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A Comparison Of UVR-Induced Mortality In Bdelloid Rotifers

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ABSTRACT

Increases in UV radiation (UVR) reaching Earth's surface as a result of anthropogenic activities and changing climate patterns are having a variety of effects on ecosystems. Such effects have been observed in aquatic environments, although certain parameters such as dissolved organic carbon (DOC) concentrations can modulate the level of exposure. Bdelloid rotifers are aquatic micro-invertebrates that pose a remarkable variety of special abilities to survive adverse conditions, including a resistance to UVR. Previous studies have suggested this resistance may have evolved in response to episodes of desiccation that they experience in their natural habitats. This characteristic may be especially important for populations living in the dynamic, ephemeral waters of the North American desert southwest, where UV radiation is pronounced due to a lack of cloud cover for most of the year, and temporary aquatic habitats can undergo extensive periods of time without water. The objective of this study is to determine the impact of UV-B radiation on aquatic biota by investigating any differences in mortality post-exposure. Bdelloids from family Philodinidae were collected from four locations in two states: Texas (a man-made lake, a temporary rock-pool, and a dust sample with high UVR exposure potential) and Wisconsin (a permanent lake with low UVR exposure potential). Water samples from the lakes and the rock-pool were collected in a summer and a winter season for DOC measurements. Bdelloids were dehydrated under three desiccation regimens, after which they were exposed to UV-B radiation using four exposure levels in a laboratory setting. The rock-pool DOC concentrations were greater than the other two locations, although significant variation could not be determined due to a low sample size. There also appeared to be a seasonal variation between these locations, with winter DOC levels in the rock-pool being over twice the levels recorded at the man-made lake. These are the first measurements of their kind for these

locations; however, since the number of samples were not sufficient to provide relevant differences, caution is advised when they are referenced. The probability of death was significantly heightened by the period of time for which the bdelloids were dried prior to exposure (X^2 = 461.24, d.f. = 2, p<0.001), their population source (X^2 = 1972.87, d.f. = 3, p<0.001), and the level of exposure UVR (X^2 = 504.57, d.f. = 3, p<0.001). The 2-way interactions of these parameters also had a significant effect on bdelloid mortality (X^2 = 34.73, d.f. = 9, p<0.001 for filter and population; X^2 = 119.69, d.f. = 6, p<0.001 for desiccation time and population). Among the four populations, bdelloids from the Wisconsin lake had a significantly higher probability of mortality than the other three populations (GLM, p<0.05). The results of this study indicate that UVR tolerance among bdelloid rotifers likely varies according to their habitats. As changes in UVR exposures are predicted to occur in the future, these observed differences may be amplified, which may in turn lead to shifts in aquatic community structure.

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CHAPTER 1: INTRODUCTION

Environmental changes have had diverse impacts on the health and structure of many ecosystems. One example is ultraviolet radiation (UVR), particularly the UV-B spectrum (280–320 nm), and its heightened effects on natural communities brought about by climate change's impacts on the interactions between atmospheric ozone, rising air temperatures, and changing precipitation patterns (Häder et al. 2015). Ozone and other gases in the atmosphere absorb shorter wave-length UV-B radiation, essentially preventing wavelengths below 290 nm from reaching the Earth's surface (Williamson et al. 2001). However, depletion of stratospheric ozone in the mid to late 20th century has resulted in alterations to this norm. The release of chlorofluorocarbons, for example, has played an important part in the depletion of total ozone levels in the atmosphere between the 1960s and 1990s, resulting in a 7-35% increase in UV-B radiation reaching Earth's surface in the northern hemisphere per year (Kerr and McElroy 1993).

In recent years, total ozone depletion seems to have stabalized over most latitudes thanks in large part to the success of the Montreal Protocol in reducing ozone-depleting substances (ODSs), which in turn has resulted in a halt to the overall increases in UVR reaching unpolluted sites that are not affected by cloud cover changes (McKenzie et al. 2011). However, year-to-year variability in these ozone levels has increased relative to before the mid-1990s, making it difficult to detect any increases in ozone that would be expected as a result in the decline of ODSs concentrations (Bais et al. 2015). Complete recovery of stratospheric ozone is also predicted to be confounded by increases in greenhouse gases (Shindell et al. 1998, McKenzie et al. 2011), which means that UV-B exposure is likely to continue to be a relevant threat to ecosystem health. Increases in UV-B irradiation can have important consequences on biota,

given that these wavelengths induce DNA damage by forming pyrimidine dimers, of which cyclobutane-pyrimidine dimers (CPDs) are the most frequent (Mitchell and Nairn 1989), and can also interfere with enzymatic reactions and physiological responses (Häder et al. 2015).

Aquatic environments have been the focus of numerous studies examining the effects brought upon by increases in UV-B radiation, ranging from the biochemical and molecular levels up to whole communities. An important variable that has significant implications for the amount of UVR that all systems will receive is their location, including elevation and relative latitude and longitude. Compared to 50 years ago, projections of future UV index (UVI) ranges from decreases by 9% in northern latitudes to increases of up to 20% in southern high latitudes (Hegglin and Shepherd 2009). In the continental United States, UVI has increased over the past three decades, with spatial distributions showing substantial variation from coastal zones to the Midwest (Gao et al. 2010; Figure 1.1). Mid-summer UV values in the southwestern United States are approximately 25% greater than values for states located at the same latitudes but along the east coast, and are also higher than those recorded in western states at higher latitudes (Fioletov et al. 2010a).

Along with location, average year-round cloud coverage can also play an important role in the quantity and nature of radiation exposure aquatic systems receive. UV attenuation by cloud cover depends on cloud properties, including optical thickness, relative position to the sun, cloud type, number of layers, etc. (Calbó et al. 2005). Ambient annual UVR is roughly about two-thirds that estimated for clear skies in temperate latitudes and up to three-fourths in the tropics (Diffey 2002). The density of cloud coverage can impact the grade of this exposure. There is little difference in UVR intensity with light scattered clouds, but complete light cloud cover reduces UVR intensity by approximately one half of that from a clear sky (Diffey 1991).

Clouds can reduce UV-A irradiance by more than 100 times under heavy thunder clouds, and this reduction can be even greater for UV-B (Fioletov et al. 2010b).

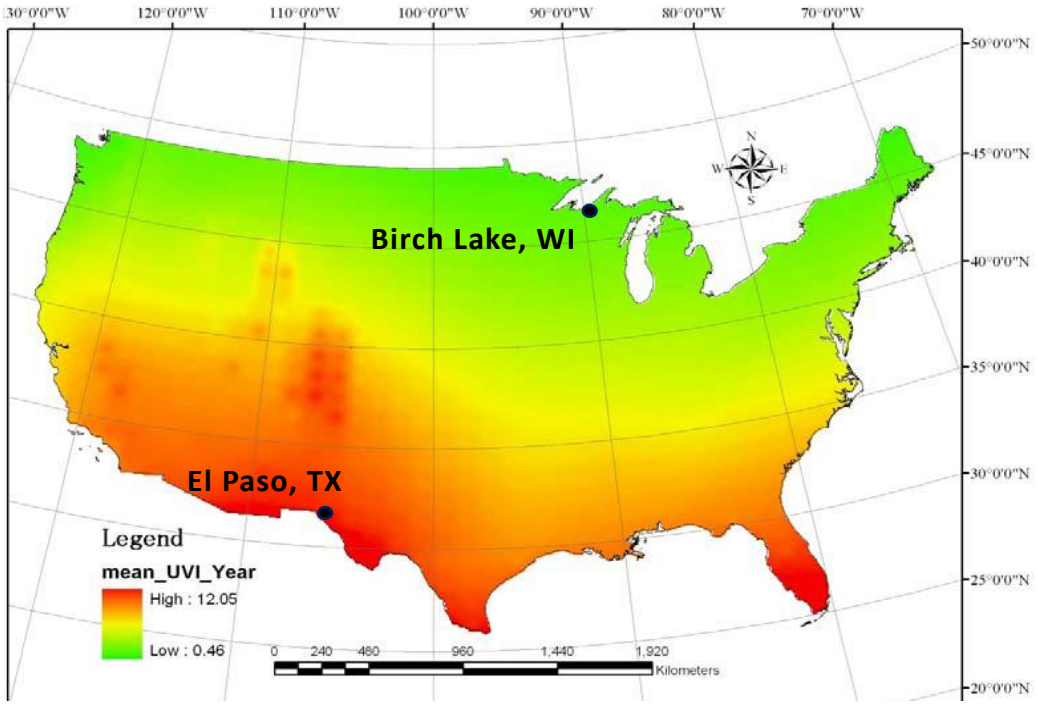

Figure 1.1. Map of the continental United States multi-year mean UV index (UVI) from 1979 to 2005 (modified from Gao et al. 2010). The two dots represent locations from which the bdelloid populations for this study were collected: UTEP Biology roof top dust, Ascarate Lake, and Hueco Tanks State Park & Historic Site in El Paso, Co., TX, and Birch Lake, Marquette Co., WI.

Projections of cloud cover changes suggest surface UV-B radiation will decrease by up to ~2% at middle latitudes and up to 7% at northern high latitudes due to greater cloud coverage by the end of the century (Bais et al. 2015). However, while the majority of studies that have investigated cloud coverage and UVR suggest that clouds play an important role in diminishing the intensity of UVR, others suggest that there is actually an enhancement effect. The magnitude and cause of this enhancement, though, are not well established (Calbó et al. 2005). These

factors have therefore instigated the need to investigate UV exposures that aquatic organisms are currently experiencing and to project future changes in them.

Freshwater ecosystems have been under scrutiny for the various effects that surface solar UVR has on aquatic organisms. Certain characteristics of aquatic systems can determine the amount of UVR exposure they receive. Only about 5% of UVR is reflected by calm waters, while up to 20% is reflected by choppy waters (Diffey 2002), meaning that water alone does not protect aquatic biota against the harmful effects of this radiation. Dissolved organic carbon (DOC) can control UV attenuation through the absorption of UV wavelengths in the water column. This attenuation is largely regulated by the concentration and absorptivity of DOC (Morris et al. 1995). Although DOC is fairly resistant to degradation, it can still be broken down into smaller subunits by UVR, thereby decreasing attenuation and allowing UVR to penetrate deeper in the water column (Häder and Sinha 2005). It can therefore be said that, generally, clear freshwater systems have a higher risk of UV-B damage (Bancroft et al. 2007).

The nature of protection that is provided by DOC varies among organisms. For fishes, DOC can protect against physiological stress associated with UVR exposure and helps maintain production of epidermal club cells, which play a role in innate immune responses (Manek et al. 2014). Zooplankton communities can also be influenced by higher UVR levels in locations where there is low DOC (Leech et al. 2005, Cooke et al. 2006). Some zooplankton species, particularly rotifers, show little to no evidence for avoidance of high UVR in low DOC habitats, while others avoid surface waters in these habitats (Cooke et al. 2006). It has been noted, however, that while DOC may be important for protection against the harmful effects that are attributed to UV-B, it can also impact the ratios of potentially beneficial to detrimental radiation. In fact, Williamson et al. (2001) noted that *Daphnia* can increase their survival in the presence of

UV-A exposure following exposure to UV-B due to the stimulation of photoenzymatic repair. Cooke et al. (2006) also mention the possible detrimental effects that high DOC levels in low UV habitats can have on some organisms, such as calanoid copepods.

Although the importance of DOC availability has been demonstrated to be critical to the survival of aquatic organisms, there are other factors that also contribute to the ability for DOC to function as a "natural sunscreen" (Porcal et al. 2009). Certain characteristics of aquatic systems, such as shallow depths (<1 m) and constant mixing, can influence photo-degradation rates exhibited and thus biota may not be as well protected as is expected even if DOC levels are relatively high (Waiser and Robarts 2004). Additionally, acidification can lead to loss of DOC in aquatic systems (Gennings et al. 2014). The estimated attenuation depths for UVR have been observed to double due to changes in DOC attributable to experimental acidification experiments (Williamson et al. 1996). Thorough investigations of DOC concentrations in aquatic systems are therefore critical in determining current levels of UVR exposure that zooplankton and other aquatic organisms may be facing and in predicting which habitats may be at higher risk as the climate continues to change.

Responses to UVR at the individual level have been observed for many aquatic organisms. The up-regulation of mycosporine-like amino acid (MAA) content in response to increases in seasonal UV-B levels appear to complement carotenoid pigmentation levels in copepods, thereby allowing these crustaceans to balance the compounds used to protect themselves against UVR while at the same time reducing their risk against predation (Hylander et al. 2009a). Rautio et al. (2009) also observed that the sources of scytonemin, carotenoids, and MAAs found in certain crustaceans from northern Canada and Alaska originated from phytoplankton or benthic algal mats, supporting the importance of diet and protection against

UVR. Additionally, UVR responses have also been observed vary among trophic levels of aquatic food webs. Differences in UV-B sensitivities between herbivores (e.g., chironimids) and primary producers suggest a significant shift affecting the balance in community structures (Bothwell et al. 1994).

Behavioral changes in aquatic organisms can also result in response to differences in UVR levels. *Daphnia* tend to show strong behavioral responses, while copepods rely mostly on the accumulation of pigments when exposed to UVR (Hansson et al. 2010). Horizontal migration by smaller zooplankton (rotifers and copepods) has been observed to be significantly higher during sunny days with high solar radiation, regardless of overall zooplankton abundance or presence of predators (Ma et al. 2013). However, such shifts in behavior may not occur or even be an option for organisms in many aquatic habitats, especially if they cannot physically engage in them because their habitats have little or no shade and/or are shallow.

As model organisms used in a variety of research areas, rotifers are anomalies of the natural world (Wallace 2002; Inaotombi et al. 2016; Moreira et al. 2016).They are found in diverse aquatic environments, including ephemeral limnoterrestrial habitats such as mosses and ephemeral water bodies that are prone to full sunlight exposure and frequent desiccation episodes. Rotifers may not play a significant role in the trophic energetics in terms of individual biomass, but in large numbers, they represent an important component in aquatic food webs by acting as a food source to a variety of invertebrate predators and the fry of many fishes (Wallace 2002). They also play important roles in nutrient cycling and linking the microbial loop to higher trophic levels (Wallace et al. 2006).

One particularly notable group of rotifers is the bdelloids (phylum Rotifera, class Bdelloidea). Bdelloidea is composed of approximately 450 recognized species that reproduce exclusively by parthenogenesis, a characteristic that has been thought to have been present for at least 40 million years (Welch and Meselson 2000, Ricci and Fontaneto 2009; but see Signorovitch et al. 2015). In very ephemeral habitats, such as rock-pools in the North American desert southwest, bdelloids can be the numerically dominant member of the zooplankton community (Walsh, personal observation).

Most bdelloids are able to undergo a dormancy stage (i.e., anhydrobiosis or cryptobiosis) that is cued by water evaporation (Ricci and Fontaneto 2009). During this time, activity is halted and their metabolism is lowered to undetectable levels, which allows for some species to survive prolonged starvation as well as desiccation (Ricci and Perletti 2006). Following this process, some authors have even suggested that offspring of some species of desiccated bdelloids seem to have increased in fitness and longevity (Ricci 1987, Ricci and Covino 2005). This stage can also serve as a vehicle for passive aeolian transport, thereby allowing them to colonize new habitats. This appears to be the case in the northern Chihuahuan Desert, where high wind and dust events are known to carry various invertebrates in resting stages from playas and other sources across vast distances (up to 150 km from source site; Rivas et al., submitted).

Studies focusing on the questions surrounding the survival of rotifers, especially bdelloids, under extreme conditions have turned to testing yet another intriguing characteristic they possess: their high tolerance to radiation from both sides of the electromagnetic spectrum (Ricci et al. 2005, Gladyshev and Meselson 2008). The monogonont rotifer *Brachionus koreanus* has a LD-50 of 2900 Gy after 24 hours of exposure, whereas *Adineta vaga* is able to survive gamma radiation levels of up to 5000 Gy, albeit with a reduction in fecundity (Krisko et al. 2012,

Won et al. 2016). Successful reproduction has been found to be much more successful in bdelloids under irradiation than at least one species of monogonont rotifer and various other metazoans, which may be due to their ability to undergo anhydrobiosis (Gladyshev and Meselson 2008). In fact, Ricci et al. (2005) demonstrated that anhydrobiotic *Macrotrachela quadricornifera* individuals had a higher survival rate to UV rays (180 nm) than did individuals who remained active. These remarkable abilities have even been the basis for recommending the use of *M. quadricornifera* as a model animal in experiments conducted in space (Ricci et al. 2005).

Behavioral avoidance to UVR in rotifers is minimal, even when their habitat has high levels of exposure (Leech et al. 2005). Bdelloids appear to rely on a variety of mechanisms that support their tolerance to environmental extremes. Radio-resistance in *A. vaga* was determined to be a consequence of an antioxidant system that allowed these animals to protect themselves against protein carbonylation induced by ionizing radiation (Krisko et al. 2012). Exposure to UV-A and UV-B was observed to cause significant double-strand DNA breaks (DSBs) in desiccated *A. vaga* individuals, with UV-B inducing a higher amount of DNA damage (Hespeels et al. 2014). These detected DSBs suggest that the genome of *A. vaga* is not protected when they undergo anhydrobiosis, but rather they possess repair mechanisms that enable them to endure UV radiation during this phase. Fischer et al. (2013) found similar results when they examined the ability of *Philodina roseola* to repair point mutations caused by induced CPDs and discovered that this bdelloid species shielded itself with uncharacterized UV-absorbing compounds to avoid CPD induction, but were mostly unable to repair UV-B-induced damage.

Though investigations in understanding how bdelloids respond to UV radiation exposure have been explored through a handful of studies, there has been a lack of inquiry into

comparisons of responses by bdelloids from populations experiencing natural variation in UVR exposure. Habitat type is likely to be a determining factor in the ability for bdelloids to survive. Having previously been considered to be cosmopolitan in distribution, bdelloids appear to have biogeography that is restricted at the continental level (Fontaneto et al. 2008). Even though this distribution is relatively extensive, there is little information on specific habitats preferred by bdelloids (Fontaneto et al. 2008). Certain environmental conditions may help predict the fitness of populations if a range in severities of stressors are acting upon them and they differ in their abilities to withstand them (Ricci et al. 2007). Ricci (1998) observed differences in the survival of bdelloids that were retrieved from a variety aquatic environments after desiccation. This study concluded that successful recovery from anhydrobiosis was more likely in bdelloids species that originated from desiccation-prone habitats than completely aquatic ones. Additionally, Fischer et al. (2013) suggested that habitat may be a potentially important confounding factor for UV tolerance in rotifers and emphasized the need for further investigations that focus on sampling in terms of both habitat and evolutionary relatedness. Therefore, it may be assumed that populations have evolved or acclimatized to specific stressor levels and that differences in stressor severities, particularly UV-B intensities, may affect the response, and even the longevity, of that population.

