2023-08-01

Diversity And Lifestyle In The Rotifera

Patrick D. Brown
University of Texas at El Paso

Recommended Citation

Brown, Patrick D., "Diversity And Lifestyle In The Rotifera" (2023). *Open Access Theses & Dissertations*. 3900.
https://scholarworks.utep.edu/open_etd/3900

DIVERSITY AND LIFESTYLE IN THE ROTIFERA

PATRICK D. BROWN

Doctoral Program in Ecology and Evolutionary Biology

Elizabeth J. Walsh, Ph.D., Chair

Igor Almeida, Ph.D.

Michael L. Moody, Ph.D.

Ming-Ying Leung, Ph.D.

Robert L. Wallace, Ph.D.

Stephen L. Crites, Ph.D.
Dean of the Graduate School

By

Patrick D. Brown

2023

DIVERSITY AND LIFESTYLE IN THE ROTIFERA

By

PATRICK DOWLING BROWN, B.S.

DISSERTATION

Presented to the Faculty of the Graduate School of
The University of Texas at El Paso
in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY
Department of Biological Sciences
THE UNIVERSITY OF TEXAS AT EL PASO
August 2023

ACKNOWLEDGEMENTS

I would like to express my gratitude to the individuals who have played a significant role in supporting and assisting me throughout this journey. Their invaluable contributions have been instrumental in the successful completion of this research.

I thank my committee members, D Igor. Almeida, Dr. Ming-Ying Leung, Dr. Michael Moody and Dr. Robert Wallace, for their support and flexibility during the challenging times marked by the trials of the COVID-19 pandemic and unforeseen adversities. Their guidance and expertise have been pivotal in navigating the complexities of this study and the PhD program.

Chapter 1 of this work dissertation was developed largely during the pandemic from dataset we analyzed during lockdown. I would like to thank my coauthors on these works, who made it possible to be productive during these times, including Thomas Schröder, Judith Ríos-Arana, Roberto Rico-Martinez, and Marcelo Silva-Briano,

I am grateful to Dr. Kyung-An Han, for providing *Drosophila* to use as flow cytometry standards, as well as providing training for working with these animals. I would like to thank Armando Varela for his kind expertise and commitment to teaching me the intricacies of flow cytometry. Without his guidance, I would have struggled to identify and rectify the numerous mistakes I encountered during the process. I received help from many graduate students working in the fifth-floor core of the Bioscience building, including Cameron Ellis, Igor Estevao Da Silva, and Brian Grajeda who helped me prepare, run and analyze rotifer proteins samples with mass spectrometry. I am still actively working on this project, although it was not included here it was an integral part of my graduate education and experience at UTEP.

Furthermore, I am deeply grateful to the graduate students in the field of evolutionary biology and ecology who have provided immense assistance throughout the program. Océane Da Cunha and Logan Miles Horne, my close friends, have been a constant source of valuable feedback and support during challenging times, including the unfortunate incident of a break-in at my apartment. I am grateful to Azar Korbachev for providing training in the lab when I first started, and her work has been a valuable resource for numerous projects we have collaborated on since then. I would like to acknowledge and express my appreciation for Maribel Baeza, who has been with me throughout this entire process as a fellow peer, and I extend my congratulations as we both approach the culmination of our academic journeys. I would also like to thank all the other graduate students who I have worked with in the Walsh lab for their spirited interest in limnology, rotifer biology and science who made working in the field and lab an enlightening and fulfilling experience.

I am thankful to the undergraduates I have had the pleasure of working with, with whom I have developed strong friendships. I would like to specifically mention Eric Suh and Vanessa Blevines for their dedication and support. Additionally, Jaime D. Guitierrez Portillo and Abigail O’hara helped to measure some of the colonies used in Chapter 2.

I need to thank my funding and financial support for these works. Funding was provided by NSF DEB-1257068 (EJW), National Institute of Health (NIH-NIMHD-RCMI 5G12MD007592), NIH 5G12RR008124, UTEP’s Dodson research grant (PDB), NSF DEB (2051704) NSF 0516031, NSF Advance #0245071 (UTEP, Department of

Education HSI STEMGROW PO31C160235 and the microplate and microplate reader were provided by grant 5U54MD007592 from the National Institute on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health (NIH), American Association for the Advancement of Science Women's International Science Collaboration (WISC) travel grant award, T& E, Inc and Funds for Faculty Development (Ripon college).

Samples collected under a variety permits and provided by many colleagues, these can be viewed in the acknowledgements within Chapters 1 & 3. Samples for Chapter 2 were collected under permits TPWD (7-21), and access to La Mancha was provided by Kevin Bixby.

I am deeply indebted to my loved ones and friends outside the realm of science. To my family, whose unwavering support has been a constant source of strength, I express my heartfelt gratitude. Hillary Schmitthenner, in addition to being there for me during challenging times, has provided me with unique and captivating sampling locations. I extend my appreciation to my friends Jessica Mirales and Katherine Alaniz whose continued support has been invaluable.

Lastly, I reserve special praise for Dr. Elizabeth Walsh, not only for their exemplary leadership within the community but also for their role as a dear friend who has always been available to lend a listening ear and provide valuable advice.

ABSTRACT

Broad questions regarding community assembly and lifestyle evolution remain unanswered. To answer these questions I used rotifers, common primary consumers present in inland waters. Rotiferan presence in nearly all freshwaters makes them an ideal study system to address questions of metacommunity ecology. Additionally, rotifers possess diverse lifestyles, including sessile, swimming, colonial and solitary forms, allowing them to be used as models of lifestyle evolution.

In Chapter 1, I address metacommunity ecology by focusing on inland waters of the Chihuahuan Desert as a study system and investigated rotifer community assembly therein through two published works, Brown et al., 2020 and 2021. These studies used species present/absence data for rotifers from sites throughout the Chihuahuan Desert collected by the Walsh lab over a 20-year period. The first of these papers investigated the general patterns of rotiferan richness across this desert and looked at the influence of scale on them. Overall, I found hotspots of rotifer species richness that become less distinct at broader scales, where some hotspots may only appear at the regional level. I found the highest rotifer richness in springs (n=175) followed by lakes (n=112) and then rockpools (n=72). I found that sampling effort was linked to observed richness per site, and that distance was linked with beta dissimilarity at small spatial scales. Rotifer communities were found to be highly nested at these sites. To interpret broad patterns of rotifer diversity, richness across the Chihuahuan Desert was predicted using empirical Bayesian kriging, pooling sites at a variety of spatial scales. In Brown et al. (2021), I used subset of the dataset used in Brown et al. (2020) to investigate rotifer community assembly in temporary waters. I found that in these habitats rotifers are

assembled into their communities stochastically. Additionally, I analyzed environmental parameters associated with the presence of particular rotifer species, finding several features such as hydroperiod and conductivity to be important, accounting for 12% of the total variation in community composition. Additionally, I found that richness was highest in the habitats with the greatest amount of aquatic vegetation.

In Chapter 2, investigate the advantages of coloniality in rotifers. Many animals form colonies, although the reasons why they do this is unclear. For rotifers, one possible explanation is that colonies may provide the individual members of the colony with an energetic advantage. One way such an advantage may manifest is through lower respiration rates of individuals in colonies with more members, which would cause colony respiration rate to scale allometrically with colony size. Additionally, genome size may be directly tied to metabolism through the metabolic hypothesis of genome size and could also be used as a related character for further validation.

. To address these questions, rotifer respiration rates were measured using a Loligo microplate and microplate reader. I measured rates within the known range of rotiferan respiration rates reported in the literature. The findings on colony size allometry were mixed; *Sinantherina socialis* respiration scaled isometrically with colony size while *Lacinularia flosculosa* and *Conochilus hippocrepis* scaled allometrically. Additionally, I examined the traits that may be associated with allometric scaling of colony respiration across all colonial taxa with published respiration rates.To do this I utilized a hierarchical mixed regression model, and found that several features, including colony shape, presence of extrazooidal structures, and an unattached lifestyle influence respiration scaling.

In Chapter 3, I explore coloniality by looking at how genome size relates to lifestyle in gnesiotrochan rotifers. Genome size is related to metabolism in some animals, such as in birds and mammals. I investigated whether genome size was associated with particular rotifer lifestyles such as coloniality and sessility. Genome sizes were measured by flow cytometry, using Propidium Iodide staining, and *Drosophila melanogaster* as a genome size standard. Genome sizes found for gnesiotrochan rotifers (0.05 – 0.16 pg) were similar to those of ploimid rotifer (0.06 – 0.46 pg). I found that genome sizes differed significantly depending on lifestyle, i.e., genome size was smaller in motile and solitary rotifers and larger in sessile and colonial rotifers.

Overall, I found that rotifers assemble stochastically and with patterns that vary by scale for rotifer communities of the Chihuahuan Desert. Both rotifer genome sizes and allometric scaling factors suggest that coloniality in rotifers does seem to be related to metabolism, suggesting that an energetic advantage for coloniality may exist. Expanding the area investigated beyond the Chihuahuan Desert and incorporating other regions would help to determine whether the patterns I found can be applied to other biomes. Likewise, expanding the number of allometric scaling factors and genome sizes measured across colonial rotifers would enhance the reliability of these conclusions.

These studies provide preliminary understandings of rotifer diversity and community assembly in one region, the Chihuahuan Desert, as well as supporting the energetic advantage hypothesis of coloniality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Despite years of advancement in ecological and evolutionary theory, fundamental concerns regarding even the most basic observations remain. Within that context there are two notable questions: (1) As Rachel Carson posed “why does an [organism] live where it does?” This is a question of community assembly. (2) “Why do some animals live together rather than independently?” This is a question concerning lifestyle evolution and ecology. Though these topics may be broad, narrowing our scope may help increase our understanding. By focusing on communities of inland freshwater systems and on the diverse lifestyles of the Rotifera, a dominant primary consumer, it may be possible to add to the growing bodies of work addressing these questions. Here I attempt to help address basic questions about rotifer diversity and community assembly as well as lifestyle evolution within the phylum; I do this by applying metacommunity ecological theory and by examining traits such as allometric scaling of respiration rate and genome size.

The notion of habitat is fundamental, differences in communities may be readily apparent, deserts sport sparce and specialized vegetation relative to forests. Upon closer inspection of these differences, it is apparent that different organisms display distinct qualities that influence where they are found. These qualities can have profound implications for their biology and evolution. For example, in coral reefs, anthozoans are both important carnivores and, when allied with symbiotic zooxanthellae, producers. These anthozoans have two notably different lifestyles, living connected sharing food through their digestive tracts as do most corals live, or living apart as solitary organisms as do many anemones. Similarly, some cnidarians live most of their lives attached to the substratum, whereas others are planktonic. Metacommunity ecology can provide insight into the processes controlling community assembly.

Metacommunity ecology refers to the study of communities that are linked by dispersal (Leibold et al., 2004). This linkage between communities allows events in one community to affect its neighbors. In these cases, the scale of investigation is important, as regional and local processes can contribute to community assembly (Logue et al., 2011). At small scale, species interactions and niche availability can determine species assembly. At larger scales, dispersal patterns, species dispersal history, climate, and other processes can influence which species are present in the larger metacommunity. Together these processes at different scales work together to shape community structure and species assembly. The factors that are important at each scale are a topic of interest in metacommunity ecology (Leibold et al., 2004; Heino, 2012; Diniz et al., 2021). Isolated systems such as islands and inland waters provide an ideal model to investigate these community interactions (Whittaker et al., 2017). By learning how communities interact with each other and their environments, we can gain insight into community assembly and species presence, and thus what shapes patterns of biodiversity.

Islands have been recognized as useful ecological models, as they are isolated and possess measurable and defined populations of species (MacArthur & Wilson, 1967; Whittaker et al., 2017). Inland waters may be used as inverse island systems for the study of metacommunity ecology. One common group of animals nearly ubiquitous in these systems are the Rotifera, a group of microscopic organisms with diverse lifestyles including sessility, coloniality, and free-living planktonic among others (Wallace, 1987).

Rotifer diversity is highest in the subtropics with hotspots in the Northeast America tropical south America, Australia, Lake Baikal, and southeast Asia (Segers, 2008). Additionally, there is low endemicity in Africa and little-studied oceanic islands. The differences in species richness among these locations is likely related to sampling effort, the rotiferologist effect, where there is higher diversity in rotifer communities where there are rotiferologists working (Fontaneto et al., 2012; Ejsmont-Karabin, 2019). This difference in sampling effort between locales is well known and actively being addressed in the field for sites in Africa, although much of Congo and DRC remains poorly sampled (Smolak et al., 2023; Fresno Lopez et al., 2023; Smolak & Walsh, 2022). In the Antarctic, rotifer diversity has been noted to decrease with increasing absolute latitude (Fontaneto et al., 2015). Given this latitudinal gradient and the relatively high diversity in the subtropics, it may mean the rotifer diversity follows this pattern globally. Overall endemicity is rare in most rotifer species, however it is well known in certain genera such as *Keratella,* and several species have known geographic ranges (Segers, 2008). This is complicated by the fact that many currently recognized species are likely cryptic species complexes, and their endemicity may be hidden because of this. For example, members within the *Brachionus plicatilis* species complex have been shown to have unique phylogeographical patterns of distribution (Gómez et al., 2000; 2007; Mills, 2006; Mills et al., 2017). Differences in dispersal capability can affect the biogeography of rotifers. These differences may be due to differences in egg morphology or deposition habits, for example eggs with extensive ridges may entrain into wind to be transported via anemochory easier than smooth or untextured eggs (Pinceel et al., 2016). This would enable these rotifers to disperse more broadly and may possess less spatial influence on their distribution. Similarly, rotifers that deposit their eggs on macrophytes or other substrata may be more prone to zoochory if those substrates are consumed by a larger animal such as a dabbling duck (Soons et al., 2016). Rotifer diet and trophi type may influence their distribution as well, malleate and malleo-ramate trophi types are generalists on small particle sizes and may be widespride, whereas virgate and forcipate trophi types may limit species to locations where their prey exist, creating a more patchy distribution in these rotifers.

The Chihuahuan Desert is known for endemism of aquatic species (Sei et al., 2009; Seidel et al., 2009; Suarez-Morales & Walsh, 2009; Briggs et al., 2020), including several recently described rotifers including *Rhinoglena ovigera* (Segers & Walsh, 2017)*, Epiphanes chihuahuaensis* (Schröder & Walsh, 2007), and a yet to be described species of *Hexarthra* (Schröder et al., 2007). Of these rotifer species, all can be found in temporary systems such as rock pools and playas. Specialization of lifestyle is widespread in the Rotifera. Extreme examples are found in Superorder Gnesiotrocha which includes sessile and colonial species. Although these forms are more common in systems with longer hydroperiods and macrophyte beds, some such as *Lacinularia flosculosa* (Müller, 1773) and *Conochilus hippocrepis* (Schrank, 1803), are commonly found in playas within the Chihuahuan Desert (personal observation, colonies can be found regularly at Album Park, El Paso Co., TX).

Repeated occurrence of lifestyles originating within a group implies a selective advantage of these lifestyles (Morris, 2003). Despite this, several widespread lifestyles such as sessility and coloniality have ambiguous or highly debated benefits (Buss, 1981; Fenchel, 1986; Wallace, 1987; Christensen-Dalsgaard & Fenchel, 2003; Kiørboe, 2011a; Burgess et al., 2017; Dyrynda, 1986; Sebens, 1987; Vasisht & Dawar, 1970; Sutherland & Weihs, 2002; Wallace et al., 2015; Lürling, 2021). What are the potential adaptive advantages for organisms to possess these lifestyles? It is possible that they provide energetic advantages to organisms. In sessile organisms, feeding ability may be improved (Jonsson & Tiselius, 1990; Jiang et al., 2002; Christensen-Dalsgaard & Fenchel, 2003; Catton et al., 2007; Kiørboe, 2011b.) whereas in colonial organisms members may work together to feed more efficiently (Jonsson & Tiselius, 1990; Jiang et al., 2002; Christensen-Dalsgaard & Fenchel, 2003; Catton et al., 2007; Kiørboe, 2011b Mackie, 1986; Jakob, 1991; Pepper et al., 2013; Roper et al., 2013; Short et al., 2006; Solari et al., 2013).

Attachment to substrata is a lifestyle feature widespread throughout aquatic life. Disadvantages to attachment are numerous: an attached organism is not actively seeking better habitat and is subject to limited available habitat and competition from other attached organisms (Edmondson, 1945; Paine, 1966; Jackson, 1977; Buss, 1990; Harmsworth & Sleigh, 1993). Despite these disadvantages the repeated occurrence of sessile lifestyles throughout Eukaryota suggests that they incur a selective advantage. A clear advantage granted by this trait is the maintenance of position within the environment (Fenchel, 1986; Fenchel, 2001). Habitats can be heterogeneous, with some areas providing higher carbon content capable of supporting denser bacterial or algal populations than the surrounding habitat. This in turn creates patches with higher food content for heterotrophs (Bell & Mitchell, 1972; Bowen et al., 1993). However, many sessile organisms attach to substrata regardless of the surrounding food concentrations or chemical cues indicative of food (Christensen-Dalsgaard & Fenchel, 2003). In some non-metazoan eukaryotes with temporary attachment capabilities, starving cells will not attach but continue to swim, suggesting that swimming in these organisms may be primarily used to migrate to better feeding sites (Christensen-Dalsgaard & Fenchel, 2003). Considering this, attaching to a substratum to maintain access to a high food concentration does not appear to be sufficient to explain the prevalence of attachment to substrata found within the eukaryotes (Christensen-Dalsgaard & Fenchel, 2003).

Increase in feeding capability or efficiency is important in explaining substrata attachment in sessile organisms. A variety of flagellates, ciliates, and rotifers are capable of both feeding while swimming and feeding while attached to a substratum (flagellates (Fenchel, 1986), choanoflagellates (Roper, 2013; Pettitt et al., 2002), *Brachionus*, *Testudinella*, and bdelloid rotifers, among others (Wallace, 1987; Felix et al., 1995; Kirkegaard & Goldstein, 2016; Gilbert, 2019)). This behavior implies that attachment benefits feeding in some way. Empirical observations of attached versus swimming feeding demonstrate that attached organisms have much faster clearance rates than their swimming counterparts. In flagellates, the clearance rate can be up to 60% higher in attached relative to swimming individuals (Christensen-Dalsgaard & Fenchel, 2003). In rotifers, those with sessile adult stages often, but not always, have higher clearance rates than those that feed while swimming (Bogdan et al., 1980; Devetter, 2009; Gilbert & Jack, 1993; Ooms-Wilms et al., 1993; Rothhaupt, 1990; Wallace & Starkweather, 1983; 1985).

In general, feeding efficiency while attached to a substratum is higher than while swimming (Tiselius & Jonsson, 1990; Jiang et al., 2002; Christensen-Dalsgaard & Fenchel, 2003; Catton et al., 2007; Kiørboe, 2010). Experiments using tracer particles to detect the feeding flow field of copepods have demonstrated that feeding while swimming produces a relatively straight flow field, while attached or anchored feeding produces a cone-shaped flow field, passing a higher volume of water per unit of work done than while swimming (Catton et al., 2007; Kiørboe, 2010). The increased feeding efficiency of tethered or hovering current generators versus those that swim through the water with their currents is increased by a factor of approximately two (Christensen-Dalsgaard & Fenchel, 2003; Tiselius & Jonsson, 1990; Jiang et al., 2002). Metabolically, swimming in some organisms is known to be costlier than feeding while attached. In rotifers, swimming can cost up to 62% of their total metabolic expenditure (Epp & Lewis, 1984). Additionally, metabolic measurements of attached and swimming rotifers suggest that attached rotifers expend somewhere between 20 to 33% of the energy used by their swimming counterparts (Vadstein et al., 2012).

Swimming organisms must compensate for drag and shear produced while swimming, which can create a tradeoff between feeding and swimming efficiency. Large ciliated structures such as the arms found in echinoderm larvae and the ciliated lobes of the corona found in sessile rotifers such as *Floscularia* spp., *Octotrocha speciosa* Thorpe, 1893, *Pentatrocha gigantea* Segers & Shiel, 2008, and others can dramatically increase clearance rate. However, large structures such as these can create shear while moving that can erratically change the direction of swimming leading to ineffective or inefficient swimming. Evidence for this disadvantage can be seen in the morphological differences found between feeding and lecithotrophic pluteus larvae of echinoderms. In these larvae feeding forms often have large ciliated laterally projecting arms, whereas the non-feeding taxa usually have reduced lateral arms, posteriorly oriented arms, or lack them entirely (Strathman & Grünbaum, 2006). The anchoring mechanisms mentioned above likely generate similar issues with shear.

Sessile organisms can benefit from the flow of water relative to their substrata, passively bringing new food to the organism (Riisgård et al., 1998; Fréchette, 1989). Planktonic organisms cannot benefit from these currents because they are swept along relative to their food, and so relative to each other remain stationary. Passive diffusion feeders can benefit significantly from attachment in this manner, as increased flow can increase clearance rate, but may bias particle capture towards smaller particles at higher velocities (Shimeta & Jumars, 1991; Shimeta & Khoel, 1997; Allen, 1998; Larsson & Jonsson, 2006).

Attachment to a substratum not only increases an organism’s ability to feed but also relieves it of predation pressure from filter feeders and suspension feeders. At a basic level, a decreased swimming rate means a reduced encounter rate with potential predators. For predators that are sessile or attached to a substratum, their encounter rate depends on the movement of prey, the currents they generate and all passive currents available. Large filter feeders can process substantial volumes of water, and in small ponds can be significant predators on plankters such as rotifers, so escape from this sort of predation may be adaptive (Wong & Levington, 2005). Gape-limited predators, if unable to remove attached organisms from their substrata will also be unable to feed on them. Despite this, attached organisms may be easier for certain predators to locate, because attached surface areas provide a smaller total search area than the open water, and attached organisms may have little ability to escape once detected. Predation like this occurs from gastropods on encrusting bryozoans (Wood et al., 2006), nudibranchs on the polyp stage of cnidarians (Hernroth & Gröndahl, 1985), and predation by sea stars on mussels in the intertidal (Paine, 1966). Additionally for small, attached organisms, they may be subject to indiscriminate grazing from consumers of periphyton such as snails (Garcia, 2004).

Several levels of individual integration are found throughout the three domains of life. Haeckel recognized at least six different levels of individual integration found in organisms (Mackie, 1986). Eukaryotic cells represent a fusion of disparate species, with some organelles (e.g., mitochondria and chloroplasts) likely derived from phagocytosis and subsequent retention of various bacteria in ancient eukaryote ancestors (Gray et al., 1999; Timmis et al., 2004). Within the eumetazoans, aggregations of multicellular individuals representing different levels of integration are widespread, typically called colonies, although there is much debate over what terms apply to colonies, aggregates, social units, and other groups (Mackie, 1986).

Using the broadest available definition, a colony of organisms is an aggregation of organisms of a higher number than expected based on environmental factors (Buskirk, 1975). This definition of coloniality would exclude aggregations occurring due to limited resources or high-quality habitat patches. Many refinements and alterations of this definition exist, seemingly to limit or redefine which taxa are classified as “colonial” or not. Such extensions to this broad definition include requirements of high levels of integration (Rosen, 1979), organic connections through bodily contactor by secreted materials (Barrington, 1967). Occasionally taxa are mentioned as not fitting the authors' definition of coloniality, when in fact they do. For example, Blackstone & Jasker (2003) state that rotifers do not fit their definition of coloniality. However, rotifers that produce a shared gelatinous matrix such as species of *Lacinularia* and *Conochilus* would fit this definition of the term coloniality. Often in mobile animals, these aggregations are called a variety of terms sometimes dependent on the level of integration of the colony members. For instance, in insects (e.g., Isoptera, Hymenopter), some crustaceans (sponge shrimps), and some mammals (e.g., naked mole rats), the term eusocial is used for colonies in which there is a division of labor at the level of reproduction, or possibly reproductive altruism, in which some colony members forgo reproduction (Burda et al., 2000). Given this confusion over the terminology, it is necessary to define what is meant by coloniality. The definition used here will be an adaption of Wallace's definition for colonialism in rotifers: members of a colony must be in direct contact either bodily, by tubes, or by other secretion (Wallace, 1987). This definition, unfortunately, excludes eusocial and other forms of aggregation in motile organisms but works well when discussing most colonial aquatic organisms, and specifically rotifers.

Colonial organisms are subject to several characteristics that can negatively impact individual fitness of the colony members. Increased intraspecific competition and increased vulnerability to density-dependent mortality from sources such as disease make group living appear to be highly disadvantageous at first glance (Allee, 1931). Under more specific cases, other problems with coloniality can arise. For instance, in small organisms the increased size due to coloniality can make them more vulnerable to predation by visual predators such as fishes (Felix et al., 1995), or organisms using feeding currents may interfere inadvertently with each other’s currents, as models of bryozoan feeding have demonstrated (Grünbaum, 1995). Ideal free distribution theory would suggest that at most food concentrations animals would divide their access to food by being in a colony (Tregenza, 1995). If food concentration is not limiting, animals may benefit from coloniality by being able to exist at higher densities without interfering with one another’s feeding. Additionally, for colonies that attach to substrata there may be very limited available surface relative to food concentration (Fairchild, 1981); in these cases, coloniality may allow better use of space and not violate the ideal free distribution.

Despite these traits, coloniality is widespread in eumetazoans, notably occurring the cnidarians (corals, hydrozoans, siphonophores, etc.), bryozoans, annelids, mollusks examples), arthropods (certain barnacles), rotifers, chordates (tunicates, salps, and doliolids), and others. This widespread occurrence suggests that colonial or group living has an adaptive value that outweighs the costs it incurs. Lifetable studies in colonial rotifers have shown that colonies outperform single individuals in several metrics (Edmondson, 1945). Thus, several hypotheses exist to explain the possible adaptive properties colonial/multicellular living may have. Coloniality/multicellularity: 1) provides protection against predation (Allee, 1912; 1931; Bonner, 1988; Diéguez & Balseiro, 1998; Gilbert, 1980; Wallace,1980), 2) increases feeding efficiency (Edmondson, 1945), 3) helps in dispersal (Bonner, 1998), 4) allows for division of labor (Simpson et al., 2017), and 5) increases dominance in cases of interspecific competition (Buss, 1981). I will not address the fourth hypothesis, as for division of labor to occur such as in specialized zooids, cells or tissues, coloniality must be present, and so it is not a reasonable explanation of the adaptive value of group living.

For colonial organisms adding additional members to the colony increases colony size. Increased size can offer an adaptive advantage to organisms in the form of defense against predation. The ancestors of two major multicellular lineages, fungi, and animals, first appear in the fossil record shortly after the appearance of eukaryotic predators and a sharp rise in oxygen concentration (Rokas, 2008). This provides circumstantial evidence that strong selective pressure from predation may be what drove the origin of multicellular/colonial organisms. Similarly, the relatively low oxygen levels prior to the sharp rise may have been limiting the evolution of larger multicellular organisms, and only when this restraint was lifted, did they evolve multicellularity. Under experimental conditions, several species of algae are known to evolve multicellular lifestyles in the presence of a predator (Boraas et al., 1998; Kampe et al., 2007; Rokas, 2008).

Colonial organisms are capable of anti-predator behaviors and inter-member communication that allows for an early warning system for the other colony members (Mackie, 1986). Mechanisms of this response vary from taxon to taxon, but some examples include a nerve net used to retract the colony, lophophores in some ectoprocts (Thorpe et al., 1975); mechanical disturbance of retraction of one colony member stimulating the retraction of all members in the rotifer *Sinatherina socialis* (Linnaeus, 1758) (Wallace, 1987); and ciliary arrest in hexactinellid sponges conducted through their trabecular tissue (Mackie & Singla, 1983).

Coloniality may provide an energetic advantage in the form of increased feeding efficiency or reduced energy expenditure in other ways. In microscopic eukaryotes that generate feeding currents through ciliary or flagellar beating, models based on far-field flow suggest that colonial morphologies can increase flux, therefore, feeding efficiency (Roper et al., 2013; Short et al., 2006; Solari et al., 2013). In contrast to models based on far-field flow, those investigating short field flow suggest feeding efficacy is directly related to swimming efficiency. This implies that feeding is optimal for single individuals and colonial lifestyles must provide another selective advantage (Kirkegaard & Goldstein, 2016). The choanoflagellate *Salpingoeca rosetta* Dayel et al. 2011 can occur as unicells or colonies shaped as balls or chains. In *S. rosetta,* colonies form when food supply is plentiful, lending support to the idea that colonial habit does not improve feeding efficiency (Kirkegaard & Goldstein, 2016). Clearance rate in colonial rotifers does not appear to be significantly higher than in solitary rotifers. Using radioactively labeled food particles or latex microspheres, Wallace (1987) measured clearance rate of colonies of differing size in *Sinantherina socialis* (Linnæus, 1758) and *Conochilus* spp., and between solitary and colonial *Floscularia conifera* (Hudson, 1886) and solitary and colonial *S. socialis*. These experiments did not reveal significant differences in clearance rate (Wallace, 1987). Colonial bryozoans and rotifers are known to produce organized flow fields, including incurrent and excurrent plumes that may increase feeding efficiency (Banta et al., 1974, Wallace, 1987). Modeling of the hydrodynamics of bryozoan feeding currents suggests that the currents produced by individual zooids may interfere with one another, leading to a decreased feeding ability (Grünbaum, 1995). If this model holds true for living bryozoans, the organized feeding currents may reduce the negative impact of interference within the colony rather than representing a selective advantage of colonial lifestyle itself.

Some colony morphologies may harbor hydrodynamic advantages to colony members. Arborescent colony morphologies such as those in certain bryozoans, tube-building colonial rotifers, and some peritrich ciliates may offer a hydrodynamic advantage to feeding by reducing wall effects since some zooids will be elevated far from the substrata (Grünbaum, 1995). Additionally, colonial organisms may benefit hydrodynamically by coordinating alternative feeding and non-feeding in adjacent zooids to reduce boundary effects (Pepper et al., 2013).

Other means of achieving an energetic advantage in colonial organisms can be seen in salps and colonial pholcid spiders. Colonial salps are arranged linearly and swim with an asynchronous pumping action of the zooids. This pumping action leads to a consistent swimming speed, and the linear nature of the colony reduces drag per zooid, in contrast to individual salps whose pumping leads to erratic swimming (Mackie, 1986). Because salps filter feed as they swim, these advantages in swimming translate directly to increased feeding efficacy. The Siphonophores often have their nectophores in an analogous arrangement to the salp zooids, leading to the same benefits over free medusa that colonial salps possess over individuals. A few pholcid spiders form colonies where the young spiders remain in the web of older spiders and eat food caught in the web. In this case, each spiderling receives less food, because it is competing against the other spiders in the colony for each catch but saves energy on producing the web; web production by a spiderling is known to be energetically expensive to produce (equated to the energy content of nine fruit flies) (Jakob, 1991). In this case, coloniality would be favored by selection so long as the energy saved through decreased web production outweighs the energy from food lost by competition with colony members (Jakob, 1991).

Rotifers display diversity with regards to coloniality, sedentary, and solitary lifestyles, with instances of colonial and solitary taxa appearing within several genera (Wallace, 1987). For example, *Floscularia ringens* (Linaeus, 1758) rarely forms colonies of more than a few individuals, whereas *Floscularia conifera* (Hudson, 1886) can form colonies of a variety of sizes (Wallace, 1987). Much of this diversity is found within the superorder Gnesiotrocha, the only rotiferan clade with facultatively sessile and colonial representatives. The diversity of lifestyles present within this clade makes the gnesiotrochan rotifers a good model for the study of lifestyle evolution.

Phylum Rotifera is a moderately diverse phylum comprised of ~2000 species of small (~50–2000 µm), free-living and parasitic invertebrates (Segers, 2007), although this number may rise considerably as cryptic species are expected to be common within the group (Gomez et al., 2002; Leasi et al., 2013; Kimpel et al., 2015; Kordbacheh et al., 2017; Mills et al., 2017; Obertegger et al., 2014; Schröder & Walsh, 2007; Walsh et al., 2009). The clade Syndermata (also Rotifera *sensu latu*) contains four clades, the Seisonidea, a group of epizootic symbionts, the Bdelloidea, a clade of obligate parthenogens with a unique dormant stage, the Acanthocephala, a group of internal parasites, and the Eurotatoria (See figure 1 for examples of species from this clad), which contains the superorders Ploima and Gnesiotrocha (Herlyn et al., 2003). The relationships among these groups is ambiguous, with gene order and transcriptomic methods supporting a grouping of seisonids and acanthocephalans (Pararotaria) sister to the Bdelloidea (Hemirotaria), whereas other sequencing methods had often confounding results (Bininda-Edmonds 2021; García-Varela & Nadler 2006; Gazi et al., 2016; Sielaf et al., 2015; Wallace et al., 2023; Wey-Fabrizius et al., 2014). Rotifers are typified as possessing an anterior region of ciliated lobes called the corona, which is used in both locomotion and feeding, and muscular pharynx, the mastax, which contains a complex jaw like structure used in processing food, the trophi (Wallace, 2002). Rotifers are typically microphagous, free-swimming members of the plankton, epiphyton, or benthos (Wallace, 2002). Exceptions to microphagy occur in the superorders Ploima and Gnesiotrocha, in which feeding on larger particle sizes, such as predation on other rotifers or small invertebrates occurs in *Asplanchna*, *Synchaeta,* and the members of Collothecaceae. Coloniality only occurs in the order Flosculariaceae, although there is at least one bdelloid, *Philodina megalotrocha* Ehrenberg, 1832, known to form temporary aggregations (Burwitz, 1977).

Figure 1. Examples from Eurotaria. A) *Plationus patulus*, a free-swimming ploimid rotifer, B) *Hexarthra* spp., a free swimming gnesiotrochan rotifer, and C) *Sinantherina socialis*, a sessile, colonial gnesiotrochan rotifer. Images courtesy of E.J. Walsh.

The order Collothecaceae is comprised mostly of rotifers with sessile adult forms, and although there are planktonic species of *Collotheca* they do not actively swim as coronate rotifers (Wallace et al., 1998). Larvae in this order swim with their coronal cilia as other rotifers, although they do not appear to use their corona to feed (Wallace, 1993). These swimming larvae seek out appropriate substrata to settle on, and once attach undergo “drastic metamorphosis” in which their corona is replaced with a new organ called the infundibulum (Hochberg; 2014; Hochberg & Hochberg, 2011; 2017; Hochberg et al., 2019; Wallace, 1980). The infundibulum may either bear setae utilized in the capture of large particles or prey as in the genera *Collotheca* and *Stephanoceros* or may form a hood which is used to enclose prey as in the genera *Cupelopagis* and *Acyclus*. The genus *Cupelopagis* is known to respond to vibrations in the water caused by prey, in which the animal can then rotate on its pedal foot to face and capture prey by enveloping them within the infundibulum (Bevington et al., 1995; Koste, 1973; Vasisht & Dawar, 1969; Preza et al., 2020). *Collotheca ferox* (Penard, 1914) may also use its infundibulum in the same manner as *Cupelopagis*, as its orientation relative to the substrata is similar to *Cupelopagis*, and it lacks the long setae found in *Collotheca* (Meksuwan et al., 2013). Substratum preference is also documented among these rotifers (Edmondson, 1944; Wallace & Edmondson, 1986; Pejler & Bērziņŝ, 1993).

The order Flosculariaceae has both free swimming and sessile members. The free-swimming members are in the families Testudinellidae, Hexarthridae, and Trochosphaeridae. The Testudinellidae are a family of loricate rotifers that are periphytic and use their pedal gland to affix themselves to substrata while feeding before detaching and swimming elsewhere. The family Hexarthridae possess appendages utilized in escape before analogous to what is seen in the ploimid genera *Polyarthra*, although the nature of these appendages is distinctly different, being feather-like and controlled by indirect musculature in the *Polyarthra* and thicker and controlled by direct musculature in the *Hexarthra* (Hochberg & Gurbuz, 2008). Some *Hexarthra* are known to be specialists in extremely ephemeral waters, in which they deviate from the typical rotifer life cycle of switching between amixis and mixis and are born capable of mictic reproduction (Schröder et al., 2007). The genus *Filinia* of the Trochosphaeridae sometimes possess long spines that can be moved via muscular action (unlike those in some loricate rotifers such as *Keratella* and *Brachionus*) that may be used as a defense against predation (Gilbert & Stemberger, 1984; Hochberg & Gurbuz, 2007; Stemberger & Gilbert, 1984).

Within the Rotifera, most work concerning lifestyle evolution has taken place in a relatively small number of model species, *Adineta vaga* (Davis, 1873), a bdelloid, and *Brachionus plicatilis* Müller, 1786, a monogonont, within the Ploima. These studies have focused on the basic life history and lifestyle, evolution of sexuality, and desiccation (for Bdelloidea) (Carmona et al., 2009; Denekamp et al., 2010; Flot et al., 2013; 2016; Mark Welch et al., 2009; Ricci et al., 1987; Serra et al., 2005; Signorovitch et al., 2016; Stelzer, 2005).

The proclivity with which gnesiotrochans express both colonial and solitary lifestyles indicates that there may be underlying characteristics that predispose them to evolve these lifestyles. The adaptive benefits of colonial over solitary lifestyle in rotifers has been explored by Wallace (1987). He puts forward two possible explanations for the evolution of colonialism within the clade, predatory avoidance (Garcia, 2004; Gilbert, 1980; Felix et al., 1995; Wallace; 1980; 1987; Walsh et al., 2006) or an energetic advantage gained by colonialism (Edmondson, 1945; Wallace, 1977; 1980; 1987). These hypotheses may not be mutually exclusive. One possible way that colonial rotifers may display an energetic advantage is by alterations to their metabolism. Colonies in other invertebrate phyla are known to possess metabolisms that scale allometrically with colony size in loose accordance with Klieber's law, that colony metabolism scales to the ¾ power as colony size increases, so that individual metabolism decreases per animal (Burgess et al., 2017). If found to occur rotifers it may be evidence that colonies allow for each individual animal to expend less energy living and growing, leading to an energetic advantage. The mechanisms behind allometric scaling of respiration rate and colony size are not well understood and are largely based upon extrapolating patterns of allometric scaling in non-colonial animals to colonial animals (Burgess et al., 2017; Hartikainen et al., 2014).

Metabolism is known to be tied to genome size, described by the metabolic rate hypothesis of genome size (Gregory, 2001; Hughes & Hughes, 1995). As metabolic rates increase in animals, genome size appears to be constrained. This pattern is well supported in some vertebrates, particularly birds but less clear in other groups (Alfsnes et al., 2017; Gregory, 2001; Gregory & Hebert, 2003; Hughes & Hughes, 1995). The exact mechanisms behind this pattern are unclear, but appears to at least be in part due to a cytogenic effect where cell size is directly related to the amount of DNA within a cell (Gregory, 2001). Genome size is heritable, and associated with basic biology such as cell size, metabolism, life cycle speed through the cell cycle. These properties may allow for genome size to be used as a quantifiable trait to investigate lifestyle. As such genome size may be another avenue to investigate patterns in the evolution of coloniality and lifestyle in these animals.

I addressed the metacommunity ecology and diversity of Rotifera as well as lifestyle evolution in this group by 1) analyzing patterns of rotifer diversity and community assembly in the Chihuahuan Desert and 2) determining how lifestyle relates to fundamental biological traits, including metabolism and genome size, with a goal of understanding whether coloniality imparts an energetic advantage. These topics are discussed through three aims arranged into chapters: 1) Patterns of rotifer diversity, 2) Genome size and lifestyle, and 3) Allometric scaling of metabolism with colony size in rotifers.

CHAPTER 1: PATTERNS OF ROTIFER DIVERSITY

Published in part as Brown, Patrick D., Thomas Schröder, Judith V. Ríos-Arana, Roberto Rico-Martinez, Marcelo Silva-Briano, Robert L. Wallace, and Elizabeth J. Walsh. "Patterns of rotifer diversity in the Chihuahuan Desert." Diversity 12, no. 10 (2020): 393.

Contribution:

In part with the coauthors: Conceptualization, validation, original draft preparation, formal analysis and editing.Formal analysis: Sampling effort, Indicator species analysis, Diversity indices, Mantel tests, Kriging.

and

Brown, P. D., Thomas Schröder, Judith. V. Ríos-Arana, Roberto Rico-Martinez, Marcelo Silva-Briano, Robert L. Wallace, and Elizabeth J. Walsh. "Processes contributing to rotifer community assembly in shallow temporary aridland waters." Hydrobiologia 849, no. 17-18 (2022): 3719-3735.

Contribution

In part with coauthors: Validation, formal analysis, original draft preparation, writing-review and editing.

Formal analysis: Species area relationships, pCCA using dbMEMs, regression analysis, DNCI.

INTRODUCTION

Inland waters, and in particular those of arid regions, can be thought of as reverse island systems, in that they are highly fragmented and isolated from one another with limited dispersal between sites (Murphy et al., 2015). This makes them ideal candidates for studying species assembly and diversity. Few groups are ubiquitous in these waters, including Branchiopoda, Copepoda, Rotifera, and ciliates. Animals of inland waters can either passively disperse via anemochory, hydrochory, or zoochory or be active dispersers, flying or traveling over land to aquatic habitats. Of these ciliates are underdescribed, with nearly 80% of species estimated to lack descriptions (Foissner et al., 2007). Rotifers however are present in most of these water bodies and have a moderate (but likely under-described) diversity of ~2000 species (Segers, 2007). Waterbodies in xeric habitats are further isolated from one another so organisms living therein may be less reliant on hydrochory for dispersal (Kobayashi et al., 2015; Stevens & Meretsky, 2008). The Chihuahuan Desert is an arid desert with many temporary and permanent water bodies scattered throughout its expanse (Wallace et al., 2005; 2008; Walsh et al.; 2008). Over its north to south range, the Chihuahuan Desert displays characteristics of both hot deserts in its Southern part in accordance with the Köppen climate classification (Peel et al., 2007). Additionally, despite being an arid region, the Chihuahuan Desert is recognized not only for its terrestrial biodiversity but also its aquatic (Dinerstein et al., 2000). This makes rotifers an ideal group to study community assembly in these habitats. The Chihuahuan Desert is a young desert that has recently undergone/is undergoing climatic change bringing about increased aridification (Ezcurra et al., 2020). This aridification has led to many of aquatic habitats becoming more isolated, and creating many temporary habitats that are wet for only a portion of each year. Some of these temporary systems, called playas can vary substantially in their salinity and when dry, produce dust that is spread by wind, potentially allowing for aeolian transport of propagules (Rivas, 2019, Rivas et al., 2018; 2019). There have been interesting species found in sites with relatively short hydroperiods, such as the rotifers *Hexarthra* sp. which has a unique life cycle (Schröder et al., 2007) as well as *Epiphanes chihuahuansis (*Schröder & Walsh, 2007*).* The undescribed *Hexarthra* species is capable of immediately undergoing mixis upon hatching from their resting eggs, all other known rotifers must go through at least one amictic generation before being capable of mixis. The very short hydroperiods of rock pools may have been selected for this shortened life cycle to ensure that diapausing eggs can be laid before the rock pool dries.

There has been substantial work on aquatic invertebrates of the Chihuahuan Desert, and for many species including rotifers endemism is known in the region (Mackay et al., 1990; Schröder et al., 2007; Sei et al., 2008; Seidel et al., 2009; Suárez-Morales et al., 2010; Wallace et al., 2005; 2008; Walsh et al., 2008; 2009; 2014). Despite this there is a lack of understanding in how these organisms are distributed and how communities in these aquatic habitats are structured regionally.

To investigate general patterns of rotifer diversity across the Chihuahuan Desert, I used a dataset spanning >20 years across 236 aquatic sites. In addition to investigating the patterns of diversity across the region I also looked at how scale impacts these patterns. Additionally, I investigated alpha (site richness), beta (species turnover), and gamma (regional diversity) across these sites. I also investigated the nestedness of the rotifer communities. This work is published in Brown et al. (2020), “Patterns of rotifer diversity in the Chihuahuan Desert.”

To learn more about how rotifer communities are structured I analyzed a subset of sites from the Chihuahuan Desert, focusing on temporary habitats. I estimated the influence of stochastic versus deterministic dispersal processes and the influence of environmental parameters in structuring rotifer community assemblies in this ecoregion. This work is published in Brown et al. (2022), “Processes contributing to rotifer community assembly in temporary aridland waters”.

PATTERNS OF ROTIFER DIVERSITY IN THE CHIHUAHUAN DESERT

Patrick D. Brown [1], Thomas Schröder [1], Judith V. Ríos-Arana [2], Roberto Rico-Martinez [3], Marcelo Silva-Briano [3], Robert L. Wallace [4] and Elizabeth J. Walsh [1,*]

[1] Department of Biological Sciences, University of Texas at El Paso, 500 West University Avenue, El Paso, Texas, 79968, USA; pdbrown3@miners.utep.edu (P.D.B.); th.schroeder@gmx.net (T.S.)

[2] Departamento de Ciencias Químico Biológicas, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Av. Benjamin Franklin no. 4650, Zona PRONAF, Cd. Juárez, 32315, Chihuahua, Mexico; jrios@uacj.mx

[3] Universidad Autónoma de Aguascalientes, Centro de Ciencias Basicas, Departamentos de Química y Biología, Avenida Universidad 940, Ciudad Universitaria, C.P., 20131, Aguascalientes, Ags., Mexico; roberto.rico@edu.uaa.mx (R.R.-M.); msilva@correo.uaa.mx (M.S.-B.)

[4] Department of Biology, 300 Seward St., Ripon College, Ripon, WI, 54971, USA; wallacer@ripon.edu

***** Correspondence: ewalsh@utep.edu; Tel.: +01 (915) 747-5421

Received: 28 August 2020; Accepted: 07 October 2020; Published: date

ABSTRACT:

Desert aquatic systems are widely separated, lack hydrologic connections, and are subject to drought. However, they provide unique settings to investigate distributional patterns of micrometazoans, including rotifers. Thus, to understand rotifer biodiversity we sampled 236 sites across an array of habitats including rock pools, springs, tanks, flowing waters, playas, lakes, and reservoirs in the Chihuahuan Desert of the USA (n = 202) and Mexico (n = 34) over a period of >20 years. This allowed us to calculate diversity indices and examine geographic patterns in rotifer community composition. Of ~1850 recognized rotifer species, we recorded 246 taxa (~13%), with greatest diversity in springs (n = 175), lakes (n = 112), and rock pools (n = 72). Sampling effort was positively related to observed richness in springs, lakes, rivers, and tanks. Nestedness analyses indicated that rotifers in these sites, and most subsets thereof, were highly nested (support from 4 null models). Distance was positively correlated with species composition dissimilarity on small spatial scales. We predicted species richness for unsampled locations using empirical Bayesian kriging. These findings provide a better understanding of regional rotifer diversity in aridlands and provide information on potential biodiversity hotspots for aquatic scientists and resource managers.

INTRODUCTION

Delineating patterns of species distributions is important for understanding basic and applied questions in biogeography, ecology, and evolutionary biology (Emerson & Gillespie, 2008; Leibold et al., 2010). Species distributions can be used in modeling current communities and in predicting outcomes to both short-term (e.g., acute pollution episodes) and long-term events (e.g., increases in temperature due to climate change). They also inform biogeography and macroecology (Guizan & Rahbek, 2011). Unfortunately, the biogeographic patterns of many small and understudied species have not been well-documented. As members of the Syndermata, rotifers offer a good example of this challenge. While they comprise an important component of freshwater ecosystems and contribute to both the microbial loop and typical aquatic food webs, it is unclear whether their distribution follows ubiquity theory (Fenchel & Finlay, 2004; Fontaneto, 2019), or whether they exhibit some level of endemicity (Dumont, 1983; Segers, 2003; Segers & Shiel, 2003; Velasco-Castrillón et al., 2014). Due to their ability to produce small resting stages that are easily transported by hydrochory (Ning et al., 2012), zoochory (Frisch et al., 2007; Vanchoenwinkel et al., 2011), or anemochory (Rivas et al., 2018; 2019), it has been assumed that most rotifers were widely dispersed by passive means and that the majority of species would have cosmopolitan distributions (Dumont, 1983; Jocqué 2007a; Rousselet 1909). However, recent studies have shown that the distribution of rotifer species encompasses the range from cosmopolitanism to biogeographies that are restricted to certain biogeographic realms, hotspots of biodiversity (Segers, 2008b; Segers & De Smet, 2008; Segers & Shiel, 2003), or habitat types (Fontaneto, 2019; Segers, 2008a; 2008b). Two examples illustrate this point. (1) In his analysis of the genus *Trichocerca*, Segers (Segers, 2003) concluded that strict cosmopolitanism was evident in >1/3rd of the species analyzed, endemism was lacking in tropical regions but that it was strongly evident in the Northern hemisphere, and latitudinal variation was evident in >25% of the species. (2) Segers and De Smet (Segers & De Smet, 2008) grouped species of *Keratella* into four categories: cosmopolitans (n = 8), Holarctic (n = 5), widespread (n = 3), and regional and local endemics, with seven subcategories: Afrotropical (n = 2), Australian (n = 6), Nearctic (n = 8), Neotropical (n = 8), Oriental (n = 2), Palearctic (n = 6), and marine (n = 5). To distinguish between the opposing views of cosmopolitanism versus endemism, additional studies are needed of larger geographic regions, with repeated sampling.

