012) studied the effect that pool size had on species diversity in rock pool habitats. They found that pool duration had an effect on the structuring of active zooplankton communities and that species present in pools increased as pool area increased. Similarly, Tavernini et al. (2005) conducted a study to assess the relationship between hydroperiod and zooplankton richness. Their results indicate that hydroperiod had an impact on active species richness, with the highest number of taxa being found in ponds that had the longest duration. A sequencing approach can help researchers answer questions on habitat biodiversity and how it is affected by biotic and abiotic factors (Harper et al., 2019). Environmental DNA (eDNA) is known to be very prevalent in aquatic sediments (Thomsen and Willerslev, 2015). In fact, evidence that DNA from organisms is well preserved in sediments regardless of water depth, geographic region, and sediment type has been explored through sequencing and metabarcoding techniques (Morard et al., 2016). Sequencing of sediments can provide an in-depth insight of species composition in temporary freshwater systems (Medinger et al., 2010; Hajibabaei et al., 2011; Shokralla et al., 2012). A single environmental sample can potentially produce thousands of sequences from numerous species (Taberlet et al., 2012; Ji et al., 2013; Fonseca, 2018). Even though taxonomic overlap oftentimes occurs when comparing results given by morphological identification and amplicon sequencing, each method usually detects taxa missed by the other (Lejzerowicz et al., 2015; Zimmermann et al., 2015; Harvey et al., 2017), therefore it is a good idea to implement both strategies. Djurhuus et al. (2018) assessed zooplankton diversity through sequencing of eDNA using the 18S and COI genes and compared the results to those obtained through morphological identification methods. Sequencing of eDNA samples detected a larger diversity of organisms when compared to morphological identification methods with each method complimenting the other by detecting unique species. While DNA metabarcoding simplifies and facilitates the assessment of resting stage diversity, comparison between traditional morphological analysis, and amplicon sequencing approaches can expand our knowledge on how primer choice and reference libraries

can influence the efficiency of calculating species richness (Bucklin et al., 2016). In this study, rotifers were focused on as model organisms due to their ability to produce resting stages and their prevalence in desert aquatic systems (Alekseev et al., 2007; Walsh et al., 2014). Rotifers inhabit both natural and artificial temporary waters that remain desiccated for indefinite periods of time and are often the first metazoans to re-emerge from resting stages as conditions become more favorable (Ricci, 2001; Gilbert and Schröder, 2004; Garcia-Roger et al., 2006; Wallace et al., 2015).

In this study, DNA was extracted from sediments for amplicon sequencing and analyzed using a bioinformatics pipeline. The first objective of this study was to compare the results from sequencing to those obtained from rehydration experiments. It was hypothesized that samples from larger, more permanent temporary ponds would have higher rotifer diversity than those that are smaller and more ephemeral. Furthermore, when compared to active communities and rehydration experiments, amplicon sequencing was hypothesized to provide the highest estimates of richness due to its ability to identify dormant stages, rare species, and taxa that are not always represented by active animals in a sample (Morey et al., 2013; Orgiazzi et al., 2015; Boscaro et al., 2017; Pawlowski et al., 2018).

3.2 Methods

3.2.1 Amplicon sequencing of sediments

Sediment samples from the USA were collected using the protocol described above with the following modifications. For playas, five samples were taken from the surface (~2.5 cm) at ~2 m intervals at each sub-site to create one composite sample. Three composite samples were taken per site. Rock pool sites were split into six equal sections and sediment was taken from the surface (~2.5 cm) of each section to create one composite sample. Composited sediments were sieved as above.

Sieved material was then placed in a sterile plastic petri dish, sealed with Parafilm, stored on ice during transportation, and then stored at -80 °C for DNA extraction. As noted above, Australian samples consisted of dried surface sediments stored in dark, cool conditions until use. DNA was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) following the manufacturer's protocol with modifications in step 1 (0.50 g of soil instead of 0.25 g) and step 10 (50 µl of solution C6 instead of 100 µl). These steps were changed to obtain higher concentrations of DNA in the final solution. Samples were quantified using QubiT® (ThermoFisher Scientific, Waltham, MA) at the UTEP Border Biomedical Research Center (BBRC) Genomic Analysis Core Facility. Samples were diluted to 10 ng/µl and sent to Molecular Research LP (MR DNA, Shallowater, TX) for massive 18S and COI rRNA tag-encoded FLX-Titanium amplicon pyrosequencing.

Briefly, PCR amplifications were conducted in triplicate for each of the sediment samples with primer pairs SSU_F04 (5'-GCTTGTCTCAAAGATTAAGCC-3') SSU_R22 (5'-GCCTGCTGCCTTCCTTGGA-3') (Blaxter et al., 1998) were used to amplify the V1/V2 a diagnostic region of the 5'and 3' end of the rRNA 18S gene and a combination of the mlCOlintF (GGWACWGGWTGAACWGTWTAYCCYCC) (Leray et al., 2013) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) to analyze the COI region. Following PCR, all amplicon products were purified using Agencourt Ampure beads (Agencourt Bioscience Corp., MA, USA). Samples were then sequenced using Roche 454 FLX titanium instruments and reagents following manufacturer's guidelines.

3.2.2 Sequencing of rotifers recovered from rehydration

DNA was extracted from approximately 15-25 individuals (cultured as clonal, asexual lineages) from rehydrated sediments. Rotifers were lyzed in 1 µl of proteinase K (20 mg/mL), and 13 µl of Chelex (Bio-Rad Laboratories, CA, USA) using a thermocycler (TECHNE TC-412)

with the following cycle conditions: 56°C for 20 min, 99°C for 10 min, and 4°C for 30 min. After cycles were complete, templates were stored at -80°C until needed for further analyses.

Amplification reactions contained 9.5 µL of DNA template, 10 µL HotStarTaq Master Mix (Qiagen), 1 µL (500 ng/µL) of each primer (18S2F and 18S2R for I8S region amplification and HCO and LCO for COI gene amplification), 1 µL Taq DNA polymerase (Invitrogen), 5 µL dNTP mix, and 22 µL HPLC grade sterile water. Some PCR amplifications were done by another protocol that consisted of 8.5 µL of extracted DNA, 12.5 µL of HotStarTaq Master Mix (Qiagen), 1 µL of $MgCl_2$ (50 mM), 1 µL (500 ng/µL) of each primer (18S2F and 18S2R for I8S region amplification and HCO and LCO for COI gene amplification), and 15 µL of mineral oil. Cycle conditions were initial denaturation at 94°C for 15 min., followed by 35 cycles of 94°C for 1 min, 47°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 7 min on a thermocycler (TECHNE TC-412).

After amplification, products were examined via electrophoresis to verify successful amplification. If successful, products were then purified using a GENECLEAN kit (MP Biomedicals, LLC) before sequencing. Purified DNA templates were sent to UTEP's BBRC Genomic Analysis Core Facility and sequenced in both directions. Sequences were viewed and cleaned in FinchTV v 1.5.0 (Geospiza, Inc., Seattle, WA, USA; http://www.geospiza.com). Contigs were made in CAP 3 using forward and reverse sequences (Huang and Madan, 1999).