Such differences in environmental stressor levels are found within the continental United States, particularly when comparing the arid southwest (i.e., the northern Chihuahuan Desert) with other parts of the country. Water sources in the Chihuahuan Desert tend to be ephemeral and separated by vast stretches of arid landscapes. Dispersal of aquatic microinvertebrates from one location to another in this environment relies upon several forms of assisted dispersal, such as aeolian transport as mentioned above. During this process, organisms may be exposed to high

amounts of UVR, especially if they are entrained to higher altitudes (Blumthaler et al. 1997). In order to address the potential high tolerance in animals undergoing transport, bdelloids collected from a dust event that occurred in the northern Chihuahuan Desert were included in this study. Bdelloids from a man-made lake located in El Paso, TX, and from a temporary rock-pool (i.e, a *hueco*) approximately 50 km northeast of El Paso were also used in order to compare the responses of populations occurring in permanent and temporary water sources from the same arid environment with those that have undergone desiccation and aeolian transport. The responses of these Chihuahuan Desert populations were compared to that of a population from a northern, temperate lake located in Marquette Co., WI, to identify any differences in survival for bdelloids from habitats that vary in natural UVR exposure. To supplement these results, concentrations of DOC were also gathered at each location in two seasons to provide a proxy for the amount of protection from UVR that these bdelloids have available in their natural habitat.

The identification of bdelloid species is difficult to assess through simple morphological determination given that many species superficially look identical to one another and defining characteristics are difficult to observe. Therefore, genetic methods were implemented to classify the species of bdelloids investigated in this study. The mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was used for this purpose, as it is useful in discriminating between closely related species (Hebert et al. 2003) and particularly within bdelloids (Fontaneto et al. 2007).

This study addresses one main objective: to examine UVR-induced mortality in bdelloids from the northern Chihuahuan Desert and from a location with lower year-round UV intensity and no episodes of desiccation. For DOC concentrations, it was predicted that the Wisconsin lake would have the highest concentrations as this lake contains dense macrophyte beds and is surrounded by more terrestrial vegetation that the other two water sources. The hypothesis for

seasonal differences in DOC concentrations was that summer concentrations would be greater than the winter samples for at all three locations, as previous studies have observed similar seasonal patterns (Waiser and Robarts 2004).

Bdelloids from all habitats were predicted to have higher mortality rates at the highest level of UVR exposure following the longest period of desiccation. Additionally, it was hypothesized that bdelloids from the northern lake would be more likely to die following desiccation and exposure to high levels of UV-B radiation, as this lake receives less UVR than the Chihuahuan Desert locations and is always filled with water. The bdelloid population from the man-made lake in El Paso Co., TX, was hypothesized to be the second most sensitive, given that this lake receives ample sun exposure for most of the year but also always has water. The rock-pool population was hypothesized to show the third highest mortality rate given that their habitat is usually dry and has full sun exposure for most daytime hours. Lastly, the bdelloids rehydrated from dust were hypothesized to be the most tolerant as this population derives from individuals who were likely exposed to both of these stressors through the process of water loss at their initial location and while they were at high altitudes as they were transported by wind.

CHAPTER 2: MATERIALS AND METHODS

2.1 COLLECTION, CULTURE OF SPECIMENS, & DOC MEASUREMENTS

Rotifers used in this project were collected from four sources: rehydration of a dust sample, two lakes (one artificial, one natural), and a rock-pool. Dust was collected using passive, standard marble dust collectors (Sow et al. 2006) from one dust-storm event at the University of Texas at El Paso Biology Building rooftop (UTEP BRT) (31° 46' 7.2768"N, 106° 30' 14.544"W; elevation 1,170 m) that occurred on November 15, 2015. This dust originated from a playa system located roughly 70 km southwest from the university (Rivas et al., submitted). Approximately 1 g of dust was rehydrated using artificial hard-water (modified MBL media, Stemberger 1981) and was checked every other day for emerging organisms. Rehydrated bdelloids were transferred to petri dishes and fed a mixture of *Chlamydomonas reinhardtii* (UTEX Culture Collection of Algae at the University of Texas Austin [UTEX] #90), *Rhodomona minuta,* and *Chlorella vulgaris* (UTEX #30) suspended in MBL. Cultures were maintained in the laboratory at room temperature until they were prepared for DNA sequencing or desiccation/ UVR exposure experiments; cultures were cleaned and fed once a week.

Bdelloid rotifers from a permanent lake (Ascarate Lake) in El Paso, TX, (31° 45' 14.5902"N, 106° 24' 14.1726"W; elevation 1,127 m) and from an ephemeral rock-pool (referred to as Kettle 4) at Hueco Tanks State Park and Historic Site (Hueco Tanks hereafter) (31°55'6.69"N, 106° 2'24.61"W; elevation 1,382 m) were collected in September 2015. Water samples were also collected on these dates, as well as in December 2016 for Ascarate Lake and January 2017 for Kettle 4. Ascarate Lake (Figure 2.1) is a lake located within the city of El Paso, TX, and neighbors a natural gas refinery and a golf course. The maximum depth is 1.8 m and it

has a surface area of 19.4 hectares ("Ascarate Lake", *fishing notes.com*). Cattails and reeds grow along some of the edges of the lake, and filamentous algae are also present. Fish stocking, typically Rainbow trout (*Oncorhynchus mykiss*) and catfish (species not specified), is conducted for recreational purposes ("Ascarate Park, El Paso Co.", *epcounty.com*). The lake tends to have high conductivity and high nutrient loading (Walsh et al., unpublished data). Frequent episodes of golden algae (*Prymnesium parvum*) blooms in the winter have affected the fish community in the lake and have led to relatively large fish-kill events in past years ("Texas Parks and Wildlife: Golden Alga Bloom Report", *tpwd.texas.gov*). Sprinkler systems have been placed within the lake to act as aerators to help increase oxygenation and improve water quality. The zooplankton community is diverse, including from those from the phylum Rotifera.

Figure 2.1. Photograph of Ascarate Lake in El Paso Co., Texas showing aeration system. Photo credit: Digital Information Gateway in El Paso, El Paso Museum of History

Kettle 4 (Figure 2.2) is a small rock-pool (a.k.a., *hueco*) located in the eastern region of Hueco Tanks in what is referred to as Mescalero Canyon. It is located on a rocky outcrop formed

of syenite rock with a depth of approximately 46 cm and a maximum length of 62 cm. It is typically filled only after rains and has no macrophytes growing in it, although algae does grow along the bedrock and detritus (leaves, sticks, etc.) is sometimes blown in from surrounding vegetation. Accumulation of this foreign organic matter sometimes leads to water chemistry changes, such as changes in water color to a yellowish-tint and increasing nutrient concentrations (Table 1). It receives direct sunlight for the majority of the day, as the closest vegetation consists of grasses and small shrubs that are approximately 1.5 m away. The high rock formations that make up the canyon in which the rock-pool is found likely shade it during the early mornings and afternoons. The rotifer community is limited; in over 20 years of survey data, only seven species have been detected (Walsh, unpublished data) and is dominated by the bdelloid species included in this study.

Figure 2.2. Photograph of Kettle 4 at Hueco Tanks State Park & Historic Site, El Paso Co., TX. Photo credit: Maite Martín

Rotifers from Birch Lake, Marquette Co., WI, (46° 12' 40.3848"N, 89° 50' 11.6412"W; elevation 501 m) were collected in June 2015 by Dr. Robert L. Wallace (Ripon College, WI,

USA) and were sent to our laboratory for identification, culturing, and subsequent analysis. Birch Lake (also referred to as Moon Lake; Figure 2.3) is 29.5 hectares in size with an approximate maximum depth of 22.3 m and a mean depth of 1.5 m (Moon Lake (Birch), *Lake-Link.com*). Vascular hydrophytes tend to be densely packed along the shoreline, and freezing events typically occur during the winter months (Wallace, personal communication). A variety of fishes are found in the lake, including Bluegill (*Lepomis macrochirus*), Largemouth Bass (*Micropterus salmoides*), and Northern Pike (*Esox lucius*) (Moon Lake (Birch), *Lake-Link.com*); there is also a diverse zooplankton community assemblage.

Figure 2.3. Photograph of Birch Lake in Marquette Co., WI. Photo courtesy of R.L. Wallace.

Unfiltered water samples were also collected from Birch Lake, Ascarate Lake, and Kettle 4 for DOC analysis. Water temperatures were recorded for all three locations, and additional water chemistry parameters were measured at Ascarate Lake and Kettle 4 using a YSI 556 multiparameter probe, a YSI 9000 field spectrometer, and YSI water test kits (see Table 3.1). Collections were made at each site twice: summer 2015 and early winter 2016 for Ascarate Lake

and Birch Lake, and summer 2015 and winter 2017 for Kettle 4. At least 1 L of water was collected at each location using brown, pre-acid washed (2% HCl acid) plastic bottles, which were stored at 4˚C for a short period (<24 hr). The water was then passed through a pre-ashed GF/F glass fiber filter (0.45 µm; Whatman, Springfield Mill, UK) and dispensed into 15 mL glass vials; care was taken so that no air bubbles were trapped inside. The vials were kept at 4˚C in the dark until they were analyzed. Three subsamples from each location/season were used to assess instrumentation error, but because there was only one water sample from each location for each season, they do not represent environmental replicates. For the 2015 samples, DOC analysis was conducted using a Lachat IL 550 TOC/TN Analyzer (Lachat Instruments, Inc.) following the manufacturer's protocol and with appropriate standards. Due to an analyzer malfunction in early 2016, the 2016 and 2017 samples were sent to University of Texas at Austin's Marine Science Institute Core Facility Laboratory in Port Aransas, TX for analysis.

2.2 Species Identification by Barcoding

Five to ten bdelloids per source population were isolated and transferred to a nine-well dish containing distilled water. Individuals were serially transferred from well to well in order to remove algae and other contaminants. DNA templates for each species were extracted using a Chelex protocol (InstaGene Matrix; Bio-Rad). While mixing, 13 µl of Chelex were dispensed into each sample tube. Samples were then placed on a heat block at 100°C for 8 min, vortexed at high speed for 10 sec, and centrifuged for 2 min. DNA templates were then placed on a TECHNE TC-412 (Cole-Parmer®, Staffordshire, UK) thermocycler and were heated at 56˚C for 1 hr, followed by a final extension step at 99˚C for 10 min. Templates were then frozen at -80°C until they were ready to be used for PCR amplification.

Templates used to sequence the COI gene were amplified by PCR using the primer pair LCO (5'-GGTCAAAAATCATAAAGATAT-3') and HCO (5'-TAAACTTCAGGGTGACCAAAA-3') (Folmer et al. 1994). Amplification, denaturation, and annealing took place in the thermocycler at the temperatures of 92°C for 1 min, 47°C for 2 min, and 72°C for 3 min, respectively, and the cycle was repeated 35 times with a final extension step at 72°C for 7 min. Gel electrophoresis was then used to visualize the DNA product. Amplified DNA was excised from the gel and purified using Gene Clean III® kits. DNA was then sequenced at UTEP's BBRC Genomics Analysis Core Facility, and retrieved sequences were visually inspected and cleaned using SeqScanner2 (Applied Biosystems) and FinchTV v 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) software.

Contiguous sequences were created using CAP3 assembly program (Huang and Madan 1999). Sequences were then compared to those in the NCBI GenBank BLAST® library to ensure that amplified products were from phylum Rotifera. Reverse complement sequences were translated using a free online tool (www.bioinformatics.org/sms/rev_comp). MUSCLE (Edgar 2004) was used through the EMBL-EBI website (www.ebi.ac.uk) to construct a multiple sequence alignment (Appendix 2).

The CIPRES Science Gateway (Miller et al. Schwartz 2010; https://www.phylo.org/portal2/home.action) was used for Bayesian tree construction using MrBayes (v3.2.6 on XSEDE; Ronquist et al. 2012). Bayesian analysis was run for 5 million generations, with sampling every 10,000 generations, using a general time reversible model with a proportion of variable sites and a gamma-shaped distribution of rates across sites (GTR+I+G), which was identified by jModelTest2 v 2.1.10 (Darriba et al. 2012) as the best-fit model for COI sequence evolution for this dataset. The monogonont rotifer species *Brachionus calyciflorus*

(GenBank accession # GU232733.1) was included as the outgroup. All other settings were left as default. The resulting consensus tree was visualized and edited using FigTree software (v 1.4.2; Institute of Evolutionary Biology, University of Edinburgh; http://tree.bio.ed.ac.uk/).

2.3 DESICCATION & UV EXPOSURES

A Spectroline® XX-15B lamp (120 v, 60 Hz, 0.7 AMPS) (Spectronics Corporation) was suspended 28.5 cm above a shelf inside a dark incubator. UV-B intensity was measured using a Digital UV Radiometer (Model 6.2 UVB, Solartech, Inc.) before each exposure experiment in order to make sure the lamp emitted a maximum intensity of 370 $\mu W/cm^2$. This radiation intensity was chosen based on the average greatest erythemal radiation (UV_{Ery}) values recorded in the summer months from 2006 to 2016 at a monitoring station in Las Cruces, NM (37 W/m^2 UV_{Ery}; Site Code NM01, UV-B Monitoring and Research Program, Colorado State University). This erythemally weighted UVR was converted to UV-B equivalents following McKenzie et al. (2004). The greatest exposure level (370 $\mu W/cm^2$) was approximately 1.4 times greater than the estimated UV-B amount for Las Cruces (279 $\mu W/cm^2$).

Rotifers were at least eight days old at the time of desiccation, as this age corresponds to the highest recovery rates after anhydrobiosis (Ricci 1998). To ensure age, individuals from each population were kept for seven days in wells containing the algae mix. Roughly 24 hr before exposure, the algae in the wells was removed and replaced with fresh MBL, which served to clear as much food from the animals as possible. Thin polycarbonate filters (0.4 µm; Whatman, Springfield Mill, UK) were cut into small pieces and placed in four glass petri dishes. Approximately 200 individuals were divided evenly among the filters (50 animals per dish); the filters were left with a thin layer of MBL to maintain moisture. To allow the filters to dry slowly,

dishes were kept in a plastic container lined with wet paper towels and maintained at approximately 97% relative humidity (RH) for 48 hr; RH was measured with a Traceable™ remote air humidity monitor (VWR®). RH was then slowly decreased to approximately 40% and maintained at 40% for the corresponding desiccation time prior to exposure (24 hr, 1 week, or 1 month) (modified from Ricci et al. 2003). Desiccation treatments were done in replicates of five for each location.

Following the desiccation period, the dishes were haphazardly assigned to four treatments in which they were either placed inside a cardboard box (negative control) or covered with a quartz disk (370 μW/cm^2, positive control), a 305 nm glass UV filter (130 μW/cm^2), or a 320 nm glass UV filter (26 μW/cm^2). All dishes were haphazardly assigned to a location below the UV lamp inside the incubator.

Rotifers were exposed to UVR for 2 hr in a dark incubator at 25˚C. This exposure time was chosen following the study conducted by Ricci et al. (2005) (1 hr 20 min). After exposure, 5 mL of MBL were added to all dishes and they were checked 48 hr after exposure, at which point animals were removed and identified as living or dead. Rotifers were considered to be "alive" if they were swimming, moving along the bottom, and/or were attached to the dish with visible movement. Animals classified as "dead" were checked again after 24 hr to make sure they were not simply immobile and wrongly classified.

2.4 Statistical Analyses

Averages and standard errors were determined for the DOC measurements for subsamples from each location/season. Due to a low sample size, comparisons were not made among locations and/or seasons. Therefore, additional statistical analyses were not possible. For

the desiccation and UV exposure experiments, logistic regressions were done using generalized linear models (GLM) to identify significant differences in mortality probabilities for main effects and their interactions: population x desiccation time, population x exposure intensity, and desiccation time x exposure intensity. Akaike information criterion (AIC) values and residual deviances from all models, except that with the 3-way interactions, were obtained and compared to determine the model with the best fit. A *post-hoc* test was carried out in a pairwise fashion to disentangle the individual contributions of the factors to a significant, combined effect using a Tukey HSD correction for multiple comparisons. Odds ratios were constructed to examine any variation in mortality estimates across combinations of the variables tested. All statistical analyses and graphs were done using RStudio™ v 3.3.2 (R Core Team 2016). The {multcomp} (v 1.4-6) package was used to conduct the pairwise comparisons Tukey HSD tests. DOC and mortality probability bar graphs were constructed using the {ggplot2} package (v 2.2.1), and the {lattice} package (v 0.20-34; Sarkar 2008) was used to make the mortality probability scatter plot.

CHAPTER 3: RESULTS

3.1 DOC ANALYSIS

Average DOC concentrations ranged from 13.4 ±0.1 to 22.2 ±0.4 mg/L in the summer samples and 16.1 ±1.6 to 36.9 ±1.6 mg/L in the winter samples, with Ascarate Lake consistently having the lowest levels and Kettle 4 having the highest (Table 3.1). It must be noted once again that the number of DOC samples used in this study acted as instrumentation subsamples, not replicates within a habitat. Although differences were observed, whether they were statistically significant cannot be determined. However, there were observable differences in DOC concentrations for Kettle 4 and Ascarate Lake, with Kettle 4 concentrations being over twice as great (Figure 3.1).

There also appeared to be seasonal differences with respect to the Kettle 4 winter concentrations. This sample had 62% greater DOC concentrations than the summer sample at the rock-pool and was almost 3 times greater than the concentrations for the Ascarate Lake summer sample, which had the lowest concentrations. Once again, these values were not able to be compared statistically.

Table 3.1. Water chemistry parameters for the three water sources compared in this study. Sites were sampled in summer 2015 and winter 2016/2017. Most water chemistry parameters were not measured for Birch Lake. SPHS= State Park and Historic Site; N/A= not available; EC= electrical conductivity; DO = dissolved oxygen.