Since deserts contain waterbodies that are often widely separated, highly fragmented, possess limited hydrologic connections, and subject to unpredictable drought (Walsh et al., 2014; Murphy et al., 2015; Sada et al., 2005), they are ideal systems to determine patterns in aquatic species distributions. However, within a basin, assemblages of aquatic habitats can be quite complex. For example, a series of spring-fed pools can lead to a stream, each with its own edaphic conditions, that support a substantial number of species (Sada et al., 2005); both can be hotspots of aquatic biodiversity, but maintain different arrays of species. Deserts also are considered ecological paradoxes. While generally low in terrestrial productivity, their varied habitats support striking levels of taxonomic diversity, often with a high degree of endemism. The Chihuahuan Desert of Mexico and the southwest USA is a prime example of such a system. This desert is a complex of intergrading plant communities arrayed across a broad series of elevational and latitudinal sequences (Hendrikson & Johnston, 1986). It covers some 6.29×10^5 km^2, largely in the central Mexican plateau, but extending northward into west Texas, south-central New Mexico, and the southeastern Arizon. This well-defined ecoregion is the only desert system included in The Global 200 conservation priority listing as being recognized for its critical biodiversity values for both terrestrial and freshwater habitats (Olsen & Dinerstein, 1998).

An analysis specific to the Chihuahuan Desert (Dinerstein et al., 2000) has designated 98 specific habitats or localities as priority sites for investigation and evaluation with respect to biodiversity resources; 37 are freshwater habitats. Of these, the highest priorities are assigned to systems with high intactness and high richness and/or endemism. An important array of these freshwater habitats is found in an arc from Big Bend National Park (BIBE, Texas) into Mexico, with the priority sites falling largely along the western boundary of the Sierra Madre Occidental, but extending as far south as the state of Hidalgo. A particularly important locality is the renowned Cuatro Ciénegas thermal spring system in Coahuila, perhaps the most studied of all Chihuahuan Desert aquatic systems (Hershler, 1985; Hershler et al., 1999; Minckley, 1968; 1978). This system of thermal springs, marshes, rivers, and large permanent lakes is home to a diversity of aquatic and mesic habitats that supports high levels of endemism in aquatic species (Minckley, 1969; 1978; Taylor, 1966). Chihuahuan Desert springs and other water sources are recognized as sites of high biodiversity with high rates of endemism of macroinvertebrates, especially springsnails (Hershler et al., 2011; Stanislawczyk et al., 2018). To complement that knowledge, more attention should be given to aquatic microinvertebrates of these systems.

While some aquatic sites in these deserts are relatively permanent over geologic time (playas and rivers), others are ephemeral over ecologic time (wet seasonally, monthly, weekly, even daily). Hydroregime (i.e., the duration, frequency, and timing of wet phases) is an important indicator of species richness, with increasing species diversity positively correlated with length of the filling cycle (Kneitel, 2014; Ripley & Simovich, 2008; Serrano & Fahd, 2005). Connectivity among sites is also an important consideration, as connected sites will likely share large portions of their species pools. In the Chihuahuan Desert, connectivity among sites in different drainage basins is reduced by vast stretches of arid landscape (Stevens & Meretsky, 2008). Thus, system isolation may be a driving force in speciation and endemism. This certainly seems to hold true for fishes (Carson & Dowling, 2005; Hurt & Hedrick, 2004; Kodric-Brown & Brown, 2007; Tobler & Carson, 2010), springsnails (Hershler et al., 2002; 2005; 2007; Johnson & Age, 2005; Moline et al., 2004), and amphipods (Adams et al., 2018; Gervasio et al., 2004). In addition, communities may be structured through recent processes such as local and regional interactions (competition and dispersal) (Cottenie et al., 2003; Mouquet & Loreau, 2003; Ricklefs, 2004), habitat permanence (Jocque et al., 2007a; 2007b), or local physiochemical conditions (Cottenie et al., 2003).

Prior to our work (Wallace et al., 2005; 2008; Walsh et al., 2007; 2008; 2009), there were few surveys of rotifers in the Chihuahuan Desert, with some notable exceptions. These mostly focused on smaller geographic areas and shorter time scales (Kubly, 1992; Mackay et al., 1990; Örstan, 1995; Rico-Martínez et al., 1993; Sarma, 1999; 2000). However, there have been numerous studies of rotifers from deserts and aridlands of the world, but in general, they have been limited to reports of species composition in specific habitats. These studies include the following: Sonoran (Örstan, 1995; Kubly, 1992; Hart et al., 1998; Kuperman et al., 2002; Riedel & Costa-Pierce, 2005; Tiffany et al., 2002; Walker, 1961), Algeria (De Ridder, 1991), Australia (Furst et al., 2014; Koste et al., 1983; Segers & Shiel, 2008; Shiel & Koste, 1992; Koste & Shiel, 1986; 1987; 1989a; 1989b; 1990a; 1990b; 1991; 1993), Kalahari (Brain & Shiel, 1995), Namib (Brain & Koste, 1993), Oman, Saudi Arabia, and Yemen (Segers & Dumont, 1993), Spain (Mazuelos et al., 1993), and Western Sahara (Dumont & Coussement, 1976). The semi-arid regions in Mongolia also have been studied by several researchers (Jersebek & Bolorsetseg, 2010).

Here we characterized patterns of rotifer species distribution in 236 aquatic systems that we sampled through a broad range of the Chihuahuan Desert. As appropriate to the system, we sampled the water column, sediments, and littoral vegetation during a period of ≥20 years. As part of our study, we tested the following hypotheses: (1) recovered richness will be positively correlated with sampling effort, (2) species are associated with particular habitats, (3) species composition will show nestedness, and (4) richness and assemblage composition possess a geographic pattern. In addition, using our dataset, we employed empirical Bayesian kriging to predict rotifer diversity across unsampled locations within the Chihuahuan Desert. Finally, we compared our results with those from five other desert systems and six studies from cool, temperate, and tropical systems. Our findings and analyses will help identify areas with high conservation value for zooplankton, including rotifers and add to our understanding of rotifer biogeography on a regional scale. They also inform the Baas-Becking (ubiquity) hypothesis in providing an indirect test of the assumption that for microinvertebrates, everything is everywhere (Fenchel & Finlay, 2004; Fontaneto, 2019).

MATERIALS AND METHODS

Collection Sites

We collected samples from 236 sites, 202 USA and 34 Mexico during 1998–2020 (Figure S1; Appendix). We sampled a variety of habitats including permanent lakes and reservoirs (n = 21), tanks (n = 11), temporary playas (n = 16), rock pools (n = 60) and artificial rock pools (n = 6), rivers and streams (n = 15), and springs (n = 95). Sampling effort varied among the sites from 1 visit to >20 visits; frequencies were used as ranks (1 = 1 sampling event; 2 = 2–5 events; 3 = 6–10 events; 4 = 11–20 events; 5 = >20 events), and at some sites only one type of sample was taken (e.g., plankton), while at others a variety of microhabitats were sampled. We compiled species lists at each site overall sampling dates using presence/absence criteria.

We described the sites at Big Bend National Park (BIBE) (Brewster Co., TX) in our previous work (Wallace et al., 2005; Walsh et al., 2004; 2007). General characteristics for rock pools sites at Hueco Tanks State Park & Historic Site (HTSPHS) (El Paso Co., Texas) were provided by Schröder and colleagues (Schröder et al., 2007) and springs in northern Mexico were described in detail by Ríos-Arana and colleagues (Ríos-Arana et al., 2019).

Sampling techniques included using plankton nets (64 µm), aspirating samplers for flocculent bottom sediments, as well as taking grab samples (i.e., aquatic macrophytes for sessile species) (Wallace et al., 2005; Walsh et al., 2007). We did not sample hyporheic habitats. The equipment was cleaned using distilled water rinses and, whenever possible, dried between uses in different systems. Although we usually took multiple samples at each site, we attempted to minimize environmental damage of the smaller systems by keeping the total amount of each sample to about 250 mL of source water. We recorded GPS coordinates using a Brunton Multi-Navigator® and used Google Earth to verify locations.

Species Identification

We identified morphospecies of rotifers (hereafter, species) primarily from live material using a Zeiss Axioscope with Neofluar objectives equipped with DIC, but when necessary, some specimens were preserved in 4% buffered formalin to view key taxonomic characters. For example, specimens of *Lecane* and *Lepadella* were fixed to view characteristics of the lorica, and in some cases trophi were examined using SEM. Keys to the Rotifera used in this study were as follows: Bdelloidea— (Donner, 2000; Koste & Shiel, 1986; Ricci & Melone, 2000); Monogononta—(Bērziņš, 1951; De Smet, 1996; Edmondson, 1949; 1959; Elliot & Rutner- Kolisko, 1976; Koste, 1978; Nogrady & Segers, 2002; Nogrady et al., 1995; Segers, 1995a, 1995b; Stemberger, 1979; Wallace et al., 2006). We identified taxa to species or, if that was not possible, to genus: e.g., *Lecane* sp. We conducted all of the analyses using the lowest level of identification that we determined. For most specimens, we took voucher images with a SPOT camera and, when possible, voucher specimens were preserved in 70% ethanol and/or 4% buffered formalin. We housed all voucher specimens in UTEP’s Biodiversity Collections.

Diversity Indices

To assess diversity of sites we calculated Hill numbers (q) of order 0 (richness, *S*), 1 (Shannon Index), and 2 (Simpson Index), and Sorensen’s Index (SI). Species incidence was characterized at a variety of spatial grains by overlaying 0.1°, 0.25°, 1.0°, 1.25°, and 2.0° grids on the site map. We calculated incidence within these grids cell from presence/absence data from each collection site occurring within the boundaries of the grid cell.

Sampling Effort

We tested the relationship between species richness and sampling effort using linear regression in R version 4.0.2 (R Core Team, 2020) for all sites combined, as well as for each habitat type separately.

Indicator Species Identification

We determined indicator species for habitat types by testing for significant associations using the indicspecies package 1.7.8 version in R version 4.0.2 (R Core Team, 2020; https://cran.r-project.org/web/packages/indicspecies/indicspecies.pdf). This analysis calculates an Indicator Value (IndVal) index to measure the association between species and sites and combinations of sites based on the methods of Dufrêne and Legendre (Dufrêne & Legendre, 1997) and De Caceres & Legendre (2009). The statistical significance is determined by permutation tests (n = 999).

Nestedness

We tested the hypothesis that smaller assemblages of rotifers are nested subsets of larger assemblages based on the habitats in which they are found by using the algorithms implemented in ANINHADO 3.0 (Bangu) (Almeida-Neto et al., 2008; Atmar & Patterson, 1993; Guimarães & Guimarães, 2006). In this program, the matrix is rearranged (packed) to achieve the densest grouping of species in the habitats (Ulrich et al., 2009). We employed both the Temperature calculator ($T°$) and nestedness metrics based on overlap and decreasing fill (NODF) (Almerida-Neto et al., 2008), but because the packing is only marginally different, here we report $T°$. We tested all packed matrices using the 4 null models described by Guimarães & Guimarães (Guimarães & Guimarães, 2006). For comparison purposes we also included a meta-analysis of 11 published datasets of rotifers from other biomes including aridlands (n = 5), cold (n = 2), temperate (n = 2), and tropical regions (n = 2). In our previous nestedness study (Ríos-Arana et al., 2019) we determined species or habitats to be idiosyncratic when their individual *T*° was ≥1 SD than the mean of the matrix *T*°. Since species and site *T*° often exhibit large variance, we decided to employ a more rigorous criterion, and here we note idiosyncratic species or habitats when their value is ≥2 SD of the mean of matrix *T*°.

Relationship between Species Richness and Geographic Distance

To determine whether distances between sites were contributing to differences in species composition, we conducted Mantel tests. Geographic distances between sites were estimated using Haversine distances based on GPS coordinates using the R package *geosphere* 1.5-10 (Hijmans, 2019). Bray-Curtis dissimilarity matrices of species composition were constructed using the *vegdist* function from the R package *vegan* 2.5-6 (Oksanen et al., 2020). We used Mantel tests, based on Spearman rank correlations, to determine whether species composition was related to (1) geographic distances between collection sites, (2) spatial scale (e.g., grid cells size), and/or (3) habitat type.

Prediction of Biodiversity Hotspots

Based on our survey data, we estimated richness throughout the Chihuahuan Desert using empirical Bayesian kriging (Krivoruchko, 2012). Using kriging as a method to predict species richness in unsampled areas has the benefit of illustrating general trends in richness across broad geographic regions. This process uses a probabilistic predictor that models spatial dependence with functions (i.e., semivariograms). A semivariogram model was estimated from the species richness data we obtained in our surveys, and then used that estimate to simulate the richness in unsampled geographic areas. From these newly simulated data, another semivariogram was estimated and evaluated against previous models using Bayes' rule. This process was iterated (n = 100) and the simulated data were used to predict richness at unsampled locations. Richness values were log-empirically transformed (a multiplicative skewing normal score approximation based on the log of our survey richness data) prior to semivariogram fitting. This process ensures that negative richness values are not predicted. Kriging was conducted on species richness at each site and for each grain size.

RESULTS

Species Composition

We identified 246 rotifer species, which represents a substantial portion of known rotifer species, genera, and families (~13, 50 & 77%, respectively) (Segers, 2007; 2008). Given that the Chihuahuan Desert comprises only about 0.35% of the global landmass (excluding the poles), it includes a large percentage of known rotifer biodiversity. Species richness ranged from 1 to 44 at a given locality. The site with the highest richness was Laguna Prieta at HTSPHS (S = 44). This site was sampled >20 times during this study. The site with the second highest richness was Lago Colina located in Chihuahua, Mexico MX (S = 43), but this site was sampled only four times over a 2-year period. Species found in all habitat types (except rock pools) include

Brachionus quadridentatus, Cephalodella catellina, Cephalodella forficula, Cephalodella gibba, Colurella obtusa, Euchlanis dilatata, Lecane bulla, Lecane hamata, Lecane luna, and *Platyias quadricornis*. *Lecane quadridentata* was found in all habitats except streams.

Diversity Indices

Of the five most common habitat types, springs had the highest richness (S = 175) while rock pools had the lowest (S = 53) (Figure 2A). Former cattle tanks also exhibited relatively low diversity (S = 53). In the few rivers (2 rivers, 26 sites) and streams (5 streams, 7 sites), sampled richness was 95 and 26, respectively. When compared to all other sites, springs also had the highest percentage of unique species (34.3%), followed by lakes and tanks (10.5%), playas (9.1%) and finally rock pools (5.7%) (Table 1). For these systems, Sorensen's Index ranged from 0.36 to 0.54, and most habitats share about 40% of their species (Table 1) with springs and lakes having the most divergent rotifer species communities. Diversity was highest at the largest spatial scale investigated, with the mean diversity for cells at the largest grid size being 48, 35, 27 for q = 0, 1, and 2, respectively. Diversity found for q = 0, 1, and 2 increased at higher spatial grains (r^2 = 0.16, 0.15, 0.12, respectively; p-value < 0.05 for each; Figure). The strength of this relationship decreased with increasing Hill number.

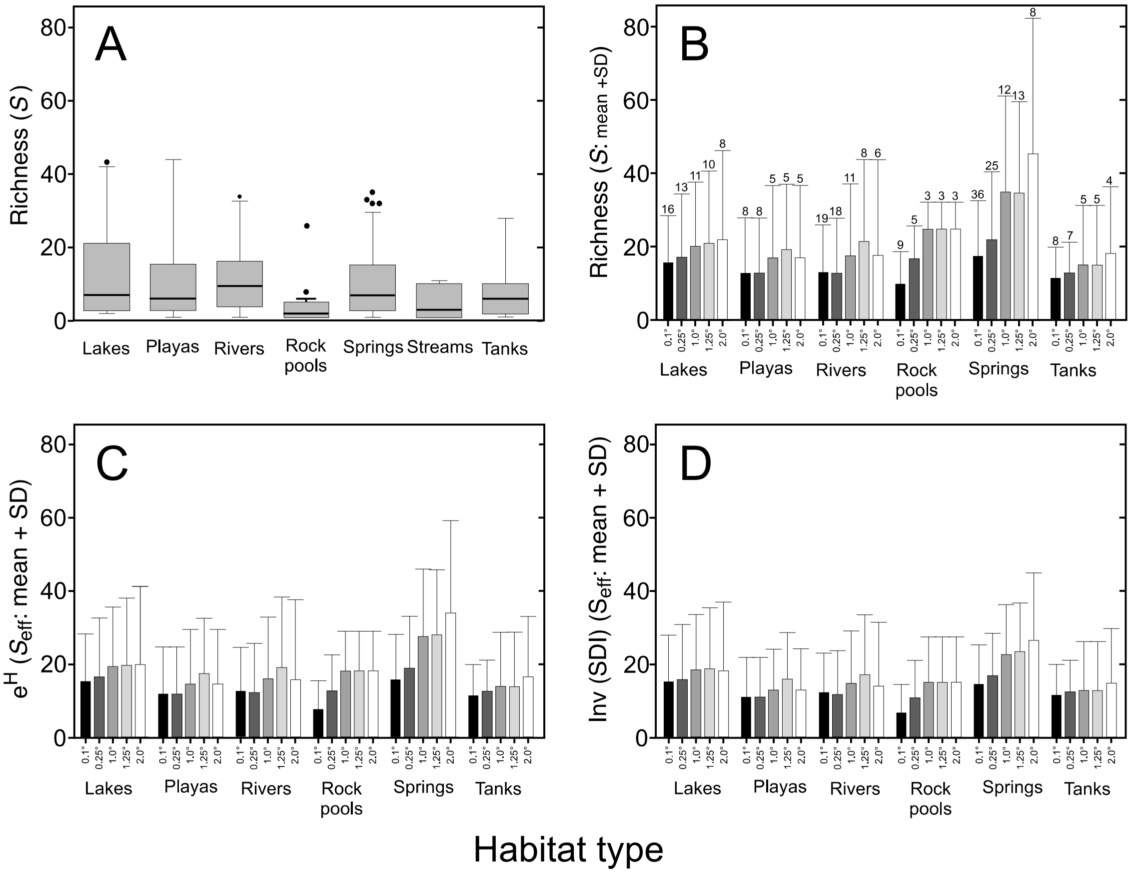

Figure 2. Observed species richness (*S*) of rotifers in 236 Chihuahuan Desert aquatic sites grouped by habitat type over >20 years. (**A**) Boxplots: horizontal lines indicate median, 95% confidence intervals are shown; dots represent outliers, (**B**) Richness at different geographic scales (grid cell sizes: 0.1°, 0.25°, 1.0°, 1.25°, 2.0°), numbers above bars are sample sizes, and are the same for panels C and D. (**C**) Effective richness e^H; Hill number, order q = 1. (**D**) Effective richness based on inverse (inv) of the Simpson's Diversity Index (SDI); Hill number, order q = 2.

Sampling Effort

There was a positive relationship between observed species richness and sampling effort when we included all sites in the analysis, although *S* is only weakly explained ($r^2 = 0.01$, $p < 0.05$; Figure 3). However, when analyzed by habitat type, the relationship was stronger ($r^2 = 0.32$, 0.17, 0.40, 0.56 for springs, lakes, rivers, and tanks, respectively). Although, in some cases, such as in rock pools, *S* was weakly explained by sampling effort ($r^2 = 0.02$, $p < 0.05$). Playas and streams did not show a significant relationship with sampling effort.

Table 1. Species richness, unique species, and Sorensen's Index (below diagonal) and number of shared species (above diagonal) of rotifers from five selected habitat types in the Chihuahuan Desert.

Habitat Type	Species Richness (*S*)	Unique Species *	Versus Lake	Versus Playa	Versus Rock Pool	Versus Spring	Versus Tank
Lake	114	12 (10.5)	—	42	36	77	33
Playa	66	6 (9.1)	0.47	—	24	45	26
Rock pool	53	3 (5.7)	0.44	0.40	—	44	20
Spring	175	60 (34.3)	0.54	0.38	0.39	—	39
Tank	57	6 (10.5)	0.39	0.42	0.36	0.34	—

*—Number of species and percentage of *S* occurring only in this habitat type compared to all sampling sites.

Indicator Species Identification

In the indicator species analysis, 144 species were associated with one habitat type, while only 4 species were associated with 6 of the 7 habitat types. Indicator species were identified for all habitat types and some combinations of habitat types

(Table 2). Playas and Lake + Tanks had the most indicator species (n = 5). While two species (*C. gibba* and *L. luna*) were indicators of all habitat types except rock pools. Not surprisingly, *Hexarthra* n. sp. is an indicator species for rock pools. Indicator species with highly significant associations (p < 0.001) include *Hexarthra* n. sp. with rock pools, *Epiphanes brachionus* with playa habitats, *B. quadridentatus* with playa + river + tank habitats, *E. dilatata* with playa + river + stream + tank habitats, and *L. bulla* with lake + playa + river + spring + stream habitats. Species that were indicators of combinations of five habitat types include: *L. bulla*, *Philodina megalotrocha*, *L. luna*, and *C. gibba.*

Table 2. Rotifer indicator species by habitat type for 236 waterbodies in the Chihuahuan Desert. Only those combinations of habitat types with significant associations are reported. Indicator value (IndVal) is the test statistic and p values were calculated using permutation tests.

Habitat Type	Number of Associated Species	Indicator Species	IndVal	*p* Value
Lake	30	*Trichocerca pusilla*	0.483	0.003
		Asplanchna priodonta	0.378	0.008
Playa	16	*Epiphanes brachionus*	0.538	0.001
		Rhinoglena ovigera	0.458	0.011
		Filinia cornuta	0.433	0.002
		Asplanchna sieboldii	0.387	0.012
		Lacinularia flosculosa	0.354	0.048
Rock Pool	6	*Hexarthra* n. sp.	0.632	0.001
Stream	3	*Dicranophorus grandis*	0.378	0.027
		Wulfertia ornata	0.378	0.027
Tank	13	*Filinia* cf. *pejleri*	0.481	0.005

		Brachionus dimidiatus	0.360	0.018
Lake + River	5	*Keratella americana*	0.432	0.011
Lake + Rock Pool	1	*Trichocerca similis*	0.514	0.004
Lake + Stream	3	*Colurella adriatica*	0.423	0.014
Lake + Tank	6	*Asplanchna brightwellii*	0.433	0.013
		Brachionus caudatus	0.354	0.031
		Brachionus havanaensis	0.350	0.041
		Euchlanis calpidia	0.345	0.042
		Mytilina ventralis	0.332	0.042
Playa + Stream	1	*Trichocerca rattus*	0.445	0.004
River + Spring	2	*Dipleuchlanis propatula*	0.396	0.034
River + Tank	6	*Plationus patulus*	0.446	0.015
		Eosphora najas	0.397	0.022
		Brachionus bidentatus	0.364	0.026
Lake + Playa + Spring	3	*Lecane closterocerca*	0.439	0.049
Lake + Playa + Stream	2	*Brachionus plicatilis*	0.486	0.006
		Notommata glyphura	0.356	0.039
Lake + River + Spring	7	*Colurella uncinata*	0.482	0.010
Lake + River + Tank	4	*Keratella cochlearis*	0.467	0.003
		Brachionus variabilis	0.431	0.011
		Polyarthra dolichoptera	0.431	0.028
		Testudinella patina	0.403	0.040
Playa + River + Tank	3	*Brachionus quadridentatus*	0.674	0.001
		Brachionus angularis	0.439	0.019

Lake + Playa + River + Stream	1	*Cephalodella catalina*	0.455	0.019
Lake + Playa + River + Tank	4	*Brachionus calyciflorus*	0.432	0.026
		Epiphanes chihuahuaensis	0.368	0.036
Playa + River + Stream + Tank	2	*Euchlanis dilatata*	0.628	0.001
		Platyias quadricornis	0.462	0.012
Lake + Playa + River + Spring + Stream	2	*Lecane bulla*	0.668	0.001
Lake + River + Spring + Stream + Tank	1	*Philodina megalotrocha*	0.598	0.007
Lake + Playa + River + Spring + Stream + Tank	4	*Lecane luna*	0.564	0.008
		Cephalodella gibba	0.495	0.017

Nestedness

We evaluated nestedness in rotifers from the 236 Chihuahuan Desert aquatic habitats at several levels: (1) the completed dataset; (2) by habitat type (lakes, playas, tanks, springs, cascading pools, and rock pools); (3) by geospatial scale (0.1°, 0.25°, 1.0°, 1.25°, and 2.0°). As a comparison, we completed a meta-analysis on data from 11 published studies that examined rotifer assemblages from other biomes (see above). We report results of these analyses in Table 3 and summarized them below.

The complete dataset exhibited nestedness, with support from 4 null models ($p < 0.001$). At this scale, only two idiosyncratic species (identified as those with a $T^\circ \geq$ 2SD above the mean matrix $T^\circ = 2.55$): *Hexarthra* n. sp. and *Trichocerca similis*. Of these two species, *Hexarthra* n. sp. (89) had the most restrictive distribution. It was confined to a group of 25 isolated rock pools at HTSPHS, indicating that it is a rock pool specialist. (See also the discussion below on rock pools.) The other idiosyncratic species, *T. similis*, was present in 24 habitats (~10% of all the sites we studied), including rock pools (n = 18), lakes (n = 4), one pond, and one spring. However, while it also seems to be a rock pool specialist, it was not present in the HTSPHS system. We found *T. similis* in two rock pool systems of BIBE possessing very different edaphic conditions. In our analysis of the complete dataset several sampling sites (n = 8) were identified as idiosyncratic habitats, but there was no common feature among them: springs (n = 2); lakes and reservoirs (n = 3); ponds (n = 2); cascading pools (n = 1).

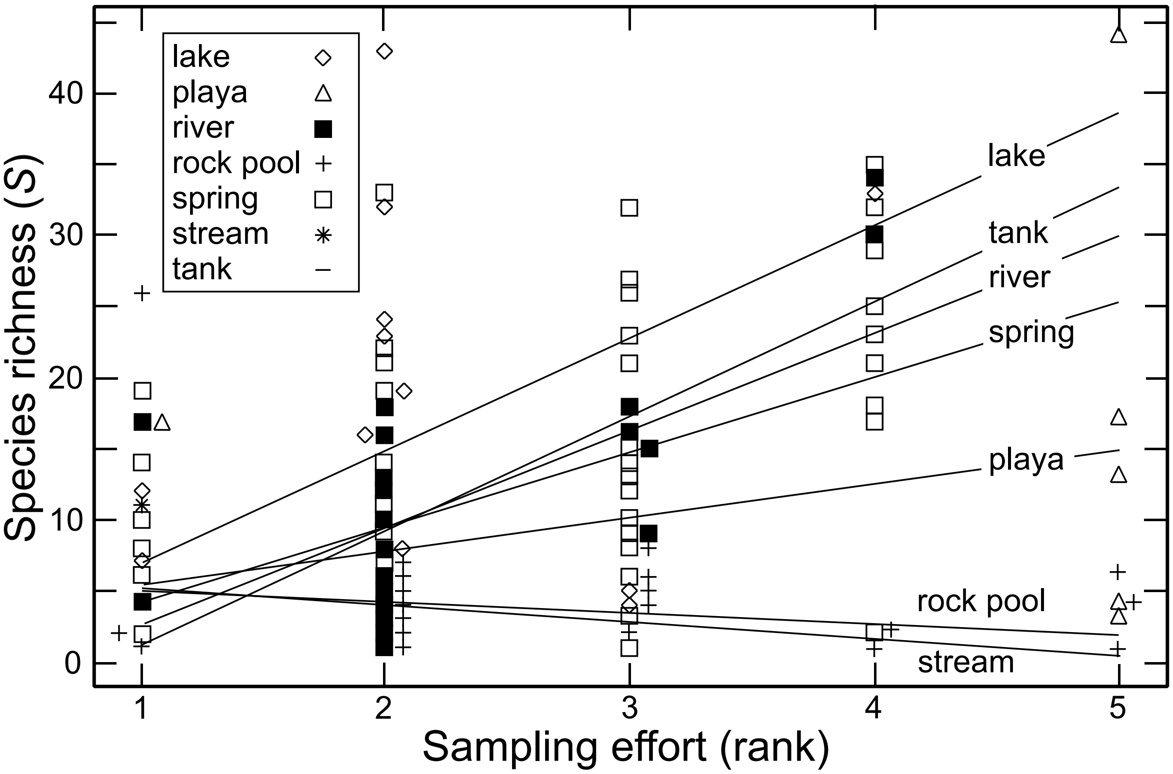

Figure 3. Observed species richness (*S*) as a function of sampling effort in 236 Chihuahuan Desert aquatic sites over 20 years. We shifted some of the data points to reveal their location; some remain obscured by other data points. Lines are linear regressions of the data analyzed separately for each site type. We ranked sampling effort as follows: 1 = 1 sampling event; 2 = 2–5 events; 3 = 6–10 events; 4 = 10–20 events; 5 = >20 events.

We subdivided the dataset by habitat type to examine the distribution of rotifers separately in lakes, playas, tanks, springs, cascading pools, and isolated rock pools at HTSPHS. Lakes and reservoirs (n = 21) possessed five idiosyncratic species (*Encentrum* cf. *algente; Lecane arcula; L. quadridentata; Polyarthra vulgaris; Synchaeta* cf. *oblonga*), but only one idiosyncratic reservoir, Presa Chihuahua. Playas (n = 16) possessed two idiosyncratic species (*Lecane hornemanni* and *L. thalera),* but no idiosyncratic habitats. There were three idiosyncratic species in the tanks (n = 11) (*Brachionus durgae, E. brachionus,* and *Lepadella patella* and one idiosyncratic habitat, Tule Cattle Tank (BIBE). The spring habitats exhibited more diversity with 10 idiosyncratic species (*Adineta vaga, Aspelta aper, C. catellina, Cephalodella tenuiseta, Colurella adriatica, Encentrum saundersiae, Filinia brachiata, Lepadella acuminata, Mytilina mucronata,* and *Notommata* cf. *haueri*). Six of the spring habitats (n = 95) idiosyncratically distinct (n = 6); these included Balmorhea Main Pool, Balmorhea wetland 2, Miller Ranch 96 Well, Oak Creek BIBE, Ojo de la Punta ANPMS, and Sitting Bull Falls LNF. In a previous study of 7 springs in Mexico (90) we found four idiosyncratic species *Cephalodella cf. graciosa* and *Cephalodella megalocephala, Pleurotrocha petromyzon,* and *Pleurotrocha sigmoidea* and one small, idiosyncratic habitat: Ojo de en Medio.

We also examined a portion of the dataset that included only BIBE habitats in which one pool cascaded into another (n = 40). In that analysis two species (*Epiphanes daphnicola* and *T. similis*) and one habitat (a pool surrounded by lush vegetation) possessed idiosyncratic *T*°. Since the edaphic conditions of these pool habitats are different, we separated them by location (n = 5) to explore whether they exhibited unique species distributions. In the Cattail Spring pools (n = 12) four species (*C. obtusa, Lecane pyriformis, Proales cryptopus,* and *Tripleuchlanis plicata*) and one small pool isolated from the main flowage yielded idiosyncratic *T*°. Surprisingly in Ernst canyon, none of the 16 species or 12 rock pools proved to be idiosyncratic. Tuff canyon pools (n = 6) also possessed no idiosyncratic species and only one idiosyncratic habitat (one small pool). In the rock pool flowage of the Window Trail pools (n = 10 sites) one species (*L. pyriformis*) and one habitat (a small tinaja nearly filled with small rocks and sediment, surrounded by plants) possessed idiosyncratic *T*°. The rock pools at HTSPHS yielded no idiosyncratic species. However, as noted above *Hexarthra* n. sp. was found in all sites except for two artificially enlarged, sheltered rock pools. Those rock pools were also possessed idiosyncratic *T*°. In a separate study of six artificial rock pools (mesocosms) placed at HTSPHS, only one species (*Lecane nana*) had an idiosyncratic *T*°. Interestingly, this species was not found in natural habitats of HTSPHS during our extensive sampling effort (n > 20 for most sites over 20 years).

Nestedness was evident across all five geospatial scales (0.1°, 0.25°, 1°, 1.25°, and 2.0°), with support from 4 null models ($P < 0.001$) at each scale. A total of 38 idiosyncratic species were identified in the geospatial analysis and of these eight were identified at more than one spatial scale: *Brachionus plicatilis; Brachionus variabilis; Cephalodella* cf. *misgurnus/pachyodon; Euchlanis calpidia; Paradicranophorus sordidus; P. vulgaris; T. similis;* and *Wulfertia ornata*. Ten regions were identified as idiosyncratic across the five geospatial grids. No obvious pattern of habitats emerged from the scale analysis.

Of the 246 species identified in this study, 59 possessed idiosyncratic *T*° in one or more of the analyses. Of that set we recorded 10 species twice (*A. vaga, B. plicatilis, B. variabilis, C.* cf. *misgurnus/pachyodon, E. calpidia, F. brachiata, L. hornemanni, L. pyriformis, S.* cf*. oblonga,* and *W. ornata*), while three other species occurred more often: three (*P. sordidus*)*,* four (*P. vulgaris*), and five (*T. similis*) times.

For comparison purposes we reviewed published datasets from four other biomes, including aridland (n = 5), tropical (n = 2), temperate (n = 2), and cold (n = 2) biomes. In 13 billabongs of Australia three species (*M. mucronata; E. daphnicola; Trichocerca rattus*), but no habitats possessed idiosyncratic *T*°. Similar results were found in the desert habitats of Oman (n = 9 sites) (*C. gibba; C. obtusa; Trichocerca tenuior*), Saudi Arabia (n = 23 sites) (*Lecane ungulata*), and Yemen (n = 12 sites) (*Brachionus urceolaris; C. forficula; C. adriatica; Lophocharis salpina*). In each of these datasets, a single habitat possessed an idiosyncratic *T*°: Ravine (Wadi O7), Sabkhat (S7), and Wet Wadi (Y30) with *Phragmites*, respectively. An analysis of 32 dune pools in Spain also yielded similar results: three idiosyncratic taxa (*L. salpina; Trichocerca bidens; T. rattus*) and two idiosyncratic habitats: mobile dune region; stable dune region and close to a salt marsh. The two tropical datasets we evaluated offered very different results. In 29 Costa Rican habitats we found six idiosyncratic species (*Ascomorpha klementi; Keratella americana; L. nana; L. patella; Resticula melandoca; Trichocerca dixonnuttalli*) and three idiosyncratic habitats (an artificial Lake; Lake Turrialba; bromeliads). On the other hand, no idiosyncratic taxa or habitats were present in five tropical fishponds. We found similar results in two temperate regions. In 31 sites on the North Island of New Zealand six species (*Filinia* cf. *pejleri; Keratella australis; Keratella tropica; Lecane flexilis; L. acuminata; Trichocerca longiseta*) and three lakes (Lake Okaro; Lake Ototoa; Lake Tutira) yielded idiosyncratic *T*°*.* In seven habitats of the Develi Plain (Turkey) three species (*L. quadridentata; Lepadella biloba; Scaridium longicauda*), but no habitats with idiosyncratic *T*°. We examined published data from two habitats in cold biomes: one each in the Antarctica (n = 14) and Arctic (n = 8 sites). These habitats yielded a moderately rich fauna of 24 and 70 taxa, with two (*B. quadridentatus; Notholca hollowdayi*) and four (*Collotheca* sp. 2; *C. catellina; Squatinella* sp.; *Trichocerca* sp.) idiosyncratic taxa, respectively.

Table 3. Comparative statistics of nestedness among selected studies based on presence/absence data of rotifer species. (See Table A1 for an explanation of the sites, including the abbreviations used here.).

Regions Analyzed [1]	# Taxa	# Genera	# Families	Packed Matrix *T°*	Null Support [2]	Idiosyncratic Species [3]	Idiosyncratic Habitats [4]
			CHIHUAHUAN DESERT (this study)				
All sites	246	59	25	2.4	4	*Hexarthra* n. sp.*; Trichocerca similis*	Caballo Reservoir, NM; Cattail Spring Pools C-D, BIBE, TX; Lake Lucero, WHSA, NM; Langford Hot Springs, BIBE, TX; Miller Ranch 2 (Spring), TX; Presa Chihuahua, MX; Rio Grande Village Cattail Pond, BIBE, TX; Rio Grande Village Upper Pond, BIBE, TX
By habitat type							
1. All lakes	112	38	24	14.2	4	*Encentrum* cf. *algente; Lecane arcula; Lecane quadridentata; Polyarthra vulgaris; Synchaeta* cf. *oblonga*	Presa Chihuahua, Chihuahua, MX
2. All playas	66	30	19	11.9	4	*Lecane hornemanni; Lecane thalera*	None
3. All tanks	57	27	14	11.1	4	*Brachionus durgae; Epiphanes brachionus; Lepadella patella*	Tule Cattle Tank, BIBE, TX

4. All springs	175	49	23	5.0	4	*Adineta vaga; Aspelta aper; Cephalodella catellina; Cephalodella tenuiseta; Colurella adriatica; Encentrum saundersiae; Filinia brachiata; Lepadella acuminata; Mytilina mucronata; Notommata* cf. *haueri*	Balmorhea State Park Main Pool, TX; Balmorhea Wetland 2, TX; Miller Ranch 96 Well, TX; Oak Creek BIBE, TX; Ojo de la Punta, ANPMS, MX; Sitting Bull Falls LNF, NM
Selected springs in Mexico	57	24	15	21.9	4	*Cephalodella* cf. *graciosa; Cephalodella megalocephala; Pleurotrocha petromyzon; Pleurotrocha sigmoidea*	One small, impounded spring: Ojo de en Medio, ANPMS
5. Cascading pools (BIBE)							
A. All rock pools	72	21	14	5.4	4	*Epiphanes daphnicola; Trichocerca similis*	Second pool of the flowage – surrounded by lush vegetation
B. Cattail Springs	65	19	11	23.7	4	*Colurella obtusa; Lecane pyriformis; Proales cryptopus; Tripleuchlanis plicata*	Small pool isolated from the main flowage at this site.
C. Ernst canyon	16	9	8	19.0	4	None	None
D. Tuff canyon	4	4	3	11.7	0	None	Shallow rock pool (Tuff Canyon Site #4)
E. Window Trail canyon	16	7	6	23.3	2	*Lecane pyriformis*	Small tinaja nearly filled with small rocks and sediment, surrounded by plants

6. Rock pools at HTSPHS							
A. Isolated rock pools	14	11	9	4.9	4	None. However, *Hexarthra* n. sp. was found in all sites except for the two artificially enlarged, sheltered rock pools noted here	Two, artificially enlarged, rock pools sheltered by an overhanging shelf
B. Mesocosms: artificial rock pools	9	6	5	22.9	1	*Lecane nana*	None
By Geospatial scale (grid size)							
1. Grid 0.1°	246	59	25	4.4	4	*Adineta vaga; Brachionus plicatilis; Brachionus variabilis; Cephalodella* cf. *misgurnus/pachyodon; Lecane hornemanni; Lecane inermis; Synchaeta* cf. *oblonga; Trichocerca similis*	20755: Northern BIBE (Cattail Springs, Window trail, Croton spring) 29355:Caballo reservoir and Percha dam 30345:BLSP
2. Grid 0.25°	246	59	25	6.0	4	*Brachionus caudatus; Brachionus variabilis; Cephalodella* cf. *misgurnus/pachyodon; Epiphanes chihuahuaensis; Paradicranophorus sordidus; Polyarthra vulgaris; Trichocerca similis; Wulfertia ornata*	3310:Northern BIBE 4842:BLSP

3. Grid 1.0°	246	59	25	11.6	4	*Brachionus bidentatus; Brachionus plicatilis; Cephalodella calosa; Euchlanis triquetra; Filinia brachiata; Keratella americana; Keratella cochlearis; Philodina acuticornis; Philodina megalotrocha; Proales cognita; Wolga spinifera; Wulfertia ornata*	177: Delicias Beisbol field pool and Presa Francisco Ignacio Madero (southern pond and reservoir respectively) 298: BLSP
4. Grid 1.25°	246	59	25	10.5	4	*Dicranophorus mesotis; Euchlanis calpidia; Hexarthra* n.sp.*; Lacinularia flosculosa; Lecane aeganea; Lecane undulata; Paradicranophorus sordidus; Polyarthra vulgaris; Proales* cf. *halophila; Squatinella lamellaris* f. *mutica; Testudinella patina; Trichocerca similis*	El Paso area including HTSPHS
5. Grid 2.0°	246	59	25	9.5	4	*Encentrum* cf. *cruentum; Euchlanis calpidia; Paradicranophorus sordidus; Plationus patulus; Polyarthra*	64: El Paso/Juarez area including ANPMS, HTSPHS, IMRS 65: GUMO and Balmorhea SP

						vulgaris; Trichocerca similis	

OTHER ARIDLAND BIOMES

1. Billabongs (Australia)	52	25	18	39.3	2	*Mytilina mucronata; Epiphanes daphnicola; Trichocerca rattus*	None
2. Various habitats (Oman)	66	20	12	45.9	3	*Cephalodella gibba; Colurella obtusa; Trichocerca tenuior*	Ravine (Wadi O7)
3. Various habitats (Saudi Arabia)	19	10	7	11.1	3	*Lecane ungulata*	Brackish water lagoon (Sabkhat S7)
4. Various habitats (Yemen)	74	26	16	11.3	4	*Brachionus urceolaris; Cephalodella forficula; Colurella adriatica; Lophocharis salpina*	Wet Wadi (Y30) with *Phragmites*
5. Dune pools (Spain)	34	18	12	16.5	4	*Lophocharis salpina; Trichocerca bidens; Trichocerca rattus*	Two pools: (1) mobile dune region; (2) stable dune region and close to a salt marsh

TROPICAL BIOMES

1. Costa Rican habitats	105	33	17	10.1	4	*Ascomorpha klementi; Keratella americana; Lecane nana; Lepadella patella; Resticula melandoca; Trichocerca dixonnuttalli*	Artificial Lake; *Bromelia*; Lake Turrialba

2. Eutrophic tropical fish ponds	57	22	15	61.8	0	None	None
TEMPERATE BIOMES							
1. North Island, NZ	79	32	20	26.3	4	*Filinia* cf. *pejleri; Keratella australis; Keratella tropica; Lecane flexilis; Lepadella acuminata; Trichocerca longiseta*	Lake Okaro; Lake Ototoa; Lake Tutira
2. Develi Plain, Turkey	84	33	17	31.6	3	*Lecane quadridentata; Lepadella biloba; Scaridium longicauda*	None
COLD BIOMES							
1. Antarctica & sub-Antarctica	24	6	3	22.7	2	*Brachionus quadridentatus; Notholca hollowdayi*	None
2. Canadian High Arctic	70	26	16	29.5	4	*Collotheca* sp. 2; *Cephalodella catellina; Squatinella* sp.; *Trichocerca* sp.	Small pool, 8 (P208)

- [1]—**PARTITIONING OF THE DATASET.** To run the nestedness analyses, we partitioned our Chihuahuan Desert dataset into units as follows. Chihuahuan Desert: All sites (n = 237). By habitat type: 1. Lakes (n = 21). 2. Playas (n = 16). 3. Tanks (n = 11). 4. Springs (n = 95). Selected springs in Mexico (n = 7) in Samalayuca, Chihuahua, Mexico; these data were previously published by Ríos-Arana, Agüero-Reyes, Wallace and Walsh (90). 5. Cascading Pools: A. All pool habitats at Big Bend National Park (BIBE) (n = 40). B. Cattail Spring (BIBE) (n = 11). C. Ernst Canyon (BIBE) (n = 12). D. Tuff Canyon (BIBE) (n = 6). E. Window Trail (BIBE) (n = 10). 6. Isolated pools: A. Isolated rock pools (n = 27) at Hueco Tanks State Park and Historical Site (HTSPHS) (El Paso, TX). B. Mesocosms–Artificial rock pools (n = 6) developed over 9 weeks at HTSPHS (Walsh et al., 2014). By scale (grid size): 1. Gridded at 0.1° (n = 83 designations). 2. Gridded at 0.25° (n

= 55 designations). 3. Grid 1.0° (n = 23 designations). 4. Gridded at 1.25° (n = 21 designations). 5. Gridded 2.0° (n = 14 designations). Other aridland biomes: 1. Billabongs (oxbows, cut–off meanders) (n = 13) in River Murray (southeastern Australia) (Shiel & Koste, 1983). 2, 3, 4. Various habitats ranging from permanent lakes and rivers to temporary pools in Oman (n = 9), Saudi Arabia (n = 19), and Yemen (n = 33), respectively (Segers & Dumont, 1993). 5. Ephemeral dune pools (n = 32) in Doñana National Park (Spain) (Mazuelos et al., 1993). Tropical biomes: 1. Costa Rica—various habitats including puddles, phytotelmata, ditches, and lakes (n = 29) (Kuczyńska-Kippen). 2. Eutrophic, tropical fish ponds (n = 5) in Darbhanga City (Bihar, India) (118). Temperate biomes: 1. Lakes on North Island, New Zealand (n = 31) (Duggan et al., 2002). 2. Develi Plain (n = 8) Middle Anatolia, Kayseri, Turkey (Kaya et al., 2010). Cold Biomes: 1. Antarctica and sub-Antarctica—various habitats (n = 14) (Hansson; 2011). 2. Canadian High Arctic (Devon Island, Northwest Territories)—pools, ponds, and a small lake (n = 8) (De Smet et al., 1995). [2]—Number of null models supporting nestedness. [3]—Comments on species with individual $T°$ ≥ 2 SD of the mean matrix $T°$. [4]—Comments on sites or gridded regions with individual $T°$ ≥ 2 SD of the mean matrix $T°$.

Of the 246 taxa identified in our Chihuahuan Desert dataset, 114 were also reported in the four comparison biomes: Aridlands (5 studies; n = 89 species); Tropical (2 studies; n = 63 species)*;* Temperate (2 studies; n = 72 species)*;* and Cold (2 studies; n = 30 species). In spite of this overlap, fewer species with idiosyncratic *T*° were found among all datasets. Of the 59 idiosyncratic species identified from the Chihuahuan Desert, only 11 also were identified as being idiosyncratic in the comparison biomes: Aridlands (n = 5) (*C. adriatica, C. obtusa, L. ungulata, M. mucronata,* and *E. daphnicola);* Tropical (n = 3) (*K. americana, L. nana,* and *L. patella);* Temperate (n = 2) (*L. quadridentata* and *L. acuminata);* Cold (n = 1) (*C. catellina*). None of those 11 species were present in more than one of the comparison biomes.

Relationship between Species Richness and Geographic Distance

Mantel tests showed a significant correlation between distance and species composition for grid cell sizes below 1.25°. The effect became progressively larger at smaller grid cell size, being the most substantial at cell size 0.1° ($p = 0.01$) and the least significant at the largest grid cell size (2°; $p = 0.1$). Species composition in springs demonstrated no significant correlation with distance at any spatial scale investigated. In contrast, playa species composition showed significant correlations with distance at all grain sizes. Tank composition was significant at all grain sizes with the exception of 0.25°. All other habitats showed significant correlation at small grain sizes, but little correlation at large grain sizes (See Table 4). Stream sites were too few (n = 3) to adequately assess using Mantel tests, and thus were not analyzed as a separate habitat.

Table 4. Mantel correlation coefficients (r) between Haversine geographic distances and Bray-Curtis dissimilarity values for rotifer communities between sites (n) at each grid size investigated. Habitat types were then analyzed separately, with the exception of streams due to low number of samples (n = 3 at grid size 0. 1°).