3.2.3 Amplicon sequencing analysis

Libraries were filtered with a minimum quality score of 25, barcodes were removed, and sequences were split using the split_libraries.py script in QIIME 1.9.1 (Caporaso et al., 2010). VSEARCH was used to dereplicate and identify chimeric sequences (Rognes et al., 2016). QIIME's pick_otus.py using the uclust algorithm (Edgar, 2010) was used to delineate molecular OTUs with 97 or 95% identity for 18S and COI respectively. The cut-off values are based on prior findings of Stefanni et al. (2018) where similarity thresholds of 97% for 18S and 95% for

COI were used to assess zooplankton diversity. Additionally, Boscaro et al. (2017) applied a 97% cut-off to assess eukaryotic diversity by sequencing freshwater sediments using the 18S gene. Using QIIME's pick_rep_set.py, representative sequences were selected for each OTU.

To assign each OTU to a taxonomic group, the SILVA 18S v. 128 reference dataset (Quast et al., 2013), was downloaded from the QIIME website (Caporaso et al., 2010). Taxonomic and associated COI sequence data were extracted from The Barcode of Life Database (BOLD, Ratnasingham and Hebert 2007, downloaded between the dates of April 13th and May 5th of 2017 from www.boldsystems.org). The fasta and taxonomic lists were modified to include information obtained by sequencing select isolates from rehydration experiments. Additionally, sequences and taxonomic classification information from rotifers obtained from the Walsh laboratory, and sequences deposited into GenBank by Dr. Diego Fontaneto, were included as well. Dr. Fontaneto's sequences were included because they are a reliable reference database due to his expertise on genomics and rotifer taxonomy. The total number of sequences included in the database for each gene consisted of 252 18S sequences and 408 COI sequences from the Walsh lab, 14 18S and 21 COI rotifer sequences from rehydration experiments, and a combination of 4,986,576 BOLD sequences. Taxonomic files were formatted for various analyses using Python.

Taxonomic identification of OTUs was assigned using the RDP option (Wang et al., 2007) in QIIME and the modified BOLD and SILVA v128 reference datasets. OTUs were then placed in BIOM formatted tables. OTU tables were curated using the R-package LULU to remove possible invalid sequences (Froslev et al., 2017). Curated OTU tables were then summarized into taxonomic abundance charts. Alpha diversity plots and Chao1 values were determined using the QIIME alpha_rarefaction.py script using default parameters. Principal Coordinates Analysis (PCoA) plots were made using Emperor (Vazquez-Baeza et al., 2013) to display beta-diversity among OTUs using the rarefied, unweighted unifrac option (Lozupone and Knight, 2005) in QIIME. PCoA plots were used to view relationships and similarities between OTUs. Non-metric multidimensional scaling (NMDS) plots were generated using rarefied OTU

tables and the *vegan* (Oksanen et al., 2018) and *ggplot2* (Wickham, 2016) packages in R version 3.4.0 (R Core Team 2013). Rotifer-specific OTUs were then filtered into a separate database. Abundance, PCoA, and NMDS analyses were repeated using this subset of the OTU tables. Finally, Australian samples were removed in rotifer-specific sequences by filtering to check the local effect of rock pool versus playa samples, and the analysis was run again.

All raw sequence reads and abundance charts (levels 10 (18S) and 7 (COI)) are deposited in the UTEP Bioinformatics data repository (http://datarepo.bioinformatics.utep.edu/getdata?acc=R4KMXDO2ZKAINXW).

3.3 Results

3.3.1 Amplicon sequencing of sediments

18S analysis: Sequencing of the 18S region resulted in 702,328 reads and 34,995 Operational Taxonomic Units (OTUs), of these, 3,610 (0.51%) corresponded to 55 OTUs (0.15%) that were identified as rotifers (Table 6). Taxonomic diversity of 18S sequences varied substantially among pond types (Figure 5). Sequences were analyzed at taxonomic level 10 in QIIME, which provides the most taxonomic resolution at the species level for 18S sequences. An assortment of annelids, arthropods, gastrotrichs, flatworms, rotifers, tardigrades, and other organisms that are generally found in freshwater systems, were identified through sequencing. HTSP playa sites East (E), Behind East (BE), and Behind Ranch House (BRH) had the most OTUs across all 12 sites (E (8,474), BE (7,525), and BRH (7,659)). However, Laguna Prieta (LP) had the fewest OTUs out of all HTSP playa sites (LP (1,665)). As seen in the rotifer-specific taxonomic abundance bar chart (Figure 6), many of the OTUs were monogonont and bdelloid rotifers that could not be further resolved taxonomically due to the limitations of using conserved, 18S primers and the available sequences in the modified SILVA database.

In rock pools (ST, V, S), the majority of OTUs were identified as Bdelloidea, Adinetida (represented by green, dark blue, orange, and red in Figure 6) or Bdelloidea, Philodinidae

(represented by light blue). Percentage of Bdelloidea OTUs in rock pools South Temp (ST), Vero (V), and Stacia (S) ranged from 0.3% (S) to 60.4% (ST) when analyzing OTU abundance at level 10. This reflects the active sample data where the bdelloid *Pleuretra lineata* (Donner, 1962) is the dominant rotifer in the rock pools and *Adineta vaga* is often found.

Table 6 Summary of 18S sequences and OTUs per site corresponding to the entire eukaryotic biome and rotifer-specific. Quality column is the number of sequences that mapped to the site and passed quality control. Curated columns contain the number of sequences after de-duplication, chimera detection, and Lulu curation steps. OTU columns contain the number of molecular OTUs identified by the uclust algorithm. OTU counts with * have the total number of OTUs found across all samples and not the individual sites added together.

18S Sample		ALL		Rotifer-specific	
	Quality	Curated	OTU	Curated	OTU
Lake Littra	119,761	28,596	2,665	213	12
Lake Limbra	81,938	26,554	3,891	107	10
Ryan's 2	116,266	41,621	4,320	69	14
404A	81,131	29,630	3,332	1,177	23
404B	83,159	31,214	3,447	60	18
East	231,316	85,805	8,474	121	17
Behind East	277,283	101,715	7,525	612	24
Behind Ranch House	171,919	91,030	7,659	63	19
Laguna Prieta	14,541	6,216	1,665	32	8
Vero	378,109	117,086	6,631	248	22
Stacia	104,416	30,262	2,865	663	22
South Temp	333,294	112,599	7,838	245	19
Total	1,715,850	702,328	34,995*	3,610	55*