Parameter	Ascarate Lake El Paso, Co., TX		Kettle 4 Hueco Tanks SPHS, El Paso Co., TX		Birch Lake, Marquette Co., WI	
	9/19/2015	12/13/2016	9/26/2015	1/10/2017	9/13/15	12/19/2016
pH	8.23	7.76	9.34	7.86	8.1	N/A
DO (%)	N/A	77.5	N/A	3.67	N/A	N/A
EC ($\mu S/cm^2$)	4363	3914	98	498	N/A	N/A
Temperature (˚C)	25.24	10.5	26.21	7.89	18	1
Average DOC (mg/L)	13.42 ±0.99	16.05 ±1.6	22.21 ±0.41	36.85 ±1.6	19.63 ±0.01	20.6 ±1.5
Turbidity (FTU)	11	8	14	29	N/A	N/A
Ammonia (mg/L NH_4-N)	0.10	0.04	0.06	Not detected	N/A	N/A
Nitrite (mg/L NO_2-N)	0.000	0.01	0.003	Not detected	N/A	N/A
Nitrate (mg/L NO_3-N)	0.072	0.085	0.089	0.68	N/A	N/A
Phosphate (mg/L PO_4-P)	0.18	0.01	0.099	Not detected	N/A	N/A
Alkalinity (mg/L $CaCO_3$)	135	168	30	140	N/A	N/A
Hardness (mg/L $CaCO_3$)	500	250	34	85	N/A	N/A
Silica (mg/L SiO_2)	38.8	N/A	38.8	N/A	N/A	N/A
Chloride (mg/L Cl^-)	232	300	Not detected	Not detected	N/A	N/A
Sulfate (mg/L SO_4)	430	390	Not detected	Not detected	N/A	N/A
Color (mg/L Pt)	10	20	70	160	N/A	N/A

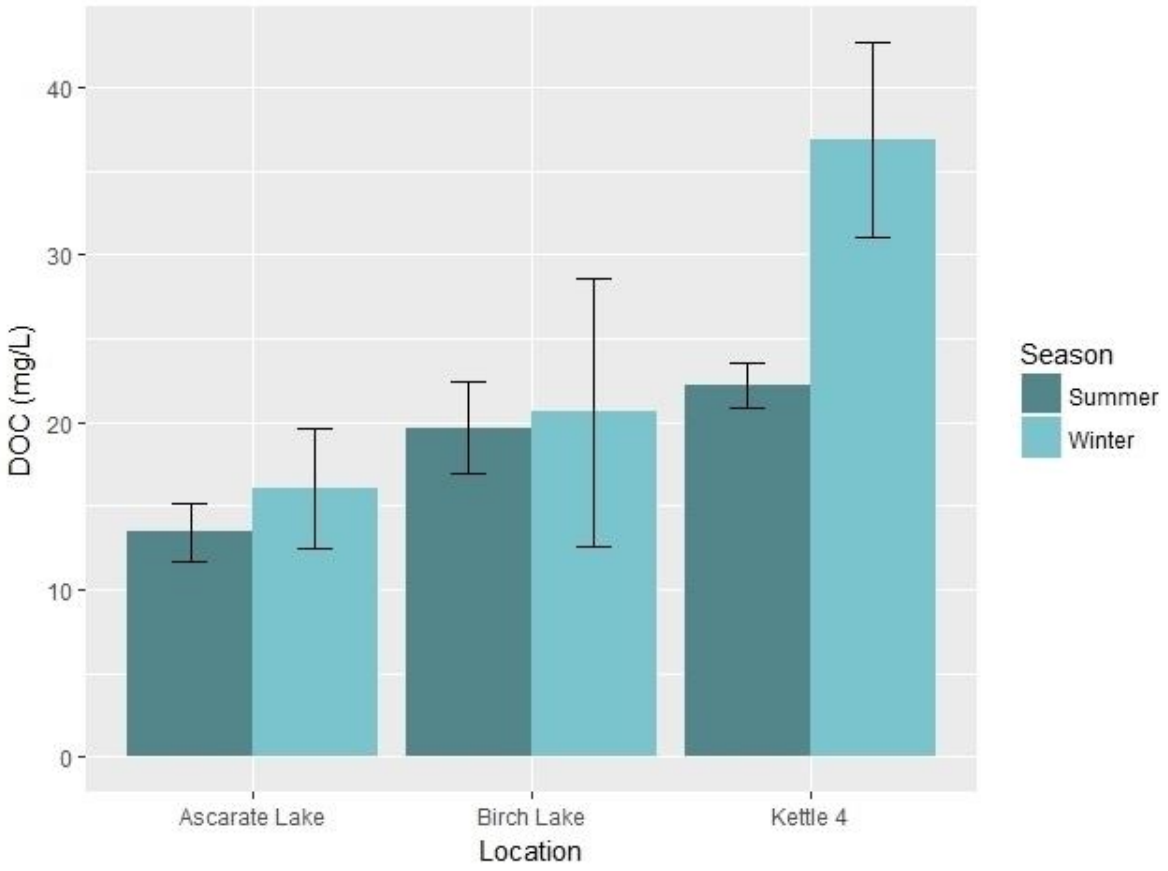

Figure 3.1. Mean dissolved organic carbon (DOC) concentrations for water from three locations collected in the summer and winter seasons. Each bar represents one sample. Error bars represents instrumentation standard errors.

3.2 Genetic Sequence Analysis

A total of 613 base pairs were sequenced for the COI gene. BLAST® searches of this gene are summarized in Table 3.2 showing the top five hits for each population sequence with E-values of <0.0001 for support. For sequences from bdelloids from both Birch Lake and BRT, all top five hits provided support for *Macrotrachela quadricornifera* matches, with a 99% identity match for the Birch Lake sequence and a 97% match for the BRT sequence (89% query cover for both). Additionally, the Kettle 4 sequence had hits with *Philodina* sp. sequences, with one match to *Philodina roseola* (97% identity match and query cover). On the other hand, the results for the Ascarate Lake sequence were not as clear, as there were multiple hits with both *Philodina* and *Abroctha* sequences. However, based on preliminary morphological identification, it is likely that this population is a species of *Philodina*.

Bayesian analysis produced a consensus tree showing high to moderate support for some of the bdelloid clades (Figure 3.1). The Kettle 4 sequence resulted in an identical match to two *Philodina* sp. sequences (posterior probability of 1.0) that had been obtained from bdelloids that had been collected by Birky Jr. et al. (2005) from a rock-pool at Hueco Tanks in 2001. Although the specific kettle name is not identified in the 2005 study, the bdelloids were either collected from the same kettle as the one studied here or 1 of the 5 nearby (<0.5 m) rock-pools (Walsh, personal communication). The BRT sequence did not cluster with any of the sequences for species available in GenBank but appears to be a sister group to *Macrotrachela quadricornifera* (posterior probability of 0.96). The results for these two sequences reinforce the BLAST® matches mentioned above.

There were some discrepancies for the other two sequences with respect to the Bayesian analysis and results obtained from the BLAST® searches, particularly for the Birch Lake

sequence. This sequence did not produce a clear match to a particular bdelloid species, although it was found in a clade within two *Rotaria* species with a moderately supported posterior probability of 0.71. Ambiguities likely result from near saturation of sequence evolution among these taxa and/or lack of representative sequences from the appropriate bdelloid species. The COI gene sequences available in GenBank that are belong to specimens identified to the species name consist of only 19% of the known bdelloid species. This was strikingly different to the *M. quadricornifera* matches that were provided in the BLAST® outputs for the BRT and Wisconsin populations. Additionally, the Ascarate Lake sequence is sister sequence to several *Philodina megalotrocha* sequences with a high level of support (posterior probability of 1.0; Figure 3.1). However, the size of the corona in these bdelloids is visibly smaller than the characteristic wide corona of *P. megalotrocha*. Although this population is likely to be *Philodina* as mentioned above, they cannot be confirmed to be this species. Additional morphological identification will be needed to determine the correct species for both of these populations.

Table 3.2. BLAST® results showing top 5 hits for sequences from each population studied.

Population	BLAST® Results Summary			
	Species	**Identity Match**	**Query Covered**	**E-value**
Birch Lake Marquette Co., Wisconsin	*Macrotrachela quadricornifera*	99%	89%	0.0
	Macrotrachela quadricornifera	99%	89%	0.0
	Macrotrachela quadricornifera	99%	89%	0.0
	Macrotrachela quadricornifera	99%	89%	0.0
	Macrotrachela quadricornifera	99%	88%	0.0
Ascarate Lake El Paso, TX	*Philodina megalotrocha*	100%	88%	0.0
	Abrochtha sonneborni	100%	87%	0.0
	Philodina sp.	98%	88%	0.0
	Abrochtha kingi	98%	87%	0.0
	Abrochtha sonneborni	98%	87%	0.0
BRT UTEP, El Paso, TX	*Macrotrachela quadricornifera*	97%	89%	0.0
	Macrotrachela quadricornifera	97%	89%	0.0
	Macrotrachela quadricornifera	97%	89%	0.0
	Macrotrachela quadricornifera	97%	89%	0.0
	Abrochtha sonneborni	96%	89%	0.0
Kettle 4 Hueco Tanks SPHS El Paso Co., TX	*Philodina* sp.	90%	100%	0.0
	Philodina roseola	97%	97%	0.0
	Philodina sp.	90%	98%	0.0
	Philodina sp.	90%	98%	0.0
	Philodina sp.	90%	97%	0.0

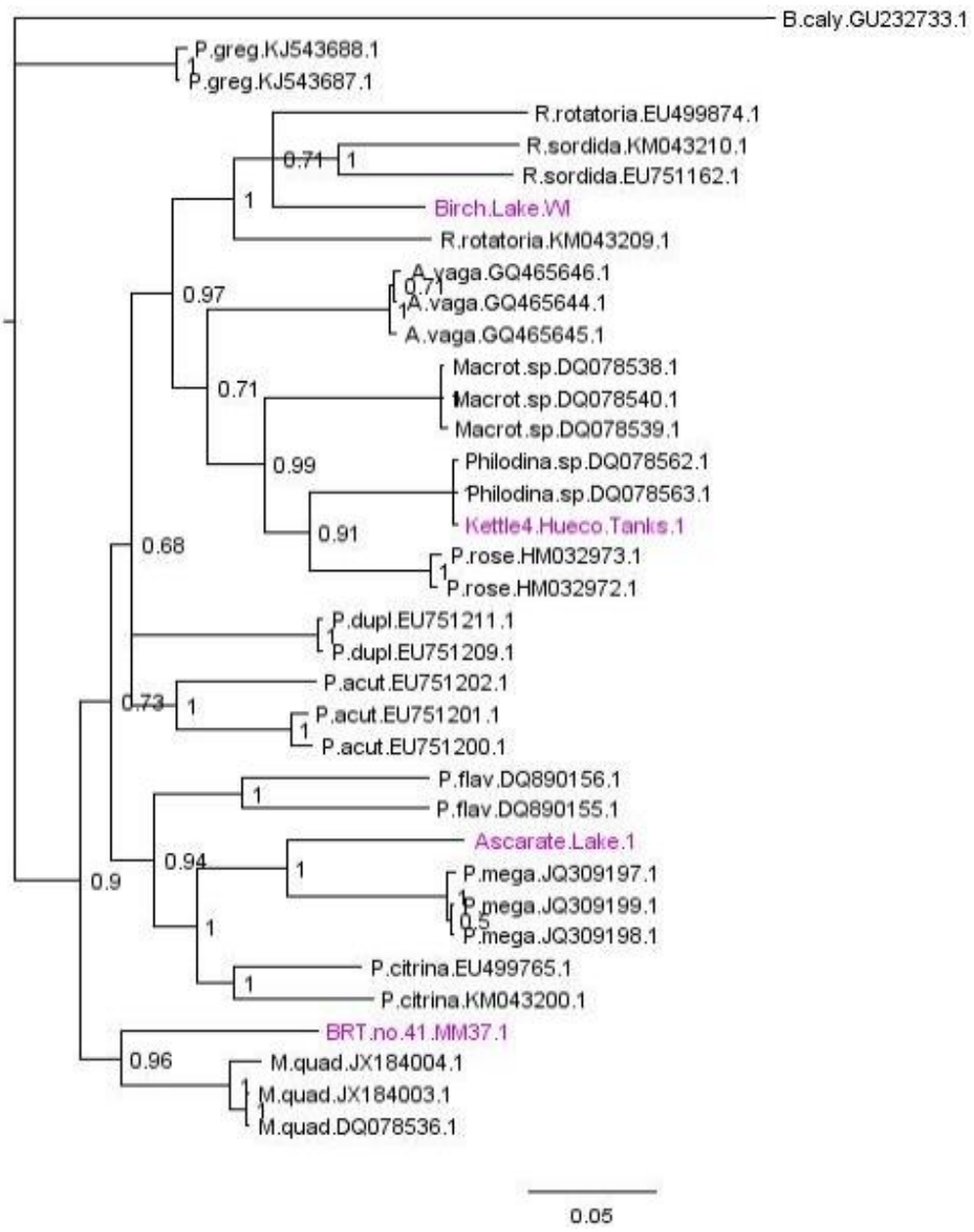

Figure 3.2. Phylogenetic relationships inferred by Bayesian analysis of COI sequences under a (GTR+I+G) substitution model for bdelloids from Ascarate Lake (El Paso Co., TX), Birch Lake (Marquette Co., WI), Kettle 4 (HTSPHS, El Paso Co., TX), and Biology Roof Top (BRT) (UTEP, El Paso Co., TX) and selected species from GenBank. One isolate of the monogonont rotifer *Brachionus calyciflorus* was used as the outgroup. Posterior probabilities are shown at nodes. Color-coded species represent lineages used in this study. Scale bar indicates substitutions/site. Accession numbers and species abbreviations for sequences obtained from GenBank are given in Appendix 1.

3.3 Interactions Between Desiccation Times & UVR Exposures

To investigate the synergistic effects of desiccation time and UVR exposure on mortality of bdelloids from different population sources, both the main effects and the interaction of these parameters were analyzed. Logistic regressions using GLM analyses with a logit link function and binomial error structure were performed for bdelloid mortality proportions measured as a response to population source, UVR exposure level, and time desiccated, along with their 2-way interactions. AIC values and residual deviances calculated from each model were compared in order to determine the model with the best fit for the data (Table 3.3). The model using all three 2-way interactions was selected for further interpretation as it had the lowest AIC value (AIC = 2464.4, residual deviance = 1594.4, d.f. =210). The second best model included the interaction between the population source and desiccation time; this model was 60 AIC units greater than the selected model (AIC = 2524.5, residual deviance = 1639.6, d.f. =255). The rest of the models had over 100 AIC units more than the selected model, with the model without any interactions had an AIC value 200 units over the selected model (AIC = 2668.9, residual deviance = 1795.9, d.f. =231).

A likelihood ratio test (LRT) was conducted using the selected model to test the overall goodness of fit for desiccation time, UVR exposure, and population origin (Table 3.4). All main effects and interactions significantly impacted the probability of mortality for bdelloid populations (X^2, p-value <0.001). These results indicate that the probability of death in bdelloids increased with the time of desiccation (X^2 = 461.24, d.f. = 2, p-value <0.001), their population source (X^2 = 1972.87, d.f. = 3, p-value <0.001), and the level of UVR exposure (X^2 = 504.57, d.f. = 3, p-value <0.001). The 2-way interaction combinations for every treatment also demonstrated a significant effect on the probability of mortality. Specifically, for bdelloids of any one

population, both time of desiccation and level of UVR exposure had a significant effect on this probability (X^2 = 34.73, d.f. = 9, *p*-value <0.001 for UVR exposure; X^2 = 119.69, d.f. = 6, *p*-value <0.001 for desiccation time). At any specific level of exposure, time of desiccation also had a significant effect on mortality, independent of population (X^2 = 92.11, d.f. = 6, *p*-value <0.001). Appendix 3 summarizes this information for all the main effects and their interactions.

Table 3.4 shows the fitted values for the probability of mortality obtained from the estimated coefficients provided by the GLM test as shown in Appendix 3. As predicted, a pattern where mortality probabilities increased as drying time and exposure levels increased was observed for all possible interactions except for Ascarate Lake and BRT at full exposure (370 μW/cm^2) following 1 week of desiccation (Figure 3.3). These values are also shown in Figure 3.3.

There appeared to be some slight decreases in mortality following 1 week of desiccation for BRT and Ascarate Lake populations at the highest level of exposure (Figure 3.3). It is unclear whether the differences between those values and the values at different desiccation times were significant (i.e. the difference between 24 hr to 1 week and 1 week to 1 month at 370 μW/cm^2 of exposure). *Post-hoc* pairwise comparisons of the interaction effects were carried out using Tukey HSD tests, but because these tests involved only 2-way interactions, they did not address interactions among all three main effects. These dips in mortality may be due to some synergistic effect between desiccation time and the highest exposure level for these two populations, but it is not yet clear as to why they occurred.

When testing the interaction between population and desiccation time, the Tukey test was applied to determine the differences between mortality probabilities using the average probabilities of the four UV exposure levels. Figure 3.4 illustrates these differences; since most

interactions appeared to be significant (Tukey HSD, *p*-value <0.05), only the non-significant relationships between the interactions are shown for clarity. For example, after 24 hr of desiccation, mortality probabilities for the Kettle 4 population did not differ significantly than either the Ascarate Lake or BRT populations after 1 week of desiccation. There was no significant difference between the averaged mortality probabilities after 24 hr and 1 week for either the Ascarate Lake or BRT populations (Tukey HSD, *p*-value=0.998 and *p*-value=0.999, respectively). However, after 1 month of desiccation, both Ascarate and BRT bdelloids had higher mortality probabilities than after only 1 week, without incorporating the effects of different exposure levels (*p*-values<0.001 for both populations). All interactions are presented in Appendix 4.

The interaction between population and UVR exposure was also examined using a Tukey HSD test. Figure 3.5 depicts average probabilities for the three desiccation times, showing only the insignificant interactions (Tukey HSD, *p*-values>0.05). Overall, the probability of mortality increased with increasing UVR exposure for all four populations. Birch Lake bdelloids were consistently significantly more likely to die as a result of treatments than bdelloids from the other populations (Tukey HSD, *p*-values<0.01), although there were some insignificant differences between some of the other populations at the next level of exposure. For example, the probability of mortality for Birch Lake individuals exposed to 130 $\mu W/cm^2$ ranged from approximately 1.3 times higher than the Kettle 4 population to 2 times higher than Ascarate Lake. The mortalities observed for the negative control (no exposure) experiments are likely due to desiccation time interactions; as mentioned above, statistically significant variation was not obtained. All interactions are summarized in Appendix 5.

The interactions between UVR levels and desiccation times were also assessed to determine the combined effects of mortality on bdelloids without the added influence of their

population source. Again, the results are presented with only the insignificant differences identified (Figure 3.6; Tukey HSD, *p*-values>0.05). Similarly to what was observed for the UVR exposure and population interaction, there was a general increase in the probability of mortality for the three desiccation regimens with the increase in exposure level (Tukey HSD, *p*-values<0.01). With the exception of the highest exposure level, the least time spent in a desiccated state (24 hr) had a significantly lower probability of death than the longer time regimes (Tukey HSD, *p*-value<0.01). All interactions are summarized in Appendix 6.