Region	Mantel r Statistic	*P*-Value	n
all sites			
sites	0.12	< 0.001	236
0.1°	0.12	0.01	84
0.25°	0.14	0.02	55
1°	0.03	0.22	24
1.25°	0.20	0.08	21
2°	0.20	0.10	14
By habitat			
Lakes			
sites	0.30	0.001	21
0.1°	0.25	0.044	16
0.25°	0.20	0.105	13
1°	0.31	0.048	11
1.25°	0.32	0.085	10
2°	0.35	0.095	8
Playas			
sites	0.55	<0.001	16
0.1°	0.60	0.009	8
0.25°	0.62	0.002	7
1°	0.74	0.008	5
1.25°	0.58	0.083	5
2°	0.80	0.008	5
Rivers			
sites	0.27	<0.001	26
0.1°	0.41	<0.001	19

0.25°	0.48	<0.001	18
1°	0.42	0.012	11
1.25°	0.13	0.271	8
2°	0.13	0.350	6
Rock pools			
sites	0.12	<0.001	60
0.1°	−0.16	0.696	9
0.25°	0.61	0.133	5
Springs			
sites	0.02	0.334	95
0.1°	−0.06	0.752	36
0.25°	−0.05	0.663	25
1°	0.06	0.321	12
1.25°	0.02	0.406	13
2°	0.16	0.253	8
Tanks			
sites	0.41	0.012	11
0.1°	0.35	0.063	8
0.25°	0.28	0.147	7
1°	0.60	0.017	5
1.25°	0.67	0.008	5
2°	0.77	0.083	4

Prediction of Biodiversity Hotspots

Generally, patterns of predicted species richness were similar among the spatial scales investigated (Figure 4). At smaller scales, localized hotspots of richness are apparent within the Chihuahuan Desert. At the site level, 0.1° and 0.25° grid cell sizes, predicted species richness was highest in a band spanning from the southern Chihuahuan Desert northward along the western border to the El Paso/Juarez area, and a band spanning from Guadalupe Mountains National Park (TX) to Balmorhea State Park (TX), with low predicted richness along the Rio Grande in this area. When we excluded the site level, a band of high predicted richness exists from Samalayuca across the Rio Grande to Balmorhea State Park, each with localized hotspots (Figure 4 B,C). Cuatro Ciénegas showed high richness at most scales (Figure 4 B–D). At grid cell sizes >0.25°, distinct hotspots are less apparent (Figure 4 D). At these higher scales, local hotspots are more difficult to resolve due to the lower number of grid cells present within the Chihuahuan Desert (n = 24 for 1° grid cells).

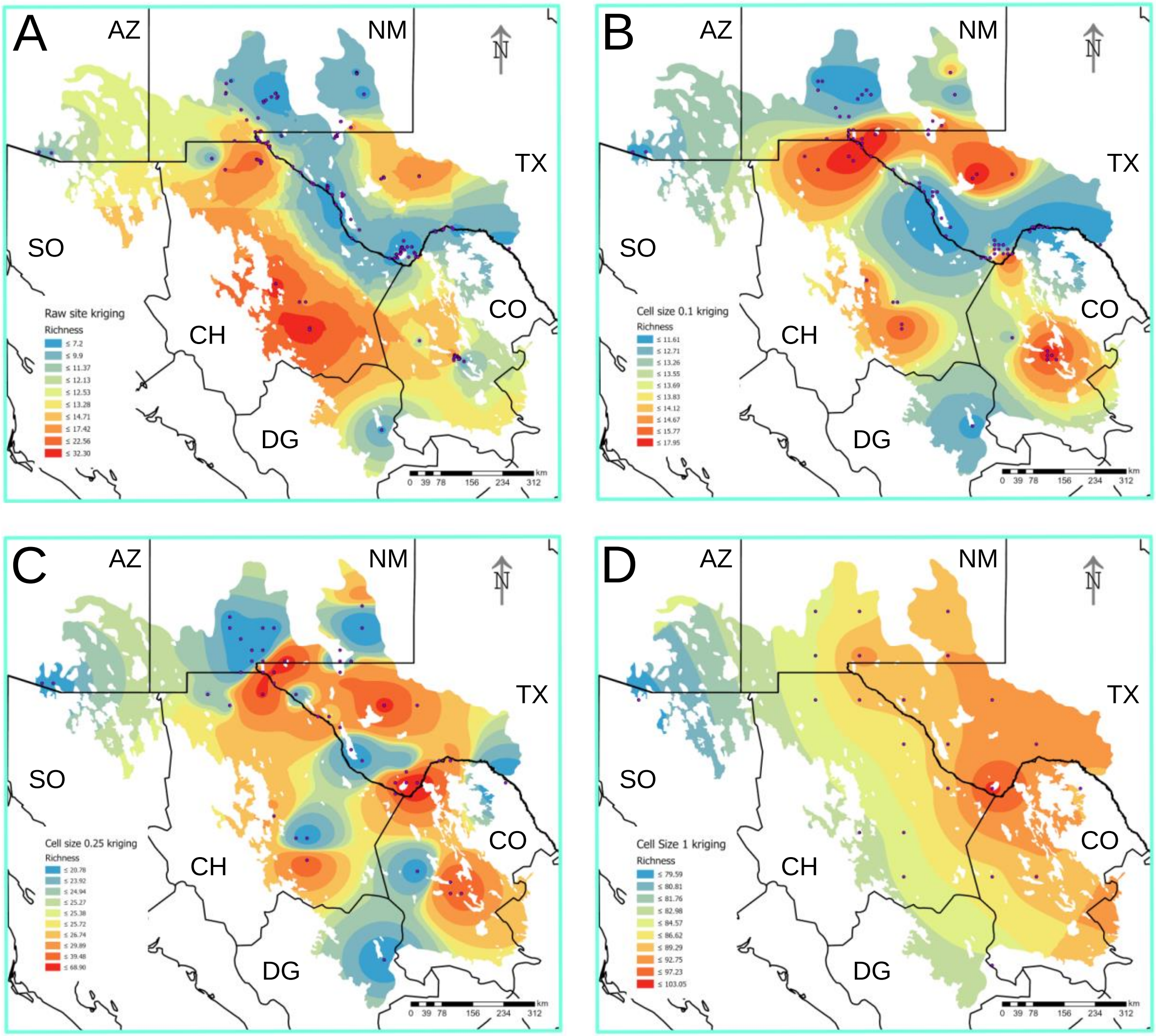

Figure 4. Empirical Bayesian kriging of predicted rotifer species richness within the Chihuahuan Desert ecoregion (Olson et al., 2001) interpolated from all sites (n = 236) and at a variety of spatial scales. (**A**) All collection sites (**B**) 0.1° grid cells, (**C**) 0.25° grid cells, and (**D**) 1° grid cells. Sites (panel A) and grid cell centroids (panels B–D) are represented by purple dots. We obtained state boundaries from the USGS and ArcGIS online (Paskevich); ArcGIS Mexican state boundary shapefile courtesy of M. Hoel (www.arcgis.com).

DISCUSSION

Comprehensive studies of rotifer distributions are common, but vary widely in their focus. For example, many emphasize long-term, ecological questions across several water bodies (Beach, 1960; May & Wallace, 2019; Špoljar et al., 2018; Vasseur et al., 2014), the dynamics in a particular lake (Hampton et al., 2006; 2008; Hampton, 2005; Herzig, 1987; Matthews et al., 2015; Molinero et al., 2005) or region (Duggan, 2007; Duggan & Barnes, 2005; Duggan et al., 2001; Kuczyńska-Kippen et al., 2020; Rivas et al., 2018; 2019; Magnuson et al., 2020; Muirhead et al., 2006; Smith et al., 2009), or examine a single taxon (Korbacheh et al., 2017; Meksuwan et al., 2015; 2018; Mills, 2006; Mills et al., 2007; 2017; Wen et al., 2016). Collectively, such studies provide insight into the biogeography of the phylum. However, to obtain a thorough understanding of the biogeography of rotifers, long-term, systematic survey data is required. Unfortunately, that level of effort is difficult to accomplish, so most studies provide a short-term, snapshot of a region or of a particular habitat (Koste & Shiel, 1987; Shiel, 1986; Shiel & Green, 1996; Shiel & Koste, 1979; Shiel et al., 1989; 2006). On the other hand, extensive regional studies have been published, which illustrate the diversity of rotifers that may be present in one area: three studies illustrate this point. (1) The study by Segers and Dumont (Segers & Dumont, 1983) of >110 sites across the Arabian Peninsula, which included five countries, yielded >115 species. (2) In examining 33 lakes on the North Island of New Zealand, Duggan and his colleagues (Duggan et al., 2001) reported 79 species. (3) In a long-term study (1982 onward) of the zooplankton of seven water bodies in the Trout Lake LTER (Magnuson et al., 2020), ~75 species have been recorded.

While our choice of collection sites was pragmatic and based on accessibility, sampling >225 diverse habitats over a 20-year period, with many sites visited multiple times, comprises an extensive survey. Due to its thorough nature, our analysis of Chihuahuan Desert aquatic systems offers additional insight to the understanding diversity of rotifers in aridlands, and it offers testable predictions regarding the presence of biodiversity hotspots at a regional level.

Among habitats, rotifer species richness was highest in springs (n = 175) and lowest in rock pools (n = 53) followed closely by tanks and playas (n = 57, 66, respectively). This difference in diversity may reflect the relative stability of these habitats in terms of hydroperiod and/or connectivity with other sites. For example, the ephemeral rock pools at HTSPHS are unique in character from all other rocky basins examined in our study. All of the HTSPHS rock pools have nearly identical edaphic conditions, and the *Hexarthra* found in these pools was identified as a strong indicator species for rock pools (Table 2). For rotifers, the use of the indicator species concept has been used mostly in regard to water quality (Wallace et al., 2006); thus, our application is somewhat unique. It should be noted that some species have been highly associated with acidic habitats (e.g., *Cephalodella hoodi* (Weithoff, 2005), *Cephalodella acidophila* (Jersabek et al., 2011), *Keratella taurocephala* (Brett, 1989)), and function as indicators. The five species with significant indicator values associated with a combination of five habitat types (*L. bulla*, *P. megalotrocha*, *L. luna*, and *C. gibba*) possess wide ecological tolerances. Another implication is that these morphospecies likely represent cryptic species complexes (Garcia-Morales & Elias-Gutierrez, 2013; Zhang et al., 2019) (see below).

Locations we identified possessing high predicted richness generally overlap the proposed wetland priority sites for the Chihuahuan Desert (Dinerstein et al., 2000). However, we found low richness in the Rio Grande and at aquatic sites in White Sands National Park (NM). Several priority areas were sparsely sampled in our study (i.e., the Apachean and the Meseta central subregions); making the predicted richness within these regions less reliable. However, some unusual outcomes occurred at various spatial scales. At our smallest scale (e.g., site level) some areas that contain highly sampled locations yielded low overall predicted richness. For example, at HTSPHS large numbers of ephemeral rock pools are in close proximity to more speciose playas such as Laguna Prieta, the site with the highest richness in our survey (n = 44). The low diversity of these rock pools decreased our predicted richness for the entire area at the smallest spatial scale. At the 0.1° grid size, the low diversity rock pools and high diversity playas of HTSPHS are combined, resulting in a hotspot on the kriging map. We found similar scenarios at Cuatro Ciénegas (Mexico), BIBE (TX) and Bottomless Lakes State Park (NM). At the largest spatial scale (grid size 1°), the pattern seemed to be more influenced by sampling intensity.

Of the 17 different ways we examined nestedness in the Chihuahuan Desert sites, only three did not exhibit nestedness. The rock pools of Tuff Canyon had no support from the null models; Window Trail Canyon had support from only two; and the artificial rock pools (mesocosms) had support from only one model. These results are not surprising as the basins within of each of these systems are quite similar: Tuff Canyon (basalt larva and tuff deposits); Window Trail (limestone); Mesocosms (plastic basins filled with artificial pond water). This indicates that, for nestedness to be present, the inclusive habitats must possess environmental heterogeneity, and if nestedness were not present, we would expect the species assembly to be random within the habitats (Dražina et al., 2017; Meksuwan et al., 2014).

In the 18 ways that we analyzed nestedness in our Chihuahuan Desert dataset, we recorded a large number of species to be idiosyncratic (n = 59; ~24%). These species are those, that within the context of the data, contributed disproportionately to the overall matrix temperature; i.e., their occurrence is, therefore, unexpected in that nested group (Table 3). It is notable that most of the idiosyncratic species are generally considered cosmopolitan or having broad environmental tolerances. Our analyses also show that rotifer assemblages are correlated with distance at smaller spatial scales but are more homogenous at the regional level (Table 4). Other papers have reported similar patterns in multiple studies analyzing species assemblages or populations of a single species (García-Morales & Elías-Gutiérrez, 2019; Kimpel et al., 2015; Kordbacheh et al., 2017; Kusumoto et al., 2020; Stendera & Johnson, 2005; Thielsch et al., 2009; Zhang et al., 2019). Thus, our results seem to support the Baas Becking Principle—"*Everything is everywhere*, but, *the environment selects"*—the ubiquity hypothesis (De Wit & Bovier, 2006). That is, organisms with small dispersal stages (<1 mm) are easily, and widely, dispersed, but arrival does not necessarily guarantee persistence in a habitat (Dumont, 1980).

We know that in rotifers, community structure may result from a combination of their high dispersal capacity and their ability to create resting egg banks (Fenchel & Finlay, 2004; Kellog & Griffin, 2006). These two traits can lead to the monopolization of local habitats if the initial colonization and subsequent production of an egg bank leads to rapid adaptation and then to the exclusion of other species. This construct has been named the monopolization hypothesis (De Meester et al., 2002; 2016). Thus, at small spatial scales, monopolization leads to high dissimilarity among sites, as may be the case of rock pools and springs in our study (lowest v. highest species richness). However, the high dispersal capability of rotifers may lead to increasing community similarity at larger spatial scales. In general, community composition of organisms with high dispersal ability are less impacted by geographic distances than those with low capacity. Local edaphic conditions, including the arrival sequence, ultimately selects the composition of assemblages that endures.

At larger spatial scales, a greater degree of habitat heterogeneity is present within each region, resulting in a reduction of assemblage differences among regions because of shared habitat types occurring within the larger geographic areas. We have previously reported that rotifer assemblages are more homogenous at the regional level, thereby supporting the relative cosmopolitan nature of dominant rotifer species (Wallace et al., 2008). However, there can be significant associations between local environmental parameters and species assemblages (Wallace et al., 2005). Here we report that Chihuahuan Desert spring assemblages were not correlated with distance at any spatial scale investigated. This may be due to the unique edaphic conditions present in each habitat. This was seen in *T. similis,* which was found in a series of small to large rock pools lying along an erosional channel of Cretaceous limestone in Ernst canyon (n = 12 sites) (Silberstorf, 2017), as well as in Tuff canyon (n = 6 sites) where the rocks pools are arrayed in a channel of eroded basalt lava and tuff deposits (Barker, 2000).

We note that our estimate of richness is likely underestimated, as we could not identify some specimens to species; this is especially true for the Bdelloidea. In addition, it is well known that many traditional species of rotifers are, in fact, complexes of cryptic species (Fontaneto, 2014; Gabaldón et al., 2017). For example, two species common in our samples, *E. dilatata* and *B. plicatilis,* are comprised of at least 4 and 15 separate lineages, respectively (Kordbacheh et al., 2017; Mills et al., 2017). Two of the four newly described species of the *E. dilatata* complex occur in the Chihuahuan Desert (Kordbacheh et al., 2017). During the surveys undertaken for this study, they were all recorded as *E. dilatata.* Finally, several new species are pending formal description.

Our research identified rotifers that exhibited distribution patterns at two extremes: either widely or narrowly distributed. Five species were widely distributed: i.e., being present in 50 or more of the sites we sampled. These species were *E. dilatata*, *L. bulla, L. luna, L. patella,* and *P. megalotrocha*. The perception in the literature is that species with wide distributions have few specific growth requirements. However, as noted above some of these species may represent cryptic species complexes: *E. dilatata* (Kordbacheh et al., 2017), *L. bulla* (Walsh et al., 2009), *P. megalotrocha* (Hamdan, 2010), and *L. luna* (Walsh, unpubl. data). On the other hand, some species were narrowly distributed. In our collections we found 70 species only once (e.g., *Asplanchna intermedia, Brachionus rotundiformis, Cephalodella dentata, Filinia limnetica, Synchaeta tremula*). These species may possess rigorous requirements for growth, be poor dispersers, and/or poor competitors, in each case restricting their distributions.

In addition, we did not sample all sites evenly. We sampled some sites only once at one station, while we sampled others >20 times and from multiple stations/microhabitats within the waterbody. We showed that for sites at BIBE, increased sampling effort increased the number of species recovered even up to seven collections (Walsh et al., 2004). Similarly, among all sampled habitat types, sampling effort increased richness found, although this relationship was weakest in rock pools, possibly due to their low diversity.

CONCLUSIONS

Understanding the biogeography of rotifers remains an important problem. Indeed, the general perception that they do not have a biogeography remains largely untested. Rousselet was the first to pose this idea; he argued that "… the Rotifera enjoy a cosmopolitan distribution which is not limited to continents, but extends to all places on the surface of the earth where suitable conditions prevail" (Rousselet, 1909). This view, which presaged that of Baas Becking, had been the prevailing view until challenged by several researchers (Dumont, 1980; 1983; Fontaneto, 2019; Fontaneto et al., 2006). Yet a large part of the question of whether rotifers possess a biogeography remains rooted in three issues. (1) There is a rotiferologist effect—that the distribution of rotifers indicates more the distribution of researchers, and the habitats that they survey, than the rotifer species themselves (Fontaneto et al., 2006). (2) Currently, there are few venues where researchers can receive training in rotifer taxonomy and identification (Clavel et al., 2011). Thus, identification is often limited to easily recognized species. (3) Recently researchers have come to the realization that cryptic speciation is widespread within the phylum (Kordbacheh et al., 2017; Michaloudi et al., 2017; Mills et al., 2017; Obertegger et al., 2014) (see also above). Thus, reports of a species from distant locations that are identified based solely by morphological characters may be insufficient to consider them as identical. Emerging science on cryptic speciation suggests that they may be genetically distinct enough to warrant the designation of separate species. Examples of previously unrecognized morphological and ecological differences in the *B. plicatilis* complex (Mills et al., 2017), among other species (Garcia-Morales & Elias-Gutierrez, 2013), support this contention. Until these issues are, to a large degree, settled, an adequate test of whether rotifers fit the ubiquity hypothesis is not possible.

Thus, our research effort addresses three important aspects in understanding species distributions and biogeography. We covered a broad geographic range, provided a long-term study, and used repeated sampling of sites. Thus, it is not surprising that our study yielded a large number of species. Supporting our previous study that focused on a smaller geographic region (i.e., BIBE), here, we found that sampling effort was positively correlated with rotifer richness in more permanent habitats (e.g., lakes, springs, rivers) and in anthropogenic tanks. In addition, for some sites our efforts spanned seasons and years. Our predictive maps show that it is probable that additional rotifer species remain undiscovered in the Chihuahuan ecoregion. They also give guidance for focusing efforts, as well as for conservation prioritization. Additional diversity also may be revealed by molecular applications such as DNA sequencing to delineate cryptic species and environmental sequencing of water and sediments to find rare species and/or to sample habitats during desiccated periods. In conjunction with environmental data (e.g., water quality data, land use patterns), our findings also can be used to determine ecological drivers of rotifer species assemblages.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure A1: Examples of Chihuahuan Desert aquatic systems.

Table A1: Site name, locations, habitat types, and sampling intensities for waterbodies included in this study.

Author Contributions: Conceptualization, E.J.W., R.L.W., P.D.B., T.S., J.V.R.-A., R.R., and M.S.-B.; validation, P.D.B., E.J.W., and R.L.W.; formal analysis, P.D.B.; R.L.W.; E.J.W.; investigation, E.J.W., R.L.W., T.S., J.V.R.-A., R.R., and M.S.-B.; resources, E.J.W., R.L.W., J.V.R.-A., R.R., and M.S.-B.; data curation, E.J.W.; writing—original draft preparation, R.L.W., P.D.B., E.J.W.; writing—review and editing, E.J.W., R.L.W., P.D.B., T.S., J.V.R.-A., R.R., and M.S.-B.; project administration, E.J.W., R.L.W.; funding acquisition, E.J.W., R.L.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in part by an American Association for the Advancement of Science Women's International Science Collaboration (WISC) travel grant award, the National Science Foundation Grant No. 0516032,NSF Advance #0245071 (UTEP), NIH 5G12RR008124, T & E, Inc., and Funds for Faculty Development (Ripon College). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the National Institutes of Health.

Acknowledgments: Assistance was provided by A. Adabache, R. Galván-De la Rosa, J. and B. Newlin, M. Sigla Arana, P.L. Starkweather, N. Lannutti and many undergraduate and graduate students in the Walsh lab A. Sosa and H.J. Gonzalez-Martínez helped us locate several of the sampling sites in Mexico. Mexican samples were collected under permit #09436 from the Secretario de Medio Ambiente y Recursos Naturales to M. Silva-Briano. We thank the Comisión Federal de Electricidad for permission to sample Presa de la Boquilla. USA samples were collected under permits (to E. Walsh) BIBE-2001-SCI-0058, BIBE-2006-SCI-0003, BIBDE-2016-SCI-0057, BIBE-2001-SCI-0012, CAVE (CAVE-2008-SCI-0005, CAVE-2-12-SCI-0001, CAVE-2016-SCI-0012), GUMO (GUMO-2009-SCI-0009, GUMO-2010-SCI-0013, GUMO-2014-SCI-0017, GUMO-2015-SCI-0017, GUMO-2016-SCI-0017), TPW 02-04, #66-99, #07-02, 2011-13, 2013-01, 2014-01, 2015-03, 2017-R1-19, WHSA-2009-SCI-0011, WHSA-2010-SCI-0008, WHSA-2009-SCI-0011, WHSA-2009-SCI-0-007, WHSA-2012-SCI-0001, WHSA-2014-SCI-0011, WHSA-2016-SCI-009, and NM State Parks Division. We thank The Nature Conservancy and J. Karges for permission to sample the East Sandia Spring Reserve. We thank the local inhabitants for permission to sample Ojo de La Casa, Ojo de La Punta (Don Bruno) and Ojo de Santa María. Jeffrey Bennett provided logistical support in sampling the hotsprings in the lower canyons downstream of BIBE. Kevin Bixby provided access to La Mancha Wetlands. H. Segers provided expert review of some of our species identifications.

Conflicts of Interest: The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

PROCESSES CONTRIBUTING TO ROTIFER COMMUNITY ASSEMBLY IN SHALLOW TEMPORARY ARIDLAND WATERS

P.D. Brown[1], T. Schröder[1], J.V. Rios-Arana [2], R. Rico-Martinez[3], M. Silva-Briano[3], R.L. Wallace[4], E.J. Walsh[1*]

[1] University of Texas at El Paso, Department of Biological Sciences, 500 West University Avenue, El Paso, Texas, 79968, USA; e-mail: pdbrown3@miners.utep.edu; ewalsh@utep.edu; th.schroeder@gmx.net

[2] Universidad Autónoma de Ciudad Juárez, Departamento de Ciencias Químico Biológicas, Instituto de Ciencias Biomédicas, Av. Benjamin Franklin no. 4650, Zona PRONAF, Cd. Juárez, 32315, Chihuahua, México; e-mail: jrios@uacj.mx

[3] Universidad Autónoma de Aguascalientes, Centro de Ciencias Básicas, Departamentos de Biología y Química, Avenida Universidad 940, Ciudad Universitaria, C.P. 20131, Aguascalientes, Ags., México; e-mail: roberto.rico@edu.uaa.mx; msilva@correo.uaa.mx

[4] Ripon College, Department of Biology, 300 Seward St., Ripon, WI, 54971, USA; e-mail: wallacer@ripon.edu

*****Correspondence: ewalsh@utep.edu; Tel.: +01 (915) 747-5421

ABSTRACT

Understanding how local conditions and dispersal dynamics structure communities of passively dispersing aquatic invertebrates remains uncertain, especially in aridland systems. In these systems, dispersal is irregular and successful colonization is subject to priority effects. To investigate these factors, we compared rotifer species composition from Chihuahuan Desert rock pools, playas, and tanks. (1) We found 132 species with high beta-dissimilarity among sites (>0.8). (2) Correlation between species richness and habitat area was significant, but weak, for all sites. (3) Dissimilarity analyses, supported by negative Dispersal-Niche Continuum Index (DNCI) values, showed that stochastic processes dominate community assembly. (4) We examined influence of three important environmental variables on richness and community structure: hydroperiod, algal mat and macrophyte development, and conductivity; we also examined how rotifer trophi type (a functional trait) affected DNCI and identified indicator species. Hydroperiod was important for playas and tanks, but not rock pools. Conductivity had a strong influence. Richness was greatest in habitats with highest amounts of vegetation. Environmental factors explained 12% of variation in community composition, indicating that while deterministic processes are significant, stochastic processes dominate in these systems. We provide a conceptual model that highlights the distinctive of nature aquatic communities in aridlands compared to temperate regions.

Funding

Our research was funded, in part, by the following> American Association for the Advancement of Science Women's International Science Collaboration (WISC) travel grant award (Walsh); NSF grants: DEB #0516032, #1257068, and # 2051704 (UTEP); NSF Advance #0245071 (UTEP); NIH 5G12RR008124; T & E, Inc.; DEB #1257116 and 2051710 (Ripon College); and Funds for Faculty Development (Ripon College). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the National Institutes of Health.

Availability of data and material

Data are available from the corresponding author, but most species data and site coordinates are available in Brown et al. (2020). Metadata are available at: https://datarepo.bioinformatics.utep.edu/getdata?acc=9UX5TMO7PXAMPZK

Ethics Approval

The appropriate agencies provided collecting permits (see acknowledgments). None of the specimens that we collected are endangered or threatened. Sampling and processing protocols followed appropriate guidelines established by state and federal parks.

Authors' contributions

Conceptualization, E.J.W., R.L.W., P.D.B; validation, P.D.B., E.J.W., R.L.W.; formal analysis, P.D.B.; R.L.W., E.J.W.; investigation, E.J.W., T.S., R.R.M., J.V.A.R., M. B.S., R.L.W.; resources, E.J.W., R.L.W., R.R.M., M.S.B., J.V.R.A.; data curation, E.J.W.; writing—original draft preparation, R.L.W., P.D.B., E.J.W.; writing—review and editing,

E.J.W., T.S., R.R.M., J.V.R.A., M. B.S., R.L.W, P.D.B; project administration, E.J.W., R.L.W.; funding acquisition, E.J.W., R.L.W. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: We thank the two reviewers and the editors who made helpful suggestions to improve the manuscript. A. Adabache, R. Galván-De la Rosa, J. and B. Newlin, M. Sigla Arana, P.L. Starkweather, N. Lannutti and many undergraduate and graduate students in the Walsh lab provided field assistance. We collected samples from Méxican sampling sites under permit #09436 from the Secretaría de Medio Ambiente y Recursos Naturales to M. Silva- Briano. USA samples were collected under permits (to E. Walsh) BIBE-2001-SCI-0058, BIBE-2006-SCI-0003, BIBE-2016-SCI-0057, BIBE-2001-SCI-0012, TPW 02-04, #66-99, #07-02, 2011-13, 2013-01, 2014-01, 2015-03, 2017-R1-19, WHSA-2009-SCI-0011, WHSA-2010-SCI-0008, WHSA-2009-SCI-0011, WHSA-2009-SCI-0-007, WHSA-2012-SCI-0001, WHSA-2014-SCI-0011, and WHSA-2016-SCI-009. We thank the local property owners for permission to sample Ojo de La Casa. H. Segers provided expert review of some of our species identifications.

INTRODUCTION

In one of her many insightful moments, Rachel Carson asked the questions "*Why does an animal live where it does? What is the nature of the ties that bind it to its world?*" This is a central goal of ecology: understanding biodiversity and how it is maintained, especially among a local suite of interacting communities comprising a metacommunity (Grainger & Gilbert, 2016; García-Girón et al., 2020). Recent advances in metacommunity theory have provided a scaffold against which we can frame questions regarding community assembly, priority effects, species functional trait distribution, area effects, dispersal, and speciation (Rizo et al., 2017; Valente-Neto et al., 2018; Gansfort et al., 2020). However, for aquatic systems most of our knowledge of community assembly comes from relatively stable (i.e., permanent) habitats. These possess long basin life, lasting centuries or at least decades (Sferra et al., 2017), relatively high surface connectivity (Chaparro et al., 2018), and frequent attendance by a diverse bird fauna, many of which are capable of carrying dispersal stages of a rich biota (Meyer-Milne et al., 2021). But metacommunity theory should include the perspective of all habitat types, not just those with long basin life. The edaphic conditions of temporary habitats are strikingly different and this may lead to pronounced differences in community structure.

The differences between small, shallow basins of permanent habitats to those in aridlands are striking. First, the wet phase in aridland basins often persist perhaps for a month, but sometimes only weeks or even days (Kulkarni et al., 2019; Walsh et al., 2014a). Second, except for a few rivers and their flood plain basins, surface connectivity is limited for most aquatic habitats to small isolated patches by vast stretches of arid landscape (Kobayashi et al., 2015). Finally, while localized zoochory by residents is likely, long-distance dispersal to these isolated habitats along flyways is probably low, but possible (de Morais Jr. et al., 2019). In addition, aridland basins are highly dependent on seasonal rainfall. Thus, these basins are subject to cyclic disassembly (drying out) and reassembly (rehydration) (O’Neill, 2016). As a result, the current population in any system predominantly arises from hatchlings of diapausing stages deposited by previous populations and/or those that arrived via anemochory. Outflow and deflation have different outcomes in these basins. Outflow is a local phenomenon. Intense rains may overfill some smaller basins, especially rock pools. If transported to a nearby basin, viable adults may reproduce while diapausing stages may hatch or sink becoming part of the propagule bank. Deflation may have local or distant consequences; winds can entrain diapausing stages along with dust and carry them 10s to 1000s of meters where they may land in a suitable basin (Rivas et al., 2018; 2019). Within a filling cycle, biotic interactions and selection pressures on life history features (e.g., high propensity for sex with concomitant ability to produce a dormant propagule) become intensified by the short hydroperiod (Schröder et al., 2007; Smith & Snell, 2012). Thus, being truly ephemeral systems, aridland basins provide exceptional opportunities to examine how communities of small-bodied, aquatic invertebrates (i.e., fairy-, clam-, tadpole shrimp, cladocerans, copepods, ostracods, rotifers) form and to test ecological theories without the confounding factors of permanence and connectivity (De Meester et al., 2005; Walsh et al., 2014a).

The Chihuahuan Desert is a large, well-defined ecoregion located in the southwest USA and northern Mexico, composed of a complex of intergrading communities arrayed across a broad series of elevation and latitudinal sequences. It is one of the few deserts recognized for its high biodiversity and high level of endemism (Dinerstein et al., 2001). This ecoregion also possesses a diverse array of aquatic habitats, including perennial and temporary waterbodies, as well as abandoned artificial basins (e.g., cattle tanks). Within this array of habitats our research has focused on rotifers for several reasons. (1) They contribute to both the food web and microbial loop (Wallace et al., 2015). (2) Habitats are usually rich in taxonomic diversity (Brown et al., 2020). (3) Rotifers produce small, desiccation resistant, propagules that resupply the sediment egg bank. These endure dry periods and yet can disperse via anemochory (Rivas et al., 2018; 2019). Thus, beginning with Rousselet (1909), researchers have argued that rotifers have a cosmopolitan distribution, following the ‘everything is everywhere’ model. Yet recent research indicates endemism for some species (Fontaneto et al., 2008a; Luo & Segers, 2020). We posit that examination of rotifer community assembly in shallow, temporary basins throughout the Chihuahuan Desert will improve our understanding of the processes that structure small passively dispersed aquatic invertebrate communities.

Our studies of aquatic habitats in the Chihuahuan Desert have shown that rotifer species diversity is high, with ~13% of all rotifer taxa occurring in this ecoregion, and that regional communities often comprise highly nested subsets of species, especially at small geographic scales (Brown et al., 2020; Ríos-Arana et al., 2019; Walsh et al., 2014b). We also have explored relationships between rotifer presence and environmental parameters for specific systems (i.e., saline systems) (Walsh et al., 2008), Mexican springs (Ríos-Arana et al., 2019), and selected aquatic sites at Big Bend (Walsh et al., 2014b). However, we still have a limited understanding of how rotifer species assembly takes place in temporary, aridland habitats across regional scales, nor do we have a firm appreciation of the relative contribution of stochastic versus deterministic processes in establishing rotifer communities in those habitats.

Researchers have recognized that both stochastic and deterministic processes are important drivers in establishing community composition (Valente-Neto et al., 2018). However, understanding their relative importance remains elusive even as researchers continue to refine these concepts (Brown et al., 2017; Fukami, 2015; Suzuki & Economo, 2021). Stochastic processes include ability to disperse, successful colonization (including overcoming priority effects), and random extirpation. Deterministic processes include species sorting and niche availability (Lopes et al., 2014; Wedderburn et al., 2013).

Stochastic processes appear to become more pronounced as dispersal becomes more difficult either due to low dispersal ability and/or increased distance between sites (De Meester et al., 2016). This may be related to increased invasibility of sites after a disturbance (Symons & Arnott, 2013; 2014). For example, initial dispersers may become established in a community, but long-term success becomes less likely over time (De Meester et al., 2016; Medeiros et al., 2021). If many species arrive approximately at the same time, such as during an intense wind event, the final assembly may include these species. However, longer time intervals between arriving colonists reduces the likelihood that a species will become established, unless it quickly adapts (Medeiros et al., 2021; Stroud et al., 2019). If species have enough time to adapt to local edaphic conditions before the arrival of subsequent immigrants, they may be able to competitively exclude newcomers, thereby creating a monopolization effect (De

Meester et al., 2002). Thus, priority effects can create patchiness in species presence among sites over time. This can lead to higher beta–diversity among systems where priority effects are important (De Meester et al., 2002; Fukami, 2015).

Many deterministic processes influence rotifers and other aquatic invertebrate community structure in shallow aridland waters. Among the most important of these are hydroperiod, conductivity, and productivity. Hydroperiod causes strong species sorting in habitats, becoming progressively stronger with shorter hydroperiods (Kulkarni et al., 2019; Sim et al., 2013; Vanschoenwinkel et al., 2010; Wellborn et al., 1996). This occurs due to the difficulty of completing a life cycle in habitats with short hydroperiods; that is, selection will exclude rotifer species with life cycles longer than the basin's hydroperiod. Thus, species sorting can create strong nestedness among assemblages, especially among those with short hydroperiods (Brown et al., 2020; Kulkarni et al., 2019). Conductivity also can create a species sorting effect by excluding species incapable of survival in certain salinity ranges (Echaniz et al., 2013; Jocque et al., 2010); this is particularly important in structuring rotifer community composition (Kaya et al., 2010; Walsh et al., 2008). Not surprisingly halophilic rotifers dominate many saline aridland systems (Nandini et al., 2019; Walsh et al., 2008). In many aquatic systems, rotifer population levels and biomass are positively correlated with productivity (Yoshida et al., 2003); however, Dodson et al. (2000) found no significant relationship of primary productivity with rotifer species richness in a survey of 33 well-studied lakes. In addition, Chase (2010) and Lopes et al. (2014) reason that regions with higher productivity are more vulnerable to priority effects, which results in greater species turnover: i.e., higher beta–diversity. However, in their study of >100 permanent and temporary lakes and ponds, Lopes et al. (2014) reported that beta–diversity was lower in the temporary habitats.

Habitat features often constrain community development such that the species assemblages are unique or contain one or more species that are indicative of the habitat. Aquatic habitats are replete with examples of indicator species (e.g., Karpowicz & Ejsmont-Karabin, 2021). The presence of indicator species likely implies that deterministic processes are important drivers of community composition.

Here, we compared rotifer species assemblages in three distinct types of shallow, temporary waters in the Chihuahuan Desert: rock pools (n = 60), playas (n = 17), and abandoned cattle tanks (n = 13). Specifically, we (1) assessed species richness among the three habitat types, (2) tested the hypothesis that species richness is determined by habitat area, (3) used an index to determine the relative strength of stochastic versus deterministic factors in the three habitat types, (4) examined relationships between three known important environmental drivers of species assemblages (hydroperiod, conductivity, and macrophyte/algae presence) and the functional trait of rotifer jaw (trophi) structure, and (5) identified indicator species for these temporary habitats.

MATERIALS AND METHODS

Sample Collection

In a large-scale survey of Chihuahuan Desert aquatic systems (2005–2020), we sampled rotifer communities in over 230 sites (Brown et al., 2020); here we analyze data from rock pools (n = 60), cattle tanks (n = 13), and temporary playas (n = 17) from the survey and with a few additional sites (Fig. 5). Although we usually took multiple samples at each site, we attempted to minimize environmental impact to smaller systems by keeping the total amount of each sample to about 250 ml of source water. We sieved all source water through netting of 64 µm. Sampling effort varied among the sites (from 1 to > 20 collections) and at some sites only one type of sample was taken (e.g., plankton), while at others a variety of microhabitats were sampled. The unbalanced effort was a result of logistical constraints of sampling a large number of sometimes widely separated temporary habitats. However as noted in Brown et al., (2020), sampling effort was not an important determinant of species richness. A rarefaction analysis was conducted on the current dataset and is included in Supplemental Information, Table A1. For each site we compiled a species list of presence/absence data over all sampling dates (Brown et al., 2020). Species were identified using the keys listed in Brown et al. (2020), Except for two sampling sites (San Francisco tank (*S* = 7; 22.0529200 N, -99.8474700 W) and Presa De La Vaca tank (*S* = 10; 22.0678055 N, -99.5843333 W)) all the sites we examined resent here are noted in Brown et al., (2020). Species lists for these sites and others are available by request.

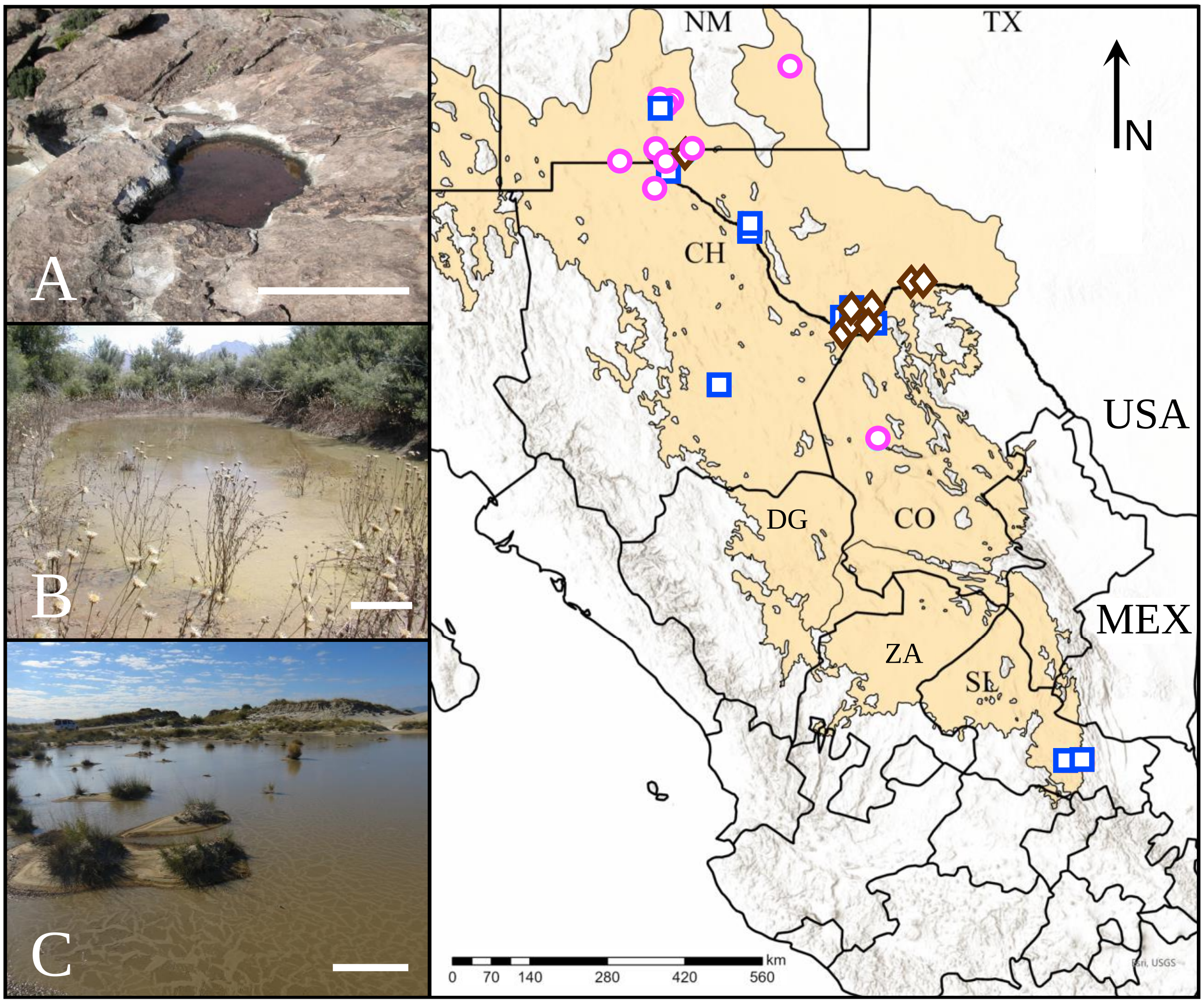

Fig. 5. Sampling sites in the U.S.A. and Mexican Chihuahuan Desert (n=90). Rock pools (n=60), diamonds; cattle tanks (n=13), squares; temporary playas (n= 17), circles. Many symbols overlap with one another. State name abbreviations in México (MEX): CH – Chihuahua, CO – Coahuila, DG – Durango, SL – San Luis Potosi, ZA – Zacatecas; in USA: NM – New Mexico, TX, Texas. Panel A: a representative rock pool, Hueco Tanks State Park & Historic Site; Panel B: abandoned cattle tank, Big Bend National Park, Panel C: temporary playa, southern New Mexico. (Bars = ~1 m)

Habitat Characterization

We analyzed selected physical and environmental parameters including habitat type and size (area), hydroperiod, conductivity, and productivity to determine relative contribution of stochastic and deterministic processes that shaped the rotifer communities. We also recorded latitude and longitude for all sites; these are given in Brown et al. (2020), except for those noted above. We measured conductivity with a pre-calibrated YSI model 556 multiprobe meter. Categorical variables included habitat type (rock pool, playa, tank), hydroperiod (ranked 1–3 (short = 1, intermediate = 2, and long = 3) based on volume and shading), and presence and relative abundance of algae, as visible mats (0 = none, 1 = some, 2 = abundant) and macrophytes (0 = none, 1 = rare, 2 = abundant, 3 = dominant). Macrophytes comprised mostly submerged cattails, grasses, and mosses. We used the level of algae and macrophytes as an indirect proxy for habitat productivity (Juračka et al., 2019). If a site had more than one sampling event, we averaged the values of the environmental parameters. We estimated area as the product of the maximum length and maximum width.

Data Analysis

We used R version 4.0.2 for statistical analyses (R Core Team, 2020). The correlation between the log area (m^2) of the habitat and log species richness (S) was determined using linear regression. We tested different models of species-area relationships using the R-package *sars,* comparing models based on Akaike's information criterion (Matthews et al., 2019). To model the influence of spatial distribution, we created distance-based Moran's eigenvector maps (dbMEMs) from the latitude and longitude of our sites with the package *adespatial* (Dray et al., 2021). Nearest neighbor trees and weights used in constructing these dbMEMs were done with the R- package *spdep* (Bivand et al., 2021). We calculated variance partitioning between our environmental predictors and significant dbMEMs with significant spatial autocorrelation using the *vegan* 2.5-6 package (Oksanen et al., 2019). To determine relationships between species distributions and environmental factors and habitat area we used partial Canonical Correspondence Analysis (pCCA) implemented in the *vegan* 2.5-6 package after removing the influence of dbMEMs with siginificant spatial autocorrelation. We decided on this unimodel approach by inspecting the first axis of a Detrended Correspondence Analysis (DCA) of our species assembly data conducted with the *vegan* package. We tested specific environmental factors for autocorrelation with dbMEMs using a Moran's I test in R. For this analysis, we excluded sites with incomplete data; after this reduction, we retained 58 rock pools, 14 playas, and 12 tanks in the dataset. Prior to running the pCCA, we tested for multicollinearity and conducted an F test (ANOVA) to determine significance of predictor variables. *We* used Sørensen's Dissimilarity Index as a measure of beta diversity (Baselga, 2012). Further, we used general linear modeling with a Poisson distribution to test for a relationship between algae/macrophytes and species richness in R version 2.5-6.

To further investigate the relative contribution of stochastic and deterministic factors on community assembly, we calculated the PER-SIMPER and Dispersal Niche Continuum Index (DNCI) (Vilmi et al., 2021) using the *DNCImper* 1.0 package with 1 000 permutations in R (Gibert et al., 2020). To account for the asymmetry in site number between habitat types (we have >50% the number of rockpool sites than other habitat types) and hydroperiods, sites of each habitat type and hydroperiod were randomly resampled to equalize the number of sites per habitat. The more negative the value of the DCNI, the more likely that stochastic processes dominate community structure. We calculated DNCI values for differences between habitat type, habitat hydroperiod, and the functional trait of rotifer jaw structure (trophi) type. Rotifers differ in how they consume food. Raptorial species with trophi of virgate, cardate, incudate, forcipate, and uncinate types tend to process one large item at a time; microphagous species with malleate, malleoramate, and ramate trophi tend to process many small particles in a short period of time (Obertegger et al., 2011). We implement an indicator species analysis using the *indicspecies* 1.7.9 package and SIMPER analysis in Community Analysis Package (CAP) version 6.2.4.

RESULTS

Species richness (*S*) among all the sites we sampled ranged from 1 to 44 with a total of 132 species in all sites. However, within each category ranges and means ($\bar{x}$ ± 1SD) varied widely: playas (1–44; $\bar{x} = 9.5 \pm 9.8$); tanks (1–28; $\bar{x} = 8.5 \pm 7.8$); rock pools (1–26; $\bar{x} = 3.2 \pm 3.6$) (Fig. 6). With the exception of one site, rock pools were relatively depauperate with *S* ranging from 1–8. The two playas with the highest richness, Laguna Prieta (*S* = 30) and Mescalero Canyon (*S* = 44), are located at Hueco Tanks State Park & Historic Site. The other playas examined in this study had wide ranging richness (*S* = 1–23). The two tanks with the highest richness were located in Big Bend National Park. A recently constructed tank at Rio Grande Village had *S* = 28. The other site (Tule Tank;

S = 21) is an artificially enhanced, natural low-lying basin near a spring and a historic settlement.

Playas had the greatest gamma-diversity (*S* = 81), with tanks and rock pools having similar levels of gamma diversity (n=65 and 61, respectively). Sørensen's dissimilarity values for our study sites are similar to those from other habitats (Table 5).