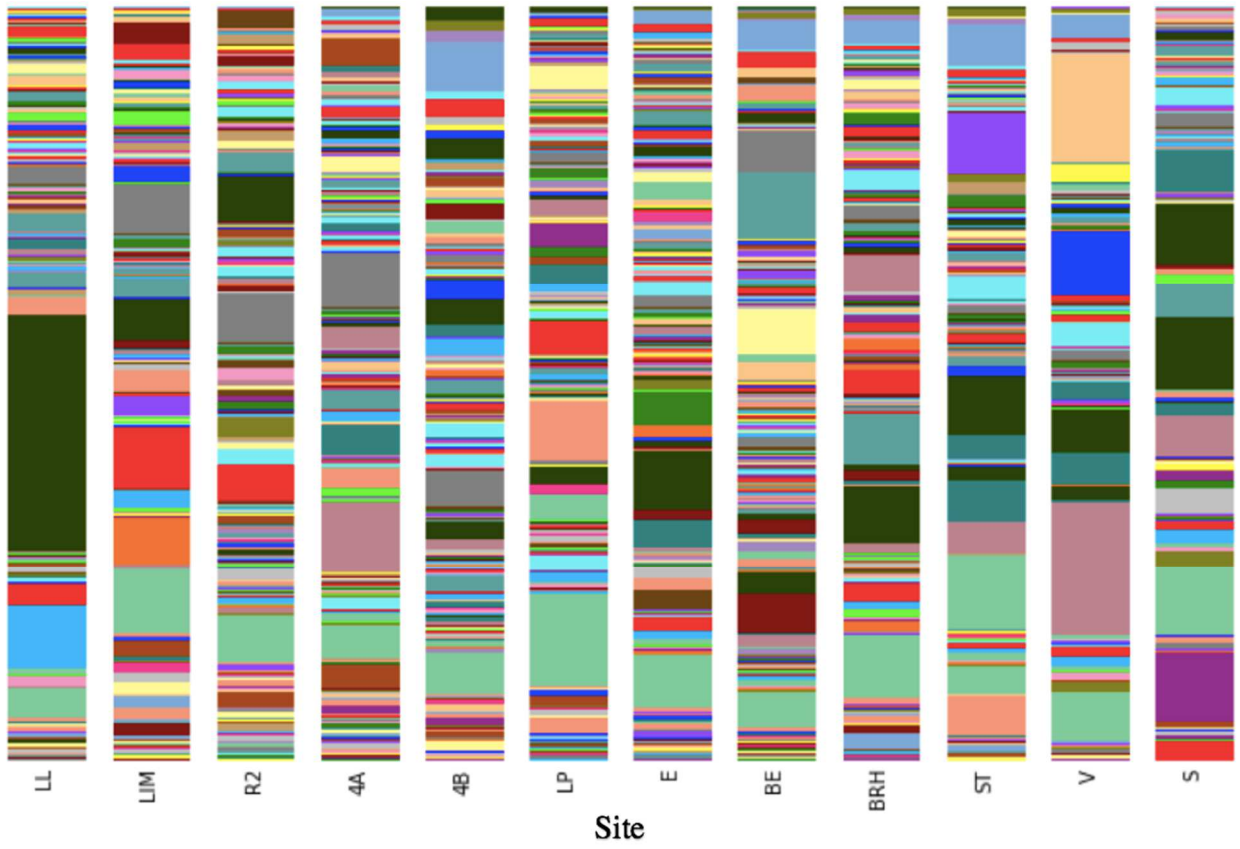

Figure 5 Abundance chart for 18S eukaryotic biome sequences at QIIME taxonomic level 10. Colors represent summarized OTUs. A key to abbreviations is given in Table 2. The legend is located in the UTEP Bioinformatics data repository (http://datarepo.bioinformatics.utep.edu/getdata?acc=R4KMXDO2ZKAINXW)

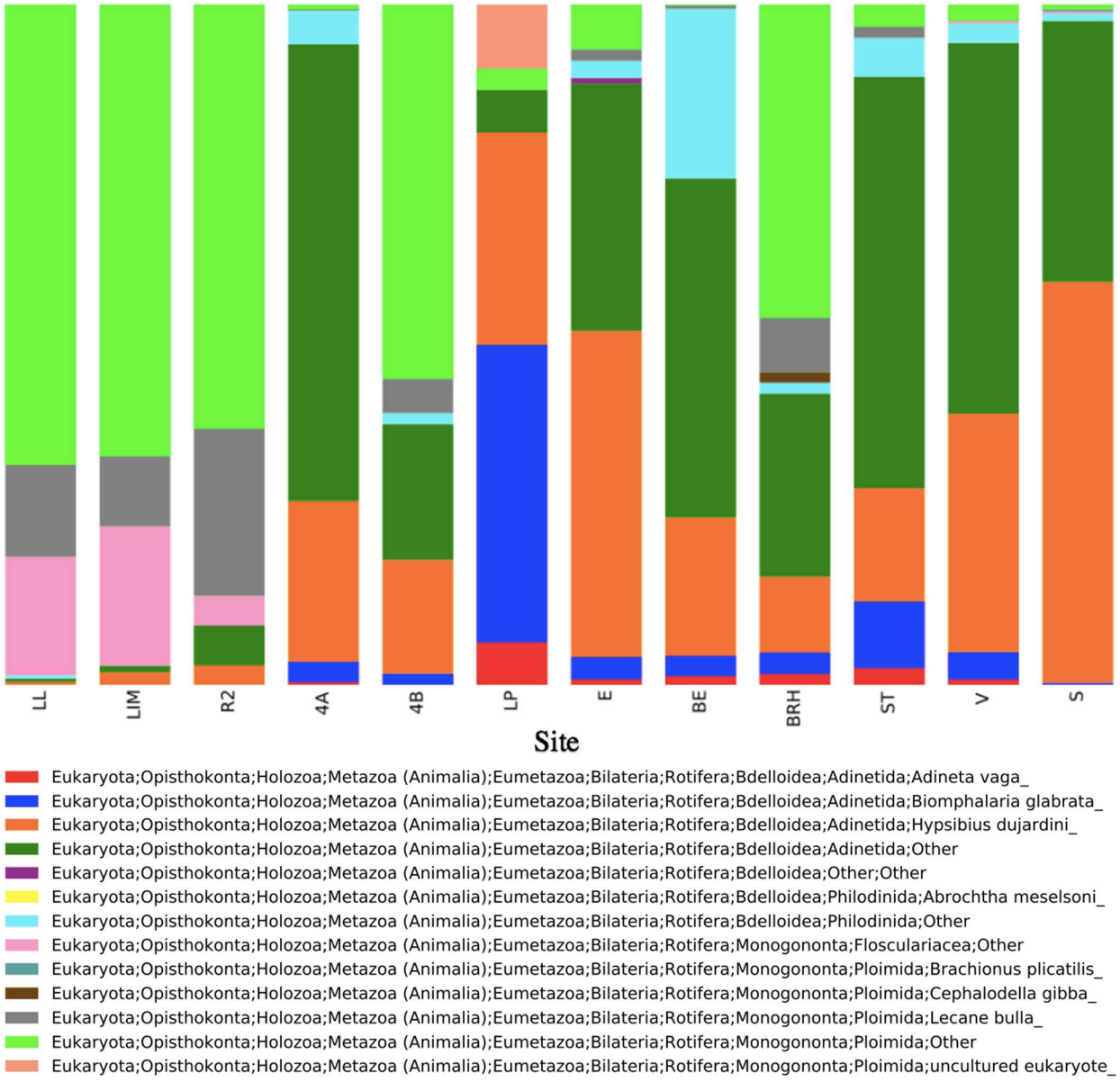

Figure 6 Abundance chart for 18S rotifer-specific sequences at QIIME taxonomic level 10.

Colors represent summarized OTUs. A key to abbreviations is given in Table 2.

In playas at HTSP, Bdelloidea sequences range from 0.8% (E) - 49.8% (BE) of OTUs. Bdelloid *Adineta vaga* (Davis, 1873) sequences were found in all larger waterbodies. For monogononts, sequences corresponding to *Lecane bulla* (Gosse, 1851) were found in two of the three larger water bodies, comprising up to 7.9% of OTUs (BRH). In addition, *Lecane bulla* was found in both active and rehydrated populations (Table 3). Monogonont OTUs for rotifers *Brachionus plicatilis (*Müller, 1786*)* were found in rock pool Stacia (0.3%), *Cephalodella gibba (*Ehrenberg, 1830*)* in Behind Ranch House (1.6%), and Floscullariacea not identified to species in rock pools Vero (0.4%) and Stacia (0.3%). For sediments from Australian sites (LL, LIM, R2), bdelloid sequences were much less common, with a maximum of 5.8% (R2) of OTUs. OTUs corresponding to Plomida, Floscullariacea, and *L. bulla* were most prevalent with *L. bulla* also being found in active populations in Ryan's 2 and Lake Limbra.