Table 3.3. Akaike Information Criterion (AIC) for GLM tests for all possible two-way models including the main effects (UVR exposure, desiccation time, population source) and their interactions. ΔAIC = differences in AIC from the best model. Residual deviances and degrees of freedom (d.f.) were also provide by each GLM test.

Model	AIC	ΔAIC	Residual Deviance	Residual d.f.
Desiccation Time x Filter + Population x Filter + Population x Desiccation Time	2464.4	-	1549.4	210
Population x Desiccation Time	2524.5	60.1	1639.6	225
Desiccation Time x Filter	2588.8	124.4	1703.8	225
Population x Filter	2652.5	188.1	1761.6	222
No interaction	2668.9	204.5	1795.9	231

Table 3.4. Results of a likelihood ratio test (LRT) applied to logistic regression model selected. Main effect coefficients are listed first followed by the 2-way interactions. All coefficients indicate significant effects in the model (*p*-values<0.001).

Coefficient	*df*	Deviance	Residual *df*	Residual Deviance	*p*-value
Null			239	4734.6	
Desiccation Time	2	461.24	237	4273.4	2.2e-16
UV Filter	3	1972.87	234	2300.5	2.2e-16
Population	3	504.57	231	1795.9	2.2e-16
Desiccation Time:UV Exposure	6	92.11	225	1703.8	2.2e-16
UV Exposure:Population	9	34.73	216	1669.1	6.7e-05
Desiccation Time:Population	6	119.69	210	1549.4	2.2e-16

Table 3.5. Fitted values for bdelloid rotifer mortality probabilities based on GLM test statistic for four populations of bdelloid rotifers. Values in red indicate unexpected probabilities that differed from the pattern observed in the rest of the data (increase in desiccation time + increase in exposure = increase in mortality probability). BRT= Biology Roof Top; Qz. = Quartz filter. Filters refer to the following exposure levels: 320 nm = 26 µW/cm^2, 305 = 130 µW/cm^2, and Quartz = 370 µW/cm^2. Box is the negative control = no exposure.

		Population			
Desiccation Time	**Filter**	**Ascarate Lk.**	**Birch Lk.**	**Kettle 4**	**BRT**
1 day	Box	0.082	0.122	0.090	0.077
	320	0.118	0.206	0.153	0.137
	305	0.173	0.395	0.248	0.219
	Qz.	0.657	0.801	0.645	0.578
1 week	Box	0.093	0.343	0.200	0.083
	320	0.151	0.528	0.345	0.166
	305	0.204	0.723	0.471	0.245
	Qz.	0.510	0.877	0.685	0.413
1 month	Box	0.171	0.305	0.209	0.214
	320	0.335	0.570	0.439	0.456
	305	0.452	0.778	0.600	0.609
	Qz.	0.793	0.916	0.807	0.794

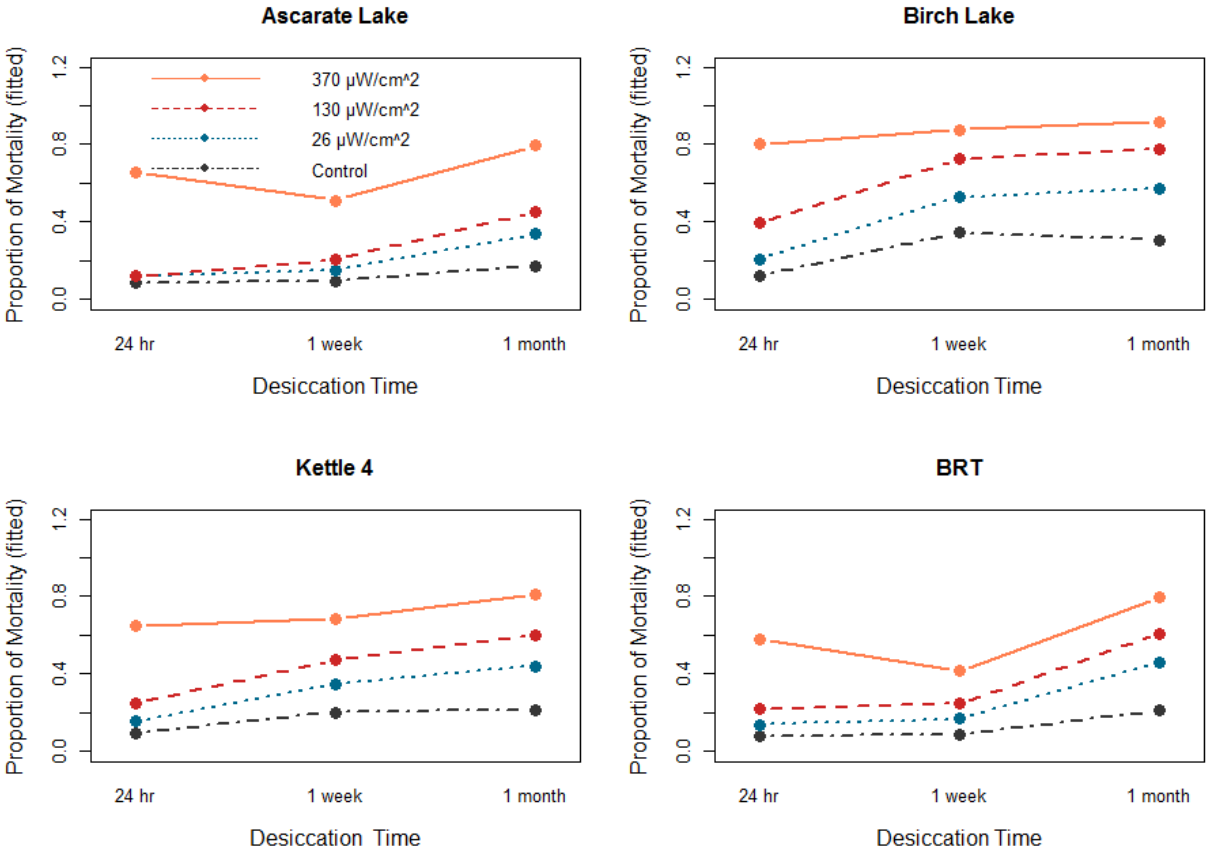

Figure 3.3. Mortality proportions for selected bdelloid rotifers from four populations under varying desiccation times and UVR exposure levels. Values represent fitted probability values based on a general linear model (GLM).

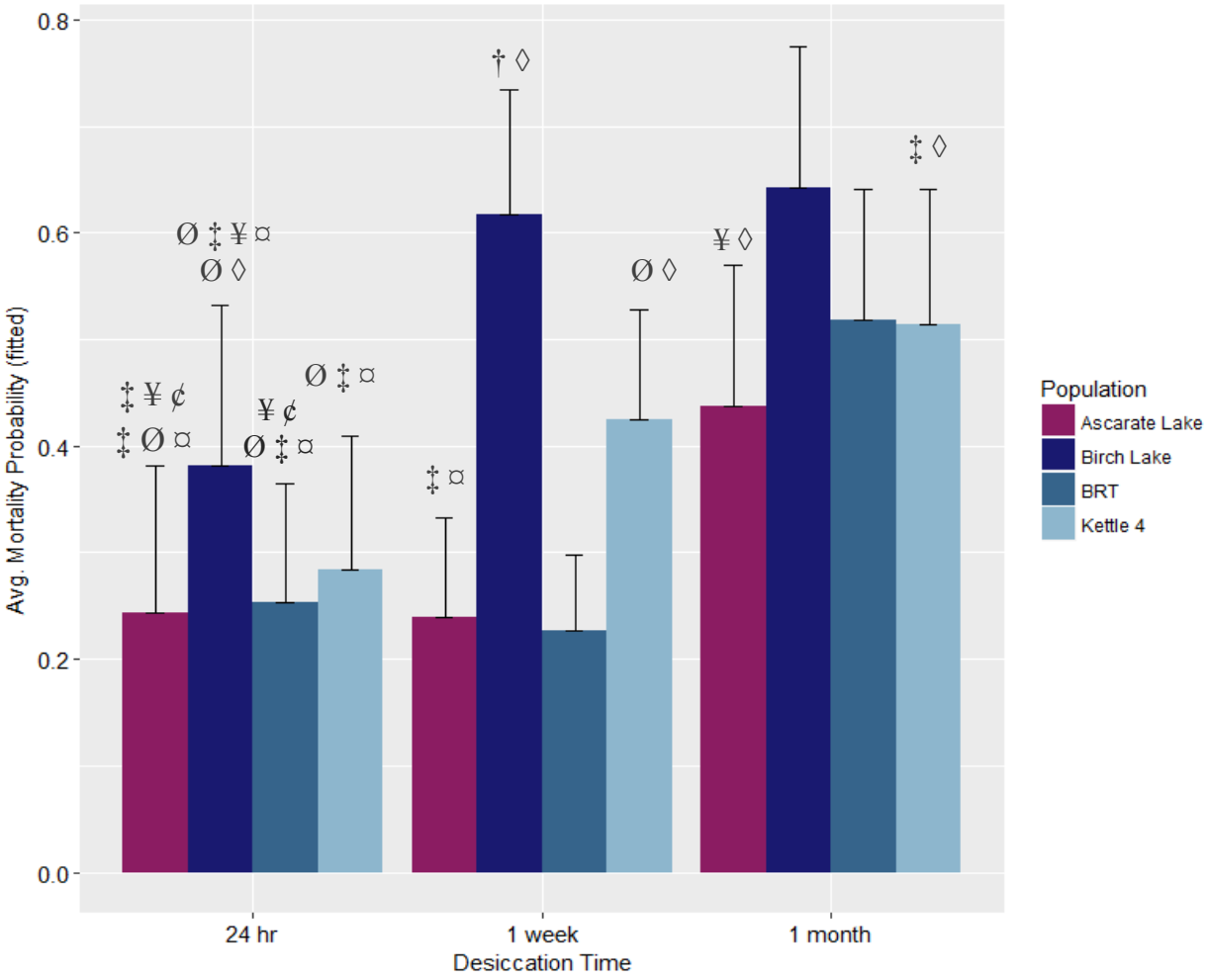

Figure 3.4. Average mortality probabilities for selected bdelloid rotifers from four populations under varying desiccation times. Each column represents fitted probability values based on a general linear model (GLM) averaged from four different UV exposures. Error bars represent standard errors. Combination of the following symbols indicate no significant (NS) differences (Tukey, $p>0.05$) to the specified parameter(s): Ø = NS difference from Ascarate Lk.; † = NS difference from Birch Lk.; ‡= NS difference from BRT; ¥ = NS difference from Kettle 4; ¢ = NS difference for 24 hr desiccation time; ¤ = NS difference for 1 week desiccation time; ◊ = NS difference for 1 month. Symbols are listed by population(s) first, followed by the dry period for which they correspond to.

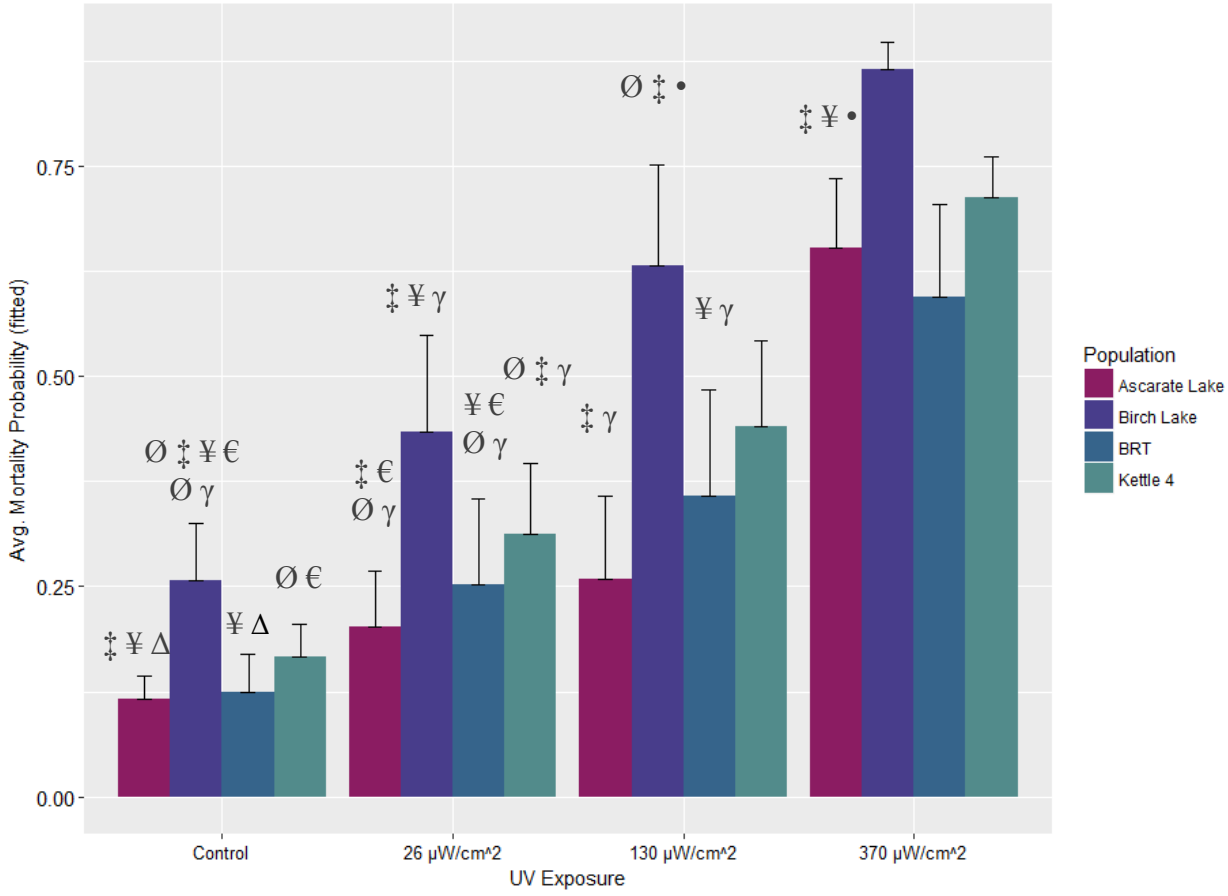

Figure 3.5. Average mortality probabilities for selected bdelloid rotifers from four populations under varying UVR exposures. Each column represents fitted probability values based on a general linear model (GLM) averaged from three desiccation durations. Error bars represent standard errors. Control treatment corresponds to no UV exposure. Combination of the following symbols indicate no significant (NS) differences (Tukey, $p>0.05$) to the specified parameter(s): Ø = NS difference from Ascarate Lk.; † = NS difference from Birch Lk.; ‡= NS difference from BRT; ¥ = NS difference from Kettle 4; Δ = NS difference for control; € = NS difference for 320 nm filter; γ = NS difference for 305 nm filter; • = NS difference for Quartz filter. Symbols are listed by population(s) first, followed by the corresponding UV exposure intensity.

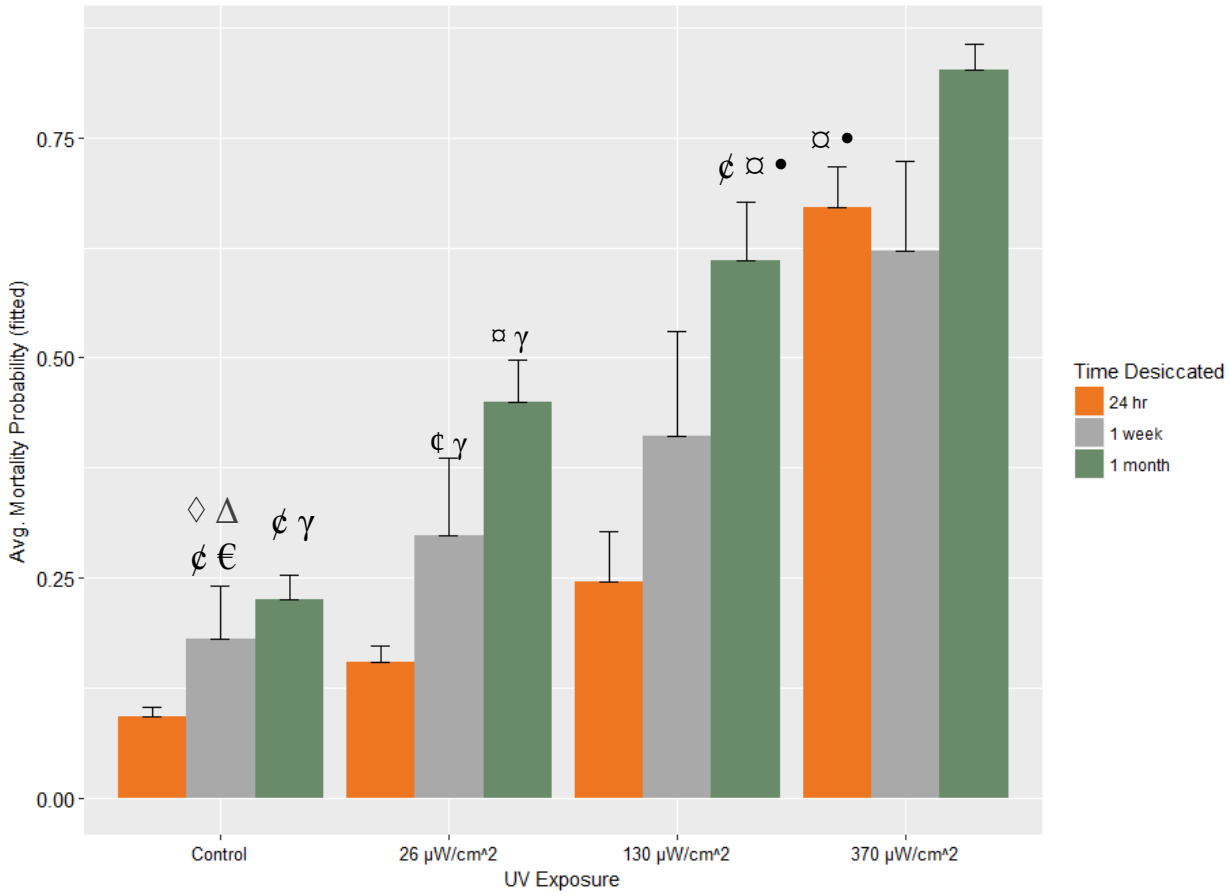

Figure 3.6. Average mortality probabilities for bdelloid rotifers under varying UVR exposures following three desiccation times. Each column represents fitted probability values based on a general linear model (GLM) averaged from four populations. Control treatment corresponds to no UV exposure. Error bars represent standard errors. Combination of the following symbols indicate no significant (NS) differences (Tukey, $p>0.05$) to the specified parameter(s): ¢ = NS difference for 24 hr desiccation time; ¤ = NS difference for 1 week desiccation time; ◊ = NS difference for 1 month; Δ = NS difference for control; € = NS difference for 320 nm filter; γ = NS difference for 305 nm filter; • = NS difference for Quartz filter. Symbols are listed by desiccation time(s) first, followed by the corresponding UV exposure intensity.