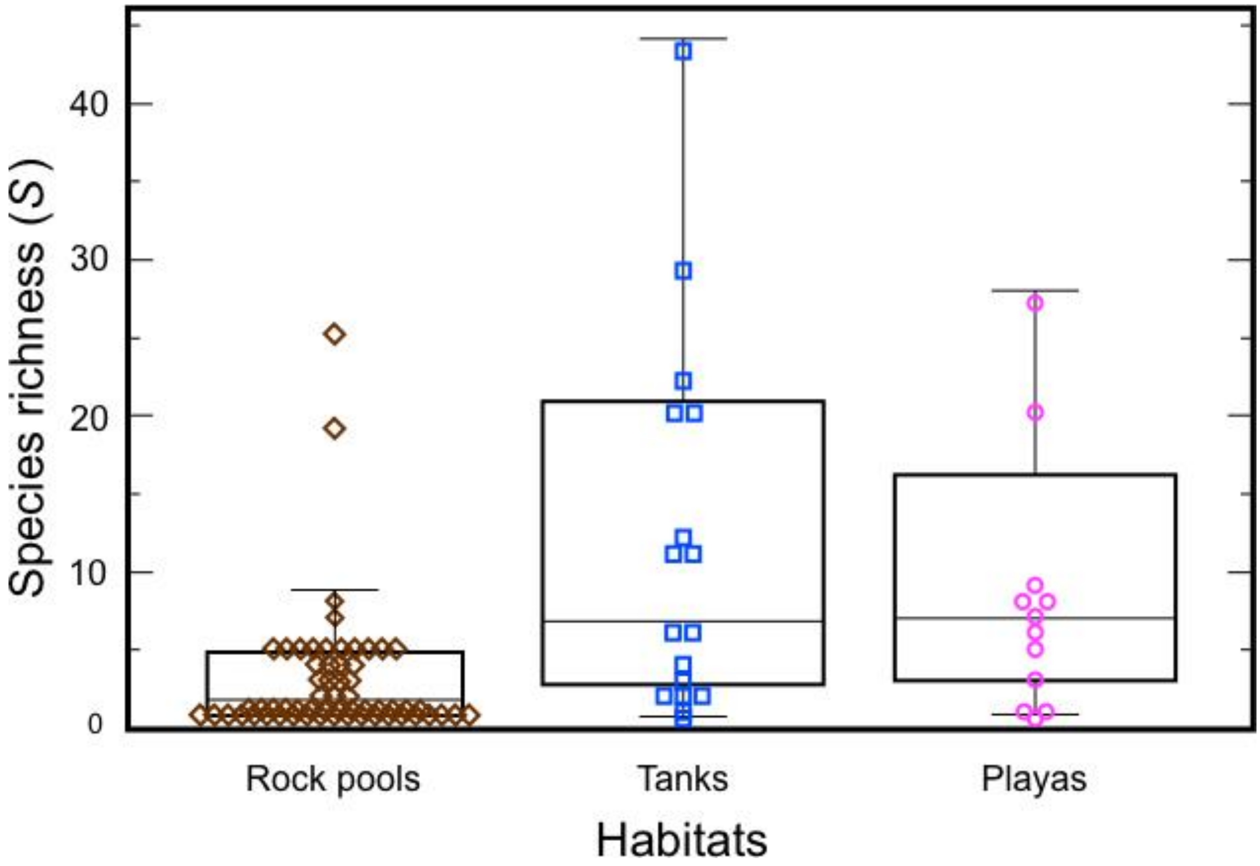

Fig. 6. Rotifer species richness (*S*) in selected habitat types in the Chihuahuan Desert. The horizontal lines within the boxes indicate their respective medians; the boxes indicated the range of lower (Q1) and upper quartiles (Q3); dots outside the boxes indicates outlying datapoints; error bars represent 2 standard deviations above the mean

Table 5. Species diversity among selected sites. Diversity calculated for all sites examined in the study: alpha = species richness (ranges); beta = mean species turnover based on Sørensen's Index (± 1SD); gamma = total regional species richness

Region	Habitat	# Sites examined	Diversity			References
			Alpha	Beta	Gamma	
Aridlands						
Chihuahuan Desert	Playas	17	1–44	0.83 (0.18)	81	1
Chihuahuan Desert	Rock pools	60	1–26	0.81 (0.30)	61	1
Chihuahuan Desert	Tanks	13	1–28	0.84 (0.13)	65	1
Chihuahuan Desert	Springs	95	1–35	0.85 (0.14)	175	2
Australia	Billabongs (River Murray)	13	8–13	0.77 (0.13)	52	3
Oman	Lakes, rivers, pools	9	10–25	0.61 (0.14)	66	4
Saudi Arabia	Lakes, rivers, pools	19	1–15	0.73 (0.26)	40	5
Spain	Dune pools	32	1–14	0.68 (0.21)	34	6
Yemen	Lakes, rivers, pools	35	1–29	0.84 (0.18)	74	7
Temperate / Tropical						
India	Eutrophic fish ponds	5	14–25	0.71 (0.21)	57	8
Subtropical China	Shallow lakes	5	26–30	0.20 (0.06)	39	9
Temperate Portugal	Eutrophic lakes	3	16–31	0.42 (0.19)	40	10
Cryogenic						

Arctic	Permafrost thaw waters	5	14–19	0.23 (0.19)	24	11
High Arctic, Canada	Pools, ponds, small lake	8	8–27	0.64 (0.12)	70	12

† – Datasets used: 1 – This study; 2 – Brown et al. (2020); 3 – R.J. Shiel, pers. commun.: discussed in Shiel and Koste (1983); 4 – Segers and Dumont (1993); 5 – Segers and Dumont (1993); 6 – Mazvelos et al. (1993); 7 – Segers and Dumont (1993); 8 – Sharma and Dudani (1992); 9 – Wen et al. (2011); 10 – Castro et al. (2005); 11 – Bégin and Vincent (2017); 12 – (De Smet and Beyens 1995).

We found that a persistence model best described our data. For each habitat type individually, we found that a negative exponential model best described rockpools, a logarithmic function best described playas and a linear function best described tanks when compared by Akaike’s information criterion. We found a significant but weak relationship ($R^2 = 0.15$; $p < 0.001$) between site area and species richness when we analyzed all habitat types together. However, when we examined the habitats separately, the sites no longer showed a significant relationship between area and species richness ($p > 0.05$) regardless of the model (Fig. 7).

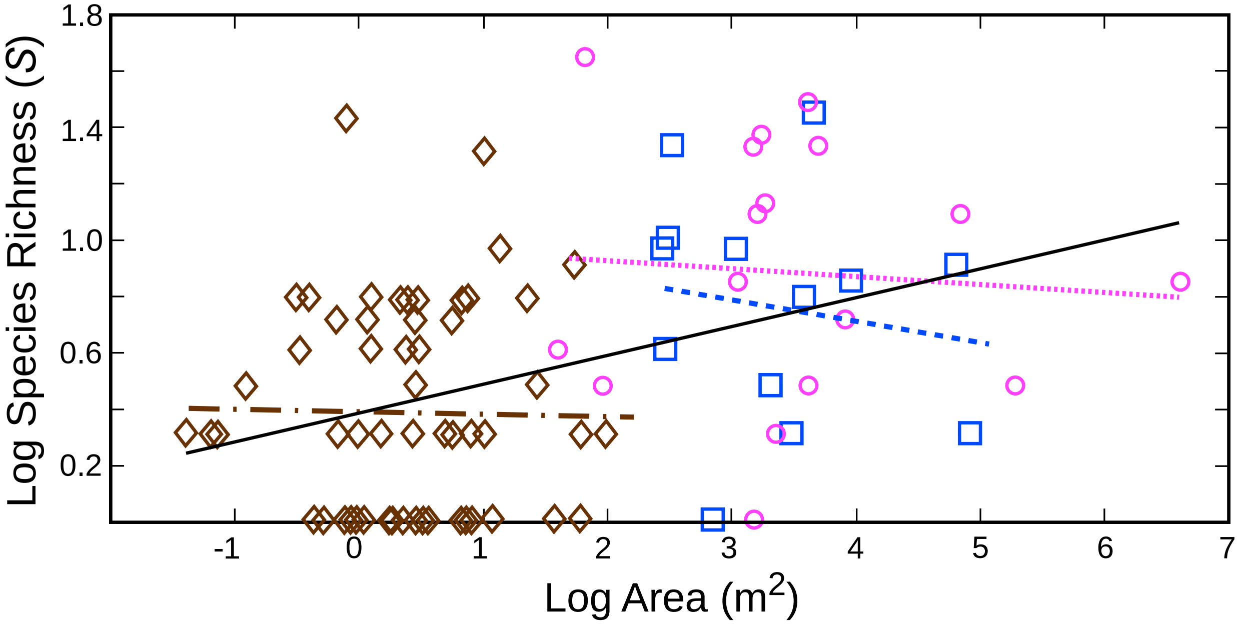

Fig. 7. Area-species richness (*S*) relationships of rotifers in selected habitat types in the Chihuahuan Desert. Some symbols overlap in their location. Symbols are as follows. Playas (n=17) circles, dotted line: *S* = –0.027 Area + 0.9862; R^2 = 0.0054; P >0.05; Cattle tanks (n=13) squares, dashed line: *S* = –0.0776 Area + 1.0288; R^2 = 0.025; P > 0.05; Rock Pools (n=60) diamonds, alternating dash-dotted line: *S* = –0.0085 Area + 0.3904; R^2 = 0.0003; P > 0.05; All sites combined (n=90) solid line: *S* = 0.103 Area + 0.3871; R^2 = 0.1514; P = 1.46 x 10^{-4}

Stochastic models most closely align with our empirical results. Models with both sites and species constrained showed the smallest deviation from our data, while constraining only sites showed the highest deviation (Fig. 8, Table 6). The mean DNCI value (± 1SD) was -6.49 ± 0.57. When we analyzed by trophi type among habitat types, raptorial feeders possessed a less negative DNCI value (-3.80 ± 0.46) than microphagous feeders (-5.42 ± 1.34). The pairwise habitat comparisons were similar to the overall results.

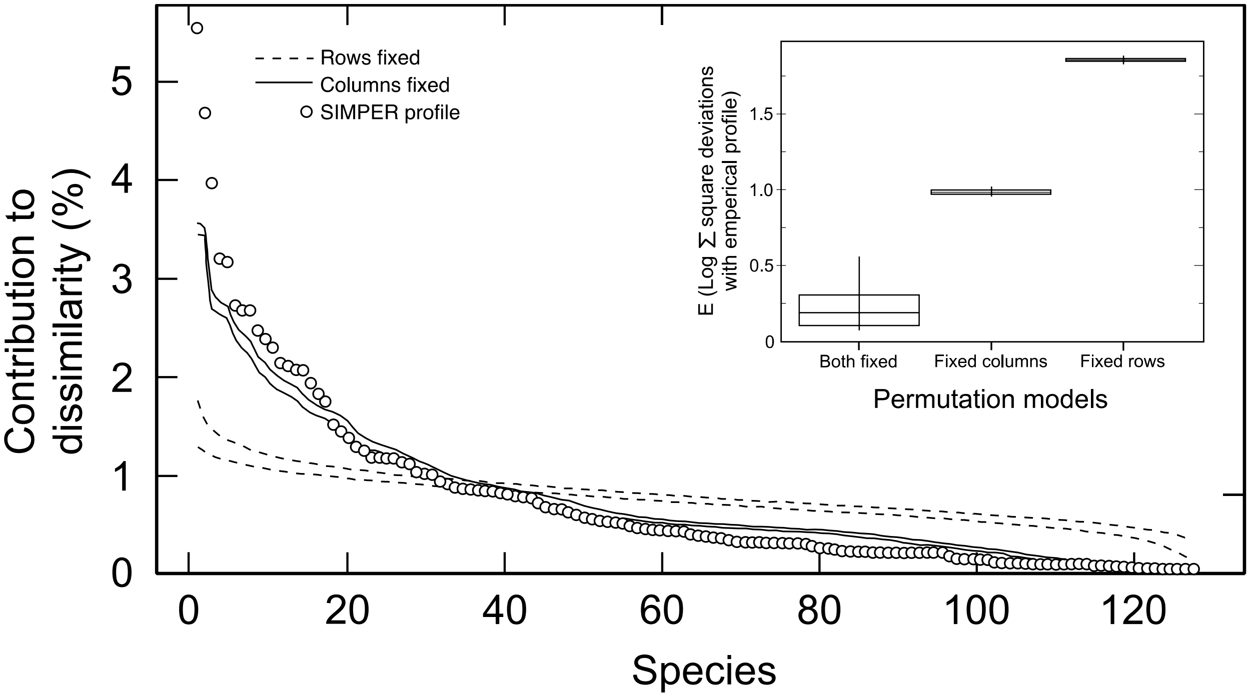

Fig. 8. Comparison of SIMPER profiles created from our empirical data (species assemblages) with permutation models representing niche-controlled distribution (rows/sites fixed, dotted lines, deterministic) and dispersal-controlled distribution (columns/species fixed, solid lines, stochastic). Inset: Box plots for the E metric of these comparisons is in the upper right corner of the graph

Table 6. Dispersal-Niche Continuum Index (DNCI) for selected rotifer communities in the Chihuahuan Desert categorized by habitat type, hydroperiod, and rotifer trophi type. Also included is an analysis based on food preference of species within a habitat. A negative value indicates the dominance of dispersal or other stochastic processes in community assembly (Vilmi et al., 2021)

Comparison	**DNCI**	**SD**
Habitat Overall	-6.17	0.57
Rock pools vs. playas	-5.18	0.95
Rock pools vs. tanks	-6.91	0.92
Playas vs. tanks	-7.47	1.16
Hydroperiod Overall	-7.20	0.93
Short vs intermediate	-5.77	0.31
Short vs long	-6.65	1.01
Intermediate vs long	-7.33	2.03
Rotifer trophi type		
Raptorial feeders	-3.80	0.46
Microphagous feeders	-5.42	1.32

Dissimilarity among the three habitat types was quite high (> 0.8). These values were similar to those for most of the habitats used as comparisons (Table 7).

Table 7. Effects of algae and macrophyte presence and abundance on rotifer species richness in desert ephemeral waters using Generalized Linear Modeling based on a Poisson distribution in R.

Coefficients	Estimated standard error	z value	Pr(>|z|)	Significance
Intercept	0.923	0.099	9.28	< 0.001
Algae	0.731	0.102	7.150	< 0.001
Macrophytes	0.690	0.071	9.67	< 0.001

The length of the first axis of our DCA analysis was 6.8, suggesting that a unimodal approach was appropriate for our data (Braak & Prentice, 1988). The ANOVA indicated that all predictor variables were significant at the 0.05 level. The pCCA explained ~12% (R^2 adjusted) of the variance observed in our species presence/absence data. The first constrained component was negatively associated with conductivity and site area, and to a lesser extent, positively associated with algal mat development (Fig. 9). The second constrained component was negatively associated with habitat type, hydroperiod, and presence of macrophytes. Several rotifer species were negatively correlated with the first constrained component (*Proales similis* Beauchamp, 1907, *Proales halophila* Remane, 1929, *Hexarthra polyodonta* (Hauer, 1957), *Encentrum putorius* Wulfert, 1936, *Brachionus plicatilis* Müller, 1786) or the second constrained component (*Euchlanis calpidia* (Myers, 1930), *Lindia torulosa* Dujardin, 1841, *Cephalodella panarista* Myers, 1924, *Sinantherina socialis* (Linnæus, 1758), *Filinia novaezealandiae* Shiel & Sanoamuang, 1993, *Cephalodella poitera* Myers, 1934, and *Brachionus havanaensis* Rousselet, 1911. On the other hand, two species (*Epiphanes macroura* (Barrois & Daday, 1894) and *Hexarthra* sp.) were positively correlated with the second constrained component. Of our predictor variables, only the presence of algal mats was significantly autocorrelated with the dbMEMs (Moran I statistic standard deviate: 5.57, $p < 0.01$). Variance was partitioned by individual fraction with environmental predictors accounting for 17% of the variance, whereas the significant dbMEMs accounted for ~2% of the observed variance (R^2 adjusted).

Poisson GLM of macrophytes and algal mat influence on species richness returned the following formula: $S = 0.73M + 0.69A + 0.92$ where M is macrophyte presence, and A is algal mat presence. All coefficients and the intercept were highly significant (Z= 9.67, 7.15, and 9.28, respectively; all had $p < 0.01$) (Table 7). Linearity of our residuals was checked by visual inspection of Q-Q plots.

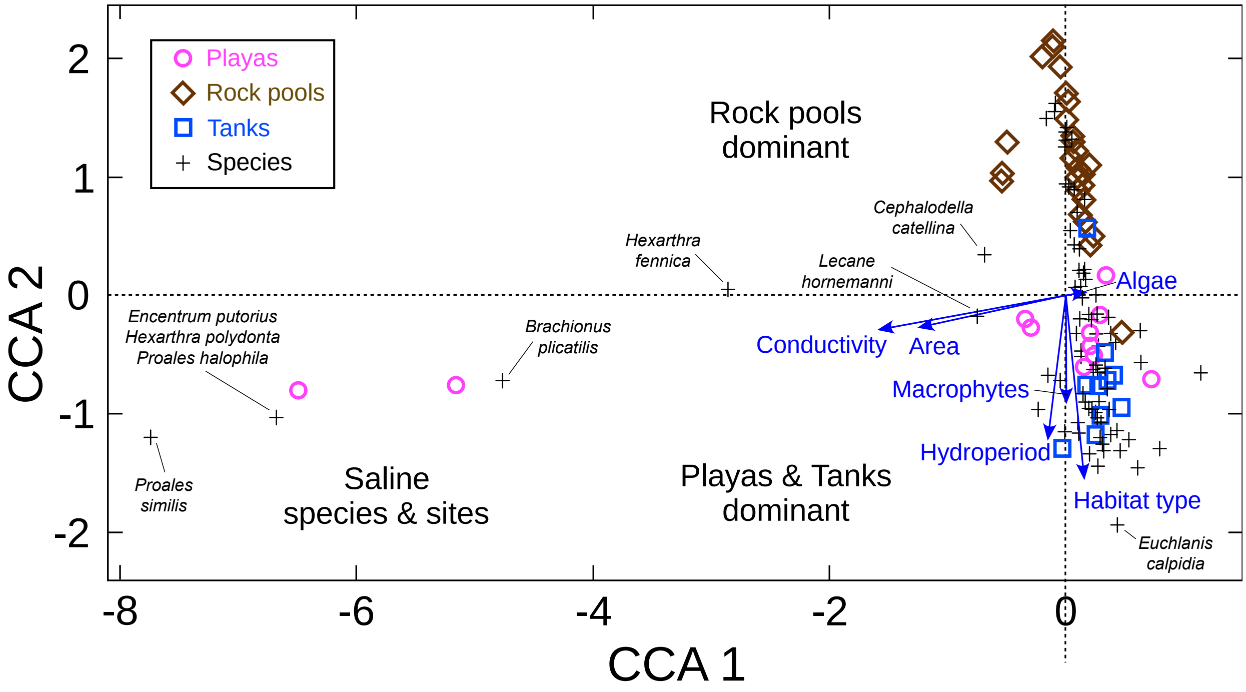

Fig. 9. Partial Canonical Correspondence Analysis (pCCA) of environmental correlates of rotifer species richness in selected Chihuahuan Desert aquatic habitats with variance due to spatial autocorrelation removed. Symbols are as in Fig 7. Note that some symbols overlap and for plotting purposes, the species and sites were scaled by eigenvalue.

We determined 29 taxa to be indicator species (*indval.g*, $p < 0.05$; Table 4). Of these, one was an indicator of rockpools (*Hexarthra* sp., $p<0.01$), 19 were indicators of playas, 6 were indicators of tanks, 5 were indicators of tanks and playas, and one was an indicator of rock pools and tanks (*Trichocerca similis* (Wierzejski, 1893)). Several species were significant indicator species ($p < 0.005$) including: playas*, Cephalodella megalocephala* (Glasscott, 1893), *Epiphanes brachionus* (Ehrenberg, 1837), *Lecane bulla* (Gosse, 1851), *Lecane luna* (Müller, 1776); tanks, *Polyarthra dolichoptera* Idelson, 1925; playas and tanks, *Brachionus angularis* Gosse, 1851, *Brachionus quadridentatus* Hermann, 1783, *Euchlanis dilatata* Ehrenberg, 1830 and *Platyias quadricornis* (Ehrenberg, 1832). SIMPER analysis also indicated that *Hexarthra* sp. was the species that most associated with rock pools but also showed *Trichocerca similis* and *Lepadella patella* made substantial contributions to differences in communities among rock pools and other habitat types. Similarly, for the other habitat types, there was some overlap with the indicator species analyses (Table 8).

Table 8. Rotifer species with highest contributions to the average between-group Sorensen dissimilarity among rotifer communities in select aquatic habitats in the Chihuahuan Desert as a function of habitat according to SIMPER analyses (species contributing at least 10% to similarity; % contribution in parentheses) and Indicator Species analyses (*indval.g* p-value)

Habitat	**Species with high SIMPER contributions**	**Indicator Species**	**p-value**
Rock pools	*Hexarthra* sp. (58%), *Trichocerca similis* (15.4%), *Lepadella patella* (10.1%)	*Hexarthra* sp.	0.01
Tanks	*Euchlanis dilatata* (50.3%), *Polyarthra dolichoptera* (14%), *Brachionus angularis* (13%)	*Polyarthra dolichoptera*	0.005
		Asplanchna brightwellii, Brachionus bidentatus, Filinia pejleri, Eosphora najas, Polyarthra vulgaris	<0.05
Playas	*Brachionus quadridentata* (24%), *Epiphanes brachionus* (10%)	*Asplanchna seiboldii, Cephalodella megalocephala, Epiphanes brachionus, Lecane bulla, Lecane luna*	0.005
		Asplanchnopus hyalinus, Brachionus calyciflorus, Brachionus plicatilis, Cephalodella gracilis, Cephalodella sterea, Filinia cornuta, Lacinularia flosculosa, Lecane thalera, Lepadella rhomboides, Notommata glyphura, Rhinoglena ovigera, Squatinella rostrum Trichocerca rattus, Trichocerca cf. *vernalis*	<0.05
Rock pools & Tanks	N/A	*Trichocerca similis*	<0.05
Rock pools & Playas	N/A	N/A	

Tanks & Playas	N/A	*Brachionus angularis, Brachionus, quadridentatus, Euchlanis dilatata, Platyias quadricornis*	0.005
		Cephalodella gibba	<0.05

DISCUSSION

In our previous study of rotifers in the Chihuahuan Desert we showed that (1) rotifer species composition is very diverse, (2) species dissimilarity among sites was correlated with distance, and (3) localized hotspots of richness are predicted across several scales of analysis (Brown et al., 2020). Here, we found that rotifer species richness varies greatly among habitat types and that stochastic processes dominate in determining community assembly for shallow ephemeral systems. While stochastic processes contribute the most to species composition, we found a small influence of deterministic effects on community assembly (i.e., hydroperiod and conductivity). Differentiating among stochastic effects could lead to further insights into community assembly in these systems. In addition, deterministic effects are more localized, so repeated sampling of individual sites may provide further support for their role in determining community structure.

We found high beta-diversity, and when compared to rotifer assemblages in other localities, they were among the highest in dissimilarity (Table 5). In our past analyses, we found that species assemblages were highly nested (Brown et al., 2020; Ríos-Arana et al., 2019), which may contribute to the high beta-diversity we observed. Nestedness may reflect the portion of beta diversity that is structured by deterministic effects. For example, nestedness in rock pools may be due to strong species sorting by hydroperiod, in which case it should reflect the influence of deterministic effects on the assembly (Ripley & Simovich, 2009). We also found that as spatial grain increases, distance influences the species assemblage less for rock pools than for other habitats (Brown et al., 2020). At larger spatial scales richness of rock pools may be more representative of the regional species pool available to these sites, leading to lower beta diversity. Additionally, at small scales rock pools may have significant hydrological connections with nearby rock pools, increasing similarity among them. Our current study supports the conclusions of Lopes et al. (2014) that species similarity should be lower in temporary habitats than in those with longer basin life. However, one should undertake comparisons among studies with caution for several reasons. (1) Sampling efforts differed among the published studies we included in Table 5. (2) Grouping sites by habitats can conflate habitats with very different edaphic conditions. For example, the rock pools comprised three different bedrocks: syenite porphyritic granite, limestone, and pyroclastic-flow deposits. (3) Studies may miss important suites of species by using snapshot datasets of communities.

Unlike Juračka et al. (2019) we found no relationship between habitat area and *S* when examining the three habitat types separately. However, when combined, there was a weak, but significant correlation. This species-area effect seems to be due to intrinsic differences in habitat size and richness between rock pools, which are smaller with relatively low diversity, and the playas and tanks which are larger with higher diversity. We sampled smaller sites (i.e., rock pools) much more frequently than the other habitats. This may account for the lack of correlation between habitat area and richness in this study. Recent studies that controlled for species abundance concluded that island species-area effects are likely a sampling bias (e.g., Gooriah & Chase, 2019; Gooriah et al., 2021). Alternatively, some studies have found that large regional species pools can cause richness scaling with habitat area due to deterministic processes (Spasojevic et al., 2018).

Our comparisons of overall DNCI scores for habitat type indicated a predominance of stochastic processes in structuring rotifer community assembly. Our values are similar to those found for passive dispersers and macroinvertebrate communities in streams by Vilmi et al. (2021). Although stochastic forces dominated overall, in our pairwise comparisons by habitat type we found slightly more deterministic indices for rock pools when compared with either playas or tanks than for tanks compared with playas. This is what we would expect to see; rock pools have multiple etiologies and their edaphic conditions differ substantially from the other habitat types. In addition, tanks and playas are separated by greater distances than rock pools which are typically clustered. Rotifer trophi structure, a functional trait, affected the DNCI scores of rotifer habitat comparisons, with raptorial feeders having a more deterministic score than microphagous feeders. Microphagous feeders are generalists relative to raptorial feeders that rely on larger prey. We speculate that reliance on particular food sources may make raptorial feeders more prone to species sorting and other deterministic processes.

Our multivariate analysis showed a small, but significant, influence of deterministic processes in shaping rotifer community assembly. Several rotifer species were highly correlated with conductivity and hydroperiod. We expected this result because hydroperiod and salinity influence rotifer richness through species sorting (Montero-Pau et al., 2011; Walsh et al., 2008). For example, several rotifers aligned to the first component are known to be halophilic species (*B. plicatilis, E. putorius, H. polydonta, P. halophila, and P. similis*) (Green, 1986; Fontaneto et al., 2008b). Spatial characteristics accounted for a very small portion of the variation (~2%) observed in rotifer species assemblages. One explanation for this small contribution may be the high passive dispersal capacity of aquatic species that inhabit temporary habitats or alternatively the relative homogeneity of these habitats. This likely leads to high stochasticity in colonists and the resulting species assemblages.

Habitat area and conductivity were both negatively aligned along the first component of the pCCA. Several of our larger playas, in particular Lake Lucero, are in locales with high water tables. When the water table is high it is more likely to interact with the playa, potentially increasing salinity (Rodríguez-Rodríguez, 2007). Additionally, Lake Lucero is located in a hot low-lying basin, so evaporites build up in the playa causing increased salination (Weir Jr., 1965). The relatively large size of playas coupled with their ground water interaction may explain the relationship we saw between conductivity and area. We also saw similar negative relationships between macrophytes and habitat type, which is likely due to the fact that most rock pool habitats lack macrophytes. We found only a small influence (~12% R^2 adjusted) of our constraining variables in structuring variation in rotifer community assemblages. This low explanatory power may indicate that deterministic effects have a relatively small role in determining community assembly in these systems or that there are other important factors that we did not measure. Despite this, given the strong gradient in conductivity found in some of these habitats, we suggest that apart from hydroperiod, salinity is the most important deterministic variable that is influencing community assembly in these habitats.

SIMPER species contributions and indicator species analysis showed overlap in species habitat associations. Indicator species analysis identified *Hexarthra* sp. as an indicator of rock pool habitats. This species is known to be adapted to short hydroperiod; it has a truncated lifecycle and is immediately capable of mixis rather than going through several amictic cycles first, the usual path for monogonont rotifers (Schröder et al., 2007). Indicator species have been reported for other specialized habitats such as acidified lakes where rotifers may occur: *e.g., Cephalodella acidophila* Jersabek, Weithoff & Weisse, 2011 (Weithoff et al., 2019) and *Keratella taurocephala* Myers, 1938 (Yan & Geiling, 1985).

While aridland basins may appear superficially similar to those in temperate systems, we have posited that constructs based on long-lived basins are insufficient to describe development of the aquatic invertebrate communities in the shallow water basins of aridlands. Nor do these basins resemble dry riverbeds that after decades of drought may receive flow from upstream (Urban et al., 2020). Thus, we need a refined conceptual model to focus attention on the processes driving habitat colonization and species assemblage in these habitats (Fig. 10).

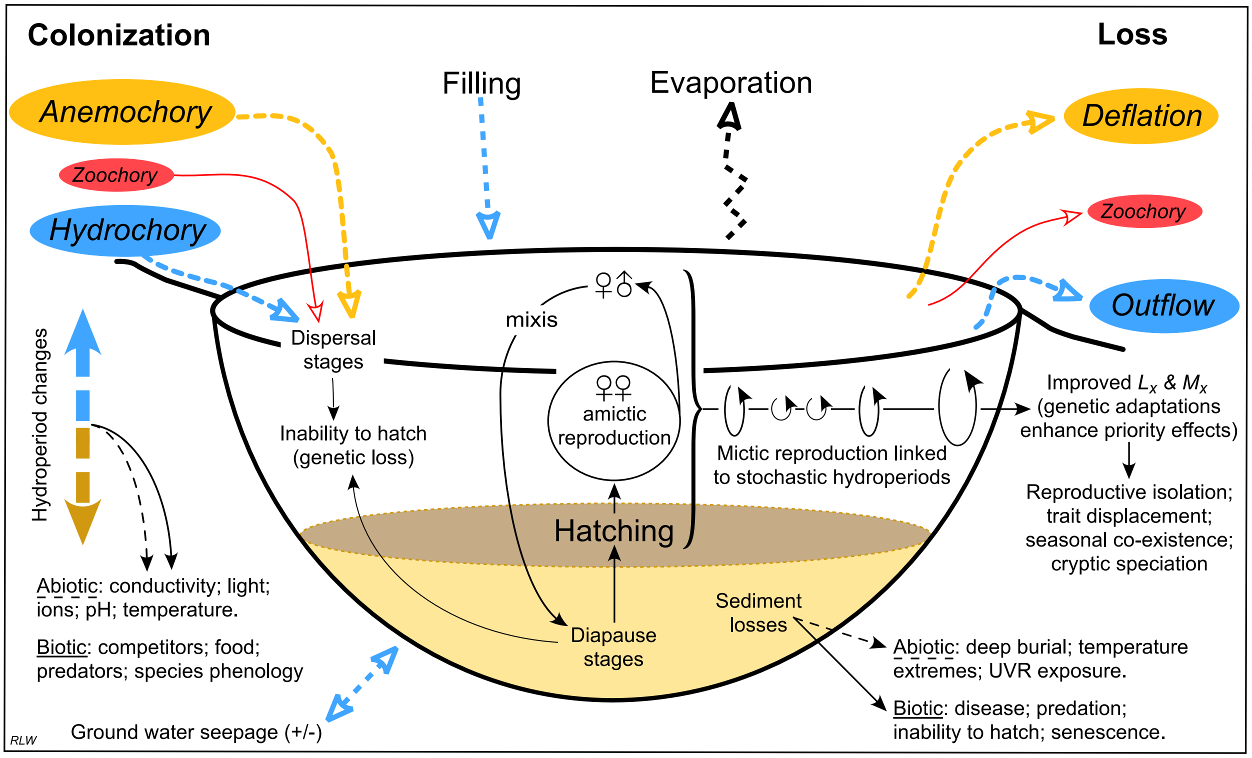

Fig. 10. Generalize conceptual model of important factors influencing community assembly of small-bodied, aquatic invertebrates including rotifers found in the isolated, temporary, shallow water basins of aridlands (e.g., playas, rock pools, tanks). Larger font size of colonization and loss processes indicates their relative importance (see text for details). Size of the circular arrows (in mictic reproduction) indicates that the length of the hydroperiods varies among filling cycles. Ground water seepage (+/-) is of minor importance in rock pools. Symbols: Biotic processes = solid lines (—), abiotic processes = dashed lines (----); closed arrows = processes within the basin

In our conceptual model we note that the distinctive factors of these aridland habitats fall into three broad categories: basin properties, sediment egg banks, and dispersal, all of which have important consequences to their invertebrate inhabitants. (1) These basins have a hydroperiod that involves stochastic filling during the wet season, followed by inevitable drying that lasts for uncertain duration. (2) Sediment depths and degree of their exposure to environmental stresses differ widely among sites. (3)

Dispersal that transport propagules to or away from basins (i.e., anemochory/deflation, hydrochory/overflow, and zoochory, including anthropogenic movement) are inherently unequal and vary among habitats.

Our conceptual model also focuses on the primary difference between shallow desert basins and those in temperate regions — ephemerality. Regardless of the edaphic conditions, shallow basins in the Chihuahuan Desert possess short hydroperiods; they fill with monsoonal rains and then lose water through evaporation and/or seepage (Scuderi et al., 2010). Thus, over the seasonal life of an aridland basin they fill rapidly and just as quickly their abiotic properties change; water temperature, conductivity, dissolved oxygen, pH, and the concentration of dissolved materials vary continuously over a short time frame. Nevertheless, the aridland basin ultimately ends in a return to dryness. Concomitant with variations in abiotic factors, biotic factors (e.g., food, competitors, and predators) also change during the basin life, reflecting the idiosyncratic nature of each basin (Fig. 10, lower left).

Sediments in desert basins also vary, ranging from nearly absent in rock pools (a few mm) to substantial (ca. 10 cm or greater) in cattle tanks and playas (authors, pers. obs.). Thus, in rock pools the diapausing stages of aquatic invertebrates experience extremes in temperature and ultraviolet radiation (Jocque et al., 2010). While sediment depth in cattle tanks and playas are more substantial, they dry to significant depths during the dry season. However, while lying deeper in the sediment may afford diapausing rotifer embryos some protection from drying, García-Roger et al. (2006) reported that percent hatching decreased as a function of sediment depth. Thus, lying deeper in the sediment probably means greater age and with that increased susceptibility to loss via abiotic and biotic processes. Collectively, these factors may impact the viability of propagules unequally in the three habitat types (Fig. 10, lower right).

Dispersal of propagules among a group of closely opposed, shallow basins can involve both gains and losses of propagules (Fig. 10, upper left and right). However, these differ among the basins we studied. Local fauna (insects and vertebrates) probably comprises the scope of zoochory, especially at the smallest sites. This is due to the fact that most of these habitats are too small and too isolated, and also because they fill during the monsoon season, which is outside the period of normal migration for avifauna. Hydrochory and outflow varies among the three habitats in our study. For cattle tanks and playas surface flow only brings in materials and potentially propagules from the surrounding landscape; water does not flow from these systems to other sites, unless it is through ground water seepage: an unknown factor in our study sites. In contrast, in rock pools hydrochory is site specific. Inter-basin connectivity between rockpools at HT occurs only as sheet-flow across a rocky surface; at best channels in these systems are poorly defined. However, connectivity among basins in the other regional rock pools we examined is much more well defined. During monsoonal rains in those systems, upstream basins systems overtop their margins and flow to the next basin in well-defined channels. Thus, they form true dispersal networks (Brown & Swan, 2010).

The construct that emerges from our studies of isolated desert basins is one of extremes. Once the basin has refilled, rotifers may begin to hatch from diapause and increase their population size, but due to an uncertain hydroperiod, mixis (which replenishes the sediment egg bank) must occur before the basin dries. This sequence repeats, but filling-drying cycles are stochastic. Therefore, because occurrence, extent, and duration of hydroperiod is not predictable, there must be a tight coupling between reproduction and short hydroperiod. As illustrated in the center of the model (Fig. 10), this process begins with amictic reproduction, but as species go through several mictic reproductive cycles each becomes genetically more well adapted to the basin's conditions. The outcome of this is a progressive genetic refinement (improved survivorship and reproduction), which enhance the resident's priority effects (De Meester et al., 2002; 2016). Over many mixis cycles this should lead to trait displacement, reproductive isolation, seasonal co-existence, and ultimately cryptic speciation (Mills et al., 2017; Kordbacheh et al., 2017).

While our understanding of aquatic invertebrate community assembly in aridland ephemeral systems is improving, we suggest that attention to the following points will advance it further. (1) While we visited many of the sampling sites repeatedly, this research only provides a snapshot survey of the rotifer fauna of these habitats. Thus, we should not construe the fact that we did not find specific species to indicate that they are not present at some other time during the hydroperiod. To circumvent this limitation, we suggest using the technique of resurrection ecology — hatching dispersal stages by rehydrating dry sediments — to assess the zooplankton fauna from the sediments of ephemeral habitats (Pinceel et al., 2017; Vargas et al., 2019) and/or by applying environmental DNA sequencing to water and sediment samples (Yang & Zhang, 2020; Zawierucha et al., 2021). (2) To differentiate impacts of stochastic effects in structuring community assembly, we recommend that researchers perform a series of mesocosm experiments in which they vary the arrival sequence of diapausing stages. That protocol could add a complicating factor of providing a sediment egg bank to some mesocosms (Langley et al., 2001; Nielsen et al., 2002). (3) To expand our understanding of community assembly we recommend the study of other aquatic invertebrates (Juračka et al., 2019), in aridland ephemeral systems, and to compare our systems to that of vernal pools (Kneitel, 2014) and prairie-potholes (McLean et al., 2020) in temperate zones. (4) The analysis of rotifer trophi should be refined by using more than two categories (Palazzo et al., 2021). (5) Additional functional traits of rotifers should be examined (Goździejewska et al., 2021; Obertegger & Flaim, 2018;). (6) An evaluation of the relative importance of zoochory versus anemochory would help further elucidate the processes structuring community assembly (Moreno et al., 2019). (7) Finally, a challenging, but next logical step would be to parameterize our conceptual model and compare its processes to that of other ephemeral systems, using microbes, protists, and other invertebrates.

Supplemental material: Table A2. Species richness estimated by rarefaction using iNEXT online.

CHAPTER 2: ALLOMETRIC SCALING OF RESPIRATION RATES IN COLONIAL INVERTEBRATES WITH NEW EXAMPLES FROM THE ROTIFERA

ABSTRACT

Coloniality may grant colony members an energetic advantage in the form of lower individual respiration rates as colony size increases. If this occurs it should be apparent as allometric scaling of respiration with colony size, and colonial organisms should have scaling factors <1. However, colonial members from phylum Rotifera have yet to be examined. To test whether or not colonial rotifers possess allometric scaling relationships between respiration rate and colony size, I measured respiration rates for four solitary and three colonial rotifer species; from these respiration rates we estimated scaling factors. I found mixed evidence for allometric scaling of respiration rate in colonial rotifers. Both rotifers with allometric scaling of respiration rate, *Conochilus hippocrepis* and *Lacinularia flosculosa*, have extensive mucilaginous coverings. These covering may represent an investment of colony members into a shared structure, lowering individual metabolic costs and thus respiratory needs. Additionally, I determined which traits are associated with allometric scaling of respiration. I compiled known scaling factors for animal phyla from a wide phylogenetic spectrum with colonial representatives, and conducted a hierarchical mixed regression that included attributes of colonies. Allometric scaling was found for two of the three colonial species measured. Traits associated with allometric scaling in colonial animals included colony shape, the presence of shared extrazooidal structures, and planktonic lifestyle. There are many other colonial rotifers and animal taxa for which allometric scaling factors have yet to be estimated, knowing these may enlighten our understanding of the benefits of coloniality in animals.

INTRODUCTION

Coloniality in animals is found across many phyla including Arthropoda, Bryozoa, Chordata, Cnidaria, and Rotifera (Blackstone & Jasker, 2003; Hiebert et al., 2021). The widespread presence of coloniality among disparate animal taxa suggests that colonial lifestyle is a convergent character and may confer an adaptive advantage, exceptions to this are cases of convergence arising due to exaptation and coincidence (Losos, 2011). Coloniality has had many different definitions depending on the study. Here, coloniality refers to groups of organisms that are physiologically integrated, either sharing organ systems, physically connected to one another, or with representative castes. This excludes more loosely integrated groups of animals such as gregarious rotifers, nesting birds, and encrusting communities of mussels and barnacles which may share some features of coloniality discussed below.

Several hypotheses have been advanced to explain the evolution of coloniality. In encrusting animals, coloniality may help monopolize limited substratum space, defend against overgrowth, share resources across the colony, optimize access to food and/or light, or help defend against predation, potentially permitting indeterminate growth (Burgess et al., 2017; Dyrynda, 1986; Sebens, 1987). For other colony morphologies, coloniality can increase mating success in some taxa (Vasisht & Dawar, 1970) or swimming efficiency (Sutherland & Weihs, 2002). Coloniality can increase the gape size needed for a predator to ingest a prey item, thus acting as a defense against gape-limited predators (Wallace et al., 2015). This holds true not just for animals, but for phytoplankton with inducible colony formation, in which colony formation presents a trade-off between feeding efficiency and predator defense (Lürling, 2021).

Some taxa display colonial characteristics while having independent gastric systems, including the Rotifera and Chordata, among others. These less integrated colonies may represent more recent origins of coloniality and may better represent the adaptive pressures that led to the evolution of coloniality. Rotiferan colonies also differ from those in modular organisms because colonies do not arise by budding, but by either coordinated egg hatching as in *Sinantherina socialis* (Linnæus, 1758) or larval settlement onto a preexisting colony as in *Beauchampi, Conochilus, Floscularia, Lacinularia, Limnias, Octotrocha*, and some *Sinantherina* species (Wallace 1987; Wallace, 2002). For rotifers, Wallace (1987) suggested two hypotheses as possible adaptive reasons behind the origin of coloniality within the phyla: (1) coloniality functions as an antipredator defense or (2) coloniality provides an energetic advantage to the colony members.

Coloniality as a defense mechanism in rotifers has been studied in *S. socialis* in which both size-selective zooplanktivorous fish and invertebrate predators which reject or fail to eat colonies (Felix et al., 1995; Garcia, 2004; Walsh et al., 2006). However, these studies are confounded by possible chemical defenses that this rotifer is hypothesized to possess (Felix et al., 1995; Hochberg et al., 2015; Walsh et al., 2006, Wallace et al., 2023). Large colony size may allow the rotifer *Conochilus hippocrepis* (Shrank, 1803) to coexist with copepod predators; however, the importance of colony size on predation success has not been directly tested (Diéguez & Balseiro, 1998). Additionally, both colony size and the gelatinous sheath appear to protect *Conochilus* colonies from predation from *Asplanchna*, a voracious rotiferan predator (Gilbert, 1980). Temporary aggregations of the rotifer *Brachionus rubens* Ehrenberg, 1838, are also known to form in response to *Asplanchna* predation, although not attached to one another and facultative, these aggregations may represent a steppingstone from solitary rotiferan lifestyles to colonial lifestyles (Gilbert, 2019).

Feeding efficiency in colonial rotifers is not higher than solitary rotifers of similar size (Wallace, 1987). Colonies of *S. socialis* show coordinated feeding currents, working together to create singular feeding excurrents over the colony (Wallace, 1987). Despite the lack of difference in feeding efficiency, an energetic advantage also may be present as a lowered metabolic rate. Metabolic rate can be inferred from release of metabolic products/by-products, such as heat, CO_2, and other metabolites (Kleiber, 1961; Niklas & Kutschera, 2015). Metabolic rate generally increases with size (Kleiber, 1947; Prosser, 1961; Ikeda, 1970; 1985). The relationship between metabolic rate and mass is allometric and is described by the power function (White & Kearney, 2011):

Formula 1. $MR = aMb$,

where MR is metabolic rate, M mass, a is the intercept, and b is the scaling factor. The nature of the exponent b is subject to an intensive and ongoing debate in ecology (Dodds et al., 2001; Glazier, 2005; 2022; Hoppeler & Weibel, 2005; Isaac & Carbone, 2010; Kozłowski et al., 2003; Niklas & Kutschera, 2015; White et al., 2011). Deviations from the ¾ scaling factor have been found for many groups of organisms and are particularly pronounced in modular colonial organisms such as bryozoans, which display high variability in their scaling factors with estimates as low as 0.5 (White et al., 2011). Studies on metabolic rate in mammals and birds suggest that explicit use of neither Kleiber's ¾ Law nor the earlier ⅔ prediction of scaling factor is appropriate (Dodds et al., 2001). Colonial animals often display allometric scaling of respiration rate with colony size (Burgess et al., 2017). In these cases, the larger size a colony obtains, the less individual respiration each colony member contributes to the colony total. In fully integrated organisms, such as non-colonial metazoans, allometric scaling with body size follow Kleiber's Law (Kleiber, 1932).

Explanations for Kleiber's Law are diverse (Glazier, 2014; 2022; Harrison et al., 2022). Historically, many of these ideas have focused on physical constraints on physiology creating the patterns of allometric scaling seen in respiration rate, as well as other systems. Notable theories are based upon physical constraints (e.g., Dynamic Energy Budget (DEB) theory (Kooijman et al., 2008; Kooijman & Kooijman, 2010); West, Brown, & Equist model (West et al., 1997), and the metabolic limitations boundary theory (Glazier 2005; 2010)). Alternatively, more recent views on the causes of allometric scaling favor a Darwinian approach. In this case, scaling factor is considered a trait that can be selected for or is the by-product of selection (Glazier, 2022; White et al., 2022). For instance, the drift-barrier hypothesis (Lynch, 2022) suggests that longer generation times seen in larger animals allow for deleterious mutations to accumulate in protein-coding genes reducing metabolic efficiencies (Lynch, 2022).

Research on the metabolic rates of rotifers has primarily focused on the Ploima, a Superorder of free-swimming solitary rotifers (Doohan, 1973; Galkovskaja, 1987; Kirk et al., 1999). In rotifers, respiration rate follows temperature closely, as is expected for poikilothermic animals (Weymouth et al., 1944). Respiration rate increases with age, which may be a function of body size since rotifers increase in volume as they age (Ruttner-Kolisko, 1972). Food particle density influences respiration rate in these animals, forming a positive relationship until a particular concentration of food particles is reached, after which respiration rate drops off suddenly (Jackson, 1980). This relationship varies at the species level (Stemberger & Gilbert, 1985; 1987). As in other animals, mass-specific respiration rate is negatively correlated with mass, whereas respiration rate and mass have a positive relationship (Starkweather, 1987). For ploimid rotifers, the scaling exponent has been found to be around 0.72, near the expectations of most allometric metabolic rate scaling models (Galkovskaya & Vinberg, 1979).

To test the hypothesis that colonial rotifers display an energetic advantage in the form of allometric scaling of respiration rate with colony size, we measured colony sizes and the respiration rates of the rotifers comprising them. We compared these rates to species with solitary lifestyles. We extend respiration rate estimates for seven additional species of rotifers (four Gnesiotrocha, three Ploima), show that microrespirometry data generated here is consistent with previous methods of measurement, and explore factors that may be associated with metabolic scaling in a variety of colonial taxa.

MATERIALS & METHODS

Seven rotifer species were collected opportunistically from sites throughout the United States (Table 9) using three methods: plankton collection with a plankton net (64 µm mesh), submerged macrophyte collection, and visible colony collection with cups. Specimens of *S. socialis* used in this study are from a long-term lab culture obtained from two sites in Texas. Rotifers were cultured in artificial hardwater (modified MBL; Stemberger, 1981) and fed a mixture of *Cryptomonas erosa* Ehrenberg 1832, *Chlorella vulgaris* Berijerinck, 1890 (Culture Collection of Algae at the University of Texas at

Austin (UTEX) strain 30), and/or *Chlamydomonas reinhardtii* Dangeard, 1888 (UTEX strain 90).

Table 9. Collection site and characteristics of species used for metabolic rate determination in this study. HTSPHS = Hueco Tanks State Park and Historic Site; NA = not available.

Rotifer Species	Collection site	Longitude	Latitude	Habitat
Asplanchna girodi Guerne, 1888	La Mancha Wetlands, Doña Ana Co., NM	-106.8286	32.2785	Planktonic
Euchlanis kingi Kordbacheh, Shapiro & Walsh, 2019	Crystal Lake, NH	- 72.0832	43.6113	Planktonic
Plationus patulus (Müller, 1786)	Rio Grande, Fabens, TX	-106.1422	31.4302	Planktonic
Hexarthra sp.	Tip top, HTSPHS, El Paso, Co., TX	-106.0245	31.5447	Planktonic
Sinantherina socialis (Linnæus, 1758)	Lab strain	NA	NA	Littoral
Lacinularia flosculosa (Müller, 1773)	Laguna Prieta, HTSPHS, El Paso, Co., TX	-106.0467	31.9249	Littoral
Conochilus hippocrepis (Schrank, 1803)	Album Park, El Paso, Co., TX	-106.3465	31.7834	Planktonic

Oxygen consumption was measured as a proxy for metabolism in colonial and solitary species. For colonial species, rotifers colonies of reproductive age were used in experiments. Before experiments, rotifers were cleaned by rinsing with modified MBL media and carefully removing debris. The rate of oxygen consumption was measured using a 24-channel oxygen sensor (SDR SensorDish® Reader) coupled with a 24-well glass microplate (200 µl; Loligo® Systems). Prior to each run the glass microplate and sensor dish were calibrated using a two-point calibration with 1% m/v sodium sulfite in MBL as a zero oxygen-containing solution, while MBL, aerated by hand with a pipette, was used as the 100% saturated oxygen solution. Glass wells were filled with MBL media. For each run, solitary rotifers of varying densities or single colonies were added to up to 18 wells, with the remaining wells containing only aerated MBL to function as controls. Measurements of oxygen concentration were taken for a 2 hr period at 20° C. All runs were conducted in the early afternoon, to ensure that daily patterns of respiration were consistent. Oxygen consumption rate was determined by subtracting the mean change in oxygen concentration in the control wells from that in wells containing rotifers and regressing these points over time in the program MicroResp™ (Loligo® Systems). The first five minutes of each run were excluded from analyzes to allow the microplate system to stabilize and the rotifers to acclimate to the experimental conditions. Wells with R^2 values under 0.5 were excluded from further analysis. Additional respiration rates for non-colonial rotifers were obtained from the literature (Galkosvkaja, 1987; Kirk et al., 1999). For comparison, rates were calculated at a density of 10 animals in 200 µl at 20° C and, in some cases, were calculated from the regression formulae provided for these species in Galkovskaja (1987).