Good's coverage results for 18S sequences using the entire eukaryotic biome showed that sampling and sequencing methods were effective at estimating taxonomic richness for all 12 sites (Figure 7A). The rarefaction index reached an asymptote at ~0.50 - 0.75, indicating that sampling and sequencing of sediments captured a considerable proportion of the taxa present. Comparatively, when filtered for rotifer-specific sequences, Good's coverage results showed that more extensive sequencing should be done in future studies to obtain a fuller representation of rotifer communities (Figure 7B). Note, all 12 sites were represented on the Good's coverage plot, but some are not distinguishable due to low sequence reads (i.e., Lake Limbra, Ryan's 2, 404B, Laguna Prieta, Behind Ranch House, South Temp). Interestingly, those that were represented on the plot, showed high rarefaction values (~0.90).

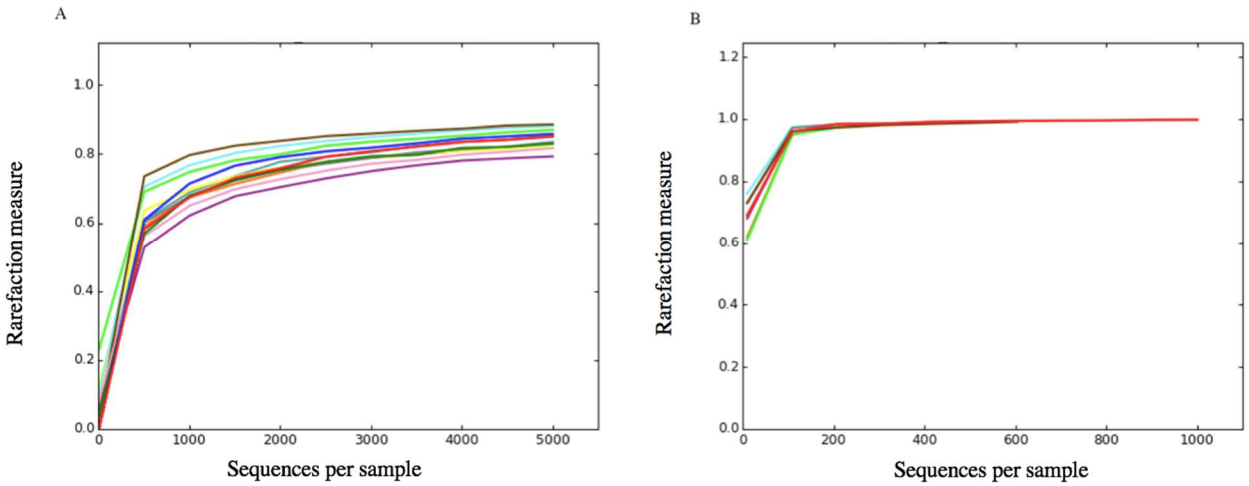

Figure 7 Good's coverage of 18S sequences considering the entire eukaryotic biome (A) compared to filtered rotifer-specific sequences (B) from 12 desert aquatic sites. Key to colors on graphs: Purple = East, HTSP, USA; Orange = Behind East, HTSP, USA; Dark Green = Behind Ranch House HTSP, USA; Pink = Laguna Prieta, HTSP, USA; Gray = South Temp, HTSP, USA; Light Green = Vero, HTSP, USA; Brown = Stacia HTSP, USA; Baby Blue = Lake Littra, Australia; Turquoise = Ryan's 2 billabong, Australia; Yellow = Lake Limbra, Australia; Red = Playa 404A, New Mexico, USA; Blue = Playa 404B, New Mexico, USA.

18S PCoA plot for entire eukaryotic biome sequences clustered sites by geographic location when considering all 12 sites (Figure 8A). Further, when local (USA) rotifer-specific sequences were analyzed independently from Australian sites, rock pools from HTSP showed some separation and clustering while sites 404A and 404B were isolated (Figure 8B). Even though 404A and 404B are in the same region and are close in geographic proximity, their separation might be due to the fact that 404A is highly disturbed by motor vehicles while 404B is more secluded, sheltered by vegetation, has more depth, and is less disturbed.

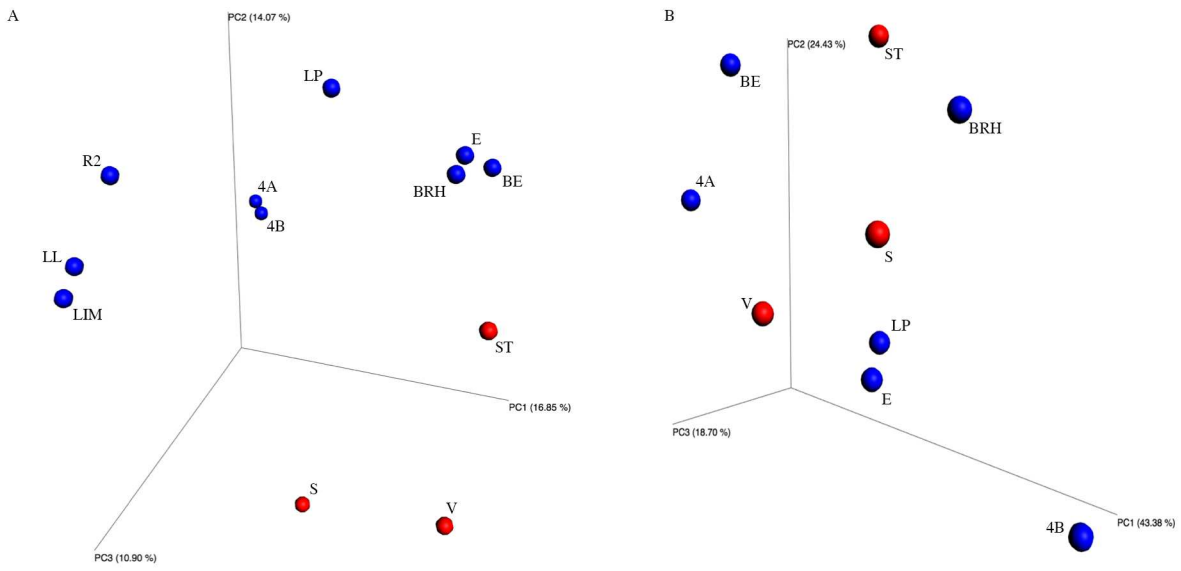

Figure 8 Principal Coordinates Analysis plots (PCoA) of 18S sequences from 12 spatially isolated, desert aquatic sites analyzed with (A) full eukaryotic biome sequences compared to (B) filtered rotifer-specific sequences from U.S. sites. Colors indicate pool type: Blue = Temporary playa; Red = Rock pool. Variance explained by each axis is shown in parentheses. A key to abbreviations is given in Table 2.