CHAPTER 4: DISCUSSION

The amount of UV radiation reaching Earth's surface is expected to change in the future as a result of various environmental and anthropogenic factors, although these changes may be location-specific (Hegglin and Shepherd 2009, Gao et al. 2010). Droughts in the western United States have been observed to have the ability to increase water transparency in lakes, thereby allowing deeper penetration of UVR (Williamson et al. 2016). Given that some areas in this region receive 25% higher UV values than other regions in the continental U.S. (Fioletov et al. 2010a), it is imperative that a baseline for UV tolerance of aquatic organisms be obtained and understood. This study was undertaken to better comprehend potential differences in tolerance to UVR by bdelloid rotifers based on geography, as well as their ability to survive various exposure levels following desiccation.

4.1 DOC

Previous studies have suggested that the amount of DOC present in a lake is potentially the most important factor controlling the attenuation of UVR (Morris et al. 1995, Morris and Hargreaves 1997). Changes in DOC concentrations may be so important to the survival of aquatic biota that they have been suggested to be even more important than stratospheric ozone depletion in regulating future changes in UVR in natural freshwater ecosystems (Williamson et al. 1996). Thus, in order to provide insights to the environmental conditions to which the bdelloid lineages being studied here are currently being exposed to, DOC concentrations from their source locations were analyzed for a summer and a winter season. All three locations had average DOC concentrations above 5 mg/L, suggesting that they are likely net heterotrophic as this value corresponds to the threshold value for the transition between lake net autotrophy to net

heterotrophy (Prairie et al. 2002). Of these locations, Kettle 4 had the greatest average DOC concentration (22.21 mg/L in the summer and 36.85 mg/L in the winter). If these values are later determined to be significant, it may be that the organisms in Kettle 4 have the advantage of added protection from harmful UVR than other locations in the region.

Although the results presented here did not identify a substantial difference in DOC levels between Birch Lake and Ascarate Lake, it is important to mention that differences in the elevations at which these two lakes are found may contribute to the UVR fluxes that the systems receive. In fact, based on estimates by Diffey (1991), Ascarate Lake is likely to receive ~3% more radiation on average than Birch Lake simply based on the difference in elevation alone. Differences in latitude also result in these two lakes being subjected to varying levels of UV intensity (Gao et al. 2010). Additionally, Arts et al. (2000) noted that UVR penetrated more deeply in saline waterbodies than in freshwater systems with similar DOC concentrations. This may be an important concern for Ascarate Lake as it has consistently high EC levels (Table 3.1).

While the samples collected from Birch Lake did not have the greatest concentrations of DOC based on the two samples, it does not necessarily suggest that the organisms in this lake are less protected from the harmful effects of UVR. Out of the three sources sampled, this lake was the deepest and possibly provides a larger area for some organisms to use for vertical migration and a lower possibility of DOC photodegradation. Furthermore, as previously mentioned, this lake has a large number of macrophytes along the shores and is surrounded by terrestrial vegetation, which may provide sources of shade for bdelloids and other zooplankton. Additional measurements of DOC throughout a yearly cycle would also be beneficial.

Biologically damaging UVR appears to have a strong seasonal dependence in temperate regions (Diffey 1991). This may be an important factor of UV-B exposure in the systems studied here, as seasonal variation in DOC were apparent for some of the samples collected. The high concentration of DOC measured in Kettle 4 in the winter is likely due to the amount of organic input flushed or blown in from the surrounding vegetation in the shallow rock-pool and the evapoconcentration of DOC. The increase in allochthonous DOC may provide added protection to bdelloids during this time of the year. While aquatic systems with shallow depths (<10 m) have been suggested to be highly susceptible to dangerous levels of UVR (Morris and Hargreaves 1997), the high levels of DOC in Kettle 4 may compensate for its shallow depth in terms of providing protection from UVR. Additionally, unlike other observations of shallow systems with high DOC photodegradation (Waiser and Robarts 2004), the evapoconcentration of DOC that may be occurring in this rock-pool may allow for DOC levels to accumulate without high photodegradation activities.

The relationship between constant mixing in shallow systems and high DOC photodegradation as noted by Waiser and Robarts (2004) may explain the lower levels of DOC measured at Ascarate Lake. As mentioned previously, this lake has artificial aeration systems in place as means to help control the accumulation of persistent harmful algal blooms. The mixing effects that these aeration systems provide may inadvertently be contributing to the photodegredation of the lake's water and hence result in lower DOC concentrations, as was suggested by Routh et al (2004) to have occurred in a Swedish lake. Mixing of the water column can also be caused by high winds, which are typical in the El Paso area during the spring and fall months and may contribute to mixing of the water in Ascarate Lake. Nevertheless, DOC concentrations measured at Ascarate Lake were similar to higher ranges of previous records at

some lakes in southeastern New Mexico (17 mg/L; Hylander et al. 2009a) and ranges measured at Pyramid Lake in Nevada (10.71 mg/L to 19 mg/L; Hamilton-Galat and Galat 1983).

If high winds are having an effect on DOC, it may also be possible that they are simultaneously contributing to the input of allochthonous DOC. Deposition of organic carbon in the form of particulate matter carried by wind events is very likely as dust storms tend to be frequent in the Paso del Norte region during the December-May dry season, where substantial dust events with visibility <10 km occur on average 15 days out of the year (Novlan et al. 2007). Additionally, it would be interesting to investigate the characteristics of the playa system from which the BRT population originated. When there is water present, these playa systems tend to be very clear and very shallow, so the concentrations of DOC are likely to be very low (Walsh, personal communication). It may also be necessary to include additional measurements, such as optical characteristics, source, and chemical composition of DOC at all the locations, as these parameters also change seasonally (Morris and Hargreaves 1997).

The results presented here are the first of their kind for the locations sampled. However, as previously mentioned, the number of DOC samples used in this study were not sufficient enough to provide a completely representative portrayal of the actual differences that these systems may have. Thus, caution is advised when interpreting these results. Additional sampling efforts should be carried out to provide a more detailed assessment and added support for any differences detected.

Climatic warming is likely to increase DOC concentration in arid and semi-arid regions due to evapoconcentration, and increased concentrations may allow for compensation of the high UVR exposure levels that aquatic systems in these regions receive (Curtis 1998). If this is the

case for aquatic systems in the Chihuahuan Desert, including those investigated in this study, it is likely that future changes brought upon by climate change may very likely result in changes to the balance of the ecosystem as a whole. Continuous and more frequent sample collections should be conducted at these locations in order to monitor changes more closely and assist in predicting the effects of UVR on the aquatic organisms in these waters.

4.2 UV Exposure & Mortality

All three treatments (population source, dessication regime, and UV exposure) investigated had a significant effect on the probability of mortality in bdelloids. There was a distinct trend for all but 2 out of the 48 experimental conditions where the probability of mortality increased with the increases in both UV exposure levels and time of desiccation prior to exposure (Figure 3.3). Tukey HSD tests also provided support for most of the significant interactions between population and time desiccated (Figure 3.4), population and UVR exposure (Figure 3.5), and desiccation time and UVR exposure (Figure 3.6), with the latter two interaction sets also having a clear trend in higher mortality probabilities with increase in exposure.

As predicted, Birch Lake bdelloids had a significantly higher probability of mortality in relation to the other three populations (GLM, p-values<0.05), with the odds of death being 92% at the highest exposure level following 1 month of desiccation (Table 3.5). With respect to the other populations, the odds of mortality for the Birch Lake bdelloids were 1.6 times higher than the Ascarate Lake population (GLM, p-value<0.01, 95% CI = 1.08, 2.25), 1.4 times higher than the BRT population (GLM, p-value<0.01, 95% CI = 1, 1.98), and 1.7 times higher than the Kettle 4 population (GLM, p-value<0.05, 95% CI = 1.17, 2.4). Thus, the hypothesis suggesting

that the Birch Lake population would have the highest predicted mortality probabilities was supported.

There were more significant interaction effects between desiccation time and populations source following 1 month of desiccation than there were for the other two time periods (Tukey HSD, p-value<0.001). Similarly, the highest level of exposure (370 $\mu W/cm^2$) resulted in the highest probabilities of death for all our populations periods (Tukey HSD, p-value<0.001). The hypotheses suggesting that mortality probabilities would increase with increases in these two stressors were therefore also supported.

The association between desiccation and UVR exposure as an effect on bdelloid rotifers has been previously studied in only a handful of studies. Following UV exposure, the bdelloid *Philodina roseola* appears to be able to repair DNA damage only through desiccation, yet the effects of UV radiation were also determined to enhance the negative effects of desiccation on the reproduction of this species as there was a threefold reduction in their reproduction between the UVR alone and in combination with desiccation (Fischer et al. 2013). This observation suggests that desiccation can be both beneficial for the survival of the individuals that have undergone high levels of UV exposure, but it can also result in detrimental consequences to their reproduction and, thus eventually to the longevity of the population.

Bdelloids used in this study belong to the family Philodinidae, with the four lineages identified as four species from three different genera (Figure 3.1). Since the bdelloids from the four populations examined in this study were different species, there may be a possibility that the results of this study were influenced by species-specific tolerances. Altiero et al. (2011) observed differences in tolerance to increasing UV dosage in two different species of eutardigrades.

However, they do mention that these two species have very different life histories, as one is carnivorous, white in color, and amphimictic, while the other is herbivorous, brown/red in color, and parthenogenetic. Additionally, two bdelloid species from two distinct families (Philodinidae and Adinetidae) had similar dose-response relations to ionizing radiation with a small indication that one was slightly more radiosensitive (Gladyshev and Meselson 2008). Regardless, various bdelloid species have demonstrated to be more tolerant than monogonont rotifers and other invertebrate metazoans, which may diminish the magnitude of difference that individual species may have with regards to their tolerance to UVR.

Though high ambient levels of UV-B exposure have been recorded in the Chihuahuan Desert (279 $\mu W/cm^2$ in Las Cruces, NM), high levels of mortality were still observed when bdelloids from this region were exposed to up to 370 $\mu W/cm^2$ of UV-B. The second highest level of exposure (130 $\mu W/cm^2$) was less than the ambient levels used as the reference, and yet the odds of mortality reached up to 61% for the BRT population. It is important to mention that the experiments in this study were carried out under extreme circumstances that are not likely to be entirely representative of what is occurring naturally in these locations. UVR was presented as an acute stressor; however, it is possible that long-term sub-lethal effects may also be important at even lower levels of UVR. Nevertheless, the level of exposure that was used as a baseline in this study was the average of the highest summer recordings for 10 years from a monitoring station in Las Cruces, NM, and thus there were higher levels of exposure, with upwards of 302 $\mu W/cm^2$ recorded. Should the predictions of increases in UV-B exposure be realized, it may deem the findings in this study even more pertinent.

The effects that mixing by wind can have on DOC levels in a lake as discussed above can also have direct consequences on the aquatic biota. Zagarese et al. (1998) observed higher

mortality of copepods when exposed to UVR under a vertical cycling experiment. Their results suggested that since it is unlikely for small organisms such as zooplankton to avoid cycling through the water column of shallow lakes, they can be exposed to potentially damaging levels of solar radiation, even in relatively turbid waters. If the same scenario is occurring at Ascarate Lake, it is likely that bdelloids from this lake are relying on means other than DOC in the water to respond to the UV stress.

Pigmentation in zooplankton also plays an important role in UVR tolerance, and it is likely that the bdelloids studied here may depend on this capability in nature. Hairston (1979) observed differences in response to changes in wavelengths by *Diaptomus nevadensis* individuals with less pigmentation. However, there may be cases in which the need for pigmentation as means for protection against UV radiation may be surpassed by other means of survival, such as predator avoidance. Copepods retain pigmentation when exposed to UVR, but will decrease the amount of pigmentation when a predatory threat is present, regardless of the level of UVR they are being exposed to (Hylander et al. 2009b). Interestingly, of the two eutardigrades Altiero et al. (2011) investigated, the species that appeared to be white in color was able to tolerate higher levels of UVR than the species with a brown/red pigmentation. Indeed, even the bdelloids from Kettle 4 had a red hue when they were first collected, although the color faded with the subsequent generations of this population likely due to a change in their food source. Thus, the role that pigmentation plays in response to UVR may be more important for some zooplankton species than others, and these differences may potentially be more prominent in certain habitats.

4.3 Conclusions & Future Directions

This study investigates how mortality differs in bdelloid rotifers exposed to combinations of UV radiation and duration of desiccation. More specifically, it addresses the variation in mortality of bdelloids derived from different natural habitat types, and thus, varying amounts of UVR exposure and with different histories of exposure to desiccation.

The aquatic systems investigated in this study and the rotifers species that inhabit them have been previously studied to address different questions (Schröder and Walsh 2007, 2010; Mills et al. 2016); however, there have not been any previous DOC measurements taken at these locations. Interestingly, while other studies have observed increases in DOC levels with increases in salinity (Curtis and Adams 1995, Waiser 2006), this trend was not evident in this study. While Kettle 4 winter samples had significantly greater DOC concentrations than Ascarate Lake, Ascarate samples had over seven times the EC levels than what was measured in the rock-pool. Although these concentrations could not be statistically compared in the current study due to the low sample size, it would be meaningful to conduct follow up studies with concurrent DOC and EC measurements to develop any sort of pattern that may contest previous assumptions, and include measurements for Birch Lake as well. Given that acidification has also been observed to impact the ability for DOC to reduce UVR attenuation (Williamson et al. 1996, Gennings et al. 2014), it would be prudent to also investigate levels of acidification that these systems may be experiencing, if any. Future sampling efforts of DOC levels should be conducted multiple times throughout the year and for multiple years in order to create a historical database for future monitoring operations and/or research applications. Other sites in the region should also be included, particularly sites with truly low DOC levels (<5 mg/L), in order to allow for a better represented comparison of any concentration changes.

Although this research assessed differences in survival following desiccation and UVR exposures as means of testing extreme tolerance abilities, other approaches to testing these differences may be interesting and beneficial to do, as they may likely provide results that are more representative to what is occurring in these natural systems. *In situ* exposure experiments at the source locations may supply additional support for UVR tolerance by these rotifers. Obtaining measurements for underwater light penetration can provide a better insight at what UVR levels these and other organisms in these systems are being exposed. Additional investigations should also be conducted to determine how these animals are able to deal with UV exposure through the evaluation of differences in behavioral, physiological, and genetic responses. Kim et al. (2011) found that UV-B radiation had a significant impact on the regulation of some important genes in DNA replication, repair process, and chaperoning for *Brachionus* sp. Thus, investigations should be carried out to identify the induction of gene expressions (e.g., heat-shock protein genes), cyclobutane-pyrimidine dimers, and other molecular activities that may result from elevated levels of UV-B radiation in bdelloid rotifers. Maintaining rotifers hydrated throughout the exposure phase and desiccating them after exposure can also be done to determine whether the negative effects of desiccation are diminished by allowing for DNA repair.

In response to the association between pigmentation and UVR protection, it would be interesting to test varying levels of pigmentation that these bdelloids may develop while being exposed to UVR in the lab or in the field. Additionally, comparing bdelloids that have developed pigmentation as a result of these exposures could be desiccated and exposed to UVR in order to determine whether the presence of pigmentation during desiccation has an effect on their survival.

One caveat that must be addressed for this investigation is that the bdelloids used in the study were all descendants from four lineages that originated from each of the corresponding population sources. Several generations of offspring from the original population were maintained in culture in the lab before the experiments began. Therefore, the rotifers used in these experiments had not been exposed to the natural stressor conditions that may occur in their habitats. However, since these stressors are likely to have played a role in the survival of the parent population, it is also likely that their fitness capabilities were passed on to their offspring, as selection acts upon the surviving, fitter individuals (Fischer et al. 2013). Nevertheless, future studies should be conducted with individuals that either originate directly from the source location or are offspring that have been kept in culture for a shorter period of time to provide additional support for the findings presented in this study.

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APPENDICES

APPENDIX 1: GENBANK ROTIFER ACCESSION NUMBERS

List of rotifer sequences obtained from GenBank BLAST® library used in this study, with their origins and GenBank accession numbers of their COI sequences.

Species	GenBank Accession Number
Adenita vaga	GQ465646.1
Adenita vaga	GQ465645.1
Adenita vaga	GQ465644.1
Brachionus calyciflorus	GU232733.1
Macrotrachela sp.	DQ078538.1
Macrotrachela sp.	DQ078539.1
Macrotrachela sp.	DQ078540.1
Macrotrachela quadricornifera	JX184004.1
Macrotrachela quadricornifera	JX184006.1
Macrotrachela quadricornifera	JX184003.1
Philodina sp.	DQ078562.1
Philodina sp.	DQ078563.1
Philodina acuticornis	EU751202.1
Philodina acuticornis	EU751201.1
Philodina acuticornis	EU751200.1
Philodina citrina	EU499765.1
Philodina citrina	KM043200.1
Philodina duplicalcar	EU751211.1
Philodina duplicalcar	EU751209.1
Philodina flaviceps	DQ890156.1
Philodina flaviceps	DQ890155.1
Philodina gregaria	KJ543688.1
Philodina gregaria	KJ543687.1
Philodina megalotrocha	JQ309197.1
Philodina megalotrocha	JQ309198.1
Philodina megalotrocha	JQ309199.1
Philodina roseola	HM032973.1
Philodina roseola	HM032972.1
Rotaria rotatoria	EU499874.1
Rotaria rotatoria	KM043209.1
Rotaria sordida	KM043210.1
Rotaria sordida	EU751162.1

APPENDIX 2: MULTIPLE SEQUENCE ALIGNMENT

Multiple sequence alignment for bdelloids in this study along with sequences taken from NCBI GenBank (accession numbers follow the species identifier) for other bdelloids and *Brachionus calyciflorus*, the outgroup. Sequences included for this study are in bold. *B. caly. = Brachionus calyciflorus*; *A.vaga = Adineta vaga*; *P.mega. = Philodina megalotrocha*; *P.greg. = Philodina gregaria*; *P.flav. = Philodina flaviceps*; *P.citrina = Philodina citrina*; *P.dupl. = Philodina duplicalcar*; *P.acut. = Philodina acuticornis*; *P.rose. = Philodina roseola*; *M.quad. = Macrotrachela quadricornifera*; *R.sordida = Rotaria sordida*; *R.rotatoria = Rotaria rotatoria*.