Rotifer body volume was estimated using methods modified from Walz et al. (1995) and Wallace et al. (1998). Briefly, body volume was assessed by determining the volume of a similar three-dimensional shape, a cylinder. Rotifer colonies were photographed with a Spot Insight camera mounted on a Zeiss Axioscope microscope. From each colony, at least five individual rotifers were chosen haphazardly and measured in ImageJ (Rasband, 1993). Rotifers were measured from the anus to the anterior end of the animal and across the widest part of the animal laterally and posterior to the corona. These measurements were then used to determine average volume/mass by approximating the three-dimensional shape as a cylinder. Colony volume was estimated by multiplying the number of individuals in the colony by the average volume of the individuals measured.

The scaling factor was estimated by linear regression of the log respiration rate and log colony size per species (both by the number of individuals and estimated volume). From this regression, the slope of the line is the allometric scaling factor (see Formula 1). For colonial animals with measured scaling factors obtained in this study or from the literature. Searches for literature containing useable scaling factors were conducted in Google Scholar between September 2022 and May 2023. We attempted an exhaustive search but acknowledge that some useable data may have been missed. Among search terms utilized were allometric scaling, colony size, respiration rate, Kleiber’s law, and terms by phyla and clade with known colonial members, such as siphonophore respiration, bryozoan colony respiration etc. Where there were cases of multiple scaling factors from the same species, these were averaged (Figure 11), and in one case two scaling factors for the same species (*Botrylloides simodensis* Saito &

Watanabe, 1981) were kept, as were estimates for two different life stages with differing characteristics during its normal and takeover phases (Anderson, 1993; Almegbel, 2018; Biggs, 1977; Delong et al., 2013; Edmunds et al., 2016; Fewel & Harrison, 2016; Hartikainen et al., 2014; Lighton, 1989; Nakaya et al., 2003; Waters et al., 2010). These two phases were kept separate since they should strengthen the statistical power to test the lifestyle characteristic that differences between them. To test the impact of lifestyle, a mixed effect model with hierarchical taxonomic ranks as mixed effects was conducted in the R package lme4 (Bates et al., 2015). Lifestyle traits tested in the model included: colony shape, the presence of shared extra-zooidal structures, shared organ systems, specialized reproduction, and whether or not the colony was attached to a substratum. Variables included in the model were selected using a stepwise approach based on Akaike's Information Criterion.

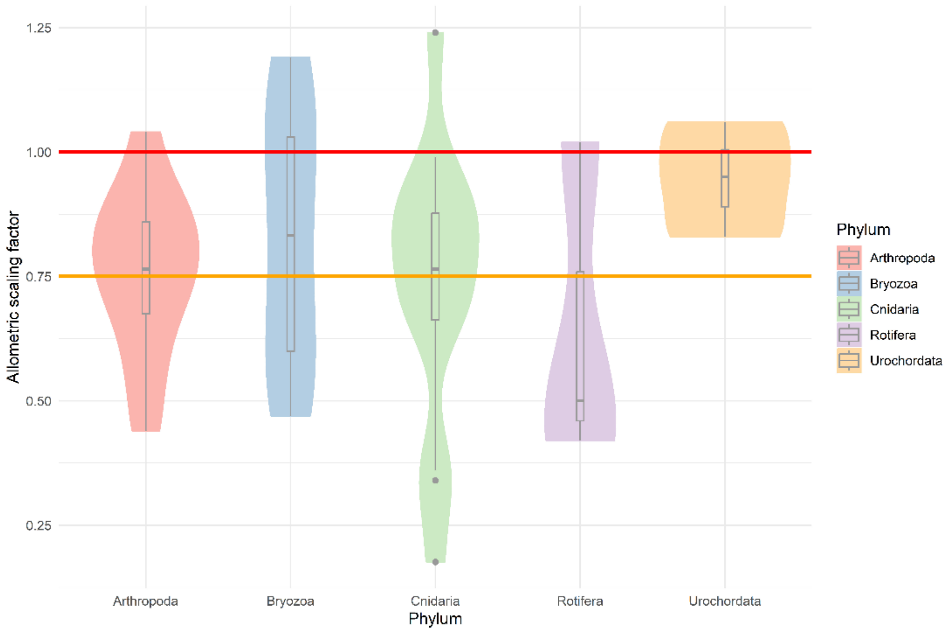

Figure 11. Allometric scaling factors of colonial invertebrates collected from the literature and three novel colonial rotifer allometric scaling factors estimated in this study. In cases where multiple allometric scaling factors existed for a single species, scaling factors were average. Scaling factors are displayed as violin plots with box plots, separated by phyla. A red line marks isometric scaling, the orange line the scaling factor expected in accordance with Kleiber's Law.

RESULTS

The oxygen consumption rate (MO^{2-}) of rotifers ranged from 0.8 pmol/min to 8.5 pmol/min per individual (Table 10). Maximum colony sizes measured were 142, 456, and 444 members for *S. socialis, Lacinularia flosculosa* (Müller, 1773), and *C. hippocrepis*, respectively. No solitary rotifers displayed a significant effect of density on individual respiration; however, *L. flosculosa* and *C. hippocrepis* displayed a negative relationship between colony size and individual respiration.

Table 10. Respiration rates of rotifer species. Respiration rates are shown for species measured in this study and values taken from the literature. Rates were calculated at a density of 10 animals in 200 µl at 20 °C. An asterisk notes that these respiration rates were calculated for 20°C from the regression formulae provided for these species in Galkovskaja (1987).

Name	Respiration rate picomoles/min	Respiration rate for 10 individuals per 200 µl	Source
Hexarthra sp.	0.797	0.761	This study
Sinantherina socialis	1.148	1.202	This study
Lacinularia flosculosa	8.511	2.692	This study
Conochilus hippocrepis	2.138	0.844	This study
Plationus patulus	6.761	5.420	This study
Euchlanis kingii	0.973	1.637	This study
Asplanchna girodi	0.851	1.107	This study
Brachionus sp.	1.767	NA	Doohan 1973
Brachionus calyciflorus	1.903*	NA	Galkovskaja 1987
Hexarthra mira	0.612*	NA	Galkovskaja 1987
Asplanchna priodonta	3.263	NA	Kirk et al. 1999
Asplanchna silvestri	11.634	NA	Kirk et al. 1999
Synchaeta pectinata	1.427	NA	Kirk et al. 1999

Allometric scaling factors for colonial rotifers by number of individuals in the colony were 1.02, 0.5, and 0.58 for *S. socialis*, *L., flosculosa*, and *C. hippocrepis*, respectively. (See Figure 12 for respiration rates calculated from these factors at different densities.) Allometric scaling factors estimated for colony volume were 1.03, 0.5, and 0.42 for *S. socialis, L. flosculosa*, and *C. hippocrepis*, respectively. Of these, only *L. flosculosa*, and *C. hippocrepis* were significantly different from isometric scaling (b = 1), indicating allometric scaling of metabolism with colony size (**Table 11**).

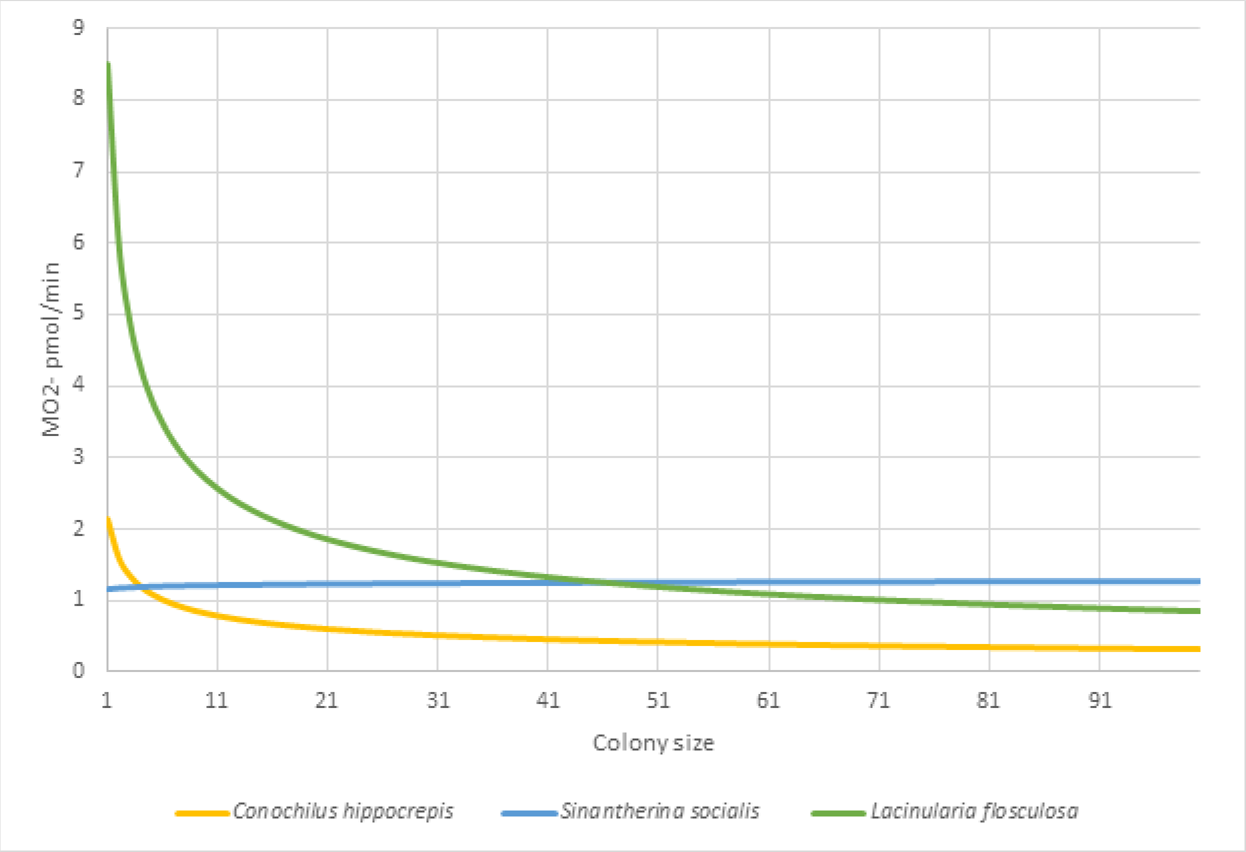

Figure 12. Respiration rate of individuals by colony size for three gnesiotrochan rotifers. Respiration rates are calculated from allometric scaling equations per species obtained by linear regression of colony respiration rates by colony size.

Table 11. Scaling factors in solitary and colonial rotifer species estimated by linear regression on density of individuals and by total volume with a 95% confidence interval. Adjusted coefficient of determination for these models are listed, as well as their lifestyle (colonial vs solitary).

Rotifer Species	**Scaling Factor by density (± 95% confidence)**	**Scaling Factor by volume (± 95% confidence)**	**Adjusted R^2 by density**	**Adjusted R^2 by volume**	**Lifestyle**
Asplanchna girodi	1.25 ± 1.2		0.18		Solitary
Euchlanis kingi	1.20 ± 0.38		0.72		Solitary
Hexarthra sp.	0.99 ± 0.51		0.25		Solitary
Plationus patulus	1.0 ± 0.93		0.15		Solitary
Sinantherina socialis	1.03 ± 0.18	1.02±0.25	0.67	0.50	Colonial
Lacinularia flosculosa	0.50 ± 0.18	0.50 ± 0.17	0.25	0.24	Colonial
Conochilus hippocrepis	0.58±0.15	0.42±0.16	0.38	0.21	Colonial

Colony shape, presence of extrazooidal structures, and attachment to a substratum significantly influenced scaling factor (marginal R^2: 0.31, conditional R^2: 0.65). The intercept was 0.86, indicating that positive coefficients on the variables measured lead to isometric or hypermetric scaling of respiration with colony size, whereas negative coefficients lead to hypometric scaling. Linear colony shape was significantly different than branching colonies and increased scaling factor, making colonies with these shapes more likely to present isometric scaling. Presence of extrazooidal structures and an unattached lifestyle decreased scaling factor, leading animals with these traits more likely to possess allometric scaling of respiration with colony size (**Table 12**).

Table 12. Regression fixed effects for colonial animals. Regression coefficients of lifestyle traits impact on scaling factor. Variables were selected via a step-wise approach (see text for details).

Fixed effects:	Estimate	Std. Error	t value	Pr(>|t|)	
Intercept	0.86	0.11	7.76	<0.001	***
Aggregate shape	0.35	0.19	1.85	0.073	.
Globular shape	0.29	0.17	1.52	0.1411	
Linear shape	0.43	0.14	2.687	0.0109	*
Sheet-like shape	-0.05	0.14	-0.381	0.7064	
Spherical shape	0.02	0.15	0.159	0.875	
Unattached to substrate	-0.28	0.13	-2.20	0.034	*
Shared extrazooidal structures	-0.22	0.09	-2.37	0.024	*

DISCUSSION

Respiration rates for rotifers measured in this study largely fall within the known ranges of those reported in the literature. One exception is *Hexarthra* sp., which has a respiration rate among the lowest measured for rotifers of 0.8 pmol/min; this is similar to that measured for *Hexarthra mira* (Hudson, 1871) (0.6 pmol/min) by Galkovskaja (1987). This species of *Hexarthra* has a relatively large body size compared to other members of the genus (12% large than *H. mira* as measured by Kak & Rao, 1998). The respiration rate of this rotifer is nearly 30% larger than *H. mira*, which cannot be accounted for by their difference in body size alone. Interestingly, it has the fastest female embryonic development time of all rotifers measured (Schröder et al., 2007), coupled with the smallest known genome size in the phylum (Brown & Walsh, 2019).

This suggests that these rotifers may be adapted for high metabolism related to their fast development. Of the solitary rotifer species studied, *Plationus patulus* (Müller, 1786) had the highest respiration rate of 6.8 pmol/min. This species produces threads of material while feeding to anchor themselves while feeding (perhaps "rotimer"; e.g., Datki et al., 2021). It is possible that the constant production of these threads and the relatively active lifestyle of *P. patulus* contribute to their relatively high metabolic rate.

Two of the colonial rotifers investigated, *L. flosculosa* and *C. hippocrepis*, had oxygen respiration that scaled allometrically with colony size. Both of these species produce an extensive mucilaginous covering that extends over their colonies. It may be that the coordinated contribution of colony members to this extrazooidal structure creates an optimization of life history that contributes to the evolution of allometric scaling factors (White et al., 2022). *Sinantherina socialis* lacks this mucilaginous covering, and this difference may account for the isometric scaling found in this species. Estimates of scaling factor by the number of individuals in a colony and estimated colony volume yielded similar results, with estimates by the number of individuals in a colony possessing better adjusted R^2 values. This indicates that colony size may be sufficient to estimate scaling factor in these animals.

If an energetic advantage were a reason that coloniality was selected for, we would expect lower respiration rates with increasing colony size. We found this to be true only for some colonial rotifers, both of which create extensive extrazooidal structures. The simplest explanation for this is that allometric scaling of respiration with colony size in these animals is a consequence of certain aspects of coloniality, and likely does not produce an energetic advantage for colony members; although this cannot yet be ruled out. Colonial rotifers may change morphology as colony size increases, a form of astogenic development, for example in *S. socialis*, animals hatching from resting eggs are solitary and have a curved morphology, which becomes straighter and longer in larger colonies (Garcia, 2004). Such morphological changes may influence the individual respiration rate in colonies of differing sizes, creating allometric scaling. However, we were unable to measure the respiration rates of very small-sized colonies.

Allometric scaling factors of colonial animals are highly variable (see **Figure 12**). We did not find shared organ systems to be an important predictor of scaling factor in our analysis, however there are empirical examples where this might be the case. Nakaya et al. (2003) estimated allometric scaling factors of *B. simodensis* during its takeover phase and standard phase. During the takeover phase this animal breaks down its shared organ systems, old zooids stop feeding, and newly budded zooids grow to replace them. They found that respiration rate scales isometrically during this takeover phase, but allometrically during the remainder of the lifecycle, with a scaling factor of 0.75. Most colonial animals are sessile, with few planktonic or unattached representatives. We found that an unattached lifestyle was associated with hypometric scaling in colonial animals. Two of the major groups of unattached animals investigated were siphonophores and eusocial insects. Siphonophores and eusocial insects both have highly specialized colony members. The degree of specialization and integration may allow these colonies to behave more similarly to individual animals in terms of scaling relationships.

Presence of shared extrazooidal structures was associated with hypometric scaling in colonial animals. These shared structures once produced contribute and can be used by other colony members. As colonies grow each member may contribute less overall to these structures leading to less work for the member and potentially less respiration. Examples of structures that once built can be utilized by new colony members without individual contribution are the nesting structures of eusocial insects, gelatinous sheaths and covers in colonial rotifers, and calcium carbonate skeletons of corals. Calcium carbonate deposition in corals is known to follow allometric scaling principles and this may reflect that process (Carlot et al., 2022).

Colony shape has been reported to influence colony scaling relationships in other studies (Hartikainen et al., 2014; White et al., 2011). We found that linear colonies were associated with isometric scaling. The other shape classes investigated were branching, sheet-like, spherical, and aggregate (eusocial insects, huddling harvestman) shapes. Many of these shapes are prone to self-shading. Self-shading has been widely studied in plants but has analogous phenomena in filter- or suspension-feeding colonial animals. In this case, some positions within the colony are better equipped to collect food and may make it more difficult for interior zooids to feed. For example, in cnidarians, individual polyp prey capture decreases as colony size increases (Kim & Lasker, 1998). Many bryozoan growth forms have evolved to mitigate this effect, such as the form of *Crystella mucedo* Cuvier, 1798, in which active zooids are added to the edges of the colony, and the middle is comprised of non-feeding individuals so that as the colony size increases all feeding zooids remain on the colony edge (Hartikainen et al., 2014). This phenomenon may be most evident in colony shapes that do not maintain an individual member's access to food, such as sheet-forming colonies where edge zooids have better access, and branching colonies where interior members may be impacted by feeding activities more exterior colony members. Because feeding organisms respire more this difference in food availability may inadvertently lead to allometric scaling in colonies possessing certain shapes. The importance of colony morphology is further exemplified in coral calcification rates, which are higher in separated coral pieces (nubbins) than in natural colonies of the same mass and in reconnected nubbins, which retain the colony morphology but lose the interconnectedness between nubbins (Edmunds et al., 2022). Our findings that linear colony shape differed significantly from branching colony shape match these expectations.

Our results of allometric scaling within colonial rotifers showed both isometric and hypometric scaling was present in the phyla. However, this is complicated by differences in lifestyle (planktonic versus sessile) and some peculiarities of the taxa measured. *S. socialis* possesses wart-like structures posterior to their corona, which are thought to function as a chemical defense/deterrent to predation (Felix et al., 1995; Wallace et al., 2023; Walsh et al., 2006). This possible antipredator defense complicates using this particular species to infer the adaptive origins of coloniality. *Sinantherina ariprepes* Edmondson, 1939, a close relative, lacks these structures and would be an ideal target for future study. Additional measurements of other species of *Conochilus* such as *Conochilus unicornis* Rousselet, 1892, which forms smaller colonies than *C. hippocrepis* would be achievable using a microplate system. Rotifers that form branching colonies are even less integrated than the colonial species we measured, and would be good targets for future study. Some rotifers form temporary aggregations, such as *Brachionus rubens* Ehrenberg, 1838, *Philodina megalotrocha* Ehrenberg, 1832, and *Philodina gregaria* Murray, 1910 (Gilbert, 2019). These congregations may be vulnerable to self-shading from feeding in a similar way as colonial rotifers and should the respiration rates of these aggregations scale hypometrically with aggregation size, it would imply that group living, rather than connectedness, promotes allometric scaling of respiration.

We attempted to measure the respiration rate of 17 rotifer species; however, many did not produce usable results. For example, we were not able to determine the rate for *Filinia longiseta* (Ehrenberg, 1834) because their long spines entrained them against the walls of the wells, often resulting in their deaths. Additionally, several species (i.e., *Cupelopagis vorax* (Leidy, 1857), *Collotheca ferox* (Penard, 1915), and *Collotheca ornata* (Ehrenberg, 1830)) did not respire sufficiently to create a measurable respiration rate at testable densities. These are all members of the Collothecaceae that utilize a sit-and-wait ambush predation strategy, and thus are likely to have lower respiration rates. In addition, we attempted to measure the respiration rates of *A. girodi* and *P. patulus* at high densities (> 60–120 individuals/200µl and > 25 individuals/200µl, respectively). At these densities, the respiration rate dropped drastically or produced respiration rates with low R^2 values. Thus, respiration rates under these conditions were not directly comparable to those at lower densities. Rotifers in the genera *Floscularia* and *Limnias* can create branching colonies. These colonies were not amenable to the microplate; they are either too large to place into the wells without damaging them — tube length ranging from 400 µm to 1500 µm in *Limnias ceratophylli* Schrank, 1803

(Wallace et al., 2018) and up to 2455 µm for *Floscularia ringens* (Linnaeus, 1758) (Fontaneto et al., 2003) — or not large enough to create measurable respiration in the chamber. For two other species (i.e., *Notommata copeus* Ehrenberg, 1934, *Sinantherina ariprepes* Edmondson, 1939), we did not have enough individuals to produce respiration rates with confidence.

Expanding our understanding of scaling processes in other colonial or social/gregarious taxa will be necessary to better understand the causes of allometric scaling and coloniality. Of particular interest are colonial choanoflagellates, which can be compared with their solitary relatives. These protists are closely related to animals, and understanding why they form colonies and how colonies impact the physiology of this group may enlighten our understanding, not just of coloniality, but also of multicellularity in general. Additionally, colonial ciliates are a common, widely available taxa not closely related to the groups covered in this work. They are in habitats and size ranges similar to colonial animals such as bryozoans and rotifers but represent colonies formed from single-celled organisms. Additionally, it would be feasible to measure colonial ciliate respiration rates using a Loligo microplate system, as these colonies are similar in size to rotiferan colonies.

Currently there are a lack of respiration studies in several groups with colonial representatives including some phoronid worms, colonial, social, and subsocial spiders, pyrosomes, pterobranchs, and doloilids. Colonial phoronids produce colonies by budding, and so present a colonial form more integrated than that in Rotifera, but still of interest to increase sample size of colonial groups (Emig, 1982). Doloilids are another colonial chordate taxon similar to salps, but possessing specialized zooids and thus have a greater degree of integration. As a whole, colonial organisms seem to violate many of the assumptions held by Newtonian theories on allometric scaling. Increased sampling of colonial animals across available taxa and sampling of gregarious taxa will help to improve our understanding of the causes of allometric scaling, colony formation, and provide additional evidence to support or reject models of allometric scaling, assuming allometric scaling in these groups has a common cause.

CHAPTER 3: GENOME SIZE AND LIFESTYLE IN GNESIOTROCHAN ROTIFERS

This work was published as Brown, Patrick D., and Elizabeth J. Walsh. "Genome size and lifestyle in gnesiotrochan rotifers." *Hydrobiologia* 844, no. 1 (2019): 105-115.

ABSTRACT

Gnesiotrochan rotifers display a variety of life styles ranging from taxa with free-swimming larval and sessile adult stages to those with motile adult stages and colonial habits. The metabolic rate hypothesis of genome size posits that genome size is correlated with lifestyle. To test this hypothesis, genomes of 11 gneisotrochan species representing nine genera were measured by flow cytometry, using *Drosophila melanogaster* as a standard. Genome sizes (1C) within Gnesiotrocha ranged from 0.05 pg (*Hexarthra mira* and *Hexarthra fennica*) to 0.16 pg (*Cupelopagis vorax*), over a three-fold difference. Within *Hexarthra*, the genome of *H. fennica* from El Huérfano, Mexico was estimated to be 15% larger than that of *H. mira* from Ojo de La Punta, Mexico and *H. fennica* from Keystone Wetland, TX, USA. Gnesiotrochan genome sizes are similar to those reported within Ploima, which range from 0.06 pg (*Brachionus rotundiformis*, *B. dimidiatus*) to 0.46 pg (*B. asplanchnoidis*). Within Gnesiotrocha, genome size was found to be significantly smaller in sessile species than those with motile adult stages (Wilcoxon rank-sum test, W = 7, p = 0.007). This study provides the first estimates of genome size within the superorder, providing a baseline for genomic and evolutionary studies within the group.

INTRODUCTION

The relationship of haploid genome size, or C-value, to a variety of biological phenomena is an ongoing topic of debate and is referred to as the C-value enigma (Elliott & Gregory, 2015). The *C*-value enigma encompasses several features of genome size including: (1) mechanisms driving growth or diminution, (2) differences among disparate taxa, and (3) biological consequences of genomic content. Factors driving genome size are multi-level and complex; at the proximate level, molecular mechanisms and population genetics likely drive genome size variation. At an evolutionary level, phylogenetic considerations and adaptation may be more important determinants of genome size (Alfsnes et al., 2017). One of the primary mechanisms of genome size expansion is the accumulation of transposable elements (TEs) (Canapa et al., 2015; Gregory, 2005; Lynch, 2007). Evidence for this includes the linear relationship between the number of TEs and genome size in eukaryotes (Kidwell, 2002) and that the loss of TEs can contribute to genome size compaction (Kapusta et al., 2017). Effective population size influences the maintenance of both TEs and duplicated DNA. In large populations, purifying selection may remove excess DNA, while in those populations with small effective sizes this constraint is lifted, and selection may even maintain additional DNA (Lynch & Conery, 2003). This model provides an explanation of genome size variation among taxa.

Genome size is related to several morphological and ecological traits, such as cell size, development time, and, in some taxa, metabolic rate (Hughes & Hughes, 1995; Gregory, 2005; Wright et al., 2014). Thus, genome size should be correlated with life history attributes. Alternatively, if TEs and effective population sizes are driving genome size evolution, then it should be independent of lifestyle or correlated only with phylogeny. For instance, independence of genome size from taxonomic rank at lower phylogenetic levels has been suggested to be the result of adaptation in arthropods (Alfsnes et al., 2017).

C-value is negatively correlated with metabolism through what appears to be a nucleotypic effect in some groups (Gregory, 2001a). For instance, genome size is relatively small in vertebrates with powered flight: bats, birds, and extinct pterosaurs (Hughes & Hughes, 1995; Organ & Shedlock, 2009; Wright et al., 2014). Additionally, these groups have relatively small cell sizes (Gregory, 2001a). The metabolic rate hypothesis is a potential explanation of the observed relationship between genome size and flight (Gregory, 2001b; Hughes & Hughes, 1995). However, this link between metabolism and genomic content does not hold for all vertebrates. For example, in amphibians and ray-finned fishes, considerations such as egg size and development time may be more important adaptive drivers of genome size (Hardie & Hebert 2003; Smith & Gregory, 2009). Our understanding of where metabolism does and does not apply to genome size is less clear for invertebrate taxa (Alfsnes et al., 2017; Gregory & Hebert 2003). Invertebrate groups that display lifestyles with differing metabolic demands such as flying and flightless insects, and sessile and swimming aquatic species are ideal targets to determine whether the metabolic rate hypothesis applies more broadly. Rotifers display a variety of lifestyles that may impose different metabolic costs; e.g., planktonic, sessile, solitary, colonial, free-living, and parasitic lifestyles (Epp & Lewis, 1984; Wallace, 1987; May, 1989; Vadstein et al., 2012).

Genome size in rotifers has been determined for several bdelloid and monogonont species (Flot et al., 2013; Kim et al., 2018; Mark Welch &

Meselson, 1998a; 2003; Nowell et al., 2018; Pagani et al., 1993; Riss et al., 2017; Stelzer, 2011; Stelzer et al., 2011). Bdelloid rotifers are likely degenerate tetraploids whereas seisonids, acanthocephalans, and most monogonont rotifers are diploid (Hur et al., 2009; Mark Welch & Meselson, 2001; Mark Welch et al., 2008). This difference in ploidy makes bdelloid genomes poor representatives of genome sizes for the phylum. Feulgen densitometry, static cell fluorometry, hybridization techniques, and full genome sequencing have been used to measure genomic DNA content in the Bdelloidea. Estimates of 1C genome size within this group range from 0.18 pg in *Rotaria magnacalcarata* (Parsons, 1892) to 1.22 pg in *Philodina roseola* Ehrenberg, 1832 (Mark Welch & Meselson 1998a; Nowell et al., 2018). Sequencing techniques have yielded smaller estimates than fluorometric techniques in the bdelloid *Adineta vaga* (Davis, 1873) (0.25 pg vs 0.36 pg, respectively) (Mark Welch & Meselson, 2003; Flot et al., 2013). Underestimates of genome size by sequencing arise due to the presence of heterochromatin and repeated regions (Bennett et al., 2003; Nishibuchi & Déjardin, 2017). Genome sizes in monogononts, as measured by flow cytometry, range from 0.06 in *Brachionus rotundiformis* Tschugunoff, 1921 and *Brachionus dimidiatus* Bryce, 1931 to 0.42 in some strains of *Brachionus asplanchnoidis* Charin, 1947 (Stelzer, 2011; Stelzer et al., 2011). Recently, an estimate of genome size in *Brachionus calyciflorus* Pallus 1766 was determined based on whole genome sequencing, 0.13 pg (Kim et al., 2018). Genome size has been well studied in the *Brachionus plicatilis* species complex (Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017). Within this complex, there is a positive relationship of genome size with body volume and egg size (Stelzer et al., 2011). This correlation may be related to the cell size, and thus potentially confirming the relationship between cell size and genome size found in other animals (Gregory, 2001b; 2005) and in accordance with the *C*-value enigma (Stelzer et al., 2011).

To date, there are no measurements of genome size within the Superorder Gnesiotrocha. Gnesiotrochan rotifers are comprised of ~ 217 species classified in two orders, the Collothecaceae and the Flosculariaceae (Segers, 2007). Gnesiotrochan rotifers possess a wide array of lifestyles, including: (1) free swimming forms as in the ploimids and bdelloids, (2) facultative sessility in some taxa, (3) sessile taxa in both orders, and (4) colonial taxa within the Flosculariaceae (Wallace, 1987; Young et al., 2018). Several families of gnesiotrochans (e.g., Hexarthridae, Testudinellidae, Trochosphaeridae) are free-swimming and unattached as adults. Colonies may either be sessile or planktonic, or facultatively planktonic as in some species of *Lacinularia* and *Sinantherina*. Unfortunately, the position of these families within the larger gnesiotrochan phylogeny is not well resolved. Given this, the evolution of lifestyle within the group cannot be disentangled from phylogenetic considerations at this point. To determine how the genome sizes of gnesiotrochan rotifers compare to other rotifers, we measured the average genome sizes of 13 gnesiotrochan rotifers representing nine genera, along with one ploimid outgroup. To determine whether there is a relationship between lifestyle and genome size, we compared the genome sizes of gnesiotrochan rotifers including sessile, motile, solitary, and colonial representatives using non-parametric pairwise tests and linear mixed models with hierarchical taxonomic ranks. Because genome size has not been reported for any gnesiotrochan rotifer, we also compared our measurements to the known genome sizes of other rotifers.

MATERIALS AND METHODS

Rotifers were collected opportunistically from the USA and Mexico (Table 1) using two methods: (1) a plankton net (64 µm mesh) to obtain planktonic rotifers, and (2) submerged macrophyte collection to obtain littoral and sessile species. In cases where waterbodies were small, a filter (20 µm mesh) was pulled through the water to concentrate plankton. Rotifers from Australia were hatched from rehydrated sediments. All rotifers were isolated, cultured in artificial hardwater (modified MBL; Stemberger, 1981), and fed a mixture of *Chlamydomonas reinhardtii* Dangeard, 1888 (Culture Collection of Algae at The University of Texas at Austin (UTEX) strain 90) and *Chlorella vulgaris* Berijerinck, 1890 (UTEX strain 30). For samples containing tube-building rotifers, carmine powder was added to provide suspended materials to aid in tube construction. Samples were cultured at room temperature (~ 21°C) under ambient lighting. Long-term cultures of some species are maintained in the laboratory. For instance, *Sinantherina socialis* (Linnaeus, 1758) is maintained and fed weekly with either *Rhodomonas minuta* Skutja 1948 or *Cryptomonas erosa* Ehrenberg 1832, depending on availability of the algal cultures.

To prepare rotifer cells for flow cytometry with propidium iodide (PI) stain, a detergent trypsin method was used following a protocol modified from Vindeløv et al., (1983). This method has been used successfully for some members of the *Brachionus plicatilis* species complex and other Ploima (Stelzer et al., 2011; Riss et al., 2017). Rotifers were not fed for 24–36 h to clear their guts of visible food. They were then cleaned by rinsing in MBL medium through serial transfer of the animals through fresh medium. Approximately, 100 to 800 rotifers were collected on a 20 µm mesh filter or placed directly into a 1 ml Dounce tissue homogenizer and re-suspended in MBL.

Excess MBL was then removed and rotifers were lysed on ice with 15 strokes. The homogenate was filtered through a 20 µm mesh sieve to remove large particulates. Next, 0.003% trypsin (dissolved in stock buffer: 3.4 mM tri-sodium citrate dihydrate, IGEPAL® at 0.1% v/v, 1.5 mM spermine tetrahydrochloride, and 0.5 mM Tris(hydroxymethyl)aminomethane at pH 7.6) was added and samples were incubated at room temperature for 15 min. Trypsin inhibitor (0.05%) and 0.01% RNAse A, both dissolved in stock buffer, were subsequently added and samples were incubated at room temperature for 15 min. Samples were then stained by addition of 0.04% PI and 0.1% spermine tetrahydrochloride dissolved in stock buffer. PI-stained samples were incubated in the dark overnight at 4°C. All chemicals were obtained from MilliporeSigma. Following incubation, samples were subjected to flow cytometry on a Gallios flow cytometer (Beckman Coulter Diagnostics) at 488 nm and subsequent fluorescence captured on a detector equipped with a 620/30 filter and analyzed with Kaluza version 1.3 (Beckman Coulter Diagnostics).

To estimate genome size, mean fluorescent intensity of rotifer cell populations were compared to those of *Drosophila melanogaster* Meigen, 1830, and approximated based on a ratio that includes the known genome size of the Oregon-R strain of *D. melanogaster*, 0.18 pg (Tavares et al., 2014). Several early cytometry runs used an available Canton-S strain. But because of the known variability in genome size among strains, Oregon-R flies were used to corroborate these results; and then used for all subsequent runs (Bosco et al., 2007). For rotifers with genome sizes nearly the same size of *D. melanogaster,* we used *Hexarthra* sp. from Hueco Tanks State Park and Historic Site (HTSPHS) as an internal standard.

Mean genome size per species was used for all analyses. For the *Brachionus plicatilis* complex, species were delineated as in Mills et al. (2017). To compare genome sizes of rotifers with sessile and motile adult forms, a Wilcoxon rank-sum test was used (R 3.5.0, R Core Team, 2018). Additionally, to account for the potential influence of phylogeny, linear mixed models with hierarchical taxonomic ranks as random effects were fitted to log-transformed genome size, lifestyle, and taxonomic rank (Bdelloida + Monogononta: superorder, order, genus; Monogononta: order). Only taxonomic ranks found to contribute to the variation in genome size were included in these models. To determine which model best explained the variance in genome size, an ANOVA was used and the model with the lowest Akaike's Information Criterion (AIC) was selected. Further, the genome sizes of gnesiotrochan rotifers were compared to those of ploimid (Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017, Kim et al., 2018) and bdelloid rotifers (Pagani et al., 1993; Mark Welch & Meselson, 1998a; 2003; Flot et al. 2013; Nowell et al., 2018) using a Kruskal–Wallis test for stochastic dominance followed by Dunn's test implemented in R (R 3.5.0, R Core Team, 2018).

RESULTS

We estimated genome size for 13 gnesiotrochan rotifer species and one ploimid species, resulting in values ranging from 0.05 pg in *Hexarthra* species to 0.25 pg in *Sinatherina ariprepes* Edmonson, 1939 and the ploimid *Plationus patulus* (Müller, 1786) (Table 13). Genome size measurements for rotifers were not normally distributed, thus non-parametric tests were used. The use of either reference standard, *Hexarthra* sp. or *D. melanogaster*, did not have a significant impact on genome size estimates for target species (Paired Wilcoxon rank-sum test, $V = 5$, $P = 0.5$). We could not distinguish fluorescent peaks for Canton-S and Oregon-R flies from each other when run together on the flow cytometer (data not shown). This indicates that the genome sizes of these strains are likely very close to one another or equivalent.

The mean genome sizes of motile and sessile gnesiotrochan rotifers, 0.07 pg and 0.15 pg, respectively, were significantly different (Wilcoxon rank-sum test, $W = 38$, $P = 0.014$). Mean genome sizes of colonial (0.18) species were significantly greater than solitary species (0.09) (Wilcoxon rank-sum test, $W = 33$, $P = 0.02$). When comparing lifestyles among all monogonont rotifers for which there is an estimate of genome size, we found no significant differences between comparisons of sessile versus free swimming or solitary versus colonial lifestyles. Linear mixed models with hierarchical taxonomic ranks showed that for Monogononta, the best model included sessility, coloniality, and taxonomic rank (genus) as predictors of genome size but was not significant (AIC = 45.3, $P = 0.123$). However, when the model was simplified to sessility + taxonomic rank or coloniality + taxonomic rank, results were significant (AIC = 48.12, 45.5; $P = 0.045$, < 0.001, respectively) (Table 14). When the model was expanded to include Monogononta + Bdelloidea, the best predictor of genome size was coloniality + taxonomic rank (superorder, order, and genus) (AIC = 72.9, $P \leq 0.001$) (Table 14).

We found a significant difference among the mean genome sizes of Ploima, Gnesiotrocha, and Bdelloidea (Kruskal–Wallis rank sum test, $\chi^2 = 18.7$, $P < 0.001$). Pairwise comparisons of genome size showed that gnesiotrochan genomes were not significantly different from those of ploimids but were significantly smaller than those of bdelloids (Dunn test, $P = 0.0002$) (Fig. 13).

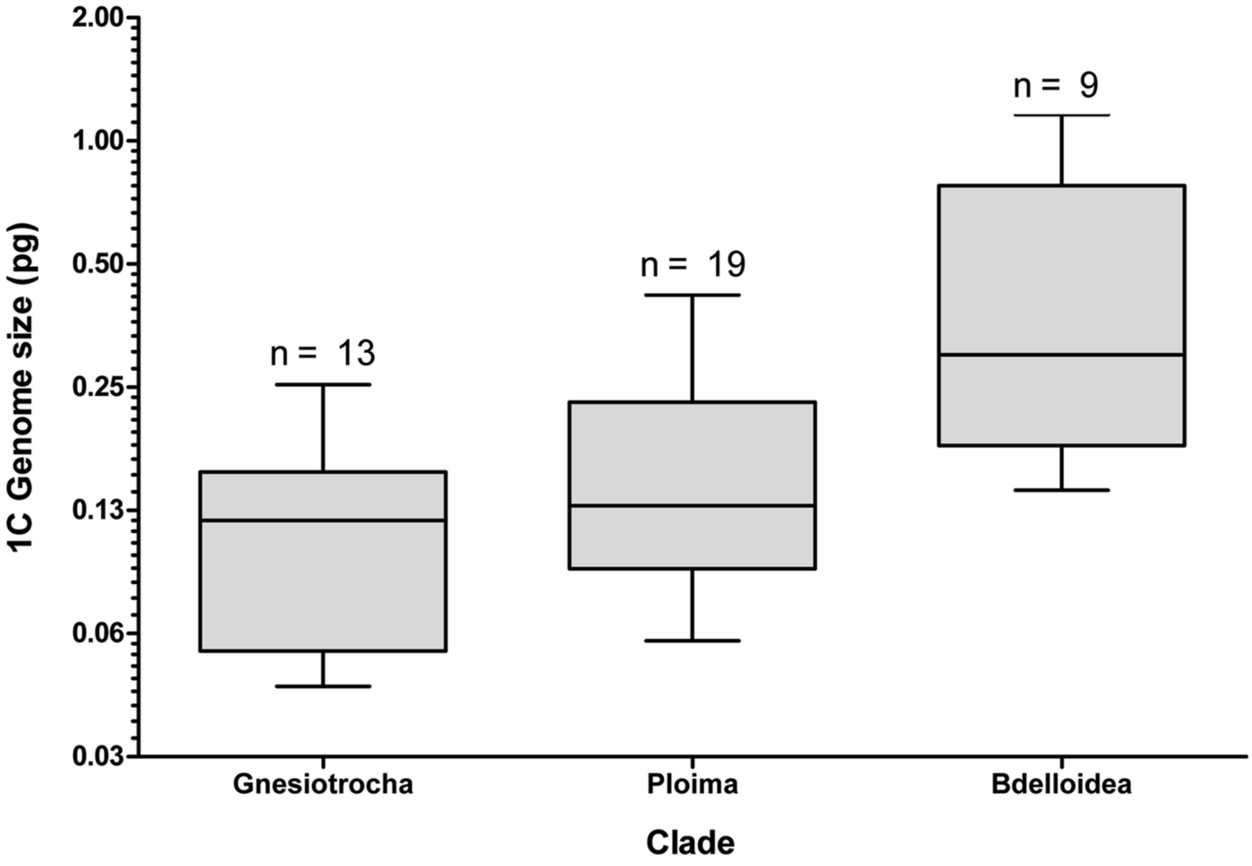

Fig. 13.: Genome sizes of representatives of gnesiotrochan, ploimid, and bdelloid rotifers. Values for Gnesiotrocha were obtained in this study; values for the Ploima and Bdelloidea are from past studies (Pagani et al., 1993; Mark Welch & Meselson, 1998; 2003; Stelzer, 2011; Stelzer et al., 2011; Riss et al., 2017).

Table 13. Summary of gnesiotrochan genome sizes (1C) determined by propidium iodide staining and lifestyle characterization. Site refers to the location from which the population was obtained. *n* = sample size; SD = standard deviation, n/a = not applicable.

Species	**Locality** **GPS Coordinates (N,W)**	***n***	**Genome Size (pg)**	**SD**	**Lifestyle**
Conochilus hippocrepis	Nockamixon State Park Fishing Pond, Bucks Co., PA 40.472833, -75.224111	1	0.1265	0.0007	Planktonic colonies
Collotheca ferox	Poza Azul, Coahuila, Mexico 26.922671, -102.122589	3	0.1410	0.0012	Sessile Solitary
C. ornata	La Mancha Wetland, Doña Ana Co., NM 32.278092, -106.828626	2	0.0616	0.0049	Sessile Solitary
Cupelopagis vorax	Staring Lake, Hennepin Co., MN 44.836781, -93.456119	1	0.1470	n/a	Sessile solitary
C. vorax	Turtle Basking Pond, Hennipin Co., MN 44.84506, -93.369538	4	0.1572	0.0089	Sessile solitary
Filinia longiseta	Ojo de la Casa, Chihuahua, Mexico 31.366033, -106.532085	2	0.0707	0.0093	Free-swimming solitary
F. longiseta	Behind Ranch House, Hueco Tanks State Park and Historic Site, El Paso Co., TX 31.923966, -106.041668	3	0.0701	0.0003	Free-swimming solitary
Hexarthra fennica	El Huérfano, Chihuahua, Mexico 31.294850, -106.511633	1	0.0564	n/a	Free-swimming solitary
H. fennica	Keystone Heritage Park, El Paso Co. TX 31.822169, -106.563532	1	0.0469	n/a	Free-swimming solitary
H. mira	Ojo de la Punta, Chihuahua, Mexico 31.385967, -106.602017	1	0.0477	n/a	Free-swimming solitary

Lacinularia flosculosa	Laguna Prieta, Hueco Tanks State Park and Historic Site, El Paso Co., TX 31.924903, -106.046654	3	0.1332	0.0101	Sessile colonies
L. flosculosa	Ryans 2 billabong, Wodonga, Australia -36.11072222, 146.96664444	2	0.1571	0.0031	Sessile colonies
Limnias melicerta	Ryans 2 billabong, Wodonga, Australia -36.11072222, 146.96664444	3	0.1182	0.0039	Sessile colonies
Sinantherina socialis	Ryans 2 billabong, Wodonga, Australia -36.11072222, 146.96664444	3	0.1227	0.0033	Sessile colonies
Testudinella patina	La Mancha Wetland, Doña Ana Co., NM 32.278092, -106.828626	1	0.0866	n/a	Free-swimming solitary
T. patina	Miller Ranch, Jeff Davis Co., TX 30.623845, - 104.674005	1	0.0749	n/a	Free-swimming solitary

Table 14. Results of linear mixed models with hierarchical taxonomic ranks, with log-transformed genome size as the response variable

Model	**df**	**AIC**	χ^2	***P***
Monogononta				
Taxonomic rank	3	50.1	n/a	n/a
Sessility + taxonomic rank	4	48.1	1.75	0.045
Coloniality + taxonomic rank	4	45.7	2.01	< 2e−16
Coloniality + sessility + taxonomic rank	5	45.3	2.35	0.123
Monogononta + Bdelloidea				
Taxonomic rank	5	74.0	n/a	n/a
Sessility + taxonomic rank	6	73.1	2.39	0.122
Coloniality + taxonomic rank	6	72.9	0.71	< 2e−16
Coloniality + sessility + taxonomic rank	7	73.5	1.43	0.233

1. Models for solely Monogononta and Monogononta + Bdelloidea were investigated separately
2. Taxonomic ranks as random variables, with superorder, order, and genus for Monogononta + Bdelloidea, and solely genus used for the Monogononta only analysis. In other models, superorder, order, family, and genus were used. Lifestyles sessility and coloniality were the fixed variables in all models

DISCUSSION

The range of genome sizes in the gnesiotrochan rotifers we investigated is similar to those found in ploimid rotifers (Mark Welch & Meselson, 1998a; Riss et al., 2017; Stelzer, 2011; Stelzer et al., 2011) but smaller than those reported for bdelloid rotifers (Mark Welch & Meselson, 1998a; 2003; Pagani et al., 1993). The genome of *P. patulus* (0.25 pg) was among the largest ploimid genome sizes, but still within the range of known genome sizes of populations of *B. asplanchnoidis* (0.22 to 0.42 pg) (Riss et al., 2017; Stelzer et al., 2011). The smallest genome sizes measured in gnesiotrochans were 0.05 for *H. mira*, *H. fennica*, and *Hexarthra* sp. (HTSPHS), which are comparable to the smallest genomes known for ploimid rotifers, 0.06 pg in *B. rotundiformis* and *B. dimidiatus* (Stelzer, 2011; Stelzer et al., 2011). The consistency of the low estimate among rotifers and other taxa may indicate a lower bound on genome size in free-living bilaterians. For instance, in the animal genome size database (Gregory, 2009), the only clades with representatives with genome sizes smaller than 0.06 pg are gastrotrichs (0.05 pg), tardigrades (0.05 pg), placozoans (0.04 pg), sponges (0.04 pg), and nematodes (as low as 0.02 pg in parasitic species) (Gregory, 2009). Additionally, several phyla have their smallest genome sizes at 0.06 pg; including platyhelminths, annelids, and chordates (Gregory, 2009). If genome size is constrained by selection for faster development in these groups, perhaps this represents a threshold value below which development cannot proceed more quickly or genome size reduction no longer appreciably speeds up development time. Non-bilaterian animals and parasitic species appear to exceptions to this phenomenon. To our knowledge, there are no genome size estimates for parasitic acanthocephalans or seisonids.

Our results are consistent with those of Stelzer et al., (2011) in that body size and genome size are correlated in rotifers. *Collotheca ornata* (Ehrenberg, 1830) was both the physically smallest of the collothecid rotifers measured and possessed the smallest genome size (0.06 pg). The largest genome size measured was that of *S. ariprepes* (0.25 pg), a moderately large gnesiotrochan with an expansive corona (Wallace & Starkweather, 1985). Given this, it is predicted that the largest known rotifer, *Pentatrocha gigantea* Segers & Shiel, 2008, will have a genome size equal to or larger than that of *S. ariprepes* (Segers & Shiel, 2008). In contrast, the *Hexarthra* species investigated in this study are rather large, yet yielded the smallest estimates of genome size (~ 0.05). This could be due to their requirement for rapid development in habitats with short hydroperiods (Schröder & Walsh, 2007), as larger genome sizes are correlated with increased development time (Gregory, 2005).