COI Analysis: COI sequences resulted in 495,643 reads and 10,241 OTUs, of these, 15,687 reads (3.16%) corresponded to 149 OTUs (1.45%) that were identified as rotifers (Table 7). HTSP sites East (E) and Behind Ranch House (BRH) had similar total number of sequences (E: 2,399; BRH: 2,255). COI sequences were analyzed at taxonomic level 7 in QIIME, which provides the most taxonomic resolution at the species level for COI. Taxonomic diversity for entire eukaryotic biome varied among sites with annelids, arthropods, nematodes, and rotifers being found in rock pools and temporary playas (Figure 9). Interestingly, when focusing on rotifer-specific sequences, Behind Ranch House had approximately 15 times more sequences than East (BRH: 857; E: 54). A total of 149 OTUs mapped to rotifers, this was more than double the rotifer OTUs resolved by 18S (55). OTUs were primarily monogonont and bdelloid rotifers that could not be further resolved taxonomically as seen in the rotifer-specific taxonomic abundance bar chart (Figure 10). In rock pools at HTSP (ST, V, S), up to 60.6% OTUs were identified as Bdelloidea, Adinetida (represented by red and blue), other unclassified Bdelloidea (represented by orange and green), and ploimids (represented by dark green and purple) (Figure 10). OTUs corresponding to Bdelloidea in rock pools (ST, V, S) were most abundant out of all taxa with percentages with up to 60.6% (S) OTUs. COI sequencing results are similar to active sample data where bdelloid rotifers are the most abundant in rock pools at HTSP (Walsh unpub. data).

Table 7 Summary of COI sequences and OTUs per site corresponding to entire eukaryotic biome and rotifer-specific. Quality column is the number of sequences that mapped to the site and passed quality control. Curated columns contain the number of sequences after de-duplication, chimera detection, and Lulu curation steps. OTU columns contain the number of molecular OTUs identified by the uclust algorithm. OTU counts with * have the total number of OTUs found across all samples and not the individual sites added together.

COI Sample		ALL		Rotifer-specific	
	Quality	Curated	OTUs	Curated	OTUs
Lake Littra	62,078	50,437	1,790	3,089	48
Lake Limbra	54,811	44,076	1,482	339	31
Ryan's 2	83,320	43,600	946	267	24
404A	56,386	43,009	1,812	1,729	16
404B	46,200	37,965	2,267	2,408	44
East	56,815	45,770	2,399	54	18
Behind East	34,152	28,277	2,520	431	38
Behind Ranch House	55,375	45,583	2,255	857	49
Laguna Prieta	35,435	29,496	2,239	715	58
Vero	42,044	30,022	1,119	301	39
Stacia	56,683	41,650	1,304	4,313	59
South Temp	76,139	55,758	1,318	1,184	17
Total	659,438	495,643	10,241*	15,687	149*

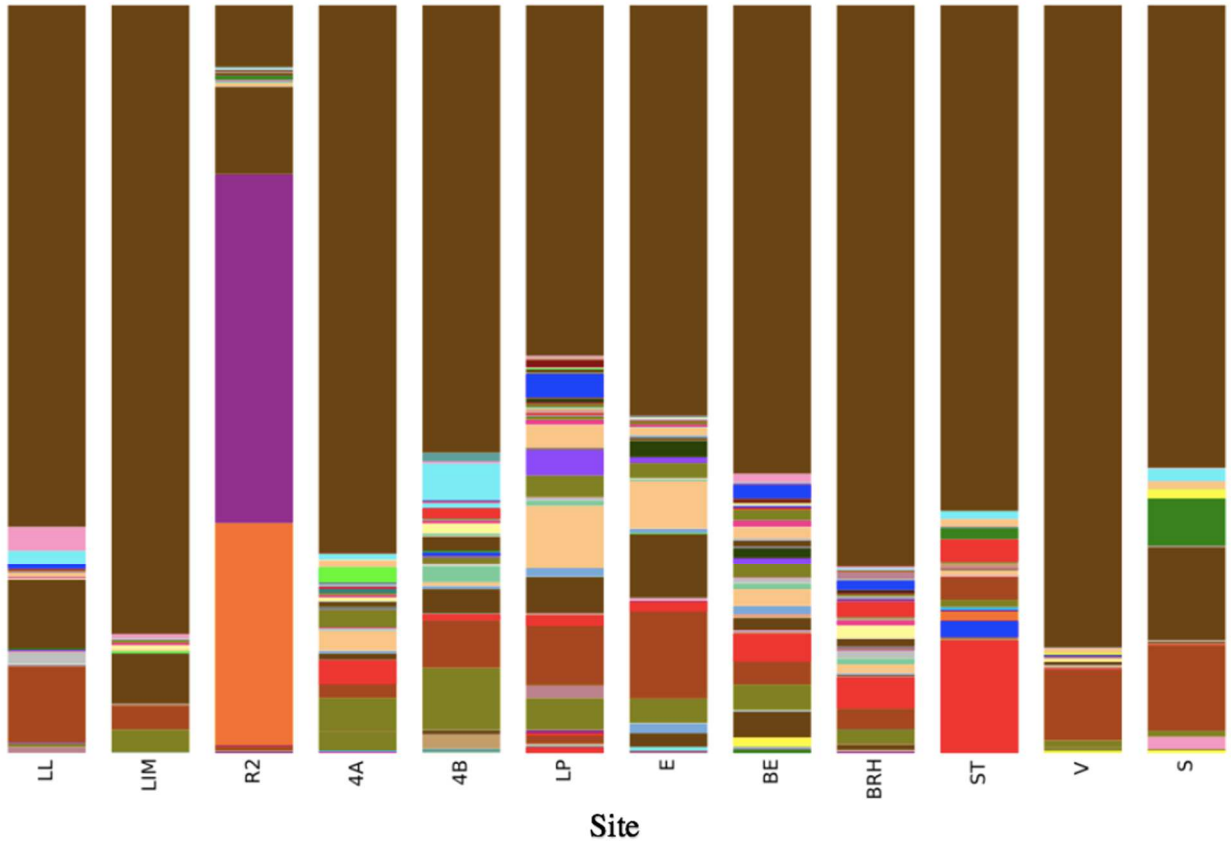

Figure 9 Abundance chart for COI eukaryotic biome sequences at QIIME taxanomic level 7. Colors represent summarized OTUs. A key to site abbreviations is given in Table 2. The legend is deposited in UTEP Bioinformatics data repository (http://datarepo.bioinformatics.utep.edu/getdata?acc=R4KMXDO2ZKAINXW).