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B.caly.GU232733.1            TTCGGAATTTGAGCCGGCTTAATTGGTCTTAGCATAAGATTCCTTATCCGCCTAGAACTA
R.rotatoria.EU499874.1       GTTGGAGTGTGATCAGGGTTTTTAGGAGCTAGAATAAGATTAATTATTCGTACTGAGTTA
R.sordida.KM043210.1         ----------------------------------------TTATTCGTACTGAATTA
R.sordida.EU751162.1         ATTGGAGTTTGATCTGGATTTTTAGGTGCTAGAATAAGTTTAATTATTCGTACTGAATTA
P.greg.KJ543688.1            ATTGGAATCTGATCCGGTTTTTTAGGAGCTAGAATAAGATTAATCATTCGTACTGAGTTA
P.greg.KJ543687.1            ATTGGAATCTGATCCGGTTTTTTAGGAGCTAGAATAAGATTAATCATTCGTACTGAGTTA
P.flav.DQ890156.1            ATTGGAATTTGATCAGGGTTTTTAGGAGCTAGGATTAGGTTAATTATTCGTACTGAGTTA
P.flav.DQ890155.1            ATTGGAATTTGATCTGGTTTCTTAGGGGCTAGAATTAGTTTAATTATTCGAACAGAGTTA
Ascarate.Lake.1              ---------------------------------AATAAGTTTAATTATTCGTACAGAGTTA
P.mega.JQ309197.1            --TGGTATTTGATCTGGGTTTTTGGGTGCAAGAATAAGATTGATTATTCGTACTGAGTTA
P.mega.JQ309199.1            ------ATTTGATCTGGGTTTTTGGGTGCAAGAATAAGATTGATTATTCGTACTGAGTTA
P.mega.JQ309198.1            ----------------GGTTTTTGGGTGCAAGAATAAGATTGATTATTCGTACTGAGTTA
R.rotatoria.KM043209.1       ATTGGGGTATGATCTGGGTTTTTAGGTGCAAGAATAAGGTTAATTATTCGTACTGAATTA
P.citrina.EU499765.1         ATTGGAATTTGATCTGGGTTTTTAGGTGCGAGAATAAGATTAATTATTCGTACAGAATTA
P.citrina.KM043200.1         ATTGGAATTTGATCTGGGTTTTTGGGTGCAAGAATAAGATTGATTATTCGTACAGAGTTA
BRT.no.41.MM37.1             ------------------------------------------------------------
P.dupl.EU751211.1            ATTGGAATTTGATCAGGGTTTTTAGGAGCTAGTATTAGTTTGATTATTCGTACTGAATTG
P.dupl.EU751209.1            ATTGGAATTTGATCAGGGTTTTTAGGAGCTAGTATTAGTTTGATTATTCGTACTGAATTG
P.acut.EU751202.1            ------------------------------------------------------GAATTA
P.acut.EU751201.1            ------------------------------------------------------GAATTA
P.acut.EU751200.1            ATTGGGATTTGATCTGGGTTTTTAGGGGCAAGAATAAGAATAATTATTCGTTCTGAATTA
Birch.Lake.WI                ---------------------------------AATAAGATTAATTATTCGTACTGAGTTA
M.quad.JX184004.1            ATTGGTGTTTGATCTGGTTTTATTGGAGCTAGATTAAGATTAATTATTCGTACTGAATTA
M.quad.JX184003.1            ----------GATCTGGTTTTATTGGAGCCAGATTAAGGTTAATTATTCGTACTGAATTA
M.quad.DQ078536.1            -------------CTGGTTTTATTGGAGCCAGATTAAGGTTAATTATTCGTACTGAATTA
A.vaga.GQ465646.1            ATTGGTGTTTGATCTGGTTTTTTAGGTGCAAGAATAAGTTTAATTATTCGTACTGAATTA
A.vaga.GQ465645.1            ATTGGTGTTTGATCTGATTTTTTAGGTGCAAGAATAAGTTTAATTATTCGTACTGAATTA
A.vaga.GQ465644.1            ATTGGTGTTTGATCTGGTTTTTTAGGTGCAAGAATAAGTTTAATTATTCGTACTGAATTA
Macrot.sp.DQ078538.1         -------------CTGGATTTTTGGGTGCAAGAATCAGATTAATTATTCGTACTGAGTTA
Macrot.sp.DQ078540.1         -------------CTGGATTTTTGGGTGCAAGAATCAGATTAATTATTCGTACTGAGTTA
Macrot.sp.DQ078539.1         -------------CTGGATTTTTGGGTGCAAGAATCAGATTAATTATTCGTACTGAGTTA
Philodina.sp.DQ078562.1      -------------CTGGTTTTTTAGGTGCGAGTATTAGTTTAATTATTCGTACTGAATTA
Philodina.sp.DQ078563.1      -------------CTGGTTTTTTAGGTGCGAGTATTAGTTTAATTATTCGTACTGAATTA
Kettle4.Hueco.Tanks.1        ------------------------------------------------------------
P.rose.HM032973.1            -------------CTGGATTTTTAAGTGCAAGTATTAGGTTAATTATTCGTACTGAGTTA
P.rose.HM032972.1            -------------CTGGATTTTTAGGTGCAAGTATTAGGTTAATTATTCGTACTGAGTTA

B.caly.GU232733.1            GGTGTAGTGGGGTCTTATCTTGGAGATGAGCATTTATACAATGTACTCGTCACAGCTCAT
R.rotatoria.EU499874.1       GGGATAGTTGGAAGAATCATTATAGATGATCAGATTTATAACTCAATGGTTACAGCTCAT
R.sordida.KM043210.1         GGAATACTTGGTAGAGTAATTATGGATGATCAGATTTATAATTCTATAATTACCGCTCAT
R.sordida.EU751162.1         GGAATAGTAGGTAGTGTAATTATGGATGATCAAATTTATAATTCTATAATTACGGCACAT
P.greg.KJ543688.1            GGAATAGTAGGTAGAATCATCATAGATGAACAAATCTATAACGCAATGGTAACTGCCCAT
P.greg.KJ543687.1            GGAATAGTAGGTAGAATCATTATAGATGAACAAATCTATAACGCAATGGTAACTGCCCAT
P.flav.DQ890156.1            GGTATAGTAGGGAGAATTATCATAGATGAACAAATTCATAATGCCATAGTTACGGCTCAT
P.flav.DQ890155.1            GGAATAGTGGGGAGAATTATTATGGATGAACAAATTTATAATGCTATGGTAACAGCACAT
Ascarate.Lake.1              GGGATAGTAGGCAGGGTTATTATAGATGAGCAAATTTATAATAGGATAGTAACTGCTCAT
P.mega.JQ309197.1            GGAATAGTAGGGAGTGTAATTATAGATGAGCAAATTTATTATAGAATAGTGACTGCTCNT
P.mega.JQ309199.1            GGAATAGTAGGGAGTGTAATTATAGATGAGCAAATTTATAATAGAATAGTGACTGCTCAT
P.mega.JQ309198.1            GGAATAGTAGGGAGTGTAATTATAGATGAGCAAATTTATAATAGAATAGTGACTGCTCAT
R.rotatoria.KM043209.1       GGTATAGTAGGTAGTGTTATTATAGATGATCAAATTTATAATTCTATAGTAACTGCTCAT
P.citrina.EU499765.1         GGTATAGTAGGTAGAATTATTATAGATGAGCAAATTTATAATAGAATAGTGACTGCTCAT
P.citrina.KM043200.1         GGGATAGTTGGGAGAATTATTATAGATGAACAAATTTATAATAGGATGGTAACTGCTCAT
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P.dupl.EU751209.1           GGAATAGTAGGTAGAATTATTATTGATGAGCAAATTTATAATGCTATGGTTACTGCTCAT
P.acut.EU751202.1           GGAATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATGCGATAGTTCCAGCTCAC
P.acut.EU751201.1           GGAATAGTTGGGAGAATTTTTATAGATGAGCAAATTTATAATGCTTTAGTAACTGCTCAC
P.acut.EU751200.1           GGAATAGTTGGTAGAATTTTTATAGATGAGCAAATTTATAATGCTTTAGTAACTGCCCAN
Birch.Lake.WI               GGTATAGTGGGAAGAATTATTATAGATGATCAAATCTATAATTCTATAGTTACAGCACAT
M.quad.JX184004.1           GGGATAGTAGGTAGAATTATTATAGATGAGCAAATTTTTAATTCTGTAGTTACTGCTCAT
M.quad.JX184003.1           GGAATAGTAGGTAGAATTATTATAGATGAGCAAATTTTTAATTCTGTAGTTACTGCTCAT
M.quad.DQ078536.1           GGAATAGTAGGTAGAATTATTATAGATGAGCAAATTTTTAATTCTGTAGTTACTGCTCAT
A.vaga.GQ465646.1           GGGATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATTCTATAGTTACTGCGCAT
A.vaga.GQ465645.1           GGGATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATTCTATAGTTACTGCGCAT
A.vaga.GQ465644.1           GGGATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATTCTATAGTTACTGCGCAT
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Macrot.sp.DQ078540.1        GGAATAGTAGGAAGGATTATTATGGATGAGCAGATTTATAATTCTTTGGTAACGGCTCAT
Macrot.sp.DQ078539.1        GGAATAGTAGGAAGGATTATTATGGATGAGCAGATTTATAATTCTTTGGTAACGGCTCAT
Philodina.sp.DQ078562.1     GGAATAGTTGGAAGTATTATTATAGATGAACAAATTTATAATTCTATAGTAACAGCTCAT
Philodina.sp.DQ078563.1     GGAATAGTTGGAAGTATTATTATAGATGAACAAATTTATAATTCTATAGTAACAGCTCAT
Kettle4.Hueco.Tanks.1       --AATAGTTGGAAGTATTATTATAGATGAACAAATTTATAATTCTATAGTAACAGCTCAT
P.rose.HM032973.1           GGGATAGTTGGAAGTATTATTATGGAGGAGCAAATTTATAATTCTATGGTAACAGCTCAT
P.rose.HM032972.1           GGGATAGTTGGAAGTATTATTATGGATGAGCAAATTTATAATTCTATGGTAACAGCTCAT

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R.sordida.EU751162.1        GCTTTTTTAATAATTTTTTTTTTTGTTATACCTGTTTCTATAGGTGGATTTGGTAATTGA
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P.greg.KJ543687.1           GCTTTCCTAATAATTTTCTTTTTTGTTATGCCTGTTGCCGTTGGTGGCTTTGGTAATTGA
P.flav.DQ890156.1           GCTTTCCTAATAATTTTTTTTTTTTGTAATGCCTGTAGCTGTGGGGGGATTTGGTAATTGG
P.flav.DQ890155.1           GCTTTCTTAATAATTTTCTTCTTTGTAATGCCTGTGGCTGTGGGGGGATTTGGAAATTGG
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P.mega.JQ309199.1           GCTTTTTTAATAATTTTTTTTTTTTGTCATACCAGTGGCTGTTGGGGGATTTGGTAATTGA
P.mega.JQ309198.1           GCTTTTTTAATAATTTTTTTTTTTTGTCATACCAGTGGCTGTTGGGGGATTTGGTAATTGA
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P.acut.EU751201.1           GCTTTTTTAAAAATTTTTTTTTTTTGTTATACCTGTTGCTGTTGGAGGCTTTGGAAATTGA
P.acut.EU751200.1           GCCTTTTTAATAATTTTTTTTTTTTGTTATACCTGTTGCTGTTGGAGGCTTTGGAAATTGA
Birch.Lake.WI               GCTTTTTTAATAATTTTTTTTTTTTGTAATACCAGTAGCTATAGGGGGTTTTGGTAATTGA
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M.quad.JX184003.1           GCTTTTTTAATGATTTTTTTTTTTTGTAATGCCTGTAGCTGTAGGAGGTTTTGGTAATTGA
M.quad.DQ078536.1           GCTTTTTTAATGATTTTTTTTTTTTGTAATGCCTGTAGCTGTAGGAGGTTTTGGTAATTGA
A.vaga.GQ465646.1           GCTTTTTTAATGATTTTTTTTTTTTGTTATACCTGTTGCAGTTGGAGGGTTTTGGAAATTGA
A.vaga.GQ465645.1           GCTTTTTTAATGATTTTTTTTTTTTGTTATACCTGTAGCAGTTGGAGGGTTTTGGAAATTGA
A.vaga.GQ465644.1           GCTTTTTTAATGATTTTTTTTTTTTGTTATACCTGTTGCAGTTGGAGGGTTTTGGAAATTGA
Macrot.sp.DQ078538.1        GCTTTTTTGATGATTTTTTTTTTTTGTTATACCTGTTGCTGTTGGGGGTTTTTGGAAATTGA
Macrot.sp.DQ078540.1        GCTTTTTTGATGATTTTTTTTTTTTGTTATACCTGTTGCTGTTGGGGGTTTTTGGAAATTGA
Macrot.sp.DQ078539.1        GCTTTTTTGATGATTTTTTTTTTTTGTTATACCTGTTGCTGTTGGGGGTTTTTGGAAATTGA
Philodina.sp.DQ078562.1     GCTTTTTTAATAATTTTTTTTTTTTGTTATACCTGTAGCTGTAGGGGGGTTTGGTAATTGG
Philodina.sp.DQ078563.1     GCTTTTTTAATAATTTTTTTTTTTTGTTATACCTGTAGCTGTAGGGGGGTTTGGTAATTGG
Kettle4.Hueco.Tanks.1       GCTTTTTTAATAATTTTTTTTTTTTGTTATACCTGTAGCTGTAGGGGGGTTTGGTAATTGG
P.rose.HM032973.1           GCTTTTTTAATAATTTTTTTTTTTTGTTATACCTGTTGCTGTAGGTGGTTTTGGTAATTGA
P.rose.HM032972.1           GCTTTTTTAATAATTTTTTTTTTTTGTTATACCTGTTGCTGTAGGTGGTTTTGGTAATTGA

B.caly.GU232733.1           CTTATTCCACTTATGTTAGGGGTAGCTGATATGGCTTTCCCTCGTATGAATAATTTATCT
R.rotatoria.EU499874.1      TTACTTCCTGTTATATTAAATGTGATAGATATGGCATTTCCTCGTTTAAATAATTTGAGG
R.sordida.KM043210.1        TTGTTACCTTTAATAATGAATGTTGCTGATATAGCTTTTCCTCGTTTAAATAATTTGAGT
R.sordida.EU751162.1        TTATTGCCTTTAATAATAAATGTAGCGGATATAGCTTTTCCTCGTTTAAATAATTTGAGA
P.greg.KJ543688.1           TTATTGCCTTTAATAATAAATGTTATGGACATAGCTTTTCCTCGATTAAATAATTTAAGG
P.greg.KJ543687.1           TTATTGCCTTTAATAATAAATGTTATGGACATAGCTTTTCCTCGATTAAATAATTTAAGG
P.flav.DQ890156.1           TTGTTGCCTTTAATAATAAATGTTATAGATATAGCTTTTCCTCGATTAAATAATTTAAGA
P.flav.DQ890155.1           TTGTTACCTTTGATAATGAATGTTATGGATATGGCATTTCCTCGTTTAAATAATTTGAGG
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Ascarate.Lake.1             TTATTGCCTTTAATAATAAATGCTATGGATATGGCATTTCCTCGTTTAAATAATTTAAGA
P.mega.JQ309197.1           CTATTACCTTTAATAATGAATGCTATAGATATAGCATTTCCTCGATTAAATAATTTAAGA
P.mega.JQ309199.1           CTATTACCTTTAATAATGAATGCTATAGATATAGCATTTCCTCGATTAAATAATTTAAGA
P.mega.JQ309198.1           CTATTACCTTTAATAATGAATGCTATAGATATAGCATTTCCTCGATTAAATAATTTAAGA
R.rotatoria.KM043209.1      CTTTTACCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGGTTAAATAATTTGAGG
P.citrina.EU499765.1        TTATTGCCTTTAATAATAAATGCTATAGATATAGCGTTTCCTCGTTTAAATAATTTGAGA
P.citrina.KM043200.1        TTGTTGCCTTTAATAATAAATGCTATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGG
BRT.no.41.MM37.1            TTATTACCTTTAATGATAAATGTTATGGATATTTTTTTTCCTCGTTTAAATAATTTGAGA
P.dupl.EU751211.1           TTATTACCTTTAATAATAAATGTGATGGATATAGCATTTCCTCGTTTGAATAATTTAAGA
P.dupl.EU751209.1           TTATTACCTTTAATAATAAATGTGATGGATATAGCATTTCCTCGTTTGAATAATTTAAGA
P.acut.EU751202.1           TTATTCCCTTTAATAATAAATGTTATAGATATAGCTTTTCCTCGCTTAAATAATTTAAGA
P.acut.EU751201.1           TTATTACCTTTAATAATGAATGTTATAGATATAGCTTTTCCTCGTTTGAATAATTTAAGA
P.acut.EU751200.1           TTATTCCCTTTAATAATGAATGTTATAGATATAGCTTTTCCTCGTTTGAATAATTTAAGA
Birch.Lake.WI               TTATTACCTTTAATGATAAATGTAATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGA
M.quad.JX184004.1           TTATTACCTTTAATGATAAATGTTATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGA
M.quad.JX184003.1           TTATTACCTTTAATGATAAATGTTATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGA
M.quad.DQ078536.1           TTATTACCTTTAATGATAAATGTTATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGA
A.vaga.GQ465646.1           TTATTGCCTTTAATAATAAATGCTATAGATATAGCTTTTCCTCGATTGAATAATTTGAGG
A.vaga.GQ465645.1           TTATTGCCTTTAATAATAAATGCTATAGATATAGCTTTTCCTCGATTGAATAATTTGAGG
A.vaga.GQ465644.1           TTATTGCCTTTAATAATAAATGCTATAGATATAGCTTTTCCTCGATTGAATAATTTGAGG
Macrot.sp.DQ078538.1        TTATTACCTTTAATAATAAATGTTATAGATATGGCTTTTCCTCGTTTAAATAATTTAAGT
Macrot.sp.DQ078540.1        TTATTACCTTTAATAATAAATGTTATAGATATGGCTTTTCCTCGTTTAAATAATTTAAGT
Macrot.sp.DQ078539.1        TTATTACCTTTAATAATAAATGTTATAGATATGGCTTTTCCTCGTTTAAATAATTTAAGT
Philodina.sp.DQ078562.1     TTATTACCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGATTAAATAATTTAAGA
Philodina.sp.DQ078563.1     TTATTACCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGATTAAATAATTTAAGA
Kettle4.Hueco.Tanks.1       TTATTACCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGATTAAATAATTTAAGA
P.rose.HM032973.1           TTACTTCCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGT
P.rose.HM032972.1           TTACTTCCTTTAATAATAAATGTAATAGATATAGCTTTTCCTCGTTTAAATAATTTAAGT