Several of the gnesiotrochan rotifers included in this study showed variation among populations. For instance, *S. socialis* hatched from Australian billabong sediments had a markedly smaller genome size than conspecifics from the US populations. *S. socialis* also showed variability among the US populations (Mean ± SE: 0.18 ± 0.02). Australian and US populations of *Lacinularia flosculosa* (Müller, 1773) had a similar pattern, with genome sizes of 0.16 and 0.13, respectively. *Cupelopagis vorax* (Leidy, 1857) displayed variability in its genome size (0.16 ± 0.02) among populations isolated from different lakes as well. These differences may either be the result of intraspecific variation or the presence of cryptic species complexes. While cryptic species complexes are relatively common in rotifers, high levels of intraspecific variation in genome size have been found in *Brachionus asplanchnoidis* so this alternative cannot be discounted. The nature of these species as possible complexes warrants further investigation, particularly between species occurring on different continents.

We found that a sessile adult lifestyle is associated with a larger genome size as compared to a motile lifestyle. Motion by ciliary action in rotifers is metabolically costly, accounting for up to 62% of total metabolic costs in the free-swimming solitary species *Brachionus plicatilis* Müller, 1786 and *Asplanchna sieboldii* (Leydig, 1854) (Epp & Lewis, 1984). Additionally, metabolic measurements of attached and swimming rotifers suggest that attached rotifers expend somewhere between 1/3 and 1/5 the energy used by their swimming counterparts (Vadstein et al., 2012). This potential relationship between lifestyle and genome size fits the pattern of what is seen in some vertebrates (Hughes & Hughes, 1995; Kapusta et al., 2017; Organ & Shedlock, 2009; Wright et al., 2014; Zhang & Edwards, 2012) and follows from the predictions of the metabolic rate hypothesis of genome size (Hughes & Hughes, 1995). Two members of the Collothecaceae, *Cupelopagis vorax* and *Collotheca ferox* (Penard, 1914), had among the largest genome sizes of the gnesiotrochan rotifers. Rotifers in this clade do not generate current by ciliary action to suspension feed as in the Flosculariaceae, but rather either wait for food to approach their setae and then either sweep prey towards their mastax as in *C. ornata* or engulf prey in their infundibulum by muscular action as in *C. vorax* (Bevington et al., 1995; Koste, 1973; Vasisht & Dawar, 1969;). *C. ferox* is a sit-and-wait ambush predator and, like *C. vorax*, the opening of its infundibulum is aligned with the substratum and it consumes metazoans smaller than itself such as *Lepadella* spp. (Meksuwan et al., 2013; pers. obs). This ambush predator lifestyle may represent a low metabolic alternative to suspension feeding by ciliary action.

However, further study into differences in metabolism between swimming and sessile adult gnesiotrochan rotifers is necessary to test the metabolic rate hypothesis.

We found that colonial lifestyle was related to an increased genome size. This may support the energetic advantage hypothesis of rotiferan coloniality. The adaptive origin of coloniality in the Gnesiotrocha has been hypothesized to be either due to predator avoidance or an increase in feeding efficacy. Clearance rates for colonial versus non-colonial rotifers do not appear to differ (Wallace & Starkweather, 1985). Despite this lack of increase, there are reports of colonies of *S. socialis* where individuals within the colony orient their coronae in the same direction and form discrete incurrent and excurrent chimneys (Wallace, 1987). If colonial rotifers are in fact working together to gather food, they may be using a smaller percentage of their total metabolic costs in feeding processes. Assuming sessile rotifers have lower metabolism, we would expect them to follow the pattern found in other organisms; i.e., that they would have larger genome sizes than rotifers with free-swimming adult stages.

The importance of taxonomic rank in predicting genome size implies that there is phylogenetic underpinning influencing lifestyle and genomic content. Unfortunately, the phylogeny of the Gnesiotrocha is not well resolved and thus cannot serve as a reliable tree for more sophisticated analyses. The most recent tree for the group had low support for the placement of several families used in this study (i.e., Hexarthridae, Testudinellidae, and Trochosphaeridae), all of which are free-swimming solitary taxa within a superorder otherwise dominated by sessility (Meksuwan et al., 2015). Due to this uncertainty, it is unclear whether the relationship found between sessile and motile taxa is an artifact of phylogenetic signal or a true relationship. For example, if motile taxa are interspersed throughout the gnesiotrochan phylogeny it would imply that genome size and lifestyle are correlated, whereas if they are sister groups or stem lineages it may mean that the relationship observed is due to common ancestry or phylogenetic inertia.

Polyploidy is a possible mechanism of genome size expansion in rotifers. Polyploidy occurs in both bdelloid and ploimid species. For example, bdelloid rotifers are degenerate tetraploids as evidenced by sequencing (Mark Welch et al., 2008; Hur et al., 2009), while in monogononts, polyploidy occurs in certain populations of the ploimid rotifer *Euchlanis dilatata* Ehrenberg, 1830 (Walsh & Zhang, 1992). Whether or not polyploidy is an important factor driving genome size likely depends on the age of the event, as over time purifying selection should diminish genome size in the absence of other factors (Lynch & Conery, 2003). Consequently, ancient chromosomal duplications have less influence on current genome sizes. The genome sizes of sessile rotifers we measured were roughly twice that of the motile rotifers, implying ploidy events may have occurred during evolution of the Gnesiotrocha. To our knowledge, there are no karyotypes for this group. Chromosome analysis would offer strong support either for or against polyploidy as a mechanism of genome size evolution within the group. It should be noted that many of these mechanisms can act in concert on populations to determine genome size, resulting in antagonistic or synergistic outcomes depending on a variety of factors. For example, polyploidy along with accumulation of TEs is antagonistic to decreases in genome size brought about by selection for faster metabolic rates. Sessile organisms may be more vulnerable to genome size increases due to these mechanisms, since their potentially lower energetic costs may lessen selection for high metabolic rates.

Increased sampling within the Gnesiotrocha may reveal the degree of variability in genome size among populations and clades. To investigate potential relationships between genome size and colonial lifestyle, increased sampling of taxa within the Flosculariaceae is needed. Rotifers to target for future genome size research include species with different colony recruitment strategies such as allorecruitive (*Floscularia,* some *Limnias,* some *Ptygura*), autorecruitive (some *Limnias, Octotrocha,* some *Ptygura,* some *Sinantherina*) and those rotifers in which colony-forming species are closely related to solitary forms (e.g., multiple species within the genera *Limnias* and *Floscularia*) (Wallace, 1987; 2002). There are several gnesiotrochans that have planktonic habits in otherwise sessile genera such as *Sinantherina spinosa* (Thorpe, 1983), *Ptygura libera* Myers, 1934, and *Collotheca libera* (Zacharias, 1894). These species along with the genus *Conochilus* either form colonies (e.g., *S. spinosa* and *Conochilus* spp.) or gelatinous tubes (e.g., *C. libera* and *P. libera*), which may serve as sources of drag to increase feeding efficiency as in other zooplankters (Kiørboe, 2011a). If the metabolic rate hypothesis of genome size holds true for these taxa we would expect them to have genome sizes intermediate between sessile and free-swimming rotifers. Our genome size estimation for *Conochilus hippocrepis* (Schrank, 1803) of 0.127 pg cautiously follows this pattern. Additional observations are needed to substantiate this hypothesis. Rotifers with other lifestyles including facultatively sessile (e.g., *Brachionus rubens* Ehrenberg, 1838, *Philodina megalotrocha* Ehrenberg, 1832) (Gilbert, 2018; Wallace, 1987), ectoparasitic (e.g., seisonids) and endoparasitic (e.g., *Albertia* spp., *Drilophaga* spp.) and the wholly parasitic Acanthocephala are interesting candidates for genome size measurement because these lifestyles have diverse metabolic demands (May, 1989). To test whether the sessile gnesiotrochan rotifers possess a metabolic advantage over free-swimming species, investigations using proxies of metabolism, such as respiration rates and the production of metabolites, should be conducted.

Acknowledgements

Funding was provided by NSF DEB-1257068 (EJW), National Institute of Health (NIH-NIMHD-RCMI 5G12MD007592), and UTEP's Dodson research grant (PDB). Samples were collected under permits TPWD 2016-03, CPDCNBSP-2016-32, and EMNRDSPD 2017. Travis LaDuc facilitated collection at Miller Ranch. Kevin Bixby provided access to La Mancha Wetland. Some samples were kindly provided by John Gilbert and Russell Shiel, Nic Lannutti, Rick Hochberg, Kevin Floyd, Sergio Samaniego, Enrique Garcia, Judith Rios-Arana, and Robert L. Wallace. We thank Armando Varela for his help with flow cytometry (BBRC CSI Core Facility, funded by NIH-NIMHD-RCMI 5G12MD007592), Kyung-An Han providing *Drosophila* and her students for help with rearing them, and Claus-Peter Stelzer for his advice on flow cytometry methods for rotifers. Robert L. Wallace made helpful comments that improved the manuscript.

GENERAL DISCUSSION

In the preceding works, I conducted studies to help answer the following questions "Why are the organisms we see where we see them?" and (2) "why might animals live together rather than independently?" by investigating metacommunity ecology and community assembly in the rotifers of the Chihuahuan Desert and by analyzing lifestyle and how it relates to genome size and allometric scaling in colonial species.

I found fundamental differences in rotifer richness and community assembly between habitat types within the Chihuahuan Desert. For example, I found the highest rotifer diversity in sites with permanent water such as springs and a strong influence of hydroperiod and conductivity in temporary waters. Despite these findings, overall community assembly appears to be dominated by stochastic processes, with environmental variation contributing relatively little to the observed variance in community composition. These communities accounted for a relatively high proportion of the known diversity of rotifers representing ~11% of described rotifer species (Segers 2003). This finding contrasts with comparable desert habitats in other continents, which are sometimes noted for their lack of diversity (Smolak et al., 2023; Wallace et al., 2005). One key difference between the Chihuahuan Desert and other deserts such as the Khalahari, is the difference in elevational heterogeneity. Another aspect of this desert is that it spans a north to south range of ~1500 kilometers, encompassing the properties of both cold and hot deserts in accordance with Köppen climate designations across this range. Despite these significant latitudinal differences, I did not observe latitudinal patterns among rotifer assemblages in the Chihuahuan Desert. Rotifer diversity found in other aridland regions is typically lower than what I found for Chihuahuan Desert sites, which had gamma diversities of 19-72 species per region

(Mazuelos et al., 1993; Seges & Dumont, 1983; Wallace et a., 2005) compared to 246 species found in the Chihuahuan Desert.

Rotifer community assembly, though largely stochastic, should also be influenced by the biological characteristics of its members. Since basic biological traits such as genome size and respiration are tied to lifestyle, environmental parameters influence these traits may play an outsized role in which rotifers are present in these habitats. One such feature is hydroperiod, which is known to be an important factor in rotifer community assembly (McDaniel, 2022; Smolak & Walsh, 2022; Smolak et al., 2023; Wellborn et al., 1996). Under short hydroperiod conditions, I would expect to see only rotifers having fast generation times, creating a nesting effect species composition based on life cycle speed. If these communities persist, smaller genome sizes and faster metabolisms may be selected. In waters with longer hydroperiods, these pressures will not be as strong, and therefore selection on life cycle speed may be relaxed which could allow for species with larger genome sizes and those with colonial sessile lifestyles to persist. Whether or not this pattern is real remains to be tested.

Rotifers are known to respond to increased temperature with faster development times in a manner similar to aquatic crustaceans (Dodson, 2000). Faster life cycle speeds may be why insects and crustaceans show differing latitudinal patterns in genome size, with crustaceans following Bergmann's rule and insects reversing it (Alfsnes et al., 2017). At higher latitudes, insects are subject to shorter development times and have smaller genome and body sizes at to accommodate faster development. In contrast, crustaceans in cold marine waters are not limited by development time and have larger body and genome sizes. To address this possible pattern in rotifer communities, it would be necessary to have a dataset over a large latitudinal gradient with features such as genome size and resting metabolism known for each rotifer species as well as hydroperiods.

In our study, genome size was significantly smaller in planktonic and solitary rotifers than in sessile and colonial species (Brown & Walsh, 2019). This aligns well with the metabolic hypothesis of genome size and the energetic advantage hypothesis of coloniality (Wallace, 1987). Under both hypotheses, I would expect larger genome sizes in less energetically taxed systems, such as colonies if they provide an energetic advantage or in sessile animals which incur do not the metabolic cost of swimming.

Respiration rates measured in this study are consistent with what was known for rotifers, ranging from 0.612 – 11.364 pm/min (Galkovskaja, 1987; Kirk et al., 1999). However, allometric scaling was variable in colonial rotifers. This variability may have been due to differences in shared extrazooidal structures present in the studied taxa; *S. socialis* does not have a muciligenous sheath while colonies of *L. flosculosa* and *C. hippocrepis* have extensive sheaths. These coverings may be generated by the colony working together to produce them, and possibly result in allometric scaling of respiration rate with colony size. However, sampling of colonial forms was limited, and further allometric scaling factors from other colonial rotifers such as *S. ariprepes* will further clarify if these extrazooidal structures are the cause of allometric scaling. On a broader taxonomic scale, I found that lifestyle traits in colonial animals were associated with allometric scaling of respiration. These included free swimming, the presence of shared zooidal structures and colony shape, specifically linear colony shape promoted isometric scaling of respiration. These findings are not surprising, as shape has been found to influence scaling processes in other studies, including linear shapes promoting isometry in bryozoans (Hartikainen et al., 2014, White et al., 2011).

A confounding factor hampering our understanding of both rotifer diversity and lifestyle evolution is the underdeveloped alpha taxonomy in the group. Cryptic species are well known in the phyla and widespread known to occur in eight commonly occurring and well-studied genera in the Monogononta (e.g., García-Morales & Domínguez-Domínguez, 2013; 2021; Gomez et al., 2002; Kimpel et al., 2015; Kordbacheh et al., 2017; Leasi et al., 2013; Mills et al., 2017; Michaloudi et al., 2017; Obertegger et al., 2014; Schröder & Walsh, 2007; Walsh et al., 2009) and eight genera within the Bdelloidea, which includes all bdelloid genera that have been investigated to date (Birky et al., 2011; Cakil et al., 2021; Fontaneto et al., 2007; 2008; 2009; 2011; Hamdan, 2010). This means that current species present-absent lists developed for rotifers will not reflect the actual diversity present at these sites. If undescribed cryptic species have distinctive biogeographic ranges or are local endemics this would be missed as well. Without voucher specimens, images, and sequencing, recovering information from these datasets for currently under-described taxa is likely impossible. Going forward some techniques may be used to ameliorate some the effects stemming from poor alpha taxonomy. Cryptic species may possess differing genome sizes, and as genome sizes are heritable these differences may be characteristic. It may be possible to use genome sizes as a trait to help differentiate future putative species prior to proper taxonomic description. For cases where inheritable small chromosomal elements are known to contribute to genome size evolution, such as in *Brachionus asplanchnoidis*, genome size as a character alone may be less valuable for this application, as these genomic traits do not appear to be species-specific and rapidly evolve (Stelzer et al., 2021).

Colonial lifestyle was both associated with allometric scaling of respiration in some rotifer species and with larger genome sizes. Taken together, these results suggest that the metabolic hypothesis of genome size holds true for rotifers and suggest that colonies provide an energetic advantage, although other possibilities such as colonies providing an anti-predator defense cannot be ruled out. We found rotifers with known fast life cycles (e.g., *Hexartha* sp., Schröder et al., 2007) to have small genome sizes. In cases where life cycle speed is dominating selection, adult metabolism may become unlinked from genome size, as these rotifers were found not to have notable respiration for their size as adults, similar to respiration rates in other *Hexarthra* that do not possess novel life histories (Galkoskavja, 1987).

Future directions

The metacommunity and diversity studies conducted here were on a small subset of all available rotifer presence/absence data. Better inferences into patterns of rotifer distribution, endemism, habitat preferences, and community assembly may be made if we expand these studies to include larger regions and/or all available data. There are still regions where there is relatively low sampling of rotifer diversity (e.g., Africa (Smolak et al., 2023), Asia, South America, and oceanic islands (Segers, 2007)) where new field studies are required. Additionally, including other regions would allow us to determine if the patterns we found for rotifers in the Chihuahuan Desert are representative of rotifers in general or specific to this ecoregion. Many such studies currently exist, that could add to our knowledge of rotifer biogeography and community assembly including studies of the rotifer fauna of several lake systems and regions, in addition to species lists from particular sites or water bodies as well as several long-term monitoring studies reviewed by May & Wallace (2019) (e.g., Duggan, 2007; Duggan & Barnes, 2005; Duggan et al., 2001; Kuczyńska-Kippen et al., 2020; May & Wallace 2019; Magnuson et al., 2020; Muirhead et al., 2006; Smith et al., 2009).

There are a number of biotic and environmental factors that are important to rotifer biology that we did not investigate. Among these are modes of rotifer dispersal, including anemochory, hydrochory, and zoochory. Anemochory is suspected to be an important mechanism of rotifer dispersal in the Chihuahuan Desert (Rivas et al., 2018; 2019). It may be possible to analyze these through modelling, or quantifying them as dust corridors, bird flyways, and watersheds. A recent mesocosms study found significant differences in zooplankton communities arising in mesocosms based on the dispersal mechanisms allowed, with predatory rotifers only arriving from resting egg banks (Parry et al., 2023). Addressing how these mechanisms of dispersal impact community assembly will improve our knowledge of the relative contributions of stochastic versus deterministic dispersal processes.

Determining what separates rotifer communities in terms of habitat is another avenue of research which could refine our understanding of community assembly. The habitat designations I used were largely subjective and based on our understanding of how environmental parameters, such as hydroperiod, affect the biota. These designations may not necessarily reflect the actual processes driving assembly of rotifer communities in these inland waters. These designations are based on assumed differences between habitats, such as a size difference between a pond and a lake, which may or may not reflect ecologically meaningful differences in communities between these systems. For example, there may be discreet types of rock pools (e.g., possessing different depths, shapes, and washout rates) in which rotifer communities assemble very differently, but were missed by our habitat designations or environmental parameters. Thus, future research could use clustering and random forest modeling techniques to define habitats empirically. This process could define new habitat classes, which could be compared with older designations to see if modeling habitat type with this approach outperforms the models used in Chapter 1. For example, cluster analysis could be used to create clusters of sites by rotifer community composition. Then random forest analysis could be used to estimate the strength of models based on these clusters against traditional habitat designations. Following this, clusters could be compared to environmental parameters and described. This technique may yield novel habitat types that are both empirically derived and better suited for describing rotifer communities.

To further explore genome size, future studies could corroborate the flow cytometry results with karyotyping and genome sequencing. Of these, karyotyping could offer further insight into ploidy level in rotifers. Several species increase genome size via ploidy, e.g., bdelloids are ancient degenerate tetraploids (Hur et al., 2009; Mark Welch et al., 2008) and *Euchlanis dilatata* Ehrenberg, 1830 can have triploid populations (Walsh & Zhang, 1992). Furthermore, as noted above *Brachionus asplanchnoidis* Charin, 1947 has been observed to change genome size by increasing the number of small accessory chromosomes they possess (Stelzer et al., 2021).

Karyotyping may also offer insight into the evolution of the chromosomal architecture in the Rotifera by providing information on the number of chromosomes and their arrangement. Genomic sequencing can confirm the measurements of genome size made by flow cytometry and additionally provide many new characters for further analysis.

Genome sizes from additional rotifer species from a variety of locations may help elucidate patterns of genome size across space and thus the evolution of genome size and lifestyle. One such area of interest would be to test if rotifer genome sizes follow Bergman's rule. Aquatic insects appear to follow the rule inversely, whereas marine crustaceans follow the rule (Alfsnes et al., 2017). The difference is thought to be related to shorter growing seasons further north for aquatic insects but longer and slower development overall in cold marine waters. For rotifers in inland waters, I would expect them to follow a similar pattern to aquatic insects, but this may be overridden by short hydroperiods which may necessitate a faster development (and possibly smaller genome size).

Expanding our understanding of scaling processes in other colonial or social/gregarious taxa will be necessary to determine the causes of allometric scaling and origins of coloniality. Of particular interest are colonial choanoflagellates, which can be compared with their solitary relatives. These protists are closely related to animals, and understanding why they form colonies and how colonies impact the physiology of this group may explain the origin of, not just of coloniality, but also of multicellularity in general. Additionally, colonial ciliates are a common, widely available taxon not closely related to the groups covered in this work. They are found in similar habitats to colonial animals such as bryozoans and rotifers, but represent colonies formed from single-celled organisms. Additionally, it would be feasible to measure colonial ciliate respiration rates using the Loligo microplate system.

Currently there are a lack of respiration studies for several other colonial groups including some phoronid worms, colonial, social, and subsocial spiders, pyrosomes, pterobranchs, and doloilids. Colonial phoronids produce colonies by budding, and so presents a colonial form more integrated than that in Rotifera (Emig, 1982). To our knowledge, respiratory scaling in web-sharing spiders has not been investigated, and there are several other groups of spiders with very loose levels of integration, described as social and subsocial. Thus, information on metabolic scaling in the subsocial species in Family Sparassidae would be insightful (Pruit & Avilés, 2018; Yip & Rayor, 2014). Spider webs, also present a case of an extrazooidal structure that may follow the pattern associated with allometric scaling of colony respiration found here. Colonial spiders share the same web and are known to spend less energy on web production as colony size increases. This relationship may be analogous to the known correspondence of spiders of increasing size spending less energy per mass on web production (Straus et al., 2022). Similarly, ant nest size increases more slowly than colony population, leading to more ants using pre-made structures (Tschinkel, 1999). Doloilids are colonial chordate taxon similar to salps, but possessing specialized zooids, meaning they may represent a greater degree of integration than salps. Comparison between doloilid and salp scaling factors may clarify our understanding of the role of zooid specialization in colony development.

Overall conclusions

In this study I characterized the diversity and community assemblies of Chihuahuan Desert rotifers as well as explored lifestyle evolution in the group throughout the lens of genome size and allometric scaling of respiration rate with colony size. Overall, through these studies I found support for the energetic advantage hypothesis of coloniality in Rotifera (Wallace, 1987), although other possibilities cannot be ruled out. These studies can hopefully serve as building blocks for developing a better understanding of rotiferan diversity and lifestyle evolution through extending the study area beyond the Chihuahuan Desert.

REFERENCES

Adams, N. E., Inoue, K., Seidel, R. A., Lang, B. K., & Berg, D. J. (2018). Isolation drives increased diversification rates in freshwater amphipods. *Molecular Phylogenetics and Evolution*, *127*, 746–757.

Alfsnes, K., Leinaas, H. P., & Hessen, D. O. (2017). Genome size in arthropods; different roles of phylogeny, habitat and life history in insects and crustaceans. *Ecology and Evolution*, *7,* 5939–5947.

Allee, Warder Clyde. (1912). *An experimental analysis of the relation between physiological states and rheotaxis in Isopoda*. University of Chicago.

Allee, W. C. (1931). *Animal aggregations: a study in general sociology*. Chicago University Press.

Allen, J. R. (1998). Suspension feeding in the brittle-star *Ophiothrix fragilis*: efficiency of particle retention and implications for the use of encounter-rate models. *Marine Biology*, *132*, 383–390.

Almegbel, M. (2018). *Role of colony integration in metabolic scaling of colonial animals*. Doctoral Dissertation, Northern Illinois University.

Almeida-Neto, M., Guimarães, P., Guimarães, P. R., Loyola, R. D., & Ulrich, W. (2008). A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos*, *117*, 1227–1239.

Anderson, J. F. (1993). Respiratory energetics of two Florida harvestmen. *Comparative Biochemistry and Physiology Part A: Physiology*, *105*, 67–72.

Atmar, W., & Patterson, B. D. (1993). The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia*, *96*, 373–382.

Banta, W. C., McKinney, F. K., & Zimmer, R. L. (1974). Bryozoan monticules: excurrent water outlets? *Science*, *185*, 783–784.

Barker, D. S. (2000). *Down to Earth at Tuff Canyon, Big Bend National Park, Texas*. Bureau of Economic Geology, the University of Texas at Austin.

Barrington, E. J. W. (1967). *Invertebrate structure and function*. Thomas Nelson and Sons.

Bartoń, K. (2010). *MuMIn: Multi-Model Inference*. Retrieved from https://cran.r-project.org/package=MuMIn.

Baselga, A. (2012). The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. *Global Ecology and Biogeography*, *21*, 1223-1232.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using Lme4. *Journal of Statistical Software*, *67*, 1–48

Beach, N. W. (1960). Study of the planktonic rotifers of the Ocqueoc River system, Presque Isle County, Michigan. *Ecological Monographs*, *30*, 339–358.

Bégin, P. N., & Vincent, W. F. (2017). Permafrost thaw lakes and ponds as habitats for abundant rotifer populations. *Arctic Science*, *3*, 354–377.

Bell, W., & Mitchell, R. (1972). Chemotactic and growth responses of marine bacteria to algal extracellular products. *The Biological Bulletin*, *143*, 265–277.

Bennett, M. D., Leitch, I. J., Price, H. J., & Johnston, J. S. (2003). Comparisons with *Caenorhabditis* (approximately 100 Mb) and *Drosophila* (approximately 175 Mb) using flow cytometry show genome size in *Arabidopsi*s to be approximately 157 Mb and thus approximately 25% larger than the Arabidopsis genome initiative estimate of approximately 125 Mb. *Annals of Botany*, *91*, 547–557

Bērziņš, B. (1951). On the Collothecacean Rotatoria with special reference to the species found in the Aneboda district, Sweden. *Arkiv För Zoologi*, *1*, 565–592.

Bevington, D. J., White, C., & Wallace, R. L. (1995). Predatory behavior of *Cupelopagis vorax* (Rotifera; Collothecea; Atrochidae) on protozoan prey. *Hydrobiologia*, *313,* 213–217.

Biggs, D. C. (1977). Respiration and ammonium excretion by open ocean gelatinous zooplankton. *Limnology and Oceanography*, *22*, 108–117.

Bininda-Emonds, O. R. P. (2021). 18S rRNA variability maps reveal three highly divergent, conserved motifs within Rotifera. *BMC Ecology and Evolution*, *21*, 1–18.

Birky, C. W., Ricci, C., Melone, G., & Fontaneto, D. (2011). Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae. *Zoological Journal of the Linnean Society*, *161*, 723–734.

Bivand, R. S., & Wong, D. W. (2018). Comparing implementations of global and local indicators of spatial association. *Test, 27*, 716–748.

Blackstone, N. W., & Jasker, B. D. (2003). Phylogenetic considerations of clonality, coloniality, and mode of germline development in animals. *Journal of Experimental Zoology, 297*, 35–47.

Bogdan, K. G., Gilbert, J. J., & Starkweather, P. L. (1979). In situ clearance rates of planktonic rotifers. *Rotatoria: Proceedings of the 2nd International Rotifer Symposium Held at Gent*, 73–77. Springer Netherlands.

Bonner, J. T. (1988). *The evolution of complexity by means of natural selection*. Princeton University Press.

Bonner, J. T. (1998). The origins of multicellularity. *Integrative Biology: Issues, News, and Reviews: Published in Association with The Society for Integrative and Comparative Biology*, *1*, 27–36.

Boraas, M. E., Seale, D. B., & Boxhorn, J. E. (1998). Phagotrophy by a flagellate selects for colonial prey: a possible origin of multicellularity. *Evolutionary Ecology*, *12*, 153–164.

Bosco, G., Campbell, P., Leiva-Neto, J. T., & Markow, T. A. (2007). Analysis of *Drosophila* species genome size and satellite DNA content reveals significant differences among strains as well as between species. *Genetics*, *177,* 1277–1290.

Bowen, J. D., Stolzenbach, K. D., & Chisholm, S. W. (1993). Simulating bacterial clustering around phytoplankton cells in a turbulent ocean. *Limnology and Oceanography*, *38*, 36–51.

Braak, C. J., & Prentice, I. C. (1988). A theory of gradient analysis. *Advances in Ecological Research*, *18*, 271 –317.

Brain, C. K., Fouriel, I., & Shiel, R. J. (1995). Rotifers of the Kalahari Gemsbok National Park, South Africa. *Hydrobiologia*, *314*, 319–324.

Brain, C. K., & Koste, W. (1993). Rotifers of the genus *Proales* from saline springs in the Namib desert, with the description of a new species. *Hydrobiologia*, *256*, 449–454.

Brett, M. T. (1989). The rotifer communities of acid-stressed lakes of Maine. *Hydrobiologia*, *187*, 181–189.

Briggs, M. K., Lozano-Cavazos, E. A., Poulos, H. M., Ochoa-Espinoza, J., & Rodriguez-Pineda, J. A. (2020). The Chihuahuan Desert: a binational conservation response to protect a global treasure. *Encycl. Worlds Biomes*, 126–138.

Brown, B. L., Sokol, E. R., Skelton, J., & Tornwall, B. (2017). Making sense of metacommunities: dispelling the mythology of a metacommunity typology. *Oecologia*, *183*, *243*–252.

Brown, B. L., & Swan, C. M. (2010). Dendritic network structure constrains metacommunity properties in riverine ecosystems. *Journal of Animal Ecology*, *79*, 571–580.

Brown, P. D., Schröder, T., Ríos-Arana, J. V., Rico-Martinez, R., Silva-Briano, M., Wallace, R. L., & Walsh, E. J. (2020). Patterns of rotifer diversity in the Chihuahuan Desert. *Diversity*, *12,* 393.

Brown, P. D., Schröder, T., Ríos-Arana, J. V., Rico-Martinez, R., Silva-Briano, M., Wallace, R. L., & Walsh, E. J. (2022). Processes contributing to rotifer community assembly in shallow temporary aridland waters. *Hydrobiologia*, *849*(17–18), 3719–3735.

Brown, Patrick D., & Walsh, E. J. (2019). Genome size and lifestyle in gnesiotrochan rotifers. *Hydrobiologia*, *844*, 105–115.

Burda, H., Honeycutt, R. L., Begall, S., Locker-Grütjen, O., & Scharff, A. (2000). Are naked and common mole-rats eusocial and if so, why? *Behavioral Ecology and Sociobiology*, *47*, 293–303.

Burgess, S. C., Ryan, W. H., Blackstone, N. W., Edmunds, P. J., Hoogenboom, M. O., Levitan, D. R., & Wulff, J. L. (2017). Metabolic scaling in modular animals. *Invertebrate Biology*, *136*(4), 456–472.

Burwitz, P. (1977). Eine sonderbare ‘Koloniebildung’ des Rädertieres *Philodina megalotrocha*. *Mikrokosmos*, *66*, 293–294.

Buskirk, R. E. (1975). Coloniality, activity patterns and feeding in a tropical orb-weaving spider. *Ecology*, *56*, 1314–1328.

Buss, L. W. (1981). Group living, competition, and the evolution of cooperation in a sessile invertebrate. *Science*, *213*, 1012–1014.

Buss, L. W. (1990). Competition within and between encrusting clonal invertebrates. *Trends in Ecology & Evolution*, *5*, 352–356.

Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology*, *90*, 3566–3574.

Cakil, Z. V., Garlasche, G., Iakovenko, N., Cesare, A., Eckert, E. M., Guidetti, R., & Hamdan, L. (2021). Comparative phylogeography reveals consistently shallow genetic diversity in a mitochondrial marker in Antarctic bdelloid rotifers. *Journal of Biogeography*, *48*, 1797–1809.

Canapa, A., Barucca, M., Biscotti, M. A., Forconi, M., & Olmo, E. (2015). Transposons, genome size, and evolutionary insights in animals. *Cytogenetic and Genome Research*, *147, 217*–239.

Carlot, J., Rouzé, H., Barneche, D. R., Mercière, A., Espiau, B., Cardini, U., & Brandl, S. J. (2022). Scaling up calcification, respiration, and photosynthesis rates of six prominent coral taxa. *Ecology and Evolution*, *12*, 8613.

Carmona, M. J., Dimas-Flores, N., García-Roger, E. M., & Serra, M. (2009). Selection of low investment in sex in a cyclically parthenogenetic rotifer. *Journal of Evolutionary Biology*, *22*, 1975–1983.

Carson, E. W., & Dowling, T. E. (2005). Influence of hydrogeographic history and hybridization on the distribution of genetic variation in the pupfishes *Cyprinodon atrorus* and *C. bifasciatus*. *Molecular Ecology*, *15*, 667–679.

Castro, B. B., Antunes, S. C., Pereira, R., Soares, A. M. V. M., & Goncalves, F. (2005). Rotifer community structure in three shallow lakes: seasonal fluctuations and explanatory factors. *Hydrobiologia*, *543*, 221–232.

Catton, K. B., Webster, D. R., Brown, J., & Yen, J. (2007). Quantitative analysis of tethered and free-swimming copepodid flow fields. *Journal of Experimental Biology*, *210*, 299–310.

Chaparro, G., Horváth, Z., O'farrell, I., Ptacnik, R., & Hein, T. (2018). Plankton metacommunities in floodplain wetlands under contrasting hydrological conditions. *Freshwater Biology*, *63,* 380–391.

Chase, J. M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. *Science*, *328*, 1388–1391

Christensen-Dalsgaard, K. K., & Fenchel, T. (2003). Increased filtration efficiency of attached compared to free-swimming flagellates. *Aquatic Microbial Ecology*, *33*, 77–86.

Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology and the Environment*, *9*, 222–228.

Cottenie, K., Michels, E., Nuytten, N., & Meester, L. (2003). Zooplankton Metacommunity structure: Regional vs. local processes in highly interconnected ponds. *Ecology*, *84*, 991–1000.

Datki, Z., Acs, E., Balazs, E., Sovany, T., Csoka, I., Zsuga, K., Kalman, J. & Galik-Olah, Z., 2021 (2021). Exogenic production of bioactive filamentous biopolymer by monogonant rotifers. *Ecotoxicology and Environmental Safety*, *208*, 111666.

De Meester, L., A. G., Okamura, B., & Schwenk, K. (2002). The Monopolization Hypothesis and the dispersal–gene flow paradox in aquatic organisms. *Acta Oecologica*, *23*, 121–135.

De Meester, Declerck, S., Stoks, R., Louette, G., Van De Meutter, F., De Bie, T., Michels, E. and Brendonck, L., (2005). Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*.

De Meester, L., J. V., Kilsdonk, L. J., & Urban, M. C. (2016). Evolving perspectives on monopolization and priority effects. *Trends in Ecology & Evolution*, *31*, 136–146.

De Smet, W. H. (1996). Rotifera: The Proalidae (Monogononta); SPB Academic Publishing bv: Amsterdam. *The Netherlands*, *4*, 102.

De Smet, W. H., & Beyens, L. (1995b). Rotifers from the Canadian High Arctic (Devon Island, Northwest Territories). *Hydrobiologia*, *313,* 29–34.

De Smet, W. H., & Pourriot, R. (1997). Rotifera.: The Dicranophoridae (Monogononta) and: The Ituridae (Monogononta); SPB Academic Publishing: Amsterdam. *The Netherlands*, *5*, 344.

Denekamp, N. Y., Reinhardt, R., Kube, M., & Lubzens, E. (2010). Late embryogenesis abundant (LEA) proteins in nondesiccated, encysted, and diapausing embryos of rotifers. *Biology of Reproduction*, *82*, 714–724.

Devetter, M. (2009). Clearance rates of the bdelloid rotifer, *Habrotrocha thienemanni*, a tree-hole inhabitant. *Aquatic Ecology*, *43*, 85–89.

Diéguez, M., & Balseiro, E. (1998). Colony size in *Conochilus hippocrepis*: defensive adaptation to predator size. *Hydrobiologia*, *387*, 421–425.

Dinerstein, E., Olson, D., Atchley, J., Loucks, C., Contreras-Balderas, S., Abell, R., Iñigo, E., Enkerlin, E., Williams, C., Castilleja, G. & Allnutt,.T. (2001). *Ecoregion-Based Conservation in the Chihuahuan Desert: A Biological Assessment*. Arlington, VA: Nature Conservancy Report.

Diniz, L. P., Petsch, D. K., & Bonecker, C. C. (2021). Zooplankton β diversity dynamics and metacommunity structure depend on spatial and temporal scales in a Neotropical floodplain. *Freshwater Biology*, *66*, 1328–1342.

Dodds, P. S., Rothman, D. H., & Weitz, J. S. (2001). Re-examination of the “3/4-law” of metabolism. *Journal of Theoretical Biology*, *209*, 9–27.

Dodson, S. I., Arnott, S. E., & Cottingham, K. L. (2000). The relationship in lake communities between primary productivity and species richness. *Ecology*, *81,* 2662– 2679.

Donner, J. (1965). *Ordnung Bdelloidea (Rotatoria, Rädertiere); Akademie-Verlag*.

Doohan, M. (1973). An energy budget for adult *Brachionus plicatilis* Muller (Rotatoria). *Oecologia*, *13*(4), 351–362.

Dražina, T., Špoljar, M., Primc, B., & Habdija, I. (2017). Distribution of rotifers and other meiofauna in the bryophytes and hyporheic zone of a karst hydrosystem – an example of a nested community. *Marine and Freshwater Research*, *68*, 43.

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs*, *67*, 345–366.

Duggan, I. C. (2007). *An Assessment of the Water Quality of Ten Waikato Lakes Based on Zooplankton Community Composition; Centre for Biodiversity and Ecology Research*. The University of Waikato, Hamilton.

Duggan, I. C., & Barnes, G. (2005). *Assessment of Trophic State Change in Selected Lakes of the Auckland Region Based on Rotifer Assemblages; Centre for Biodiversity and Ecology Research*. University of Waikato, Hamilton.

Duggan, I. C., Green, J. D., & Shiel, R. J. (2002). Distribution of rotifer assemblages in North Island, New Zealand, lakes: relationships to environmental and historical factors. *Freshwater Biology*, *47*, 195–206.

Dumont, H. J. (1980). Workshop on taxonomy and biogeography. In *Rotatoria* (Vol. 73, pp. 205–206).

Dumont, H. J. (1983). Biogeography of rotifers. *Hydrobiologia*, *104*, 19–30.

Dumont, H. J., & Coussement, M. (1976). Rotifers from Rio de Oro (North-Western Sahara). *Hydrobiologia*, *51*, 109–112.

Dyrynda, P. E. J., J., P., & Burgess, S. C. (1986). Defensive strategies of modular organisms. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *313*, 227–243.

Echaniz, S. A., Cabrera, G. C., Rodríguez, C., & Vignatti, A. M. (2013). Do temporary lakes vary from year to year? A comparison of limnological parameters and zooplankton from two consecutive annual cycles in an Argentine temporary saline lake. *International Journal of Aquatic Science*, *4,* 41–61.

Edmondson, W. T. (1944). Ecological studies of sessile Rotatoria: Part I. Factors affecting distribution. *Ecological Monographs*, *14*, 31–66.

Edmondson, W. T. (1945). Ecological studies of sessile Rotatoria, Part II: Dynamics of populations and social structures. *Ecological Monographs*, *15*, 141–172.

Edmondson, W. T. (1949). A formula key to the rotatorian Genus *Ptygura. Transactions of the American Microscopical Society*, *68*, 127.

Edmondson, W. T. (1959). *Rotifera*. New York, NY, USA: John Wiley & Sons, Inc.

Edmunds, P. J., Johnson, K. W., & Burgess, S. C. (2022). Branching coral morphology affects physiological performance in the absence of colony integration. *Biology Letters*, *18*, 20220414.

Elliott, J. M., & Ruttner-Kolisko, A. (1976). Plankton Rotifers: Biology and Taxonomy. *The Journal of Animal Ecology*, *45*, 617.

Elliott, T. A., & Gregory, T. R. (2015). What's in a genome? The C-value enigma and the evolution of eukaryotic genome content. *Philosophical Transactions of the Royal Society B*, *370,* 20140331.

Emerson, B. C., & Gillespie, R. G. (2008). Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology & Evolution*, *23*(11), 619–630.

Emig, C. C. (1982). The Biology of Phoronida. In *Advances in Marine Biology* (Vol. 19, pp. 1–89).

Epp, R. W., & Lewis, W. M. (1984). Cost and speed of locomotion for rotifers. *Oecologia*, *16,* 289–292.

Ezcurra, E., Martínez-Berdeja, A., & Villanueva-Almanza, L. (2020). The evolution of North American deserts and the uniqueness of Cuatro Ciénegas. In *Plant Diversity and Ecology in the Chihuahuan Desert: Emphasis on the Cuatro Ciénegas Basin* (pp. 45–60).

Fairchild, G. W. (1981). Movement and microdistribution of *Sida crystallina* and other littoral microcrustacea. *Ecology, 62*, 1341–1352.

Felix, A., Stevens, M. E., & Wallace, R. L. (1995). Unpalatability of a colonial rotifer, *Sinantherina socialis*, to small zooplanktivorous fishes. *Invertebrate Biology*, *125*, 139–144.

Fenchel, T. (1986). The ecology of heterotrophic microflagellates. In *Advances in Microbial Ecology* (pp. 57–97). Boston, MA: Springer US.

Fenchel, T. O. M. (2001). How dinoflagellates swim. *Protist*, *152*, 329–338.

Fenchel, T., & Finlay, B. J. (2004). The ubiquity of small species: Patterns of local and global diversity. *Bioscience*, *54*, 777.

Fewell, J. H., & Harrison, J. F. (2016). Scaling of work and energy use in social insect colonies. *Behavioral Ecology and Sociobiology*, *70*, 1047–1061

Flot, J. F., Hespeels, B., Li, X., Noel, B., Archipova, I., Danchin, E. G. J., E.G., Hejnol, A., Henrissat, B., Koszul, R., Aury, J.M. & Barbe, V. Koszul, R. (2013). Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. *Nature*, *500*, 453–457.

Flot, J.-F., Debortoli, N., Hallet, B., & Doninck, K. (2016). Response to Signorovitch et al. *Current Biology*, *26*, 755.

Foissner, W. (2007). Dispersal and biogeography of protists: recent advances. *Japanese Journal of Protozoology*, *40*, 1–16.

Fontaneto, D. (2014). Molecular phylogenies as a tool to understand diversity in rotifers. *International Review of Hydrobiology*, *99*, 178–187.

Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, *7*, 10.

Fontaneto, D., Melone, G., & Wallace, R. L. (2005). Morphology of *Floscularia ringens* (Rotifera, Monogononta) from egg to adult. *Invertebrate Biology*, *122*, 231–240.

Fontaneto, D., Ficetola, G. F., Ambrosini, R., & Ricci, C. (2006). Patterns of diversity in microscopic animals: are they comparable to those in protists or in larger animals? *Global Ecology and Biogeography*, *15*, 153–162.

Fontaneto, D., Herniou, E. A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C., & Barraclough, T. G. (2007). Independently evolving species in asexual bdelloid rotifers. *PLoS iology*, *5*, 87.

Fontaneto, D., Barraclough, T. G., Chen, K., Ricci, C., & Herniou, E. A. (2008a). Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. *Molecular Ecology*, *17,* 3136– 3146.

Fontaneto, D., Smet, W. H., & Melone, G. (2008b). Identification key to the genera of marine rotifers worldwide. *Meiofauna Marina*, *16,* 75–99.

Fontaneto, D., Kaya, M., Herniou, E. A., & Barraclough, T. G. (2009). Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, *53*, 182–189.

Fontaneto, D., Iakovenko, N., Eyres, I., Kaya, M., Wyman, M., & Barraclough, T. G. (2011). Cryptic diversity in the genus Adineta Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. *Hydrobiologia*, *662*, 27–33.

Fontaneto, D., Barbosa, A. M., Segers, H., & Pautasso, M. (2012). The ‘rotiferologist’ effect and other global correlates of species richness in monogonont rotifers. *Ecography*, *35*, 174–182.

Fontaneto, D., Iakovenko, N., & Smet, W. H. (2015). Diversity gradients of rotifer species richness in Antarctica. *Hydrobiologia*, *761*, 235–248.

Fréchette, M., Butman, C. A., & Geyer, W. R. (1989). The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, Mytilus edulis L. *Limnology and Oceanography*, *34*, 19–36.

Lopez, F., Cancellario, T., Fontaneto, D., Kamburska, L., Karimullah, K., Wallace, R.L., Walsh, E.J. & Smolak, R. (2023). A georeferenced dataset for occurrence records of the phylum Rotifera in Africa. *Journal of Limnology*, *82*.

Frisch, D., Green, A. J., & Figuerola, J. (2007). High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, *69*, 568–574.

Fukami, T. (2015). Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics*, *46,* 1–23.

Furst, D., Aldridge, K., Shiel, R., Ganf, G., Mills, S., & Brookes, J. (2014). Floodplain connectivity facilitates significant export of zooplankton to the main River Murray channel during a flood event. *Inland Waters*, *4*, 413–424.

Gabaldón, C., Fontaneto, D., Carmona, M. J., Montero-Pau, J., & Serra, M. (2017). Ecological differentiation in cryptic rotifer species: what we can learn from the *Brachionus plicatilis* complex. *Hydrobiologia*, *796*, 7–18.

Galkovskaja, G. A. (1987). Planktonic rotifers and temperature. *Hydrobiologia*, *147*, 307–317.

Galkovskaya, G. A., & Vinberg, G. G. (1979). The relationship of oxygen consumption rate and rotifer body mass. In *General Studies in Aquatic Systems* (pp. 21–25). Nauka.

Gansfort, B., Fontaneto, D., & Zhai, M. (2020). Meiofauna as a model to test paradigms of ecological metacommunity theory. *Hydrobiologia*, *847,* 2645–2663.

Garcia, M. A. (2004). *The asexual life history of the colonial rotifer Sinantherina socialis* (Linnaeus). Doctoral Dissertation, Yale University.

García-Girón, J., Heino, J., García-Criado, F., Fernández-Aláez, C., & Alahuhta, J. (2020). Biotic interactions hold the key to understanding metacommunity organization. *Ecography*, *43,* 1180–1190.

García-Morales, A. E., & Elías-Gutiérrez, M. (2013). DNA barcoding of freshwater Rotifera in Mexico: Evidence of cryptic speciation in common rotifers. *Molecular Ecology Resources*, *13* 1197–1107.

García-Morales, D.-D. (2013). Cryptic molecular diversity in the morphologically variable rotiferan *Brachionus quadridentatus* (Rotifera: Monogononta). *Revista de Biología Tropical*, *67*, 1114–1130.

García-Morales, Alma E., Domínguez-Domínguez, O., & Elías-Gutiérrez, M. (2021). Uncovering hidden diversity: Three new species of the *Keratella* Genus (Rotifera, Monogononta, Brachionidae) of high altitude water systems from central Mexico. *Diversity*, *13*, 676.

García-Roger, E. M., Carmona, M. J., & Serra, M. (2006). Hatching and viability of rotifer diapausing eggs collected from pond sediments. *Freshwater Biology*, *51,* 1351–1358.

García-Varela, M., & Nadler, S. A. (2006). Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, *40*, 61–72.

Gazi, M., Kim, J., García-Varela, M., Chungoo Park, D. T. J. L., & Park, J. (2016). Mitogenomic phylogeny of Acanthocephala reveals novel C lass relationships. *Zoologica Scripta*, *45*, 437–454.

Gervasio, V., Berg, D. J., Lang, B. K., Allan, N. L., & Guttman, S. I. (2004). Genetic Diversity in the *Gammarus pecos* species complex: implications for conservation and regional biogeography in the Chihuahuan Desert. *Limnology and Oceanography*, *49*, 520–531.

Gilbert, J. J. (1979). Observations on the susceptibility of some protists and rotifers to predation by *Asplanchna girodi*. *Rotatoria: Proceedings of the 2nd International Rotifer Symposium*, 87–91. Springer Netherlands.

Gilbert, J. J. (2018). Attachment behavior in the rotifer *Brachionus rubens*: induction by *Asplanchna* and effect on sexual reproduction. *Hydrobiologia, 844,* 9–20.

Gilbert, J. J., & Stemberger, R. S. (1984). *Asplanchna*-induced polymorphism in the rotifer *Keratella slacki*. *Limnology and Oceanography*, *29*, 1309–1316.

Gilbert, J. J., & Jack, J. D. (199). Rotifers as predators on small ciliates. *Rotifer Symposium VI: Proceedings of the Sixth International Rotifer Symposium,*, 247–253. Spain: Springer Netherlands.

Glazier, D. S. (2005). Beyond the “3/4-power law”: variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews*, *80*, 611.

Glazier, D. S. (2010). A unifying explanation for diverse metabolic scaling in animals and plants. *Biological Reviews*, *85*, 111–138.

Glazier, D. S. (2014). Metabolic scaling in complex living systems. *Systems*, *2*, 451–540.

Glazier, D. S. (2022). Variable metabolic scaling breaks the law: from ‘Newtonian’ to ‘Darwinian’ approaches. *Proceedings of the Royal Society B: Biological Sciences 289,* 20221605.

Gómez, A., Carvalho, G. R., & Lunt, D. H. (2000). Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting egg banks. *Proceedings of the Royal Society of London. Series B: Biological Sciences 267*, 2189–2197.