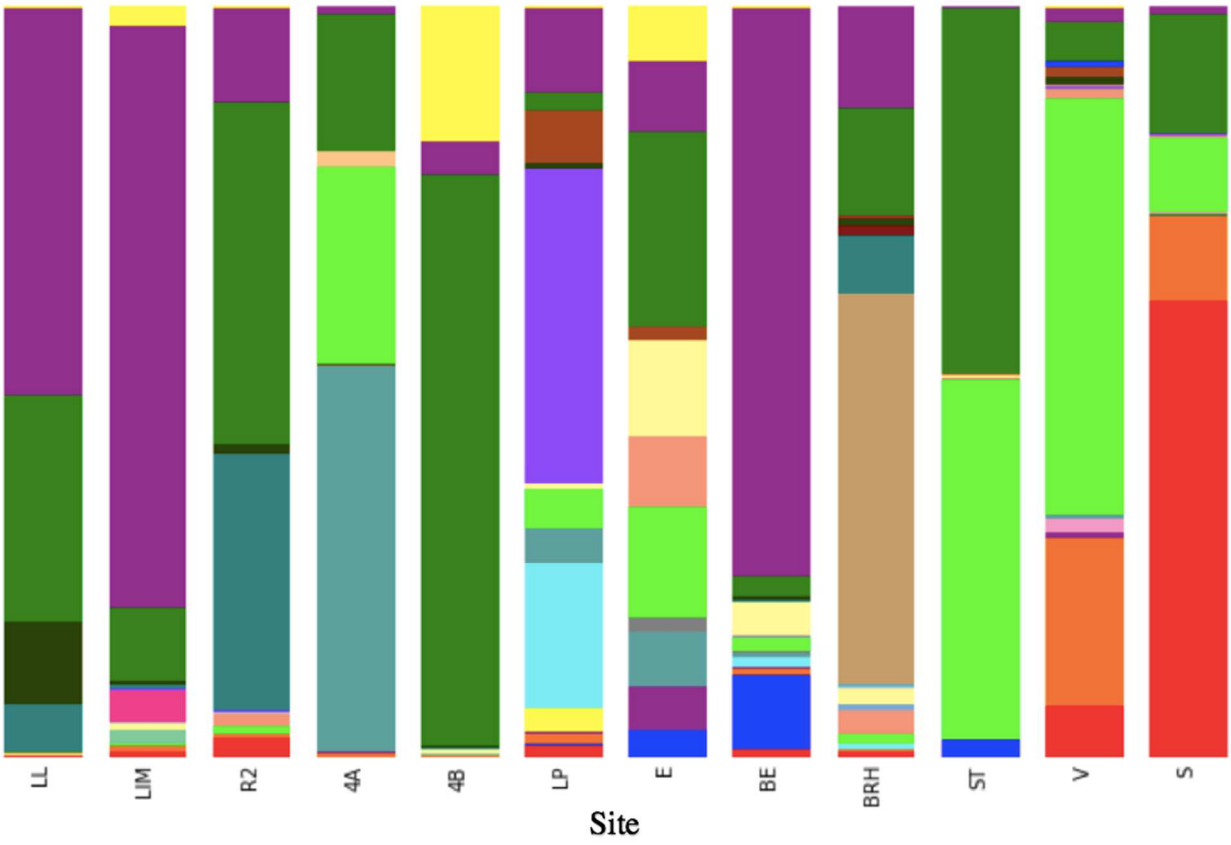
LL
LIM
R2
4A
4B
LP
E
BE
BRH
ST
V
S
Site

Figure 10 Abundance chart for COI rotifer-specific sequences at QIIME taxanomic level 7. Colors represent summarized OTUs. A key to site abbreviations is given in Table 2.

In larger playas at HTSP, Bdelloidea sequences represented from 0.1% (BRH) - 14.8% (E) of OTUs. Moreover, unclassified Bdelloidea species were found in all larger water bodies. For monogonont rotifers, sequences corresponding to unclassified ploimids were found in all larger waterbodies with up to 75.9% of OTUs (BE). Additionally, OTUs corresponding to rotifers *Cupelopagis vorax* (Leidy, 1857) and *Collotheca campanulata* (Dobi, 1849), not present in active species lists, were found in playas East (E) and Behind Ranch House (BRH) at HTSP. In Australian sites (LL, LIM, R2), bdelloid sequences were much less common, with a maximum of 2.6% (R2) of OTUs. Comparatively, OTUs corresponding to unclassified ploimids were most prevalent with a maximum of 77.6% OTUs. Unique monogonont rotifers not found in active or recovered species lists were also found through sequencing (i.e., *Collotheca tenuilobata* (Anderson, 1889) (4A, 4B, LIM), *Floscularia melicerta* (Ehrenberg, 1832) (BE)*, Sinantherina ariprepes* (Edmondson, 1939) (BRH, LIM, R2)*, Sinantherina semibullata* (Thorpe, 1889) (LIM)).

Estimation of taxonomic richness for all 12 sites using COI primers had better resolution than results provided by 18S primers. Rarefaction indices reached an asymptote at ~0.80 - 0.95, suggesting that our sampling method captured a large proportion of the taxa present in all 12 sites (Figure 11A). Furthermore, when filtered for rotifer sequences only, Good's coverage results indicated that rotifer communities were also effectively sequenced (~0.85 - 0.99) (Figure 11B). Only one out of the 12 sites sequenced, East, was not distinguishable due to low sequence reads.

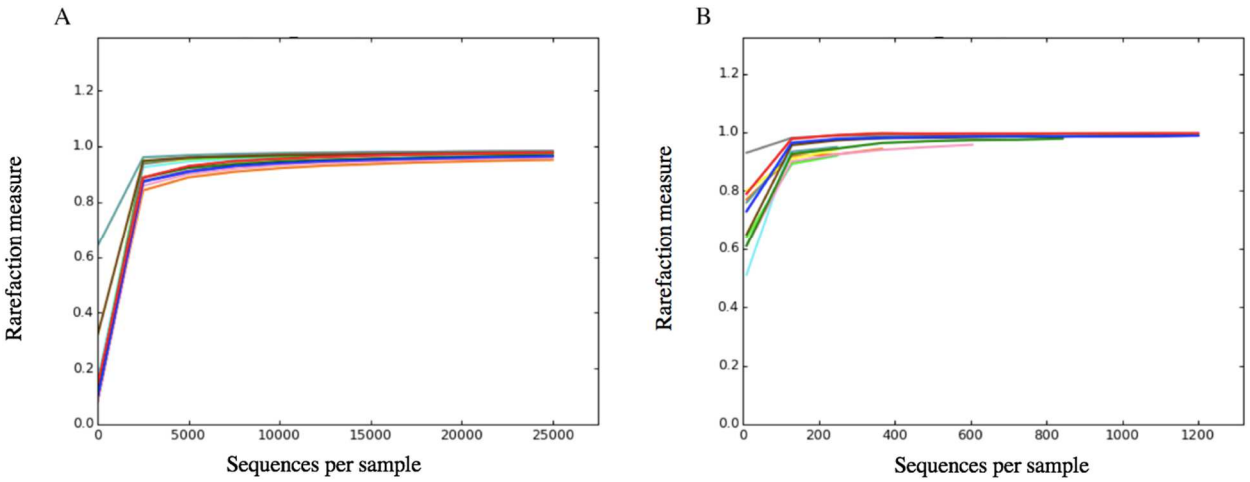

Figure 11 Good's coverage of COI sequences considering the entire eukaryotic biome (A) compared to filtered rotifer-specific sequences (B) from 12 desert aquatic sites. Key to colors on graphs: Purple = East, HTSP, USA; Orange = Behind East, HTSP, USA; Dark Green = Behind Ranch House HTSP, USA; Pink = Laguna Prieta, HTSP, USA; Gray = South Temp, HTSP, USA; Light Green = Vero, HTSP, USA; Brown = Stacia HTSP, USA; Baby Blue = Lake Littra, Australia; Turquoise = Ryan's 2 billabong, Australia; Yellow = Lake Limbra, Australia; Red = Playa 404A, New Mexico, USA; Blue = Playa 404B, New Mexico, USA.

When taking all 12 sites into consideration, the COI PCoA plot for entire eukaryotic biome sequences clustered sites by both pool type and geographic location (Figure 12A). Larger playas from the USA (LP, E, BE, BRH, 404A, 404B) clustered in close proximity to rock pools (ST, V, S) but some separation between pool types was obtained. Australian sites (LL, Lim, R2) clustered separately along the component 1 axis. When rotifer-specific sequences from local sites (USA) were analyzed separately from rotifer-specific sequences from Australian sites, rock pools (ST, V, S) from HTSP began to isolate and cluster (Figure 12B). Similar to 18S results, when assessing rotifer specific sequences, sites 404A and 404B were isolated at opposite ends of each coordinate with no sign of clustering (Figure 12B). These results suggest that habitat type, which is related to water body depth, temperature, and surface area, may influence rotifer species richness.