B.caly.GU232733.1           TTCTGGCTTTTAGTCCCTG---CATTTATGTTTTTACTTCTGTCTTCCGCTATTGATGCT
R.rotatoria.EU499874.1      TTTTGA---TTAGTGCCTATTTCTTTGTTATTTATGAGAATAAGATTATTAGTAGGGTTG
R.sordida.KM043210.1        TTTTGG---TTAGTACCTGTTTCTTTATTATTTATAATTATAAGCTTATTAGTTGGATTA
R.sordida.EU751162.1        TTTTGG---TTAATTCCTGTTTCTTTGTTATTTATAATTATAAGGTTATTAGTTGGTTTA
P.greg.KJ543688.1           TTTTGA---TTAGTACCTGTAGCTCTCTTATTTATATCCTTAAGATTATTAGTAGGATTA
P.greg.KJ543687.1           TTTTGA---TTAGTACCTGTAGCTCTCTTATTTATATCCTTAAGATTATTAGTAGGATTA
P.flav.DQ890156.1           TCTTGA---TTGGTTCCTGTAGCTTTGTTATTTATGACCATAAGGTTGTTAGTGGGTTTG
P.flav.DQ890155.1           TTTTGA---TTAGTTCCTGTTGCTTTATTATTTATAACCATAAGCTTATTAGTTGGATTA
Ascarate.Lake.1             TTTTGA---TTAGTTCCTGTTGCTTTATTATTTTTATAGCTATAAGATTATTGATTGGTTTG
P.mega.JQ309197.1           TTTTGA---TTAGTACCAGTTGCTTTATTTTTTATAGCTATAAGATTATTGATTGGGTTA
P.mega.JQ309199.1           TTTTGA---TTAGTACCAGTTGCTTTATTTTTTATAGCTATAAGATTATTGATTGGGTTA
P.mega.JQ309198.1           TTTTGA---TTAGTACCAGTTGCTTTATTTTTTATAGCTATAAGATTATTGATTGGGTTA
R.rotatoria.KM043209.1      TTTTGA---TTAGTTCCTATTTCTTTATTATTTATGTGTATAAGATTATTAGTAGGGTTA
P.citrina.EU499765.1        TTTTGG---TTAGTTCCTGTAGCTTTATTATTTATAATTATAAGTTTGTTAGTTGGTTTA
P.citrina.KM043200.1        TTTTGA---TTAGTTCCAGTAGCTTTATTATTTATAATTATAAGATTATTGGTTGGTTTA
BRT.no.41.MM37.1            TTTTGA---TTAGTGCCAGTAGCATTATTATTTATAACATTGAGTTTATTAGTTGGCTTA
P.dupl.EU751211.1           TTTTGA---TTAGTACCTGTTGCTTTATTATTTATAATAATAAGGTTATTAATTGGTTTA
P.dupl.EU751209.1           TTTTGA---TTAGTACCTGTTGCTTTATTATTTATAATAATAAGGTTATTAATTGGTTTA
P.acut.EU751202.1           TTTTGA---TTAGTCCCGGTAGCTTTATTATTTATAATTATAAGTTTGTTAGTTGGTTTA
P.acut.EU751201.1           TTTTGG---TTAGTTCCGGTTGCTTTATTATTTATAATTATAAGTTTATTGGTGGGATTA
P.acut.EU751200.1           TTTTGG---TTAGTTCCGGTTGCTTTATTATTTATAATTATAAGTTTATTGGTGGGATTA
Birch.Lake.WI               TTTTGA---TTATTACCTGTTTCTTTATTATTTATAGTGATAAGTTTATTAGTTGGGCTA
M.quad.JX184004.1           TTTTGA---TTAGTACCTGTGGCTTTATTATTTATAACTTTAAGATTATTAGTAGGTTTA
M.quad.JX184003.1           TTTTGA---TTAGTACCTGTGGCTTTATTATTTATAACTTTAAGATTATTAGTAGGTTTA
M.quad.DQ078536.1           TTTTGA---TTAGTACCTGTGGCTTTATTATTTATAACTTTAAGATTATTAGTAGGTTTA
A.vaga.GQ465646.1           TTTTGA---TTAGTCCCAGTTGCTTTGTTATTTATGAGTTTAAGTTTGTTAGTTGGATTA
A.vaga.GQ465645.1           TTTTGA---TTAGTCCCAGTTGCTTTGTTATTTATGAGTTTAAGTTTGTTAGTTGGATTA
A.vaga.GQ465644.1           TTTTGA---TTAGTCCCAGTTGCTTTGTTATTTATGAGTTTAAGTTTGTTAGTTGGATTA
Macrot.sp.DQ078538.1        TTTTGA---TTAGTTCCTGTTGCATTATTATTTATAAGTTTAAGACTATTAGTAGGTTTA
Macrot.sp.DQ078540.1        TTTTGA---TTAGTTCCTGTTGCATTATTATTTATAAGTTTAAGACTATTAGTAGGTTTA
Macrot.sp.DQ078539.1        TTTTGA---TTAGTTCCTGTTGCATTATTATTTATAAGTTTAAGACTATTAGTAGGTTTA
Philodina.sp.DQ078562.1     TTTTGA---TTAGTTCCTGTTGCTTTATTGTTTATAAGTTTAAGTTTATTAATTGGATTG
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Kettle4.Hueco.Tanks.1       TTTTGA---TTAGTTCCTGTTGCTTTATTGTTTATAAGTTTAAGTTTATTAATTGGATTG
P.rose.HM032973.1           TTTTGA---TTAGTTCCTGTAGCTTTATTATTTATAAGTTTAAGTTTATTAGTAGGTTTA
P.rose.HM032972.1           TTTTGA---TTAGTTCCTGTAGCTTTATTATTTATAAGTTTAAGTTTATTAGTAGGTTTA

B.caly.GU232733.1           GGAGCCGGTACAGGGTGGACTGTTTACCCTCCCCTTTCAGATTCGAGATACCATAGTGGT
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R.rotatoria.EU499874.1      GGACCTGGGACAGGTTGAACTGTTTATCCTCCATTATCTAATTCTGTTTATCATTTTGGA
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R.sordida.EU751162.1        GGTTCTGGAACTGGATGGACTGTTTATCCTCCTTTATCGAATTCTATTTATCATTTTGGA
P.greg.KJ543688.1           GGAGCAGGAACTGGATGAACAGTATACCCTCCTTTATCTAATTCTGTCTATCATTTTGGA
P.greg.KJ543687.1           GGAGCAGGAACTGGATGAACAGTATACCCTCCTTTATCTAATTCTGTCTATCATTTTGGA
P.flav.DQ890156.1           GGTGCAGGAACGGGTTGGACTGTTTACCCTCCTTTATCTAATTCTGTGTATCATTTTGGG
P.flav.DQ890155.1           GGGGCAGGTACGGGGTGAACAGTGTATCCTCCTTTATCTAATTCTGTGTATCATTTTGGC
Ascarate.Lake.1             GGTGCTGGTACAGGGTGAACAGTTTATCCTCCTCTTTCTAATTCTGTGTATCATTTTGGA
P.mega.JQ309197.1           GGGGCTGGGACAGGTTGAACTGTTTATCCTCCTTTATCTAATTCGGTTTATCATTTTGGA
P.mega.JQ309199.1           GGGGCTGGGACAGGTTGAACTGTTTATCCTCCTTTATCTAATTCGGTTTATCATTTTGGA
P.mega.JQ309198.1           GGGGCTGGGACAGGTTGAACTGTTTATCCTCCTTTATCTAATTCGGTTTATCATTTTGGA
R.rotatoria.KM043209.1      GGGGCTGGGACTGGTTGGACAGTATACCCTCCTTTGTCTAATTCTGTGTATCATTTTGGT
P.citrina.EU499765.1        GGAGCGGGGACAGGTTGAACCGTCTATCCTCCTTTATCTAATTCTGTTTATCATTTTGGA
P.citrina.KM043200.1        GGTGCAGGTACAGGTTGAACAGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGA
BRT.no.41.MM37.1            GGGCCAGGGACAGGGTGAACTGTTTACCCGCCGTTATCAAATTCTATTTATCATTTTGGA
P.dupl.EU751211.1           GGAGCGGGTACTGGTTGAACTGTTTATCCTCCATTATCTAATTCTGTTTATCATTTTGGT
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P.acut.EU751202.1           GGTGCAGGAACTGGTTGAACAGTTTATCCTCCTTTATCTAATTCTGTATATCATTTTGGA
P.acut.EU751201.1           GGAGCTGGAACAGGTTGAACGGTTTATCCTCCTTTATCAAATTCCGTTTATCATTTTGGA
P.acut.EU751200.1           GGAGCTGGAACAGGTTGAACGGTTTATCCTCCTTTACCAAATTCCGTTTATCATTTTGGA
Birch.Lake.WI               GGACCTGGAACAGGTTGAACAGTTTATCCTCCTTTATCTAATTCTGTTTATCATTTTGGA
M.quad.JX184004.1           GGACCAGGAACTGGTTGAACAGTGTATCCTCCTTTATCAAATTCTATTTATCATTTTGGA
M.quad.JX184003.1           GGGCCAGGAACTGGTTGAACAGTATACCCTCCTTTATCAAATTCTATTTATCATTTTGGA
M.quad.DQ078536.1           GGGCCAGGAACTGGTTGAACAGTATACCCTCCTTTATCAAATTCTATTTATCATTTTGGA
A.vaga.GQ465646.1           GGTCCAGGCACAGGTTGAACTGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGT
A.vaga.GQ465645.1           GGTCCAGGCACAGGTTGAACTGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGT
A.vaga.GQ465644.1           GGTCCAGGCACAGGTTGAACTGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGT
Macrot.sp.DQ078538.1        GGTCCTGGAACTGGATGAACTGTTTACCCACCTTTATCTAATTCTGTTTATCATTTTGGA
Macrot.sp.DQ078540.1        GGTCCTGGAACTGGATGAACTGTTTACCCACCTTTATCTAATTCTGTTTATCATTTTGGA
Macrot.sp.DQ078539.1        GGTCCTGGAACTGGATGAACTGTTTACCCACCTTTATCTAATTCTGTTTATCATTTTGGA
Philodina.sp.DQ078562.1     GGTCCTGGGACTGGATGAACAGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGA
Philodina.sp.DQ078563.1     GGTCCTGGGACTGGATGAACAGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGA
Kettle4.Hueco.Tanks.1       GGTCCTGGGACTGGATGAACAGTTTATCCTCCTTTGTCTAATTCTGTTTATCATTTTGGA
P.rose.HM032973.1           GGTCCTGGAACAGGGTGAACTGTATATCCTCCTTTATCTAATTCTGTTTATCATTTTGGC
P.rose.HM032972.1           GGTCCTGGAACAGGGTGAACTGTATATCCTCCTTTATCTAATTCTGTTTATCATTTTGGC

B.caly.GU232733.1           ATTTCGGTTGATTTAGCGATTTTTAGTCTTCACTTATCTGGGGTCTCTTCTATCTTAGGT
R.rotatoria.EU499874.1      GGTTCTATTGATTTGGCTATTTTTAGATTACATATTGCTGGTGTGTCGTCTATTTTAGCT
R.sordida.KM043210.1        GGTTCTGTTGATTTAGCAATTTTTAGATTACATGTTGCTGGTGTTTCTTCTATTTTAGCT
R.sordida.EU751162.1        GGGTCTGTTGATTTAGCAATTTTTAGGTTACATGTAGCTGGTGTATCATCTATTTTAGCT
P.greg.KJ543688.1           GGTTCTGTTGATTTAGCTATTTTTAGATTACATGTAGCAGGAGTTTCTTCTATTTTAGGT
P.greg.KJ543687.1           GGTTCTGTTGATTTAGCTATTTTTAGATTACATGTAGCAGGAGTTTCTTCTATTTTAGGT
P.flav.DQ890156.1           GGATCAGTGGATTTAGCAATTTTTAGATTGCATGTGGCAGGCGTTTCTTCTATTTTAGGA
P.flav.DQ890155.1           GGGTCTGTTGATTTAGCTATTTTTAGTTTGCATGTAGCAGGGGTTTCTTCTATTTTGGGG
Ascarate.Lake.1             GGGTCTGTTGATTTTGCTATTTTTAGATTACATGTGGCCGGGGTATCTTCTATTTTAGGG
P.mega.JQ309197.1           GGGTCAGTTGATTTTGCTATTTTTAGTCTACATGTTGCGGGTGTTTCTTCTATTTTAGGT
P.mega.JQ309199.1           GGGTCAGTTGATTTTGCTATTTTTAGTCTACATGTTGCGGGTGTTTCTTCTATTTTAGGT
P.mega.JQ309198.1           GGGTCAGTTGATTTTGCTATTTTTAGTCTACATGTTGCGGGTGTTTCTTCTATTTTAGGT
R.rotatoria.KM043209.1      GGGTCAGTTGATTTAGCTATTTTTAGCCTTCATATTGCAGGTGTGTCTTCTATTTTAGGG
P.citrina.EU499765.1        GGATCTGTAGATTTGGCTATTTTTAGTTTACATGTAGCTGGAGTTTCATCAATTTTAGGA
P.citrina.KM043200.1        GGTTCTGTAGATTTAGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCGATTTTAGGA
BRT.no.41.MM37.1            GGTTCTGTAGATTTAGCAATTTTTAGTTTGCATGTTGCTGGGGTTTCTTCTATTTTAGGG
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P.dupl.EU751209.1           GGTTCAGTTGATTTTGCTATTTTTAGATTACATGTTGCTGGTGTGTCATCTATTTTAGGA
P.acut.EU751202.1           GGATCTGTTGATTTAGCTATTTTTAGACTACATGTTGCAGGTGTTTCATCAATTTTAGGA
P.acut.EU751201.1           GGTTCTGTGGATTTAGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCTATTTTAGGA
P.acut.EU751200.1           GGTTCTGTTGATTTAGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCTATTTTAGGA
Birch.Lake.WI               GGATCAGTTGATTTAGCTATTTTTAGATTACATGTTGCGGGGGTATCTTCTATTTTAGCT
M.quad.JX184004.1           GGATCTGTTGATTTTGCTATTTTTAGGTTACATGTTGCTGGGGTTTCTTCTATTTTGGGG
M.quad.JX184003.1           GGATCTGTTGATTTTGCTATTTTTAGATTACATGTTGCTGGGGTTTCTTCTATTTTAGGG
M.quad.DQ078536.1           GGATCTGTTGATTTTGCTATTTTTAGATTACATGTTGCTGGGGTTTCTTCTATTTTAGGG
A.vaga.GQ465646.1           GGTTCTGTTGATTTGGCTATTTTTAGTTTACATGTAGCTGGAGTTTCTTCTATTTTAGGA
A.vaga.GQ465645.1           GGTTCTGTTGATTTGGCTATTTTTAGTTTACATGTAGCTGGAGTTTCTTCTATTTTAGGA
A.vaga.GQ465644.1           GGTTCTGTTGATTTGGCTATTTTTAGTTTACATGTAGCTGGAGTTTCTTCTATTTTAGGA
Macrot.sp.DQ078538.1        GGTTCAATTGATTTTGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCAATTTTGGGT
Macrot.sp.DQ078540.1        GGTTCAATTGATTTTGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCAATTTTGGGT
Macrot.sp.DQ078539.1        GGTTCAATTGATTTTGCTATTTTTAGTTTACATGTTGCTGGAGTTTCTTCAATTTTGGGT
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Philodina.sp.DQ078563.1      GGTTCTATTGATTTTGCTATTTTTAGATTACATGTCGCTGGGGTTTCATCTATTTTAGGT
Kettle4.Hueco.Tanks.1        GGTTCTATTGATTTTGCTATTTTTAGATTACATGTCGCTGGGGTTTCATCTATTTTAGGT
P.rose.HM032973.1            GGTTCTATTGATTTAGCTATTTTTAGTTTACATGTAGCTGGTGTTTCTTCAATTTTAGGG
P.rose.HM032972.1            GGTTCTATTGATTTAGCTATTTTTAGTTTACATGTAGCTGGTGTTTCTTCAATTTTAGGG

B.caly.GU232733.1            AGGATTAACTTCTTGACCACTATTATTTGCTCACGTACTACAAAAAGA------ATCTCG
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R.sordida.KM043210.1         AGTATTAATTT------TATTACTACGTGTATAAAAGGTAAGATTAGYYMTATTTTAAGA
R.sordida.EU751162.1         AGTATTAATTT------TATTACTACTTGTATAAAGGGTAAAATTAGATATGTATTGAGT
P.greg.KJ543688.1            TCTATTAATTT------CATTACTACTTGTCTAAAAGGTAAAATCAGTTACGTCTTATCT
P.greg.KJ543687.1            TCTATTAATTT------CATTACTACTTGTCTAAAAGGTAAAATCAG---TGTCTTATCT
P.flav.DQ890156.1            GGAATTAATTT------TATTACGACATGTTTGAAAGGAAAAATTAGTTATGTTTTATCT
P.flav.DQ890155.1            GGGATTAATTT------TATTACAACTTGTTTAAAAGGGAAAATTAGTTATGTTTTATCT
Ascarate.Lake.1              GCTATTAATTT------TATTACTACATGTTTAAAAGGTAAGGCTAGATATGTCTTGTCT
P.mega.JQ309197.1            GCAATTAATTT------TATTACTACGTGTTTAAAAGGTAAGGCAAATTATATTTTATCT
P.mega.JQ309199.1            GCAATTAATTT------TATTACTACGTGTTTAAAAGGTAAGGCAAATTATATTTTATCT
P.mega.JQ309198.1            GCAATTAATTT------TATTACTACGTGTTTAAAAGGTAAGGCAAATTATATTTTATCT
R.rotatoria.KM043209.1       GGGATTAATTT------TATTACTACTTGTATAAAAGGTAAAATTAGTTTTATTTTAAGG
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P.citrina.KM043200.1         GGAATTAATTT------TATTACAACATGTATAAAAGGGAAAATTAGTTATGTACTTTCT
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P.acut.EU751202.1            GCAATTAATTT------TATTACTACTTGTTTAAAAGGAAAAATAAGTTATATTTTATCT
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P.acut.EU751200.1            GCTATTAATTT------TATTACTCCATGTTTAAAAGGAAAAATCAGAAATGTTTTATCT
Birch.Lake.WI                AGGATTAATTT------TATTACAACTTGTATGAAAGGTAAGATTAGGTATATTATAAGA
M.quad.JX184004.1            GGAATTAATTT------TATTACAACTTGTTTAAAAGGGAAAATTAGATACGTTATAAGA
M.quad.JX184003.1            GGAATTAATTT------TATTACAACTTGTTTAAAAGGGAAAATTAGATACGTTATAAGA
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A.vaga.GQ465646.1            GGTATTAATTT------TATTACTACTTGTTTGAAAGGTAAAATTAGTTATGTTTTAAGT
A.vaga.GQ465645.1            GGTATTAATTT------TATTACTACTTGTTTGAAAGGTAAAATTAGTTATGTTTTAAGT
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Macrot.sp.DQ078538.1         GGGATTAATTT------TATTACTACTTGTTTAAAAGGAAAAGTTAGTTTTGTAATAAGA
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Macrot.sp.DQ078539.1         GGGATTAATTT------TATTACTACTTGTTTAAAAGGAAAAGTTAGTTTTGTAATAAGA
Philodina.sp.DQ078562.1      GGGATTAATTT------CATTACGACTTGTTTAAAAGGAAAGATTAGATATGTATTATCT
Philodina.sp.DQ078563.1      GGGATTAATTT------CATTACGACTTGTTTAAAAGGAAAGATTAGATATGTATTATCT
Kettle4.Hueco.Tanks.1        GGGATTAATTT------CATTACGACTTGTTTAAAAGGAAAGATTAGATATGTATTATCT
P.rose.HM032973.1            GGGATTAATTT------TATTACGACTTGTTTAAAAGGTAAAATTAGATATGTTTTATCT
P.rose.HM032972.1            GGGATTAATTT------TATTACGACTTGTTTAAAAGGTAAAATTAGATATGTTTTATCT