Gómez, A., Serra, M., Carvalho, G. R., & Lunt, D. H. (2002). Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera. *Evolution*, *56*, 1431–1444.

Gómez, A., Montero-Pau, J., Lunt, D. H., Serra, M., & Campillo, S. (2007). Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. *Molecular Ecology*, *16*, 3228–3240.

Gooriah, L. D., & Chase, J. M. (2019). Sampling effects drive the species–area relationship in lake zooplankton. *Oikos*, *129,* 124– 32.

Gooriah, L., Blowes, S. A., Sagouis, A., Schrader, J., Karger, D. N., Kreft, H., & Chase, J. M. (2021). Synthesis reveals that island species–area relationships emerge from processes beyond passive sampling. *Global Ecology and Biogeography*, *30,* 2119–2131.

Goździejewska, A. M., Koszałka, J., Tandyrak, R., Grochowska, J., & Parszuto, K. (2021). Functional responses of zooplankton communities to depth, trophic status, and ion content in mine pit lakes. *Hydrobiologia*, *848,* 2699–2719.

Grainger, T. N., & Gilbert, B. (2016). Dispersal and diversity in experimental metacommunities: linking theory and practice. *Oikos*, *125,* 1213–1223.

Gray, M. W., Burger, G., & Lang, B. F. (1999). Mitochondrial evolution. *Science*, *283*, 1476–1481.

Green, J. (1986). Associations of zooplankton in six crater lakes in Arizona, Mexico and New Mexico. *Journal of Zoology*, *208,* 135–159.

Gregory, T. R. (2001a). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biological Reviews of the Cambridge Philosophical Society*, *76,* 65–101.

Gregory, T. R. (2001b). The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates. *Blood Cells, Molecules and Disease*, *27,* 830–843.

Gregory, T. R., & Hebert, P. D. (2003). Genome size variation in lepidopteran insects. *Canadian Journal of Zoology*, *81,* 1399–1405.

Gregory, T. R. (2005). Genome size evolution in animals. In *The Evolution of the Genome*. San Diego, CA: Elsevier.

Guimarães P. & Jr. Guimarães, P. (2006). Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling & Software*, *21*, 1512–1513.

Guisan, A., & Rahbek, C. (2011). SESAM - A new framework integrating macroecological and species distribution models for predicting spatio-temporal patterns of species assemblages. *Journal of Bigeography*, *38*, 1433–1444.

Grünbaum, D. (1995). A model of feeding currents in encrusting bryozoans shows interference between zooids within a colony. *Journal of Theoretical Biology*, *174*, 409–425.

Hamdan, L. K. (2010). *Ecology and genetics of Philodina megalotrocha (Rotifera, Bdelloidea) from Chihuahuan Desert populations.* Master Thesis: University of Texas at El Paso.

Hampton, S. E. (2005). Increased niche differentiation between two *Conochilus* species over 33 years of climate change and food web alteration. *Limnology and Oceanography*, *50*, 421–426.

Hampton, S. E., Scheuerell, M. D., & Schindler, D. E. (2006). Coalescence in the Lake Washington story: Interaction strengths in a planktonic food web. *Limnoogy and. Oceanography*, *51*, 2042–2051.

Hampton, S. E., Izmest'eva, L. R., Moore, M. V., Katz, S. L., Dennis, B., & Silow, E. A. (2008). Sixty years of environmental change in the world's largest freshwater lake - Lake Baikal, Siberia. *Global Change Biology*, *14*, 1947–1958.

Hansson, L.-A., Hylander, S., Dartnall, H. J. G., Lidström, S., & Svensson, J.-E. (2012). High zooplankton diversity in the extreme environments of the McMurdo Dry Valley lakes, Antarctica. *Antarctic Science*, *24*, 131–138.

Hardie, D. C., & Hebert, P. D. N. (2003). The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. *Genome*, *46,* 683–706.

Harmsworth, G. C., & Sleigh, M. A. (1993). Colonization of non-living surfaces in streams by peritrich ciliates. *European Journal of Protistology*, *29*, 294–301.

Harrison, J. F., Biewener, A., Bernhardt, J. R., Burger, J. R., Brown, J. H., Coto, Z. N., & Duell, M. E. (2022). White Paper: An integrated perspective on the causes of hypometric metabolic scaling in animals. *Integrative and Comparative Biology*, *62*, 1395–1418.

Hart, C. M., González, M. R., Simpson, E. P., & Hurlbert, S. H. (1998). Salinity and fish effects on Salton Sea microecosystems: Zooplankton and nekton. *Hydrobiology*, *381*, 129–152.

Hartikainen, H., Humphries, S., & Okamura, B. (2014). Form and metabolic scaling in colonial animals. *Journal of Experimental Biology*, *217*, 779–786.

Heino, J., Grönroos, M., Soininen, J., Virtanen, R., & Muotka, T. (2012). Context dependency and metacommunity structuring in boreal headwater streams. *Oikos*, *121*, 537–544.

Hendrickson, J., & Johnston, M. C. (1986). Vegetation and Community Types of the Chihuahuan Desert. *Second Symposium of Resources of the Chihuahuan Desert Region: U.S. and Mexico"; Chihuahuan Desert Research Institute*, *II*. Alpine, TX, USA: Sul Ross State University.

Herlyn, H., Piskurek, O., Schmitz, J., Ehlers, U., & Zischler, H. (2003). The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, *26*, 155–164.

Hernroth, L., & Gröndahl, F. (1985). On the biology of *Aurelia aurita* (L.) 3. Predation by *Coryphella verrucosa* (Gastropoda, Opisthobranchia), a major factor regulating the development of *Aurelia* populations in the Gullmar Fjord, western Sweden. *Ophelia*, *24*, 37–45.

Hershler, R. (1984). Systematic revision of the hydrobiid snails (Gastropoda: Rissoacea) of the Cuatro Ciénegas Basin, Coahuila. *Mexico. Malacologia*, *26*, 31–123.

Hershler, R., Liu, H.-P., & Mulvey, M. (1999). Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American Southwest. *Molecular Phylogenetics and Evolution*, *13*, 377–391.

Hershler, R., Liu, H.-P., & Stockwell, E. A. (2002). A new genus and species of aquatic gastropods (Rissooidea: Hydrobiidae) from the North American Southwest: Phylogenetics relationships and biogeography. *Proceedings of the Biological Society of Washington*, *115*, 171–188.

Hershler, R., Mulvey, M., & Liu, H.-P. (2005). Genetic variation in the Desert springsnail (*Tryonia porrecta*): implications for reproductive mode and dispersal. *Molecular Ecology*, *14*, 1755–1765.

Hershler, R., Liu, H.-P., & Lang, B. K. (2007). Genetic and morphologic variation of the *Pecos assiminea*, an endangered mollusk of the Rio Grande region, United States and Mexico (Caenogastropoda: Rissooidea: Assimineidae). *Hydrobiologia*, *579*, 317–335.

Hershler, R., Liu, H.-P., & Landye, J. J. (2011). New species and records of springsnails (Caenogastropoda: Cochliopidae: *Tryonia*) from the Chihuahuan Desert (Mexico and United States), an imperiled bio-diversity hotspot. *Zootaxa*, *3001*, 1.

Herzig, A. (1987). The analysis of planktonic rotifer populations: A plea for long-term investigations. *Hydrobiologia*, *147*, 163–180.

Hiebert, L. S., Simpson, C., & Tiozzo, S. (2021). Coloniality, clonality, and modularity in animals: The elephant in the room. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *336*, 198–211.

Hijmans, R. J. (2017). *Introduction to the "geosphere" package*. Retrieved from https://rdrr.io/cran/geosphere/.

Hochberg, A. (2014). *Comparative myoanatomy of collothecid rotifers (Rotifera: Gnesiotrocha: Collothecaceae) with details on larval metamorphosis and development of the infundibulum in species of* Stephanoceros. Master Thesis: University of Massachusetts Lowell.

Hochberg, A., & Hochberg, R. (2017). Musculature of the sessile rotifer *Stephanoceros fimbriatus* (Rotifera: Gnesiotrocha: Collothecaceae) with details on larval metamorphosis and development of the infundibulum. *Zoologischer Anzeiger*, *268*, 84–95.

Hochberg, R., & Gurbuz, O. A. (2008). Comparative morphology of the somatic musculature in species of *Hexarthra* and *Polyarthra* (Rotifera, Monogononta): its function in appendage movement and escape behavior. *Zoologischer Anzeiger-A Journal of Comparative Zoology*, *247*, 233–248.

Hochberg, R., Hochberg, A., & Chan, C. (2015). Ultrastructure of the rotifer integument: Peculiarities of *Sinantherina socialis* (Monogononta: Gnesiotrocha). *Invertebrate Biology*, *134*, 181–188.

Hochberg, R., Yang, H., Walsh, E. J., & Wallace, R. L. (2019). Fine structure of the subitaneous eggshell of the sessile rotifer *Stephanoceros millsii* (Monogononta) with observations on vesicle trafficking in the integument during ontogeny. *Invertebrate Reproduction & Development*, *63*, 122–134.

Hoppeler, H., & Weibel, E. R. (2005). Scaling functions to body size: theories and facts. *Journal of Experimental Biology*, *208*, 1573–1574

Hughes, A. L., & Hughes, M. K. (1995). Small genomes for better flyers. *Nature*, *377,* 391–391.

Hur, J. H., Doninck, K., Mandigo, M. L., & Meselson, M. (2009). Degenerate tetraploidy was established before bdelloid rotifer families diverged. *Molecular Biology and Evolution*, *26,* 375–385.

Hurt, C., & Hedrick, P. (2004). Conservation genetics in aquatic species: General approaches and case studies in fishes and springsnails of arid lands. *Aquatic Sciences*, *66*, 402–413.

Ikeda, T. (1970). Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. *Bulletin of the Faculty of Fisheries Sciences, Hokkaido University*, *21*, 91–112.

Ikeda, T. (1985). Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. *Marine Biology*, *85*, 1–11.

Isaac, N. J. B., & Carbone, C. (2010). Why are metabolic scaling exponents so controversial? Quantifying variance and testing hypotheses. *Ecology Letters*, *13*, 728–735.

Jackson, J. B. C. (1977). Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *The American Naturalist*, *111*, 743–767.

Jackson, J. V. (1980). *The effect of food density on the net assimilation values, respiration and swimming speeds of the rotifer, Brachionus calyciflorus*. Master Thesis University Nevada.

Jakob, E. M. (1991). Costs and benefits of group living for pholcid spiderlings: losing food, saving silk. *Animal Behaviour*, *41*, 711–722.

Jersabek, C. D., Weithoff, G., & Weisse, T. (2011). *Cephalodella acidophila* n. sp. *Zootaxa*, *2939*, 50–58.

Jersabek, C. D., & Bolortsetseg, E. (2010). Mongolian rotifers (Rotifera, Monogononta) — a Checklist with Annotations on Global Distribution and Autecology. *Proceedings of the Academy of Natural Sciences of Philadelphia 159*, 119–168.

Jiang, H., Osborn, T. R., & Meneveau, C. (2002). The flow field around a freely swimming copepod in steady motion. Part I: Theoretical analysis. *Journal of Plankton Research*, *24*, 167–189.

Jocqué, M., Graham, T., & Brendonck, L. (2007a). Local structuring factors of invertebrate communities in ephemeral freshwater rock pools and the influence of more permanent water bodies in the region. *Hydrobiologia*, *592*, 271–280.

Jocqué, M., Riddoch, B. J., & Brendonck, L. U. C. (2007b). Successional phases and species replacements in freshwater rock pools: towards a biological definition of ephemeral systems. *Freshwater Biology*, *52*, 1734–1744.

Jocqué, M., Vanschoenwinkel, B., & Brendonck, L. U. C. (2010). Freshwater rock pools: a review of habitat characteristics, faunal diversity and conservation value. *Freshwater Biology*, *55*.

Johnson, S. G. (2005). Age, phylogeography and population structure of the microendemic banded spring snail, *Mexipyrgus churinceanus*. *Molecular Ecology*, *14*, 2299–2311.

Jonsson, P. R., & Tiselius, P. (1990). Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Marine Ecology Progress Series*, 35–44.

Juračka, P. J., Dobiáš, J., Boukal, D. S., Šorf, M., Beran, L., Černý, M., & Petrusek, A. (2019). Spatial context strongly affects community composition of both passively and actively dispersing pool invertebrates in a highly heterogeneous landscape. *Freshwater Biology*, *64,* 2093–2106.

Kak, A., & Rao, T. R. (1998). Does the evasive behavior of *Hexarthra* influence its competition with cladocerans? *Hydrobiologia*, *387*, 409–419.

Kampe, H., König-Rinke, M., Petzoldt, T., & Benndorf, J. (2007). Direct effects of *Daphnia*-grazing, not infochemicals, mediate a shift towards large inedible colonies of the gelatinous green alga *Sphaerocystis schroeteri*. *Limnologica*, *37*, 137–145.

Kapusta, A., Suh, A., & Feschotte, C. (2017). Dynamics of genome size evolution in birds and mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *114,* 1460–1469.

Karpowicz, M., & Ejsmont-Karabin, J. (2021). Diversity and structure of pelagic zooplankton (Crustacea, Rotifera) in NE Poland. *Water*, *13,* 456.

Kaya, M., Fontaneto, D., Segers, H., & Altindağ, A. (2010). Temperature and salinity as interacting drivers of species richness of planktonic rotifers in Turkish continental waters. *Journal of Limnology*, *69*, 297–304.

Kellogg, C. A., & Griffin, D. W. (2006). Aerobiology and the global transport of desert dust. *Trends in Ecology & Evolution*, *21*, 638–644.

Kidwell, M. G. (2002). Transposable elements and the evolution of genome size in eukaryotes. *Genetica*, *115,* 49–63.

Kim, H. S., Lee, B. Y., Han, J., Jeong, C. B., Hwang, D. S., Lee, M. C., & Kang, H. M. (2018). The genome of the freshwater monogonont rotifer *Brachionus calyciflorus*. *Molecular Ecology Resources*, *18,* 646–655.

Kim, K., & Lasker, H. R. (1998). Allometry of resource capture in colonial cnidarians and constraints on modular growth. *Functional Ecology*, *12*, 646–654.

Kimpel, D., Gockel, J., Gerlach, G., & Bininda-Emonds, O. R. P. (2015). Population structuring in the monogonont rotifer *Synchaeta pectinata* : high genetic divergence on a small geographical scale. *Freshwater Biology*, *60*, 1364–1378.

Kiørboe, T. (2011a). How zooplankton feed: mechanisms, traits and trade-offs. *Biological Reviews of the Cambridge Philosophical Society*, *86,* 311–399.

Kiørboe, T. (2011b). What makes pelagic copepods so successful? *Journal of Plankton Research*, *33*, 677–685.

Kirk, K. L., Ellis, J., & Taylor, J. (1999). Physiological responses to variable environments: storage and respiration in starving rotifers. *Freshwater Biology*, *42*, 637–644.

Kirkegaard, J. B., & Goldstein, R. E. (2016). Filter-feeding, near-field flows, and the morphologies of colonial choanoflagellates. *Physical Review E*, *94*, 052401.

Kleiber, Max. (1947). Body size and metabolic rate. *Physiological Reviews*, *27*(4), 511–541.

Kleiber, M. (1961). *The fire of life. An introduction to animal energetics*. Krieger.

Kneitel, J. M. (2014). Inundation timing, more than duration, affects the community structure of California vernal pool mesocosms. *Hydrobiologia*, *732*, 71–83.

Kobayashi, T., Ralph, T. J., Ryder, D. S., Hunter, S. J., Shiel, R. J., & Segers, H. (2015). Spatial dissimilarities in plankton structure and function during flood pulses in a semi-arid floodplain wetland system. *Hydrobiologia*, *747,* 19–31.

Kodric-Brown, A., & Brown, J. H. (2007). Native fishes, exotic mammals, and the conservation of desert springs. *Frontiers in Ecology and the Environment*, *5*, 549–553.

Kooijman, S. A. L. M., Sousa, T., Pecquerie, L., Meer, J., & Jager, T. (2008). From food-dependent statistics to metabolic parameters, a practical guide to the use of dynamic energy budget theory. *Biological Reviews*, *83*, 533–552.

Kooijman, B., & Kooijman, S. A. L. M. (2010). *Dynamic energy budget theory for metabolic organisation*. Cambridge University Press.

Kordbacheh, A., Garbalena, G., & Walsh, E. J. (2017). Population structure and cryptic species in the cosmopolitan rotifer *Euchlanis dilatata*. *Zoological Journal of the Linnean Society*, *181,* 757–777.

Koste, W. (1973). Das Rädertier-Porträt. Ein merkwürdiges festsitzendes Rädertier: *Cupelopagis vorax*. *Mikrokosmos*, *62*.

Koste, W. R. (1978). *Die Rädertiere Mitteleuropas*. Stuttgart: Gebrüder Borntraeger.

Koste, W., Shiel, R. J., & Brock, M. A. (1983). Rotifera from Western Australian wetlands with descriptions of two new species. In *Biology of Rotifers* (pp. 9–17).

Koste, W., & Shiel, R. (1986). Rotifera from Australian inland waters. I. Bdelloidea (Rotifera: Digononta). *Marine and Freshwater Research*, *37*, 765–792.

Koste, W., & Shiel, R. (1987a). Rotifera from Australian inland waters. II. Epiphanidae and Brachionidae (Rotifera: Monogononta). *Invertebrate Systematics*, *1*, 949–1021.

Koste, W., & Shiel, R. (1987b). Tasmanian rotifer: Affinities with the Australian fauna. *Hydrobiologia*, *147*, 31–43.

Koste, W., & Shiel, R. J. (1989a). Rotifera from Australian inland waters. III. Euchlanidae, Mytilinidae and Trichotriidae (Rotifera: Monogononta). *Transactions of the Royal Society of South Australia*, *113*, 85–114.

Koste, W., & Shiel, R. J. (1989b). Rotifera from Australian inland waters. IV. Colurellidae (Rotifera: Monogononta). In *Transactions of the Royal Society of South Australia* (pp. 119–143).

Koste, W., & Shiel, R. J. (1990a). Rotifera from Australian inland waters. V. Lecanidae (Rotifera: Monogononta). *Transactions of the Royal Society of South Australia*, *114*, 1–36.

Koste, W., & Shiel, R. J. (1990b). Rotifera from Australian inland waters. VI. Proalidae (Rotifera: Monogononta). *Transactions of the Royal Society of South Australia*, *114*, 129–143.

Koste, W., Shiel, R. J., J., M. K., & Gawelczyk, A. T. (1991). Rotifera from Australian inland waters. VII. Notommatidae (Rotifera: Monogononta). Transactions of the Royal Society of South Australia 115. *Proceedings of the National Academy of Sciences*, *100*, 111-159.

Krivoruchko, K. (2012). Empirical Bayesian kriging. *Esri*. Retrieved from http://www.esri.com/news/arcuser/1012/empirical-byesian-kriging.html.

Kubly, D. M. (1992). Aquatic invertebrates in desert mountain rock pools: The White Tank Mountains, Maricopa County, Arizona. *Limnology Aquat. Biol. Southwest*, *2*, 55–69.

Kuczyńska-Kippen, N., & Basińska, A. (2014). Habitat as the most important influencing factor for the rotifer community structure at landscape level. *International Review of Hydrobiology*, *99*, 58–64.

Kuczyńska-Kippen, N., & Ejsmont-Karabin, J. (2020). Rotifera of various aquatic environments of Costa Rica in reference to Central American rotifer fauna. *Turkish Journal of Zoology*, *44*, 238–247.

Kulkarni, M. R., Padhye, S. M., Rathod, R. B., Shinde, Y. S., & Pai, K. (2019). Hydroperiod and species-sorting influence metacommunity composition of crustaceans in temporary rock pools in India. *Inland Waters*, *9,* 320–333.

Kuperman, B. I., Matey, V. E., Dexter, D. M., & Tiffany, M. A. (2002). Invertebrates of the Salton Sea: a scanning electron microscopy portfolio. *Hydrobiology*, *473*, 203–216.

Kusumoto, B., Costello, M. J., Kubota, Y., Shiono, T., Wei, C., Yasuhara, M., & Chao, A. (2020). Global distribution of coral diversity: Biodiversity knowledge gradients related to spatial resolution. *Ecological Research*, *35*, 315–326.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*, 1–26.

Langley, J. M., Shiel, R. J., Nielsen, D. L., & Green, J. D. (2001). Hatching from the sediment egg-bank, or aerial dispersing?—the use of mesocosms in assessing rotifer biodiversity. *Hydrobiologia*, *446,* 203–211.

Larsson, A. I., & Jonsson, P. R. (2006). Barnacle larvae actively select flow environments supporting post-settlement growth and survival. *Ecology*, *87*, 1960–1966.

Laumer, C.E., Fernández, R., Lemer, S., Combosch, D., Kocot, K.M., Riesgo, A., Andrade, S.C., Sterrer, W., Sørensen, M.V. & Giribet, G. (2019). Revisiting metazoan phylogeny with genomic sampling of all phyla. *Proceedings of the Royal Society B: Biological Sciences 286*, 20190831.

Leasi, F., Tang, C. Q., De Smet, W. H., & Fontaneto, D. (2013). Cryptic diversity with wide salinity tolerance in the putative euryhaline *Testudinella clypeata* (Rotifera, Monogononta. *Zoological Journal of the Linnean Society*, *168*, 17–28.

Leibold, M. A., Economo, E. P., & Peres-Neto, P. (2010). Metacommunity phylogenetics: separating the roles of environmental filters and historical biogeography. *Ecology Letters*, *13*, 1290–1299.

Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., & Holt, R. D. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*, *7*, 601–613.

Lighton, J. R. B. (1989). Individual and whole-colony respiration in an African formicine ant. *Functional Ecology*, *3*, 523.

Lopes, P. M., Bini, L.M., Declerck, S.A., Farjalla, V.F., Vieira, L.C., Bonecker, C.C., Lansac-Toha, F.A., Esteves, F.A. & Bozelli, R.L. (2014). Correlates of zooplankton beta diversity in tropical lake systems. *PLoS ONE*, *9,* 109581.

Losos, J. B. (2011). Convergence, adaptation, and constraint. *Evolution*, *65*, 1827–1840.

Luo, Y. H., & Segers, H. (2020). Eight new Lepadellidae (Rotifera, Monogononta) from the Congo bring to level endemism in Africa's rotifers. *Zootaxa*, *47,* 371–387.

Lürling, M. (2021). Grazing resistance in phytoplankton. *Hydrobiologia*, *848*, 237–249.

Lynch, M. (2007). *The Origins of Genome Architecture*. Sunderland: Sinauer Associates Inc.

Lynch, M., & Conery, J. S. (2003). The origins of genome complexity. *Science*, *302,* 1401–1404.

Lynch, M., Trickovic, B., & Kempes, C. P. (2022). Evolutionary scaling of maximum growth rate with organism size. *Scientific Reports*, *12*, 22586.

Mackie, G. O. (1986). From aggregates to integrates: physiological aspects of modularity in colonial animals. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *313*, 175–196.

Mackie, G. O., & Singla, C. L. (1983). Studies on hexactinellid sponges. I. Histology of *Rhabdocalyptus dawsoni* (Lambe, 1873. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *301*, 365–400.

MacKay, W. P., Loring, S. J., Frost, T. M., & Whitford, W. G. (1990). Population dynamics of a playa community in the Chihuahuan Desert. *The Southwestern Naturalist*, *35*, 393.

Magnuson, J., Carpenter, S., & Stanley, E. (2020). North temperate lakes LTER: zooplankton–trout lake area 1982 - current ver 34. *Environmental Data Initiative*.

Mark Welch, D. B., & Meselson, M. (2001). Rates of nucleotide substitution in sexual and anciently asexual rotifers. *Proceedings of the National Academy of Sciences of the United States of America 98,* 6720–6724.

Mark Welch, D. B., & Meselson, M. (2003). Oocyte nuclear DNA content and GC proportion in rotifers of the anciently asexual Class Bdelloidea. *Biological Journal of the Linnean Society*, *79,* 85–91.

Mark Welch, D. B., Welch, J. L. M., & Meselson, M. (2008). Evidence for degenerate tetraploidy in bdelloid rotifers. *Proceedings of the National Academy of Sciences of the United States of America*, *105,* 5145–5149.

Mark Welch, M., B., D., Ricci, C., & Meselson, M. (2009). Bdelloid rotifers: progress in understanding the success of an evolutionary scandal. *Lost Sex: The Evolutionary Biology of Parthenogenesis*, 259–279.

Mark Welch, D. M., & Meselson, M. (1998). Measurements of the genome size of the monogonont rotifer *Brachionus plicatilis* and the bdelloid rotifers *Philodina roseola* and *Habrotrocha constricta*. *Hydrobiologia*, *387,* 395–402.

Mark Welch, J. L., & Meselson, M. (1998). Karyotypes of bdelloid rotifers from three families. *Hydrobiologia*, *387,* 403–407.

Martin, P. J. (1991). Respiration of the ant *Leptothorax unifasciatus* (Hymenoptera, Formicidae) at individual and society levels. *Journal of Insect Physiology*, *37*, 311–318.

Matthews, D., Effler, S., Prestigiacomo, A., & O'Donnell, S. (2015). Trophic state responses of Onondaga Lake, New York to reductions in phopshorus loading from advanced wastewater treatment. *Inland Waters*, *5*, 125–138.

Matthews, T., Triantis, K., Whittaker, R., & Guilhaumon, F. (2019). sars: an R package for fitting, evaluating and comparing species–area relationship models. *Ecography*, *42,* 1446–1455.

May, L. (1989). Epizoic and parasitic rotifers. *Hydrobiologia*, *186,* 59–67.

May, L., & Wallace, R. L. (2019). An examination of long-term ecological studies of rotifers: comparability of methods and results, insights into drivers of change and future research challenges. *Hydrobiologia*, *844*, 129–147

Mazuelos, N., Toja, J., & Guisande, C. (1993). Rotifers in ephemeral ponds of Doñana National Park. *Hydrobiologia*, *256*, 429–434.

McDaniel, J. L. (2022). *Chihuahuan Desert rock pool community assemblages: Patterns of Taxonomic Diversity*. Master Thesis, The University of Texas at El Paso.

McLean, K. I., Mushet, D. M., Sweetman, J. N., Anteau, M. J., & Wiltermuth, M. T. (2020). Invertebrate communities of prairie-pothole wetlands in the age of the aquatic Homogenocene. *Hydrobiologia*, *847,* 3773–3793.

Medeiros, L. P., Boege, K., Del-Val, E., Zaldívar-Riverón, A., & Saavedra, S. (2021). Observed ecological communities are formed by species combinations that are among the most likely to persist under changing environments. *The American Naturalist*, *197,* 17–29.

Meksuwan, P., Pholpunthin, P., & Segers, H. (2013). The Collothecidae (Rotifera, Collothecacea) of Thailand, with the description of a new species and an illustrated key to the Southeast Asian fauna. *ZooKeys*, *315,* 1.

Meksuwan, P., Pholpunthin, P., Walsh, E. J., Segers, H., & Wallace, R. L. (2014). Nestedness in sessile and periphytic rotifer communities: A meta-analysis. *International Review of Hydrobiology*, *99*, 48–57.

Meksuwan, P., Pholpunthin, P., & Segers, H. H. (2015). Molecular phylogeny confirms Conochilidae as ingroup of Flosculariidae (Rotifera, Gnesiotrocha). *Zoologica Scripta*, *44*, 562–573.

Meksuwan, P., Jaturapruek, R., & Maiphae, S. (2018). Two new species of genus *Limnias* from Thailand, with keys to congeners (Rotifera, Gnesiotrocha). *ZooKeys*, *787*, 1–15.

Meyer-Milne, E., Brendonck, L., & Pinceel, T. (2021). Egg morphology may underpin the successful distribution of large branchiopods in temporary waters. *Aquatic Ecology*, *55,* 237–251.

Michaloudi, E., Mills, S., Papakostas, S., Stelzer, C.P., Triantafyllidis, A., Kappas, I., Vasileiadou, K., Proios, K. & Abatzopoulos, T. (2017). Morphological and taxonomic demarcation of *Brachionus asplanchnoidis* Charin within the *Brachionus plicatilis* cryptic species complex (Rotifera, Monogononta). *Hydrobiologia*, *796*, 19–37.

Mills, S. (2006). *Investigations of the Brachionus plicatilis species complex, with particular reference to southwest Western Australia*. Doctoral Dissertation, The University of Western Australia.

Mills, S., Lunt, D. H., & Gómez, A. (2007). Global isolation by distance despite strong regional phylogeography in a small metazoan. *BMC Evolutionary Biology*, *7*, 225.

Mills, S., Alcántara-Rodríguez, J.A., Ciros-Pérez, J., Gómez, A., Hagiwara, A., Galindo, K.H., Jersabek, C.D., Malekzadeh-Viayeh, R., Leasi, F., Lee, J.S. & Mark Welch, D.B. (2017). Fifteen species in one: deciphering the *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy. *Hydrobiologia*, *796,* 39–58.

Minckley, W. L. (1969). *Environments of the Bolsón of Cuatro Ciénegas, Coahuila, Mexico, with special reference to the aquatic biota*. Texas Western Press: El Paso.

Minckley, W. L. (1974). Endemic fishes of the Cuatro Ciénegas Basin, Northern Coahuila, Mexico. *Symposium on the Biological Resources of the Chihuahuan*

Desert Region, United States and Mexico. In U.S. National Park Service Transactions and Proceedings Series 3, Government Printing Office (pp. 383–404).

Moline, A. B., Shuster, S. M., Hendrickson, D. A., & Marks, J. C. (2004). Genetic variation in a desert aquatic snail (*Nymphophilus minckleyi*) from Cuatro Ciénegas, Coahuila, Mexico. *Hydrobiologia*, *522*, 179–192.

Molinero, J. C., Anneville, O., Souissi, S., Balvay, G., & Gerdeaux, D. (2006). Anthropogenic and climate forcing on the long-term changes of planktonic rotifers in Lake Geneva. *Europe. Journal of Plankton Research*, *28*, 287–296.

Montero-Pau, J., Ramos-Rodríguez, E., Serra, M., & Gómez, A. (2011). Long-term coexistence of rotifer cryptic species. *PLoS ONE*, *6,* 21530.

Morais, C. S., Diniz, L. P., Sousa, F. D. R., Gonçalves-Souza, T., Elmoor-Loureiro, L. M. A., & Júnior, M. M. (2019). Bird feet morphology drives the dispersal of rotifers and microcrustaceans in a Neotropical temporary pond. *Aquatic Sciences*, *81,* 1–9.

Moreno, E., Pérez-Martínez, C., & Conde-Porcuna, J. M. (2019). Dispersal of rotifers and cladocerans by waterbirds: seasonal changes and hatching success. *Hydrobiologia*, *834,* 145–162.

Morris, S. C. (2003). The navigation of biological hyperspace. *International Journal of Astrobiology*, *2*, 149–152.

Mouquet, N., & Loreau, M. (2003). Community patterns in source-sink metacommunities. *The American Naturalist*, *162*, 544–557.

Muirhead, J. R., Ejsmont-Karabin, J., & Macisaac, H. J. (2006). Quantifying rotifer species richness in temperate lakes. *Freshwater Biology*, *51*, 1696–1709.

Murphy, A. L., Pavlova, A., Thompson, R., Davis, J., & Sunnucks, P. (2015). Swimming through sand: connectivity of aquatic fauna in deserts. *Ecology and Evolution*, *5*, 5252–5264.

Nakaya, F., Saito, Y., & Motokawa, T. (2003). Switching of metabolic–rate scaling between allometry and isometry in colonial ascidians. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*, 1105–1113.

Nandini, S., Peña-Aguado, F., Rebolledo, U. A., Sarma, S. S. S., & Murugan, G. (2019). Molecular identity and demographic responses to salinity of a freshwater strain of *Brachionus plicatilis* from the shallow Lake Pátzcuaro, Mexico. *Fundamental and Applied Limnology*, *192,* 319–329.

Nielsen, D. L., Hillman, T. J., Smith, F. J., & Shiel, R. J. (2002). The influence of seasonality and duration of flooding on zooplankton in experimental billabongs. *River Research and Applications*, *18* ,227–237.

Ning, N. S. P., Gawne, B., Cook, R. A., & Nielsen, D. L. (2013). Zooplankton dynamics in response to the transition from drought to flooding in four Murray–Darling Basin rivers affected by differing levels of flow regulation. *Hydrobiologia*, *702*, 45–62.

Nishibuchi, G., & Déjardin, J. (2017). The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals. *Chromosome Research*, *25,* 77–87.

Nogrady, T., Pourriot, R., & Segers, H. R. (2002). The Notommatidae and: The Scaridiidae; SPB Academic Publishing: The Hague. *The Netherlands*, *3*, 248.

Nowell, R. W., Almeida, P., Wilson, C. G., Smith, T. P., Fontaneto, D., Crisp, A., Micklem, G., Tunnacliffe, A., Boschetti, C & Barraclough, T. G. (2018). Comparative genomics of bdelloid rotifers: insights from desiccating and nondesiccating species. *PLoS Biology*, *16,* 2004830.

O'Neill, B. J. (2016). Community disassembly in ephemeral ecosystems. *Ecology*, *97,* 3284–3292.

Obertegger, U., Smith, H. A., Flaim, G., & Wallace, R. L. (2011). Using the guild ratio to characterize pelagic rotifer communities. *Hydrobiologia*, *662*, 157–162.

Obertegger, U., Flaim, G., & Fontaneto, D. (2014). Cryptic diversity within the rotifer *Polyarthra dolichoptera* along an altitudinal gradient. *Freshwater Biology*, *59*, 2413–2427.

Obertegger, U., & Flaim, G. (2018). Taxonomic and functional diversity of rotifers, what do they tell us about community assembly? *Hydrobiologia*, *823,* 79–91.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Solymos, P. (2015). Vegan: An introduction to ordination. *Community Ecology Package*. Retrieved from https://rdrr.io/cran/vegan/.

Olsen, D. M., & Dinerstein, E. (1998). The Global 200: A representation approach to conserving the Earth's most biologically valuable ecoregions. *Conservation Biology*, *12*, 502–515.

Olson, D. M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C. & Loucks, C.J. (1998). Terrestrial ecoregions of the world: A new map of life on Earth. *Bioscience*, *51*, 933–938.

Ooms-Wilms, A. L., Postema, G., & Gulati, R. D. (1991, June). Clearance rates of bacteria by the rotifer *Filinia longiseta* (Ehrb.) measured using three tracers. *Rotifer Symposium VI: Proceedings of the Sixth International Rotifer Symposium, Held in Banyoles*, 255–260. Spain: Springer Netherlands.

Organ, C. L., & Shedlock, A. M. (2009). Palaeogenomics of pterosaurs and the evolution of small genome size in flying vertebrates. *Biology Letters*, *5,* 47–50.

Örstan, A. (2003). A new species of bdelloid rotifer from Sonora. *Mexico. Southwest. Nat*, *40*, 255–258.

Pagani, M., Ricci, C., & Redi, C. A. (1993). Oogenesis in *Macrotrachela quadricornifera* (Rotifera, Bdelloidea)—I. Germarium eutely, karyotype and DNA content. *Hydrobiologia*, *255,* 225–230.

Paine, R. T. (1966). Food web complexity and species diversity. *The American Naturalist*, *100*(910), 65–75.

Palazzo, F., Bomfim, F. F., Dias, J. D., Simões, N. R., Lansac-Tôha, F. A., & Bonecker, C. C. (2021). Temporal dynamics of rotifers' feeding guilds shaped by chlorophyll-a, nitrate, and environmental heterogeneity in subtropical floodplain lakes. *International Review of Hydrobiology*, *106,* 95–105.

Parry, V., Kiemel, K., Pawlak, J., Eccard, J., Tiedemann, R., & Weithoff, G. (2023). Drivers of zooplankton dispersal in a pond metacommunity. *Hydrobiologia*, 1–19.

Paskevich, V. U. S. (2003). *STATE_BOUNDS.SHP: Internal US state boundaries*. US Geological Survey Open-File Report, 2005, 1018.

Peel, M. C., Finlayson, B. L., & McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, *11*, 1633–1644.

Pejler, B., & Bērziņš, B. (1993). On relation to substrate in sessile rotifers. *Hydrobiologia*, *259*, 121–124.

Pepper, R. E., Roper, M., Ryu, S., Matsumoto, N., Nagai, M., & Stone, H. A. (2013). A new angle on microscopic suspension feeders near boundaries. *Biophysical Journal*, *105*, 1796–1804.

Pettitt, M. E., Orme, B. A. A., Blake, J. R., & Leadbeater, B. S. C. (2002). The hydrodynamics of filter feeding in choanoflagellates. *European Journal of Protistology*, *38*, 313–332.

Pinceel, T., Brendonck, L., & Vanschoenwinkel, B. (2016). Propagule size and shape may promote local wind dispersal in freshwater zooplankton—a wind tunnel experiment. *Limnology and Oceanography, 61*, 122–131.

Pinceel, T., Vanschoenwinkel, B., Hawinkel, W., Tuytens, K., & Brendonck, L. (2017). Aridity promotes bet hedging via delayed hatching: a case study with two temporary pond crustaceans along a latitudinal gradient. *Oecologia*, *184,* 161–170.

Preza, E., Walsh, E. J., & Hochberg, R. (2020). Remodeling of the nervous system of the indirectly developing rotifer *Cupelopagis vorax* (Gnesiotrocha, Collothecaceae. *Invertebrate Biology*, *139*(4), 12301.

Prosser, C. L., Pruitt, J. N., & Avilés, L. (2018). Oxygen: respiration and metabolism. *Comparative Animal Physiology*, *143*, 165--211.

R: A language and environment for statistical computing. (2020). Vienna, Austria: R Foundation for Statistical Computing.

Rasband, W. S. I. U. S. (1997-2023). *National Institutes of Health*. Bethesda, Maryland, USA, https: //imagej.nih.gov/ij.

Ricci, C., & Melone, G. (2000). Key to the identification of the genera of bdelloid rotifers. *Hydrobiologia*, *418*, 73–80.

Ricci, C., Vaghi, L., & Manzini, M. L. (1987). Desiccation of rotifers (*Macrotrachela quadricornifera*): survival and reproduction. *Ecology*, *68*, 1488–1494.

Ricklefs, R. E. (2004). A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, *7*, 1–15.

Rico-Martínez, R., & Silva-Briano, M. (1993). Contribution to the knowledge of the Rotifera of Mexico. *Hydrobiologia*, *256*, 467–474.

Ridder, M. (1974). Rotifers from Algeria. *Journal of African Zoology*, *105*, 473–483.

Riedel, R., & Costa-Pierce, B. A. (2005). Feeding ecology of Salton Sea Tilapia (*Oreochromis* spp). In *Buletin of the Southern California Academy of Sciences* (Vol. 104, pp. 26–36).

Ríos-Arana, J. V., Carmen Agüero-Reyes, L., Wallace, R. L., & Walsh, E. J. (2019). Limnological characteristics and rotifer community composition of Northern Mexico Chihuahuan Desert Springs. *Journal of Arid Environments*, *160*, 32–41.

Ripley, B., & Simovich, M. A. (2009a). Species richness on islands in time: variation in ephemeral pond crustacean communities in relation to habitat duration. *Hydrobiologia*, *617*, 181–196.

Riisgård, H. U., Jensen, A. S., & Jürgensen, C. (1998). Hydrography, near-bottom currents, and grazing impact of the filter-feeding ascidian *Ciona intestinalis* in a Danish fjord. *Ophelia*, *49*(1), 1–16.

Riss, S., Arthofer, W., Steiner, F. M., Schlick-Steiner, B. C., Pichler, M., Stadler, P., & Stelzer, C. P. (2017). Do genome size differences within *Brachionus asplanchnoidis* (Rotifera, Monogononta) cause reproductive barriers among geographic populations? *Hydrobiologia*, *796,* 59–75.

Rivas, J. A., Mohl, J. E., Van Pelt, R. S., Leung, M. Y., Wallace, R. L., Gill, T. E., & Walsh, E. J. (2018). Evidence for regional aeolian transport of freshwater biota in an arid region. *Limnology and Oceanography Letters*, *3,* 320–330.

Rivas, J. A., Schröder, T., Gill, T. E., Wallace, R. L., & Walsh, E. J. (2019). Anemochory of diapausing stages of microinvertebrates in North American drylands. *Freshwater Biology*, *64*, 1303–1314.

Rizo, E. Z. C., Gu, Y., Papa, R. D. S., Dumont, H. J., & Han, B. P. (2017). Identifying functional groups and ecological roles of tropical and subtropical freshwater Cladocera in Asia. *Hydrobiologia*, *799,* 83–99.

Rodríguez-Rodríguez, M. (2007). Hydrogeology of ponds, pools, and playa-lakes of southern Spain. *Wetlands*, *27,* 819–830.

Rokas, A. (2008). The origins of multicellularity and the early history of the genetic toolkit for animal development. *Annual Review of Genetics*, *42*, 235–251.

Roper, M., Dayel, M. J., Pepper, R. E., & Koehl, M. A. R. (2013). Cooperatively generated stresslet flows supply fresh fluid to multicellular choanoflagellate colonies. *Physical Review Letters*, *110*, 228104.

Rothhaupt, K. O. (1990). Differences in particle size-dependent feeding efficiencies of closely related rotifer species. *Limnology and Oceanography*, *35*, 16–23.

Rousselet, C. F. (1909). On the geographic distribution of the Rotifera. *J. Quekett Microscopy. Cl*, *2*, 465–470.

Ruttner-Kolisko, A. (1974). Rotatoria. *Die Binnengewässer*, *26*, 99–234.

Sada, D. W., Fleishman, E., & Murphy, D. D. (2005). Associations among spring-dependent aquatic assemblages and environmental and land use gradients in a Mojave Desert mountain range. *Diversity and Distributions*, *11*, 91–99.

Sanoamuang, L. (1998). Rotifera of some freshwater habitats in the floodplain of the River Nan, northern Thailand. *Hydrobiologia*, *387*, 27–33.

Sarma, S. S. S. (1999). Checklist of rotifers (Rotifera) from Mexico. *Environmental Ecology*, *17*, 978–983.

Sarma, S. S. S., & Elias-Gutierrez, M. (2000). Rotifers from Mexico: new records in high altitude ponds. *The Southwestern Naturalist*, *45*, 366.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675.

Schröder, T. E. J., & Walsh, E. J. (2007). Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of new species. *Hydrobiologia*, *593,* 129–140.

Schröder, T., Howard, S., Arroyo, M. L., & Walsh, E. J. (2007b). Sexual reproduction and diapause of *Hexarthra* sp. (Rotifera) in short-lived ponds in the Chihuahuan Desert. *Freshwater Biology*, *52*(6), 1033–1042.

Scuderi, L. A., Laudadio, C. K., & Fawcett, P. J. (2010). Monitoring playa lake inundation in the western United States: modern analogues to late-Holocene lake level change. *Quaternary Research*, *73,* 40–58.

Sebens, K. P. (1987). The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics*, *18*, 371–407.

Segers, H., & Dumont, H. J. (1993). Rotifera from Arabia, with descriptions of two new species. *Fauna of Saudi Arabia*, *13,* 3–26.

Segers, H. R. (1995a). The Lecanidae (Monogononta); SPB Academic Publishing bv: Amsterdam. *The Netherlands*, *2*, 226.

Segers, H. (1995b). A reappraisal of the Scaridiidae (Rotifera, Monogononta). *Zoologica Scripta*, *24*, 91–100.

Segers, H. (2003). A biogeographical analysis of rotifers of the genus *Trichocerca* Lamarck, 1801 (Trichocercidae, Monogononta, Rotifera), with notes on taxonomy. *Hydrobiologia*, *500*, 103–114.

Segers, H. (2007a). Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, *1564,* 1–104.

Segers, H. (2007b). Global diversity of rotifers (Rotifera) in freshwater. In *Freshwater Animal Diversity Assessment* (Vol. 198, pp. 49–59).

Segers, H., & Shiel, R. J. (2003). Microfaunal diversity in a biodiversity hotspot: new rotifers from southwestern Australia. *Zoological. Studies*, *42*, 516–521.

Segers, H., & De Smet, W. H. (2008). Diversity and endemism in Rotifera: a review, and *Keratella* Bory de St Vincent. *Biodiversity and Conservation*, *17*, 303–316.

Segers, H., & Shiel, R. J. (2008). Diversity of cryptic metazoa in Australian freshwaters: a new genus and two new species of sessile rotifer (Rotifera, Monogononta, Gnesiotrocha, Flosculariidae). *Zootaxa 1750*, 19

Segers, H., & Walsh, E. J. (2017). The genus *Rhinoglena* (Rotifera, Monogononta, Ephiphanidae) in North America, with the description of two new species. *Zootaxa*, *4290*, 113–122.

Sei, M., Lang, B. K., & Berg, D. J. (2009). Genetic and community similarities are correlated in endemic-rich springs of the northern Chihuahuan Desert. *Global Ecology and Biogeography*, *18*, 192–201.

Seidel, R. A., Lang, B. K., & Berg, D. J. (2009). Phylogeographic analysis reveals multiple cryptic species of amphipods (Crustacea: Amphipoda) in Chihuahuan Desert springs. *Biological Conservation*, *142*, 2303–2313.

Serra, M., Snell, T. W., & Gilbert, J. J. (2005). Delayed mixis in rotifers: an adaptive response to the effects of density-dependent sex on population growth. *Journal of Plankton Research*, *27*, 37–45.

Serrano, L., & Fahd, K. (2005). Zooplankton communities across a hydroperiod gradient of temporary ponds in the Doñana National Park (SW Spain). *Wetlands*, *25*, 101–111.

Sferra, C. O., Hart, J. L., & Howeth, J. G. (2017). Habitat age influences metacommunity assembly and species richness in successional pond ecosystems. *Ecosphere*, *8,* 01871.

Sharma, B. K., & Dudani. (1992). Rotifers from some tropical ponds in Bihar: species composition, similarities and trophic indicators. *Journal of the Indian Institute of Science*, *72,* 121.

Shiel, R. J. (1983). Rotifer communities of billabongs in northern and south-eastern Australia. *Hydrobiologia*, *104*, 41–47.

Shiel, R. H. (1986). Zooplankton of the Murray-Darling system. In B. R. Davies, K. F. Walker, & D. W. J. P (Eds.), *The Ecology of River Systems*. Dordrecht, The Netherlands.

Shiel, R. J. (2006). Zooplankton diversity and assembly in arid zone rivers of the Lake Eyre basin, Australia. *Marine and Freshwater Research*, *57*, 49–60.

Shiel, R. J., & Green, J. D. (1996). Rotifera recorded from New Zealand, 1859–1995, with comments on zoogeography. *New Zealand Journal of Zoology*, *23*, 191–207.

Shiel, R. J., & Koste, W. (1979). Rotifera recorded from Australia. *Transactions of the Royal Society of South Australia*, *103*, 57–68.

Shiel, R. J., & Koste, W. (1983). Rotifer communities of billabongs in northern and south-eastern Australia. *Hydrobiologia*, *104,* 41–47.

Shiel, R. J., & Koste, W. (1993). Rotifera from Australian inland waters. IX. Gastropodiae, Synchaetidae, Asplanchnidae (Rotifera: Monogononta. *Transactions of the Royal Society of South Australia*, *117*, 111–139.

Shiel, R. J., Koste, W., & Tan, L. W. (1989). Tasmania revisited: Rotifer communities and habitat heterogeneity. *Hydrobiologia*, *186*, 239–245.

Shimeta, J., & Koehl, M. A. R. (1997). Mechanisms of particle selection by tentaculate suspension feeders during encounter, retention, and handling. *Journal of Experimental Marine Biology and Ecology*, *209*, 47–73.

Shimeta, J. E. F. F., & Jumars, P. A. (1991). Physical mechanisms and rates of particle capture by suspension feeders. *Oceanogr. Mar. Biol. Annu. Rev*, *29*, 191–257.

Short, M. B., Solari, C. A., Ganguly, S., Powers, T. R., Kessler, J. O., & Goldstein, R. E. (2006). Flows driven by flagella of multicellular organisms enhance long-range molecular transport. *Proceedings of the National Academy of Sciences*, *103*, 8315–8319.

Sielaff, M., Schmidt, H., Struck, T. H., Rosenkranz, D., Welch, D. B. M., Hankeln, T., & Herlyn, H. (2016). Phylogeny of Syndermata (syn. Rotifera): Mitochondrial gene order verifies epizoic Seisonidea as sister to endoparasitic Acanthocephala within monophyletic Hemirotifera. *Molecular Phylogenetics and Evolution*, *96*, 79–92.