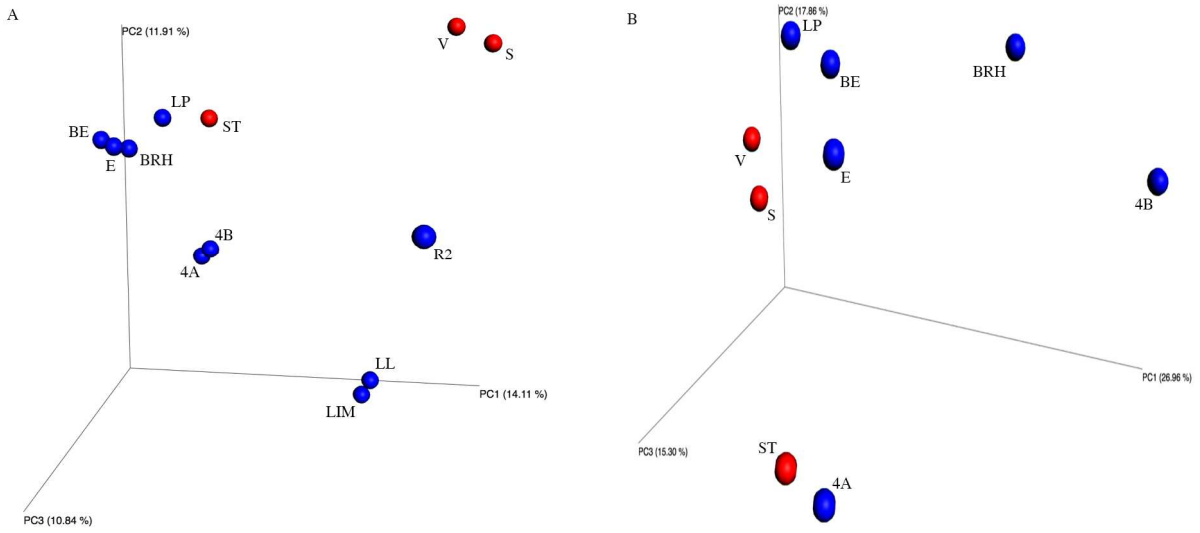

Figure 12 Principal Coordinates Analysis plots (PCoA) of COI sequences from 12 spatially isolated, desert aquatic sites analyzed with (A) full eukaryotic biome sequences compared to (B) filtered rotifer-specific sequences excluding Australian sites. Colors indicate pool type Blue = Temporary playa; Red = Rock pool. Variance explained by each principal coordinate axis is shown in parentheses. Key to abbreviations is given in Table 2.

3.4 Discussion

Sequencing of sediments using 18S and COI primers retrieved 3,610 and 15,687 rotifer-specific sequences respectively. Rotifer diversity of 18S and COI sequences varied substantially among sites. As expected, 18S primers retrieved fewer rotifer-specific OTUs than COI primers due to it having lower taxonomic resolution at the species level. Additionally, according to the Good's coverage index, COI primers had better coverage than 18S primers when estimating taxonomic richness. When analyzing similarities in the rotifer communities, PCoA plots for both primers suggested that pool types may influence rotifer species richness recovered from sequencing. Furthermore, amplicon sequencing of sediments was able to detect taxa missed by sediment rehydration experiments and active populations.

Capturing past and present diversity of wetlands through sequencing of sediments is a good option when assessing biodiversity. Sequencing of eDNA can provide information on resting stage diversity and fill in gaps of knowledge regarding the diversity of species at any given site (Hajibabaei et al., 2011; Shokralla et al., 2012; Shokralla et al., 2014; Gibson et al., 2015). Sequencing techniques can facilitate the identification of species without the need of taxonomic expertise that is rapidly declining (Hopkins and Freckleton, 2002; Wheeler et al., 2004; Thomsen et al., 2012). In rotifers, this is especially helpful due to the high level of difficulty in identifying them to species (Segers, 2007). To our knowledge, this is the first study to specifically look for rotifers in sediments using DNA metabarcoding.

The 18S and COI primer sets were implemented in order to get a full representation of what is present at each site and what may be affecting rotifer diversity. Similar studies assessing diversity in sediments have been conducted in the past; but different sampling tools, methods, and primer sets have been used (Thomsen et al., 2012; Machler et al., 2015; Deiner et al., 2015; Geerts et al., 2018; Wei et al., 2018). For example, Andújar et al. (2017) assessed the effects that a pesticide spill in the River Kennet, located in Southern England, had on invertebrate freshwater assemblages by applying metabarcoding techniques. The authors used the same SSU 18S primer pairs applied in this study and two different fragments of the COI primer. Each primer set was

able to recover unique species that the other missed. Rotifers were exclusively amplified with COI primers while Platyhelminthes and Nematoda were only amplified with 18S primers. Here, rotifers were amplified by both genes.

Combining rehydration experiments and sequencing approaches can be beneficial because they complement each other by capturing richness that the other does not. In this investigation, sequencing of sediments serves as further support for the results provided by rehydration experiments where playas have more richness than rock pools. In general, sequencing results were consistent with the results from rehydration experiments sharing several rotifer OTUs to rotifers recovered from sediments. Additionally, species that were not found after sediment rehydration were presented in sequencing results. Similar to this study, the combination of molecular and morphological approaches to assess taxa diversity in various ecosystems has been explored by others. Yang et al. (2017) conducted a study where the genetic diversity of zooplankton communities found in a freshwater lake ecosystem were characterized through DNA metabarcoding using the same COI primers used here as well as morphological identification techniques. The authors noted that when compared with traditional morphology-based identification, DNA metabarcoding identified more taxa, with more than half of the total OTUs obtained corresponding to rotifers. Also, using diatom specific primers, Rivera et al. (2018) found that morphological and molecular inventories of diatoms provided similar structure of littoral benthic communities suggesting that these methods can be used to obtain a full and precise characterization of these communities. Applying an integrated approach that combines morphological and molecular techniques for the assessment of rotifer diversity, or zooplankton in general, should be adopted because each method can identify taxa possibly missed by the other.

In general, bdelloid rotifers were more prominent in rock pools than monogonont rotifers when analyzing sequencing results. Comparatively, in larger playas, monogonont rotifers were more frequent than bdelloid rotifers. Bdelloid rotifers are known to inhabit these types of extremely ephemeral pools (Ricci and Melone, 2000, Wallace et al., 2005; Walsh et al., 2014).

Interestingly, rock pools had a large number of rotifer-specific OTUs, more than are usually present in active species lists. The high turnover of sediment that is deposited into rock pools by larger animals, wind, rain overflow might have been the reason why there was a high number of rotifer OTUs in rock pools (Vanschoenwinkel et al., 2008a; Vanschoenwinkel et al., 2008b; Moreno et al., 2016). As mentioned above and seen in Rivas et al. (2018), taxa like rotifers, nematodes, ostracods, and gastrotrichs are transported from temporary playas to great distances after wind events. The effect that habitat types have on rotifer diversity was also shown by PCoA plots. When analyzing rotifer-specific sequences, sites clustered by pool type suggesting that pool type might have an effect on rotifer community composition. More sequencing of similar pool types should be done to further explore and confirm this result.