Signorovitch, A., Hur, J., Gladyshev, E., & Meselson, M. (2016). Evidence for meiotic sex in bdelloid rotifers. *Current Biology*, *26*, 754–755.

Silberstorf, R. R. (2017). *Structural Analysis of Ernst Tinaja Canyon Focusing on Strike-slip Faulting and Folding*. Texas: Big Bend National Park.

Sim, L. L., Davis, J.A., Strehlow, K., McGuire, M., Trayler, K.M., Wild, S., Papas, P.J. O'Connor, J (2013). The influence of changing hydroregime on the invertebrate communities of temporary seasonal wetlands. *Freshwater Science*, *32,* 327–342.

Simpson, C., Jackson, J. B., & Herrera-Cubilla, A. (2017). Evolutionary determinants of morphological polymorphism in colonial animals. *The American Naturalist*, *190*(1), 17–28.

Smith, E. M., & Gregory, T. R. (2009). Patterns of genome size diversity in the ray-finned fishes. *Hydrobiologia*, *625,* 1–25.

Smith, H. A., & Snell, T. W. (2012). Rapid evolution of sex frequency and dormancy as hydroperiod adaptations. *Journal of Evolutionary Biology*, *25*, 2501–2510.

Smith, H. A., Ejsmont-Karabin, J., Hess, T. M., & Wallace, R. L. (1922). Paradox of planktonic rotifers: similar structure but unique trajectories in communities of the Great Masurian Lakes (Poland). *Verhandlungen des Internationalen Verein Limnologie*, *30*, 951–956.

Smolak, R., & Walsh, E. J. (2022). Rotifer species richness in Kenyan waterbodies: contributions of environmental characteristics. *Diversity*, *14*, 583.

Smolak, R., Walsh, E. J., Brown, P. D., & Wallace, R. L. (2023). A synthesis of the biogeographical distribution of Rotifera in Africa. *Journal of Plankton Research*, *45*, 65–81.

Solari, C. A., Kessler, J. O., & Goldstein, R. E. (2013). A general allometric and life-history model for cellular differentiation in the transition to multicellularity. *The American Naturalist*, *181*, 369–380.

Soons, M. B., Brochet, A. L., Kleyheeg, E., & Green, A. J. (2016). Seed dispersal by dabbling ducks: an overlooked dispersal pathway for a broad spectrum of plant species. *Journal of Ecology, 104*, 443–455.

Spasojevic, M. J., Catano, C. P., Lamanna, J. A., & Myers, J. A. (2018). Integrating species traits into species pools. *Ecology*, *99*, 1265–1276.

Špoljar, M., Dražina, T., Lajtner, J., Sertić, M. D., Radanović, I., Wallace, R. L., … Tomljanović, T. (2018). Zooplankton assemblage in four temperate shallow waterbodies in association with habitat heterogeneity and alternative states. *Limnologica*, *71*, 51–61.

Stanislawczyk, K., Walters, A. D., Haan, T. J., Sei, M., Lang, B. K., & Berg, D. J. (2018). Variation among macroinvertebrate communities suggests the importance of conserving desert springs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*, 944–953.

Starkweather, P. L. (1987). Rotifera. Chap. 5. In Pandian & Vernberg (Eds.), *Animal Energetics* (pp. 159–183). Academic press.

Stelzer, C.-P. (2005). Evolution of rotifer life histories. *Hydrobiologia*, *546*, 335–346.

Stelzer, C. P. (2011). A first assessment of genome size diversity in monogonont rotifers. *Hydrobiologia*, *662*, 77–82.

Stelzer, C. P., Riss, S., & Stadler, P. (2011). Genome size evolution at the speciation level: the cryptic species complex *Brachionus plicatilis* (Rotifera). *BMC Evolutionary Biology*, *11,* 1–11.

Stemberger, R. S. (1979). *A Guide to Rotifers of the Laurentian Great Lakes*. Cincinnati, OH: US Environmental Protection Agency, National Technical Information Service.

Stemberger, R. S. (1981). A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences*, *38,* 721–724.

Stemberger, R. S., & Gilbert, J. J. (1984). Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. *Freshwater Biology*, *14*(6), 639–647.

Stemberger, R. S., Gilbert, J. J., S., R., & Gilbert, J. J. (1987). Rotifer threshold food concentrations and the size-efficiency hypothesis. *Ecology*, *68*, 181–187.

Stendera, S. E. S., & Johnson, R. K. (2005). Additive partitioning of aquatic invertebrate species diversity across multiple spatial scales. *Freshwater Biology*, *50*, 1360–1375.

Stevens, L. E., & Meretsky, V. J. (2008). *Aridland Springs in North America: Ecology and Conservation* (M. L.e. & Vj, Eds.). Tucson, AZ, USA: The University of Arizona Press.

Strathmann, R. R., & Grünbaum, D. (2006). Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology*, *46*, 312–322.

Straus, S., González, A. L., Matthews, P., & Avilés, L. (2022). Economies of scale shape energetics of solitary and group-living spiders and their webs. *Journal of Animal Ecology*, *91*, 255–265.

Stroud, J. T., Giery, S. T., Outerbridge, M., & Feeley, K. J. (2019). Ecological character displacement alters the outcome of priority effects during community assembly. *Ecology*, *100,* 02727.

Suárez-Morales, E., Gutiérrez-Aguirre, M. A., & Walsh, E. J. (2010). Freshwater Copepoda (Crustacea) from the Chihuahuan Desert with comments on biogeography. *The Southwestern Naturalist*, *55*, 525–531.

Sutherland, K. R., & Weihs, D. (2017). Hydrodynamic advantages of swimming by salp chains. *Journal of The Royal Society Interface*, *14*, 20170298.

Suzuki, Y., & Economo, E. P. (2021). From species sorting to mass effects: spatial network structure mediates the shift between metacommunity archetypes. *Ecography*, *44,* 715–726.

Symons, C. C., & Arnott, S. E. (2013). Regional zooplankton dispersal provides spatial insurance for ecosystem function. *Global Change Biology*, *19,* 1610–1619.

Symons, Celia C., & Arnott, S. E. (2014). Timing is everything: priority effects alter community invasibility after disturbance. *Ecology and Evolution*, *4*, 397–407.

Tavares, S., Ramos, A. P., Pires, A. S., Azinheira, H. G., Caldeirinha, P., Link, T., & Abranches, R. (2014). Genome size analyses of Pucciniales reveal the largest fungal genomes. *Frontiers in Plant Science*, *5,* 422.

Taylor, D. W. (1967). A remarkable snail fauna from Coahuila, México. *The Veliger*, *9*, 152–228.

Thielsch, A., Brede, N., Adam Petrusek, L. U. C. de M., & Schwenk, K. (2009). Contribution of cyclic parthenogenesis and colonization history to population structure in *Daphnia*. *Molecular Ecology*, *18*, 1616–1628.

Thorpe, J. P., Shelton, G. A. B., & Laverack, M. S. (1975). Electrophysiology and co-ordinated behavioural responses in the colonial bryozoan *Membranipora membranacea* (L. *Journal of Experimental Biology*, *62*, 389–404.

Tiffany, M. A., Swan, B. K., Watts, J. M., & Hurlbert, S. H. (2002). Metazooplankton dynamics in the Salton Sea, California, 1997–1999. *The Salton Sea*, *473*, 103–120.

Timmis, J. N., Ayliffe, M. A., Huang, C. Y., & Martin, W. (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature reviews genetics*, *5*, 123–135.

Tobler, M., & Carson, E. W. (2010). Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. *Journal of Evolutionary Biology*, *23*, 1475–1489.

Tregenza, T. (1995). Building on the ideal free distribution. *Advances in Ecological Research, 26*, 253–307.

Tschinkel, W. R. (1999). Sociometry and sociogenesis of colony-level attributes of the Florida harvester ant (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, *92*, 80–89.

Ulrich, W., Almeida-Neto, M., & Gotelli, N. J. (2009). A consumer's guide to nestedness analysis. *Oikos*, *118*, 3–17.

Urban, M. C., Strauss, S.Y., Pelletier, F., Palkovacs, E.P., Leibold, M.A., Hendry, A.P., De Meester, L., Carlson, S.M., Angert, A.L. and Giery, S.T., (2020). Evolutionary origins for ecological patterns in space. *Proceedings of the National Academy of Sciences*, *117,* 17482–17490.

Vadstein, O., Olsen, L. M., & Andersen, T. (2012). Prey-predator dynamics in rotifers: density-dependent consequences of spatial heterogeneity due to surface attachment. *Ecology*, *93,* 1795–1801.

Valente-Neto, F., Durães, L., Siqueira, T., & Roque, F. O. (2018). Metacommunity detectives: confronting models based on niche and stochastic assembly scenarios with empirical data from a tropical stream network. *Freshwater Biology*, *63,* 86–99.

Vanschoenwinkel, B., Seaman, M., & Brendonck, L. (2010). Hatching phenology, life history and egg bank size of fairy shrimp *Branchipodopsis* spp. (Branchiopoda, Crustacea) in relation to the ephemerality of their rock pool habitat. *Aquatic Ecology*, *44,* 771–780.

Vanschoenwinkel, Bram, Waterkeyn, A., Tamuka Nhiwatiwa, T. O. M. P., Spooren, E., Geerts, A., Clegg, B., & Brendonck, L. U. C. (2011). Passive external transport of freshwater invertebrates by elephant and other mud-wallowing mammals in an African savannah habitat. *Freshwater Biology*, *56*, 1606–1619.

Vargas, A. L., Santangelo, J. M., & Bozelli, R. L. (2019). Recovery from drought: viability and hatching patterns of hydrated and desiccated zooplankton resting eggs. *International Review of Hydrobiology*, *104,* 26–33.

Vasisht, H. S., & Dawar, B. L. (1969). Anatomy and histology of the rotifer *Cupelopagis vorax* Leidy. *Research Bulletin (N. S.) Panjab University*, *20,* 205.

Vasisht, H. S., & Dawar, B. L. (1970). The anatomy and histology of the rotifer *Lacinularia flosculosa* Müller *Research Bulletin (N. S.) Panjab University*, *21*, 361–377.

Vasseur, D. A., Fox, J. W., Gonzalez, A., Adrian, R., Beisner, B. E., Helmus, M. R., & Johnson, C. (2014). Synchronous dynamics of zooplankton competitors prevail in temperate lake ecosystems. *Proceedings of the Royal Society B: Biological Sciences, 281*, 20140633.

Velasco-Castrillón, A., Page, T. J., Gibson, J. A. E., & Stevens, M. I. (2014). Surprisingly high levels of biodiversity and endemism amongst Antarctic rotifers uncovered with mitochondrial DNA. *Biodiversity*, *15*, 130–142.

Vilmi, A., Gibert, C., Escarguel, G., Happonen, K., Heino, J., Jamoneau, A., & Passy, S. I. (2021). Dispersal–niche continuum index: a new quantitative metric for assessing the relative importance of dispersal versus niche processes in community assembly. *Ecography*, *44,* 370–379.

Vindeløv, L. L., Christensen, I. J., & Nissen, N. I. (1983). A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry*, *3,* 323–327.

Walker, B. W. (1961). The Ecology of the Salton Sea, California, in Relation to the Sportfishery. The resources agency of California. *Department of Fish and Game, Fish Bulletin*, (113), 199–204.

Wallace, R. L. (1987). Coloniality in the phylum Rotifera. *Hydrobiologia*, *147*, 141–155.

Wallace, R. L. (2002). Rotifers: exquisite metazoans. *Integrative and Comparative Biology*, *42,* 660–667.

Wallace, R. L., Cipro, J. J., & Grubbs, R. W. (1998). Relative investment in offspring by sessile Rotifera. *Hydrobiolog*, *387*, 311–316.

Wallace, R. L., Hochberg, R., & Walsh, E. J. (2023). The undiscovered country: ten grand challenges in rotifer biology. *Hydrobiologia*, 1–24.

Wallace, R. L., Kordbacheh, A., & Walsh, E. J. (2018). Key to the currently recognized species of *Limnias* Schrank, 1803 (Rotifera, Monogononta, Gnesiotrocha, Flosculariidae). *Zootaxa*, *4442*, 307–318.

Wallace, R. L., & Edmondson, W. T. (1986). Mechanism and adaptive significance of substrate selection by a sessile rotifer. *Ecology*, *67*, 314–323.

Wallace, R. L., Snell, T. W., Ricci, C., & Nogrady, T. R. (2006). *Biology, Ecology and Systematics* (2nd ed., Vol. 1). Backhuys Publishers: Leiden.

Wallace, R. L., Snell, T. W., & Smith, H. A. (2015). Phylum Rotifera. In J. H. Thorp & D. C. Rogers (Eds.), *Thorp and Covich's Freshwater Invertebrates*.

Wallace, R. L., & Starkweather, P. L. (1985). Clearance rates of sessile rotifers: *In vitro* determinations. *Hydrobiologia*, *121,* 139–144.

Wallace, R. L., Walsh, E. J., Arroyo, M. L., & Starkweather, P. L. (2005). Life on the Edge: Rotifers from springs and ephemeral waters in the Chihuahuan Desert, Big Bend National Park (Texas, USA). *Hydrobiologia*, *546*, 147–157.

Wallace, R. L., Walsh, E. J., Schröder, T., Rico-Martinez, R., & Rios-Arana, J. V. (2008). Species composition and distribution of rotifers in Chihuahuan Desert waters of México: is everything everywhere? *Verhandlungen des Internationalen Verein Limnologie*, *30*, 73–76.

Walsh, E. J., Arroyo, M. L., Schröder, T., & Wallace, R. L. (2004). Species richness and species turnover (complementarity) of Rotifera in selected aquatic systems of Big

Bend National Park, Texas. *Proceedings of Sixth Symposium on the Natural Resources of the Chihuahuan Desert Region*, 185–204. Fort Davis, TX, USA.

Walsh, E. J., Schröder, T., Wallace, R. L., & Rico-Martinez, R. (2009). Cryptic speciation in *Lecane bulla* (Monogononta: Rotifera) in Chihuahuan Desert waters. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *30*(7), 1046–1050.

Walsh, E. J., Smith, H. A., & Wallace, R. L. (2014). Rotifers of temporary waters. *International Review of Hydrobiology*, *99,* 3–19.

Walsh, E. J., Salazar, M., Remirez, J., Moldes, O., & Wallace, R. L. (2006). Predation by invertebrate predators on the colonial rotifer *Sinantherina socialis*. *Invertebrate Biology*, *125*(4), 325–335.

Walsh, E. J., Schröder, T., Arroyo, M. L., & Wallace, R. L. (2007). How well do single samples reflect rotifer species diversity? A test based on interannual variation of rotifer communities in Big Bend National Park (Texas, USA). *Hydrobiologia*, *593*, 39–47.

Walsh, E. J., Schröder, T., Wallace, R. L., Ríos-Arana, J. V., & Rico-Martínez, R. (2008). Rotifers from selected inland saline waters in the Chihuahuan Desert of México. *Saline Systems*, *4*, 7.

Walsh, E. J., Smith, H. A., & Wallace, R. L. (2014). Rotifers of temporary waters. *International Review of Hydrobiology*, *99*, 3–19.

Walsh, E. J., & Zhang, L. (1992). Polyploidy in a natural population of the rotifer *Euchlanis dilatata*. *Journal of Evolutionary Biology*, *5,* 345–353.

Walz, N., Sarma, S. S. S., & Benker, U. (1995). Egg size in relation to body size in rotifers: an indication of reproductive strategy? *Hydrobiologia*, *314*, 165–170.

Wedderburn, S. D., Hillyard, K. A., & Shiel, R. J. (2013). Zooplankton response to flooding of a drought refuge and implications for the endangered fish species *Craterocephalus fluviatilis* cohabiting with alien *Gambusia holbrooki*. *Aquatic Ecology*, *47,* 263–275.

Weibel, E. R., & Hoppeler, H. (2005). Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *Journal of Experimental Biology*, *208*, 1635–1644.

Weithoff, G., Neumann, C., Seiferth, J., & Weisse, T. (2019). Living on the edge: reproduction, dispersal potential, maternal effects and local adaptation in aquatic, extremophilic invertebrates. *Aquatic Sciences*, *81,* 1–9.

Weithoff, G. (2005). On the ecology of the rotifer *Cephalodella hoodi* from an extremely acidic lake. *Freshwater Biology*, *50*, 1464–1473.

Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, *27,* 337–363.

Wen, X. L., Xi, Y. L., Qian, F. P., Zhang, G., & Xiang, X. L. (2011). Comparative analysis of rotifer community structure in five subtropical shallow lakes in East China: role of physical and chemical conditions. *Hydrobiologia*, *661,* 303–316.

Wen, X., Xi, Y., Zhang, G., Xue, Y., & Xiang, X. (2016). Coexistence of cryptic *Brachionus calyciflorus* (Rotifera) species: Roles of environmental variables. *Journal of Plankton Research*, *38*, 478–489.

West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science*, *276*, 122–126.

Wey-Fabrizius, A.R., Herlyn, H., Rieger, B., Rosenkranz, D., Witek, A., Welch, D.B.M., Ebersberger, I. and Hankeln, T. (2014). Transcriptome data reveal syndermatan relationships and suggest the evolution of endoparasitism in Acanthocephala via an epizoic stage. *PloS One*, *9*, 88618.

Weymouth, F. W., Crismon, J. M., Hall, V. E., Belding, H. S., & Field, J. (1944). Total and tissue respiration in relation to body weight a comparison of the kelp crab with other crustaceans and with mammals. *Physiological Zoology*, *17*, 50–71.

White, C. R., Alton, L. A., Bywater, C. L., Lombardi, E. J., & Marshall, D. J. (2022). Metabolic scaling is the product of life-history optimization. *Science*, *377*, 834–839.

White, C. R., & Kearney, M. R. (2014). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Comprehensive Physiology*, *4*, 231–256.

White, C. R., Kearney, M. R., Matthews, P. G. D., Kooijman, S. A. L. M., & Marshall, D. J. (2011). A Manipulative test of competing theories for metabolic scaling. *The American Naturalist*, *178*, 746–754.

Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K., & Triantis, K. A. (2017). Island biogeography: taking the long view of nature's laboratories. *Science*, *357*, 8326.

Wit, R., & Bouvier, T. (2006). Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? Environ. *Microbiol*, *8*, 755–758.

Wong, W. H., & Levinton, J. (2005). Consumption rates of two rotifer species by zebra mussels *Dreissena polymorpha*. *Marine and Freshwater Behaviour and Physiology*, *38*, 149–157.

Wood, T. S., Chaichana, P. A. R., Mahujchariyawong, J., & Satapanajaru, T. (2006). Heavy predation on freshwater bryozoans by the golden apple snail, *Pomacea canaliculata* Lamarck, 1822 (Ampullariidae. *Tropical Natural History*, *6*, 31–36.

Wright, N. A., Gregory, T. R., & Witt, C. C. (2014). Metabolic "engines" of flight drive genome size reduction in birds. *Proceedings of the Royal Society B*, *281,* 20132780.

Yan, N. D., & Geiling, W. (1985). Elevated planktonic rotifer biomass in acidified metal-contaminated lakes near Sudbury, Ontario. *Hydrobiologia*, *120*.

Yang, J., & Zhang, X. (2020). eDNA metabarcoding in zooplankton improves the ecological status assessment of aquatic ecosystems. *Environment International*, *134,* 199–205.

Yip, E. C., & Rayor, L. S. (2014). Maternal care and subsocial behaviour in spiders. *Biological Reviews*, *89*(2), 427–449.

Yoshida, T., Urabe, J., & Elser, J. J. (2003). Assessment of ‘top-down’ and ‘bottom-up’ forces as determinants of rotifer distribution among lakes in Ontario, Canada. *Ecological Research*, *18,* 639–650.

Young, A. N., Hochberg, R., Walsh, E. J., & Wallace, R. L. (2018). Modeling the life history of sessile rotifers: larval substratum selection through reproduction. *Hydrobiologia, 844,* 62–82.

Zawierucha, K., Porazinska, D. L., Ficetola, G. F., Ambrosini, R., Baccolo, G., Buda, J., & Ceballos, J. L. (2021). A hole in the nematosphere: tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing. *Journal of Zoology*, *313,* 18–36.

Zhang, Q., & Edwards, S. V. (2012). The evolution of intron size in amniotes: a role for powered flight? *Genome Biology and Evolution*, *4,* 1033–1043.

Zhang, W., Lemmen, K. D., Zhou, L., Papakostas, S., & Declerck, S. A. J. (2019). Patterns of differentiation in the life history and demography of four recently described species of the *Brachionus calyciflorus* cryptic species complex. *Freshwater Biology*, *64*, 1994–2005

Zweerus, N. L., Sommer, S., Fontaneto, D., & Ozgul, A. (2017). Life-history responses to environmental change revealed by resurrected rotifers from a historically polluted lake. *Hydrobiologia*, *796*, 121–130.

APPENDIX

Figure A1: Examples of Chihuahuan Desert aquatic systems. Left to right. Upper panel: stream – Lost River (WHSA, NM, USA); reservoir – Presa de al., Boquilla (Chihuahua, MX). Middle panel: rock pool – Ernst Tinaja 4 (BIBE, TX, USA); playa – Columbus Playa (NM, USA). Lower panel: tank – Tule Cattle Tank (BIBE, TX, USA); Poza Azul (Cuatro Ciénegas, Coahuila, MX)

Table A1. Site name, locations, habitat types, and sampling intensities for waterbodies included in this study. APFFC = Área de Protección de Flora y Fauna Cuatrociénegas, ANPMS = Área Natural Protegida Médanos de Samalayuca, BANWR = Buenos Aires National Wildlife Refuge, BIBE = Big Bend National Park, CAVE = Carlsbad Caverns National Park, GUMO = Guadalupe Mountains National Park, HTSHPS = Hueco Tanks State Park and Historic Site; WHSA = White Sands National Park. Sampling effort: 1 = 1 sample date only, 2 = 2–5 sampling dates, 3 = 6–10 sampling dates, 4 = 11–20 sampling dates, 5 = >20 sampling dates.

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Arizona					
Triangle Pond, BANWR	spring	31.55	−111.533889	6	2
Lake Arivaca, BANWR	lake	31.531896	−111.253136	6	1
New Mexico					
Lazy Lagoon, BLSP	playa	33.3541666	−104.3417666	3	2
Cottonwood Lake, BLSP	lake	33.3388666	−104.3340277	6	2
Mirror Lake, BLSP	lake	33.3363666	−104.3327333	2	2
Figure Eight Lake, BLSP	lake	33.3339333	−104.3324666	2	2
Pasture Lake, BLSP	lake	33.3310666	−104.3295666	16	2
Lea Lake, BLSP	lake	33.3170833	−104.3303666	8	2
Elephant Butte Reservoir	lake	33.1607361	−107.1885194	2	2
Rio Grande, Williamsburg	river	33.10335	−107.293983	12	2
Caballo Reservoir	lake	32.8977222	−107.2985583	13	1
Dune Pond 1, WHSA	playa	32.7243	−106.393367	1	1
Dune Pond 3, WHSA	playa	32.72365	−106.394917	3	1

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Lost River, WHSA	stream	32.8802	−106.1708833	3	1
Lower Lost River Pool, WHSA	stream	32.8775333	−106.1789333	1	1
Lake Holloman	lake	32.80745	−106.1227833	6	1
Backcountry Trailhead, WHSA	playa	32.797	−106.26965	2	1
Garton Spring, WHSA	spring	32.775067	−106.145267	1	2
Lake Lucero, WHSA	playa	32.6976333	−106.4511666	7	2
Cattle Tank, WHSA	tank	32.67485	−106.44345	4	2
Dripping Springs	spring	32.3231888	−106.5725138	6	2
La Mancha Wetlands	river	32.278092	−106.828626	13	2
Red Lake	lake	32.8615027	−104.1771791	2	
Sitting Bull Falls, LNF	spring	32.243666	−104.696599	7	1
Sitting Bull Falls, LNF	spring	32.2434916	−104.6962916	19	2
Sitting Bull Falls Pool 1, LNF	spring	32.2390333	−104.7025333	19	1
Sitting Bull Falls Pool 2, LNF	spring	32.2385	−104.702667	3	1
Rattlesnake Spring, CAVE	spring	32.1097	−104.471625	33	2
404A Playa	playa	32.0125844	−106.523427	16	
404B Playa	playa	32.022586	−106.508957	17	1
McKittrick Creek, GUMO	stream	31.985783	−104.769383	1	2

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Smith Spring, GUMO	spring	31.9186111	−104.806667	3	1
Manzanita Spring, GUMO	spring	31.9103194	−104.79855	23	3
Chosa Spring south side, GUMO	spring	31.9065333	−104.7821166	5	2
Chosa Spring north side, GUMO	spring	31.906397	−104.782996	4	2
Upper Pine Spring Pool #1, GUMO	spring	31.9032666	−104.81785	4	2
Upper Pine Spring Pool #2, GUMO	spring	31.9029666	−104.81765	7	2
Guadalupe Canyon Seepage 1, GUMO	spring	31.869527	−104.8380166	3	1
Guadalupe Canyon Seepage 3, GUMO	spring	31.8696	−104.8377833	5	1
Columbus Playa, NM	playa	31.805433	−107.103833	12	1
NM Highway 180	river	32.508553	−106.957176	10	2
Rio Grande, Percha Dam	river	32.868149	−107.304454	5	2
Rio Grande, Anthony	river	32.005933	−106.639733	9	3
Texas					
BRH, HTSPHS	playa	31.927081	−106.041142	4	5
Heart, HTSPHS	rock pool	31.924848	−106.042467	2	5
Hex, HTSPHS	rock pool	31.924734	−106.04221	2	5

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Stacia, HTSPHS	rock pool	31.924685	−106.042592	1	5
North Temp, HTSPHS	rock pool	31.924682	−106.042347	4	5
Vero, HTSPHS	rock pool	31.924675	−106.042662	2	5
Boo’s Pond, HTSPHS	playa	31.9246611	−106.045825	3	5
South Temp, HTSPHS	rock pool	31.924658	−106.042285	6	5
Cammie, HTSPHS	rock pool	31.924642	−106.042669	1	5
Laguna Prieta, HTSPHS	playa	31.9246388	−106.046675	17	5
Al, HTSPHS	rock pool	31.924634	−106.042674	1	5
Walsh, HTSPHS	rock pool	31.924628	−106.042628	2	5
Julie, HTSPHS	rock pool	31.924622	−106.042497	1	5
Luisa, HTSPHS	rock pool	31.924768	−106.042617	1	5
Jamie, HTSPHS	rock pool	31.92456	−106.042433	1	5
Behind East, HTSPHS	playa	31.919195	−106.041106	13	5
Mescalero Canyon, HTSPHS	playa	31.9188166	−106.040366	44	5
Clammation, HTSPHS	rock pool	31.922556	−106.042508	1	4
Shelby, HTSPHS	rock pool	31.924622	−106.042668	1	5

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Pia, HTSPHS	rock pool	31.924544	−106.042239	1	4
Monica, HTSPHS	rock pool	31.925051	−106.045727	1	4
Kettle 1, HTSPHS	rock pool	31.918455	−106.040106	2	4
Kettle 2, HTSPHS	rock pool	31.918455	−106.040107	2	4
Kettle 3, HTSPHS	rock pool	31.918455	−106.040101	2	4
Kettle 4, HTSPHS	rock pool	31.918446	−106.040105	4	5
Kettle 5, HTSPHS	rock pool	31.918484	−106.040087	2	4
Behind Picnic, HTSPHS	rock pool	31.924831	−106.045855	2	3
1 of 4, HTSPHS	rock pool	31.924826	−106.045663	2	4
2 of 4, HTSPHS	rock pool	31.92482	−106.04567	1	4
3 of 4, HTSPHS	rock pool	31.924813	−106.045669	1	4
4 of 4, HTSPHS	rock pool	31.924799	−106.045673	1	4
Abelex, HTSPHS	rock pool	31.924624	−106.042526	1	3
Iceskating Pond, HTSHPS	playa	31.924729	−106.045909	4	3
Rio Grande, Borderland	river	31.8859527	−106.5988777	12	1
Crossroads Pond	lake	31.836988	−106.580518	4	2

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Keystone Heritage Park Wetland	spring	31.8224694	−106.5642444	5	2
Rio Grande, American Dam	river	31.786506	−106.526992	15	3
Ascarate Lake	lake	31.7501777	−106.4047527	33	4
Ascarate Duck Pond	lake	31.7473027	−106.4035527	7	1
Feather Lake	lake	31.6890972	−106.305	24	2
Rio Bosque Wetland Cell 1	tank	31.64202	−106.315503	2	1
Rio Bosque Wetland Cell 2	tank	31.636467	−106.310833	8	2
Rio Grande, San Elizario	river	31.669737	−106.337114	18	3
Rio Grande, Fort Quitman	river	31.087533	−105.60933	4	2
Rio Grande, Presidio	river	29.60365	−104.45197	2	2
Rio Grande, C 50	river	30.585217	−104.892833	5	2
Rio Grande, C 20	river	30.36695	−104.8118	3	2
Rio Grande, Candelaria	river	30.133417	−104.69	1	2
Rio Grande, Guadalupe POE	river	31.431854	−106.148343	4	2
Rio Grande, Montoya Drain	river	31.799933	−106.556490	11	3
Montoya and Doniphan	river	31.873037	−106.592262	4	2
Rio Grande Fabens	river	31.430277	−106.14222	18	2
Album Park	playa	31.783419	−106.346349	5	3

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
McNary Reservoir	lake	31.2242138	−105.7890083	12	1
Diamond Y Roadside	spring	31.0088	−102.922533	13	2
Diamond Y Spring	spring	31.0010666	−102.9242833	18	2
East Sandia Flow	spring	30.9910833	−103.7286	10	2
East Sandia Spring	spring	30.9909666	−103.7288666	22	2
Balmorhea Lake	lake	30.9663333	−103.7134	5	2
Balmorhea Main Pool	spring	30.9445833	−103.7876666	5	2
Balmorhea Wetland 1	spring	30.9449166	−103.7835	27	3
Balmorhea Wetland 2	spring	30.945413	−103.785982	5	2
Balmorhea Canal	spring	30.9444472	−103.7851583	32	3
Roadside Wetland	river	30.8551333	−105.3608833	17	1
Soda Spring	spring	30.8276388	−105.3173055	10	1
Beauty Spring B	spring	30.8243333	−105.3148611	2	2
Stump Spring A	spring	30.8225883	−105.3151466	7	1
Masims Spring	spring	30.8219666	−105.314733	2	1
Dynamite Spring	spring	30.8218833	−105.31545	6	1
Squaw Spring	spring	30.7972166	−105.0111833	2	2
Corral Tank, IMRS	tank	30.785263	−104.984084	9	2
Peccary Tank, IMRS	tank	30.755556	−105.004167	3	1
Rattlesnake Tank, IMRS	tank	30.743611	−105.008333	1	1

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Red Tank, IMRS	tank	30.7303083	−104.9891083	2	2
Miller Ranch 96 Well	spring	30.6238533	−104.6739988	9	2
Miller Ranch 2 (Spring)	spring	30.55025	−104.66645	13	1
Miller Ranch Glidewell	spring	30.571483	−104.657317	8	1
Pinto Canyon Stream	stream	30.0308666	−104.468433	10	1
Kimball Hole Miller Ranch	spring	30.585278	−104.626667	5	1
Sanderson Canyon	rock pool	29.8472	−102.1837055	6	1
La Mesa Canyon Tule 2	rock pool	29.829091	−102.360993	26	1
Rio Grande, Above Dryden	river	29.8090277	−102.1481138	1	1
Lower Madison Falls Seep 1	spring	29.7967666	−102.3779333	7	2
Silber Hotspring 2	spring	29.76835	−102.5635833	2	1
Below Hotsprings Texas	spring	29.7484	−102.5406833	3	1
Fuentes Ranch Shafter	stream	29.7936833	−104.27665	11	1
Buttrill Springs, BIBE	spring	29.54585	−103.2738	6	2
McKinney Spring 1, BIBE	spring	29.4090166	−103.08715	3	1
Grapevine Spring, BIBE	spring	29.4075666	−103.19085	1	1

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
McKinney Wall Spring, BIBE	spring	29.407466	−103.0885166	1	1
McKinney Tinaja, BIBE	rock pool	29.4073666	−103.0886833	1	1
Dripping Spring Cliff, BIBE	spring	29.4066833	−103.3103166	1	1
Dripping Spring, BIBE	spring	29.4049666	−103.3078583	1	2
Dripping Spring Upper, BIBE	spring	29.4049491	−103.3078470	1	1
Onion Tinaja, BIBE	rock pool	29.4014	−103.32585	1	1
Paint Gap Tank, BIBE	tank	29.3878555	−103.302675	10	3
San Felipe Creek Del Rio	stream	29.36985	−100.8838166	1	1
Croton Spring, BIBE	spring	29.3446166	−103.3471166	10	3
Croton Stream, BIBE	spring	29.3437833	−103.3465	4	2
Government Spring 2, BIBE	spring	29.3406167	−103.2559833	2	2
Government Spring 1, BIBE	spring	29.3405666	−103.2560833	2	4
Oak Creek, BIBE	spring	29.2828666	−103.3421833	6	3
Window Trail Pool A, BIBE	rock pool	29.28003	−103.3299472	2	2
Window Trail Pool B, BIBE	rock pool	29.28003	−103.33	4	2
Window Trail Pool C, BIBE	rock pool	29.28009	−103.33018	1	2

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Window Trail Pool D, BIBE	rock pool	29.2802	−103.33038	2	2
Window Trail Pool E, BIBE	rock pool	29.28025	−103.33043	6	3
Window Trail Pool F, BIBE	rock pool	29.28031	−103.3305	6	3
Window Trail Pool G, BIBE	rock pool	29.28035	−103.3305388	4	3
Window Trail Pool H, BIBE	rock pool	29.2804138	−103.3305388	4	2
Window Trail Pool I, BIBE	rock pool	29.2804611	−103.3305388	6	2
Window Trail Pool Donut, BIBE	rock pool	29.2802722	−103.330475	5	2
Carlota Tinaja, BIBE	rock pool	29.2790833	−103.0354166	1	1
Cattail Spring A, BIBE	spring	29.2731805	−103.3355138	35	4
Cattail Spring B, BIBE	spring	29.2731833	−103.33555	25	4
Cattail Spring C, BIBE	spring	29.2731833	−103.3355861	17	4
Cattail Spring C’, BIBE	spring	29.2731833	−103.3356305	9	3
Cattail Spring C’’, BIBE	spring	29.2731833	−103.335675	8	3
Cattail Spring C-D, BIBE	spring	29.2731555	−103.3357336	13	3
Cattail Spring D, BIBE	spring	29.2731527	−103.3358277	17	4
Cattail Spring E, BIBE	spring	29.2731444	−103.3359666	18	4

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Cattail Spring F, BIBE	spring	29.2731333	−103.3360833	21	4
Cattail Spring G, BIBE	spring	29.2731666	−103.3361638	29	4
Cattail Spring H, BIBE	spring	29.2731694	−103.3362388	23	4
Ernst Tinaja 1, BIBE	rock pool	29.2568666	−103.0100833	6	3
Ernst Tinaja 2, BIBE	rock pool	29.2567416	−103.0103583	5	3
Ernst Tinaja 3, BIBE	rock pool	29.2567415	−103.0104	6	2
Ernst Tinaja 4, BIBE	rock pool	29.2562666	−103.0112916	2	2
Ernst Tinaja 4A, BIBE	rock pool	29.2563611	−103.0111083	6	2
Ernst Tinaja 5, BIBE	rock pool	29.2560416	−103.0117361	8	3
Ernst Tinaja 6, BIBE	rock pool	29.2559972	−103.0119166	6	3
Ernst Tinaja 7, BIBE	rock pool	29.2559944	−103.01195	5	3
Ernst Tinaja 8, BIBE	rock pool	29.2559888	−103.0119694	1	2
Ernst Tinaja 9, BIBE	rock pool	29.2559805	−103.0119972	5	3
Ernst Tinaja 10, BIBE	rock pool	29.255975	−103.0120138	3	2
Ernst Tinaja Hueco, BIBE	rock pool	29.2551	−103.0148833	6	1
Ward Spring 2, BIBE	spring	29.24445	−103.3505833	1	1

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Tule Cattle Tank, BIBE	tank	29.2424333	−103.4438305	21	3
Tule Spring A, BIBE	spring	29.2422833	−103.4426666	6	3
Tule Spring B, BIBE	spring	29.24155	−103.4428333	3	3
Burro Spring, BIBE	spring	29.2373	−103.4259	14	3
Rio Grande Village Cattail Pond, BIBE	tank	29.189	−102.9716166	28	3
Rio Grande Village Canal, BIBE	river	29.18615	−102.97225	6	2
Rio Grande Rio Grande Village, BIBE	river	29.18555	−102.979666	16	3
Langford Hot Springs, BIBE	spring	29.1794944	−102.995466	3	2
Rio Grande Village Pump House, BIBE	river	29.17945	−102.95325	16	2
Rio Grande Village Upper Pond, BIBE	river	29.1785472	−102.9531833	30	4
Rio Grande Village Lower Pond, BIBE	river	29.1785166	−102.95375	34	4
Glenn Springs, BIBE	spring	29.1744166	−103.1575	21	3
Trap Spring, BIBE	spring	29.1636333	−103.4194166	3	2
Mule Ears Spring (Middle), BIBE	spring	29.1624	−103.4082666	2	1

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Mule Ears Spring (Lower), BIBE	spring	29.16235	−103.4082833	5	2
Rio Grande, Santa Elena	river	29.15415	−103.598683	4	1
Tuff Canyon Falls (wall), BIBE	rock pool	29.15115	−103.4855	2	1
Tuff Canyon 1, BIBE	rock pool	29.1507666	−103.48605	1	2
Tuff Canyon 3, BIBE	rock pool	29.1507666	−103.4859	2	2
Tuff Canyon 4, BIBE	rock pool	29.15077	−103.4857666	3	2
Tuff Canyon 5, BIBE	rock pool	29.1509	−103.48575	2	2
Tuff Canyon 6, BIBE	rock pool	29.15095	−103.485389	1	1
Mexico					
Presa Chihuahua	lake	28.5762166	−106.1711833	32	2
Delicias Beisbol Field Pool	tank	28.1648166	−105.498500	6	1
Presa Francisco Ignacio Madero	lake	28.1626166	−105.6321833	19	2
Lago Colina	lake	27.5724	−105.4004666	43	2
Presa de la Boquilla	lake	27.5361333	−105.4011333	23	2
Laguna La Leche	playa	27.2860833	−102.9161666	7	1
San Jose del Anteojo, APFFC	spring	26.9693166	−102.1208166	21	2
Tio Julio, APFFC	spring	26.9462833	−102.0592	10	1
Poza Tortugas, APFFC	spring	26.93145	−102.1247	27	3

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Poza Azul, APFFC	spring	26.9226666	−102.1226333	3	2
Rio Mesquites, APFFC	river	26.9222222	−102.1083333	8	2
Poza Marcelo, APFFC	spring	26.9104	−102.0363166	6	2
Las Playitas, APFFC	spring	26.9085166	−102.01745	7	2
Los Gatos, APFFC	spring	26.88875	−101.9980333	14	2
Poza la Becerra, APFFC	spring	26.8784166	−102.1377666	13	2
Los Hundidos Main pool, APFFC	spring	26.8711666	−102.0204166	13	2
La Campana, APFFC	spring	26.8683666	−102.0278333	3	1
Poza El Arco B, APFFC	spring	26.8683333	−102.0228	6	1
Poza Churince, APFFC	spring	26.8404166	−102.1342333	15	3
Ejido El Venado Entrance, APFFC	spring	26.9146333	−102.047	14	1
Ejido El Venado Grande, APFFC	spring	26.8199	−101.904833	1	1
Ejido El Venado A, APFFC	spring	26.8194666	−101.9053166	7	1
Presa Francisco Zarco Durango	lake	25.2693055	−103.7727222	2	1
Ojos Altos A	spring	31.40685	−107.6181833	1	3
Ojos Altos B	spring	31.4068	−107.6179666	1	2
Ojos Altos C	spring	31.4035166	−107.616	12	3

Site Name/Location	Habitat Type	Latitude	Longitude	Species Richness	Sampling Effort
Ojos Altos D	spring	31.4032666	−107.6163	9	3
Ojo de la Punta, ANPMS	spring	31.3859166	−106.6022666	32	4
Ojo de en Medio ANPMS	spring	31.37885	−106.5877833	26	3
Ojo de la Casa ANPMS	spring	31.3656166	−106.5322333	21	3
DunasCampestre ANPMS	spring	31.335967	−106.491333	8	3
El Huerfano ANPMS	spring	31.294817	−106.511017	10	3
Ojo de Santa Maria	spring	31.1552777	−107.3172222	22	2
Upper Mexican Hotsprings	spring	29.7460833	−102.5455666	11	2

Table A2. Species richness estimated by rarefaction using iNEXT online (Chao et al., 2014, Chao et al., 2016). Displayed are all sites extrapolated to approximately the same sampling effort (20 collections). Site designation

	Habitat type	Method	Rarefied richness / Observed richness
404A Playa	playa	extrapolated	25
404B Playa	playa	extrapolated	13
Album Park	playa	extrapolated	7.4
Backcountry Trailhead, WSNP	playa	extrapolated	2
Behind East, HTSPHS	playa	observed	21
Behind Picnic, HTSPHS	playa	extrapolated	2.5
Boo's Pond, HTSPHS	playa	observed	3
Columbus Playa	playa	extrapolated	12
Dune Pond 1, WSNP	playa	extrapolated	1
Dune Pond 3, WSNP	playa	extrapolated	3
Laguna La Leche	playa	extrapolated	7
Laguna Prieta, HTSPHS	playa	observed	30
Lake Lucero, WSNP	playa	extrapolated	13.1
Lazy Lagoon, BLSP	playa	extrapolated	4.3
Mescalero Canyon, HTSPHS	playa	observed	44
Ojo de la Casa, ANPMS	playa	extrapolated	34.0
1 of 4, HTSPHS	rock pool	extrapolated	2.3
2 of 4, HTSPHS	rock pool	extrapolated	1
3 of 4, HTSPHS	rock pool	extrapolated	1
4 of 4, HTSPHS	rock pool	extrapolated	1
Abelex, HTSPHS	rock pool	extrapolated	1
Al, HTSPHS	rock pool	observed	1

BRH, HTSPHS	rock pool	observed	23
Cammie, HTSPHS	rock pool	observed	1
Carlota Tinaja, BIBE	rock pool	extrapolated	1
Clammation, HTSPHS	rock pool	extrapolated	1
Ernst Tinaja 10, BIBE	rock pool	extrapolated	4.3
Ernst Tinaja 1, BIBE	rock pool	extrapolated	9.1
Ernst Tinaja 2, BIBE	rock pool	extrapolated	7.4
Ernst Tinaja 3, BIBE	rock pool	extrapolated	10.8
Ernst Tinaja 4A, BIBE	rock pool	extrapolated	10.8
Ernst Tinaja 4, BIBE	rock pool	extrapolated	2.5
Ernst Tinaja 5, BIBE	rock pool	extrapolated	12.4
Ernst Tinaja 6, BIBE	rock pool	extrapolated	9.1
Ernst Tinaja 7, BIBE	rock pool	extrapolated	7.4
Ernst Tinaja 8, BIBE	rock pool	extrapolated	1
Ernst Tinaja 9, BIBE	rock pool	extrapolated	7.4
Ernst Tinaja Hueco	rock pool	extrapolated	6
Heart, HTSPHS	rock pool	observed	4
Hex, HTSPHS	rock pool	observed	2
Ice skating Pond, HTSPHS	rock pool	extrapolated	5.8
Jamie, HTSPHS	rock pool	observed	1
Julie, HTSPHS	rock pool	observed	1
Kettle 1, HTSPHS	rock pool	extrapolated	2.3
Kettle 2, HTSPHS	rock pool	extrapolated	2.3
Kettle 3, HTSPHS	rock pool	extrapolated	3.6
Kettle 4, HTSPHS	rock pool	observed	6
Kettle 5, HTSPHS	rock pool	extrapolated	2.3

La Mesa Canyon Tule 2	rock pool	extrapolated	26
Luisa, HTSPHS	rock pool	observed	1
McKinney Tinaja, BIBE	rock pool	extrapolated	1
Monica, HTSPHS	rock pool	extrapolated	1
North Temp, HTSPHS	rock pool	observed	9
Onion Tinaja 1, BIBE	rock pool	extrapolated	1
Pia, HTSPHS	rock pool	extrapolated	1
Sanderson Canyon	rock pool	extrapolated	6
San Francisco Cattle Tank	rock pool	extrapolated	7
Shelby, HTSPHS	rock pool	observed	1
South Temp, HTSPHS	rock pool	observed	20
Stacia, HTSPHS	rock pool	observed	1
Tuff Canyon 1, BIBE	rock pool	extrapolated	1
Tuff Canyon 3, BIBE	rock pool	extrapolated	2.5
Tuff Canyon 4, BIBE	rock pool	extrapolated	4.3
Tuff Canyon 5, BIBE	rock pool	extrapolated	2.5
Tuff Canyon 6, BIBE	rock pool	extrapolated	1
Tuff Canyon Falls (Wall), BIBE	rock pool	extrapolated	2
Vero, HTSPHS	rock pool	observed	2
Walsh, HTSPHS	rock pool	observed	2
Window Trail Pool A, BIBE	rock pool	extrapolated	2.5
Window Trail Pool B, BIBE	rock pool	extrapolated	6.4
Window Trail Pool C, BIBE	rock pool	extrapolated	1
Window Trail Pool D, BIBE	rock pool	extrapolated	2.5
Window Trail Pool Donut, BIBE	rock pool	extrapolated	8.5
Window Trail Pool E, BIBE	rock pool	extrapolated	9.1

Window Trail Pool F, BIBE	rock pool	extrapolated	9.1
Window Trail Pool G, BIBE	rock pool	extrapolated	5.8
Window Trail Pool H, BIBE	rock pool	extrapolated	6.4
Window Trail Pool I, BIBE	rock pool	extrapolated	10.8
Cattle Tank, WSNP	tank	extrapolated	6.4
Corral Tank, IMRS	tank	extrapolated	17.8
Delicias Beisbol Field Pool	tank	extrapolated	6
Paint Gap Tank, BIBE	tank	extrapolated	15.7
Peccary Tank, IMRS	tank	extrapolated	3
Presa De La Vaca Tank	tank	extrapolated	9
Rattlesnake Tank, IMRS	tank	extrapolated	1
Red Tank, IMRS	tank	extrapolated	2.5
Rio Bosque Wetland Cell 1	tank	extrapolated	2
Rio Bosque Wetland Cell 2	tank	extrapolated	15.4
Rio Grande Village Cattail Pond, BIBE	tank	extrapolated	45.7
Tule Cattle Tank, BIBE	tank	extrapolated	34.0

Brown, P. D., Schröder, T., Ríos-Arana, J. V., Rico-Martinez, R., Silva-Briano, M., Wallace, R. L., & Walsh, E. J., 2020. Patterns of rotifer diversity in the Chihuahuan Desert. Diversity 12: 393.570. doi:10.3390/d12100393.

Chao, A., Ma, K. H., and Hsieh, T. C., 2016. iNEXT (iNterpolation and EXTrapolation) Online. Program and User's Guide published at http://chao.stat.nthu.edu.tw/wordpress/software_download/

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., and Ellison, A. M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs 84: 45–67. doi: 10.1890/13-0133.1

VITA

Patrick Brown graduated from Cornell university class of 2013 with a Bachelor of Science in Entomology and Biology with a concentration in Marine Biology. Patrick was admitted to the Evolution and Ecology program at The University of Texas at El Paso in Fall 2015. As a member of the Walsh lab at UTEP Patrick worked on projects related to lifestyle evolution in the gnesiotrochan rotifers as well as metacommunity ecology using long term collection data from the lab. These projected culminated in experiments focusing on genome size as it relates to lifestyle in the gnesiotrochan rotifers and scaling or respiration rate with colony size in animals. While a student at UTEP Patrick was supported by funds from NSF DEB-1257068 (EJW), two Dodson awards (2016, 2017), the UTEP travel grants (2019), Summer Graduate Grant (2020) and STEMGROW program (2017-2021) and received TA and RA funding throughout the length the program. Patrick presented his research at eight conferences including Rotifera (2015, 2022) Association for the study of Limnology and Oceanography (2020, 2023) Ecological Society of America (2020), Shallow Lakes (2020), SICB (2023,) and Evolution (2021) including receiving third place for young scientist presentation at Rotifera XVI 2022 based on his work on allometric scaling in colonial rotifers.