Half of the sites were not represented due to low sequence reads when assessing Good's coverage for 18S sequences. Comparatively for COI sequences, only one site was not represented. An explanation for the low sequence reads might be that the similarity threshold for 18S primers was set at 97%, also being the reason that many rotifers in this study could not be further resolved to the species level. Tang et al. (2012) assessed meiofaunal diversity using 18S and COI markers. Their result showed that 18S reduced diversity estimates whereas COI increased them and suggested that the use of COI for biodiversity surveys in environmental DNA could provide more accurate estimates of species richness. Mohrbeck et al. (2015) further showed that the use of 18S markers was not ideal and reliable for assessing biodiversity testing artificially prepared samples to assess the accuracy of species detection and found that species detection by this marker results in poor resolution of taxa. Many sequences mapped to unidentified monogonont or bdelloid when analyzing COI sequences, this might have been due to the availability of rotifer-specific sequences in the reference dataset. Even though the reference database was reinforced with rotifer-specific sequences, and sequences recovered from rehydration experiments, having more rotifer sequences added to the reference dataset might have yielded more matches. This is one limitation that should be taken into consideration; public databases oftentimes contain sparse and missing data that might affect the assessment of certain

taxa. The results given herein indicate that COI identified more rotifer specific OTUs than 18S; but both genes are capable of identifying rotifers and should be considered in diversity assessment studies going further.

A total of 55 OTUs corresponding to rotifer taxa were identified through amplicon sequencing using 18S primers. Comparatively, 149 OTUs were matched to rotifers using COI primers. The small amount of sediment required by the PowerSoil® DNA Isolation Kit might have been a limiting factor in the number of rotifer taxa that was recovered through this approach. Only 0.50 g of sediment were used in the DNA extraction process. This is a very small amount that more than likely did not represent all rotifer resting stages found at the sites. Using a kit that allows the extraction of eDNA from more sediment may provide a better representation of rotifer communities. Also, the use of sediments from the same collection date may also minimize variability and provide better resolution when it comes to identifying rare rotifer species. Many limnological and ecological studies rely on sporadic live sampling to capture species diversity of aquatic systems; this study demonstrates the added effectiveness of rehydration and amplicon sequencing sampling strategies in recovering and accurately estimating local and regional species pools without the need of multiple visits.

Chapter 4: Conclusions

This investigation was conducted to more fully assess rotifer biodiversity in temporary Chihuahuan Desert wetlands through rehydration experiments and amplicon sequencing. This approach was also tested in a second desert system by including samples from Australian wetlands. In arid regions, large reservoirs of resting stages can be found in the sediment banks of temporary aquatic pools (Hairston and Kearns, 2002; Brock et al., 2003; Brendonck and De Meester, 2003). Here, the focus was on the recovery of rotifers due to their ability to produce resting stages and their successful strategies that allow them to persist in extreme desert aquatic environments (Alekseev et al., 2007; Walsh et al., 2014). To further assess rotifer diversity and complement the results given by rehydration experiments, sequencing of sediments was applied. DNA in environmental samples such as water and sediments is abundant, therefore this tool is often used when assessing biodiversity (Taberlet and Coissac, 2012; Rees et al., 2014; Bista et al., 2018).

Data collected here extends our knowledge of rotifer species present in these Chihuahuan Desert and Australian wetlands and shows the effectiveness of rehydration experiments to assess rotifer diversity. We found that sediments from larger temporary playas resulted in highest species richness and rock pools had less. These findings strengthen the existing data on rotifer diversity from the 12 sites. Similarities between recovered communities and those found during wet periods were found between sites that are located in close geographic proximity. Additionally, unique taxa that were not present in active species lists studied here, were found through rehydration experiments. Thus, the possibility of future researchers to discover additional biodiversity at a given site is possible through the application of these methods potentially expanding our knowledge on rare, elusive, and overall species composition.

Sequencing of sediments using 18S and COI primers resulted in numerous rotifer OTUs from all 12 sites. Data provided in this research complements existing records of rotifer diversity in the Chihuahuan Desert and Australian sites through a sequencing approach. To our knowledge

this was one of the first projects that specifically focused on rotifer biodiversity in sediments through the use of amplicon sequencing. As expected, 18S primers retrieved fewer rotifer-specific OTUs than COI primers due to it having less taxonomic resolution at lower levels. Interesting results were found when analyzing similarities between sequence data. PCoA plots began to cluster sequences by pool type, this should be explored through additional sequencing by future researchers to further confirm the influence that these have on rotifer species richness. By applying this sequencing technique, taxa missed by sediment rehydration experiments and active sampling techniques were detected, reinforcing the effectiveness of this approach to assess rotifer diversity in desert wetlands.

Further exploring rehydration of sediments collected at different time periods would be valuable to determine if storage time has an effect on hatching success of resting eggs. More evidence is needed on which environmental parameters, mesocosm types, and overall rehydration methods optimize hatching conditions to capture the most rotifer species as possible from sediments. It is well known that rotifer species hatch under different conditions (Pourriot and Snell, 1983; May, 1987; Garcia-Roger et al., 2008; Gilbert, 2017). Constructing an online database similar to the one proposed by Walsh et al. (2017), containing information on resting stages such as morphology, when and how they were collected, and includes species-specific hatching conditions would be helpful. Based on results provided here, the use of COI gene to explore rotifer diversity in sediments should be considered for future investigations. Using a combination of rehydration experiments and a sequencing approach as performed here, can provide interesting insight not only on the recovery and assessment of rotifers, but other resting stage producing species inhabiting temporary desert wetlands worldwide.

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Appendix

Appendix 1: 18S ribosomal RNA (rRNA) gene sequences of rotifers recovered from rehydration experiments

>SS7-9_18S L.luna_BE
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ATGTGGTAGCCATTTCTCATGCTCC

>SS7-10_18S C.campanulata_BE_inside
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GTATTGTTATTTTTCGTCACTACCTCCCCGATCTAGGAGTGGGTAATTTGCGCGCCTGCTGCCTTCCGTA
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>SS7-11_18S E.brachionous_BE
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>SS16-3_18S C.ornata_BE6/3/99
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>SS18-7_18S B.quadridentatus_BE98
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>SS18-8_18S Eosphora_BE98
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>SS20-5_18S E.brachionus_BE2000
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>SS21-6_18S E.brachionus_BE2000
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>SS23-9_18S C.ornata_Lakelittra
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Appendix 2: Mitochondrial cytochrome c oxidase subunit COI sequences of rotifers recovered from rehydration experiments

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Vita

Sergio David Samaniego graduated from the University of Texas at El Paso with a Bachelor of Environmental Science in Spring 2016. He worked as a Research Assistant funded by the National Science Foundation (DEB-1257068) in the Walsh laboratory from 2014-2017. As a graduate student, he was also a Teaching Assistant for ESCI 1101 Environmental Science Lab, ESCI 1310 Methods in Environmental Science, ESCI 2204 Research Experience in Environmental Science, and ESCI 2105 Research Experience in Environmental Science. He presented posters on his research at various conferences including UTEP COURI symposium in El Paso, TX on August 1, 2015; SACNAS National Conference, in Washington, DC on October 29-31, 2015; Society of Wetland Scientists annual meeting, in Corpus Christi, TX on May 31-June 4, 2016 (was the recipient of a 2016 Society of Wetland Scientists travel award); Evolution meeting, in Portland, OR on June 23-27, 2017; and XV International Rotifer Symposium, in El Paso, TX on June 3-9, 2018. Sergio was also the recipient of the Dodson Research Grant award from the UTEP Graduate School on December 13, 2017. Sergio graduated with his Master of Science in Environmental Science degree in May 2019.

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This thesis was typed by Sergio David Samaniego.