B.caly.GU232733.1            TTAGACCGTCTTCCTCTCTTCTTATGGGCTATTGCTGTAACAGCAGTGCTCTTGATTACA
R.rotatoria.EU499874.1       TTGGAATATTTAACATTGTTTGTTTGAGCTATAATTGTTACAAGATTTCTATTAGTTCTT
R.sordida.KM043210.1         TTAGAATATTTAACTTTGTTTGTTTGGGCTATGATTGTCACTAGATTTTTATTGCTTTTA
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R.rotatoria.KM043209.1       TTAGAATATTTGACTTTATTTGTTTGGGCTATAATTGTTACTAGATTTTTATTAGTTTTA
P.citrina.EU499765.1         TTTGAGTTTTTAAATTTATTTGTTTGGGCAATAATTGTAACGAGATTTTTATTAGTCTTG
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BRT.no.41.MM37.1             GTTGAGTTTTTAACATTATTTGTTTGAGCAATAATTGTTACAAGATTTTTATTAGTTTTA
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P.dupl.EU751209.1            TTCGAGTTTTTAACTTTATTTGTTTGAGCAATAATTGTAACA------------------
P.acut.EU751202.1            TTTGAATTTTTAACTTTATTTGTTTGAGCTATAATTGTTACTAGATTTTTATTAGTGTTG
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P.acut.EU751200.1            TTTGAATTTTTAACTTTATTTGTTTGAGCAATAATTGTTACTAGATTTTTATTAGTTCTA
Birch.Lake.WI                TTAGAATATTTAACCTTGTTTGTTTGAGCCATAATTGTTACTAGGTTTTTATTAGTTTTG
M.quad.JX184004.1            TTTGAATTTTTAACTTTATTTGTTTGAGCAATAATTGTTACTAGATTTTTATTAGTTTTA
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M.quad.DQ078536.1           TTTGAATTTTTAACTTTATTTGTTTGAGCAATAATTGTTACTAGATTTTTATTAGTTTTA
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Macrot.sp.DQ078538.1        GTTGAATTTTTAACTTTATTTTCTTGAGCTATGGTGGTTACTAGTTTTTTATTAGTATTA
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Philodina.sp.DQ078562.1     TTGGAGTTTTTAACTTTATTTAGATGGGCAATGGTAGTAACTAGGTTTTTACTAGTCTTA
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Kettle4.Hueco.Tanks.1       TTGGAGTTTTTAACTTTATTTAGATGGGCAATGGTAGTAACTAGGTTTTTACTAGTCTTA
P.rose.HM032973.1           TTAGAATTTTTAACTTTGTTTAGTTGGGCGATGGTTGTTACTAGATTTTTGTTAGTCTTG
P.rose.HM032972.1           TTAGAATTTTTAACTTTGTTTAGTTGGGCGATGGTTGTTACTAGATTTTTGTTAGTCTTG

B.caly.GU232733.1           AGGCTTCCCGTGTTAGCTGGGGCTATTACTATGTTACTTACCGATCGTAATTTTAATACC
R.rotatoria.EU499874.1      AGATTACCTGTTTTAGCTGGTGGGATTACTATATTATTATTAGATCGTAATTTTGGGTCT
R.sordida.KM043210.1        AGTTTACCAGTGTTAGCTGGTGGGATTACTATATTGTTGTT-------------------
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P.greg.KJ543688.1           AGATTACCTGTCTTGGCAGGGGGAATTACAATATTACTATTAGATCGTAATTTTGGATCT
P.greg.KJ543687.1           AGATTACCTGTCTTGGCAGGGGGAATTACAATATTACTATTAGATCGTAATTTTGGATCT
P.flav.DQ890156.1           AGGTTACCTGTTTTAGCGGGTGGTATTACTATGCTATTGTTAGATAATAATTTTGGCTCC
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P.mega.JQ309197.1           AGTTTACCAGTGTTAGCCGGAGGTATTACTATGTTATTATTAGATCGAAACTTTGGTTCT
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P.mega.JQ309198.1           AGTTTACCAGTGTTAGCCGGAGGTATTACTATGTTATTATTAGATCGAAACTTTGGTTCT
R.rotatoria.KM043209.1      AGTTTACCGGTATTAGCTGGAGGAATTACTATGCTTTTATTAGATCGTAATTTTGGTTCT
P.citrina.EU499765.1        AGGCTTCCTGTTTTAGCAGGAGGTATTACAATGTTATTGTTAGATCGTAATTTTGGATCC
P.citrina.KM043200.1        AGTCTTCCTGTATTAGCAGGAGGAATTACAATATTATTACTTGATCGTAATTTTGGTTCT
BRT.no.41.MM37.1            AGATTACCTGTTTTAGCTGGAGGAATTACAATATTATTGTTGGATCGTAATTTTGGTTCT
P.dupl.EU751211.1           AGTTTACCTGTTTTGGCTGGAGGTATTACGATATTAT-----------------------
P.dupl.EU751209.1           ------------------------------------------------------------
P.acut.EU751202.1           AGATTCCCAGTATTCGCTGGTGGGATTCCAATATTATTATTAGATCG-------------
P.acut.EU751201.1           AGATTCCCTGTTTTAGCTGGAGGTATTCCAATGTTATTATTAGATCGAAATTT-------
P.acut.EU751200.1           AGATTCCCTGTTTTAGCTGGAGGTA-----------------------------------
Birch.Lake.WI               AGTCTTCCAGTTTTAGCTGGAGGGATTACTATACTATTATTAGATCGTAATTTTGGTGCT
M.quad.JX184004.1           AAACTTCCAGTTTTAGCTGGTGGAATTACTATGTTGTTATTAGATCGTAATTTTGGGGCT
M.quad.JX184003.1           AGACTTCCAGTTTTAGCTGGTGGAATTACTATGTTATTATTAGATCGTAATTTTGGGGCT
M.quad.DQ078536.1           AGACTTCCAGTTTTAGCTGGTGGAATTACTATGTTATTATTAGATCGTAATTTTGGGGCT
A.vaga.GQ465646.1           AGTTTACCTGTTTTAGCGGGGGGTATTACTATGTTGTTATTAAATCGTAATTTTGGTTCT
A.vaga.GQ465645.1           AGTTTACCTGTTTTAGCGGGGGGTATTACTATGTTGTTATTAGATCGTAATTTTGGTTCT
A.vaga.GQ465644.1           AGTTTACCTGTTTTAGCGGGGGGTATTACTATGTTGTTATTAGATCGTAATTTTGGTTCT
Macrot.sp.DQ078538.1        AGTCTTCCTGTATTAGCTGGAGGTATTACTATATTGTTATTAGATCGAAATTTTGGTTCT
Macrot.sp.DQ078540.1        AGTCTTCCTGTATTAGCTGGAGGTATTACTATATTGTTATTAGATCGAAATTTTGGTTCT
Macrot.sp.DQ078539.1        AGTCTTCCTGTATTAGCTGGAGGTATTACTATATTGTTATTAGATCGAAATTTTGGTTCT
Philodina.sp.DQ078562.1     AGTTTACCTGTATTAGCTGGTGGGATTACTATGTTATTATTAGATCGTAACTTTGGTTCG
Philodina.sp.DQ078563.1     AGTTTACCTGTATTAGCTGGTGGGATTACTATGTTATTATTAGATCGTAACTTTGGTTCG
Kettle4.Hueco.Tanks.1       AGTTTACCTGTATTAGCTGGTGGGATTACTATGTTATTATTAGATCGTAACTTTGGTTCG
P.rose.HM032973.1           AGATTGCCTGTATTAGCAGGTGGTATTACTATATTGTTGTTAGATCGTAATTTTGGTTCT
P.rose.HM032972.1           AGATTGCCTGTATTAGCAGGTGGTATTACTATATTGTTGTTAGATCGTAATTTTGGTCCT

B.caly.GU232733.1           TCTTTCTTTGACC
R.rotatoria.EU499874.1      TCTTTTTTTGATC
R.sordida.KM043210.1        -------------
R.sordida.EU751162.1        -------------
P.greg.KJ543688.1           TCTTTCTTTGATC
P.greg.KJ543687.1           TCTTTCTTTGATC
P.flav.DQ890156.1           TCTTTTTTTGAT-
P.flav.DQ890155.1           TCTTTTTTTGAT-
Ascarate.Lake.1             TCTTTTTTTGATC
P.mega.JQ309197.1           TCTTTTTTTGATC
P.mega.JQ309199.1           -------------
P.mega.JQ309198.1           TCTTTTTTTGATC
R.rotatoria.KM043209.1      TCTTTTTTTGATC
P.citrina.EU499765.1        TCATTTTTTGATC
P.citrina.KM043200.1        TCATTTTTTGATC
BRT.no.41.MM37.1            TCTTTTTTTGATC
P.dupl.EU751211.1           -------------
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P.dupl.EU751209.1            -------------
P.acut.EU751202.1            -------------
P.acut.EU751201.1            -------------
P.acut.EU751200.1            -------------
Birch.Lake.WI                TCGTTTTTTGATC
M.quad.JX184004.1            TCTTTTTTTGATC
M.quad.JX184003.1            TCTTTTTTTGACC
M.quad.DQ078536.1            TCTTTTTTTGACC
A.vaga.GQ465646.1            TCTTTTTTTGATC
A.vaga.GQ465645.1            TCTTTTTTTGATC
A.vaga.GQ465644.1            TCTTTTTTTGATC
Macrot.sp.DQ078538.1         TCCTTTTTTGATC
Macrot.sp.DQ078540.1         TCCTTTTTTGATC
Macrot.sp.DQ078539.1         TCCTTTTTTGATC
Philodina.sp.DQ078562.1      TCTTTTTTTGATC
Philodina.sp.DQ078563.1      TCTTTTTTTGATC
Kettle4.Hueco.Tanks.1        TCTTTTTTTGATC
P.rose.HM032973.1            TCTTTTTTTGACC
P.rose.HM032972.1            TCTTTTTTTGACC
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Appendix 3: GLM Summary Results for All Three 2-Way Interactions

Output from GLM analysis of bdelloid rotifer mortality as a response to various UVR exposure levels and drying times with a logit link function and binomial error structure. Odds ratios and their confidence intervals (95% CI) were calculated by an exponential transformation of the coefficient estimates. In the model, reference coefficients were set to the Birch Lake population, 24 hr desiccation time, and no exposure (negative control). Values in bold represent significant *p*-values. S.E. = Standard error. z = Wald statistic.

Fixed Effect	Coefficient Estimate	S.E.	z	*p*-value	Odds Ratio	95% CI
Intercept	-1.97	0.15	-13.48	**<0.01**	0.14	0.10, 0.84
1 week desiccation	1.32	0.16	0.76	**<0.01**	3.76	2.73, 5.19
1 month desiccation	1.15	0.16	7.02	**<0.01**	3.15	2.29, 4.35
26 µW/cm^2	0.62	0.17	3.59	**<0.01**	1.86	1.33, 2.61
130 µW/cm^2	1.54	0.17	9.29	**<0.01**	4.68	3.39, 6.50
370 µW/cm^2	3.36	0.18	18.51	**<0.01**	28.83	20.3, 41.39
Ascarate Lk.	-0.45	0.19	-2.40	**0.06**	1.56	1.09, 2.25
BRT	-0.51	0.18	-2.79	**0.01**	1.67	1.17, 2.4
Kettle 4	-0.34	0.17	-1.98	**0.05**	1.4	1, 1.98
1 week desiccation:26 µW/cm^2	0.14	0.18	0.77	0.44	1.15	0.80, 1.65
1 month desiccation:26 µW/cm^2	0.49	0.18	2.77	**0.01**	1.63	1.15, 2.29
1 week desiccation:130 µW/cm^2	0.06	0.18	0.36	0.72	1.07	0.75, 1.50
1 month desiccation:130 µW/cm^2	0.53	0.17	3.11	**<0.01**	1.70	1.22, 2.38
1 week desiccation:370 µW/cm^2	-0.75	0.18	-4.19	**<0.01**	0.47	0.33, 0.67
1 month desiccation:370 µW/cm^2	-0.15	0.18	-0.82	0.414	0.86	0.61, 1.22
26 µW/cm^2:Ascarate Lk.	0.21	0.20	1.09	0.275	0.81	0.55, 1.19
130 µW/cm^2:Ascarate Lk.	0.69	0.19	3.59	**<0.01**	0.50	0.34, 0.73
Quartz filter:Ascarate Lk.	0.30	0.21	1.42	0.16	0.74	0.49, 1.12
26 µW/cm^2:BRT	0.02	0.19	0.11	0.91	1.02	0.70, 1.49
130 µW/cm^2:BRT	-0.33	0.19	-1.75	0.08	0.72	0.50, 1.04
370 µW/cm^2:BRT	-0.56	0.21	-2.75	**0.01**	0.57	0.38, 0.85
26 µW/cm^2:Kettle 4	-0.02	0.18	-0.10	0.92	0.98	0.69, 1.39
130 µW/cm^2:Kettle 4	-0.34	0.18	-1.89	0.06	0.71	0.50, 1.01
370 µW/cm^2:Kettle 4	-0.45	0.20	-2.27	**0.02**	0.64	0.43, 0.94
1 week desiccation:Ascarate Lk.	-1.19	0.17	7.11	**<0.01**	0.31	0.22, 0.42
1 month desiccation:Ascarate Lk.	-0.31	0.17	1.89	0.06	0.73	0.53, 1.01
1 week desiccation:BRT	-1.24	0.16	-7.62	**<0.01**	0.29	0.21, 0.40
1 month desiccation:BRT	0.03	0.16	0.21	0.84	1.03	0.75, 1.42
1 week desiccation:Kettle 4	-0.39	0.16	-2.53	**0.01**	0.67	0.50, 0.91
1 month desiccation:Kettle 4	-0.17	0.16	-1.03	0.30	0.85	0.62, 1.16

Appendix 4: Summary Results for Interactions between Populations & Desiccation

Output from the *post-hoc* Tukey HSD test conducted on the GLM analysis to compare the general linear hypotheses of bdelloid mortality for the interactions between bdelloid population and desiccation times. Linear hypotheses are set up with the population first and desiccation time next, and assume that the differences between the interactions is equal to 0. Desiccation times are represented by the following: 24 hr = 1, 1 week = 2, and 1 month = 3. Values in bold represent significant values (<0.05). S.E. = Standard error; z-value = standard score; Pr(>|z) = significance adjusted for multiple tests.

Linear Hypotheses	Coefficient Estimate	S.E.	z-value	Pr(>|z)
Ascarate.1 – Birch.1	-0.61	0.10	-6.09	**<0.01**
BRT.1 – Birch.1	-0.62	0.10	-6.13	**<0.01**
Kettle.1 – Birch.1	-0.47	0.10	-4.75	**<0.01**
Birch.2 – Birch.1	0.94	0.09	9.97	**<0.01**
Ascarate.2 – Birch.1	-0.72	0.10	-6.96	**<0.01**
BRT.2 – Birch.1	-0.71	0.10	-7.06	**<0.01**
Kettle.2 – Birch.1	0.17	0.09	1.82	0.81
Birch.3 – Birch.1	1.11	0.10	11.56	**<0.01**
Ascarate.3 – Birch.1	0.24	0.09	2.59	0.28
BRT.3 – Birch.1	0.57	0.09	6.23	**<0.01**
Kettle.3 – Birch.1	0.53	0.09	5.63	**<0.01**
BRT.1 – Ascarate.1	-0.01	0.12	-0.07	1.00
Kettle.1 – Ascarate.1	0.15	0.10	1.40	0.96
Birch.2 – Ascarate.1	1.55	0.10	15.31	**<0.01**
Ascarate.2 – Ascarate.1	-0.11	0.11	-0.96	1.00
BRT.2 – Ascarate.1	-0.10	0.11	-0.95	1.00
Kettle.2 – Ascarate.1	0.78	0.10	7.74	**<0.01**
Birch.3 – Ascarate.1	1.72	0.10	16.72	**<0.01**
Ascarate.3 – Ascarate.1	0.85	0.10	8.46	**<0.01**
BRT.3 – Ascarate.1	1.19	0.10	11.94	**<0.01**
Kettle.3 – Ascarate.1	1.14	0.10	11.28	**<0.01**
Kettle.1 – BRT.1	0.15	0.11	1.46	0.95
Birch.2 – BRT.1	1.56	0.10	15.32	**<0.01**
Ascarate.2 – BRT.1	-0.10	0.11	-0.89	1.00
BRT.2 – BRT.1	-0.09	0.10	-0.88	1.00
Kettle.2 – BRT.1	0.79	0.10	7.78	**<0.01**
Birch.3 – BRT.1	1.73	0.10	16.72	**<0.01**
Ascarate.3 – BRT.1	0.86	0.10	8.49	**<0.01**
BRT.3 – BRT.1	1.19	0.10	11.96	**<0.01**
Kettle.3 – BRT.1	1.15	0.10	11.30	**<0.01**
Birch.2 – Kettle.1	1.40	0.10	14.24	**<0.01**
Ascarate.2 – Kettle.1	-0.25	0.11	-2.35	0.44
BRT.2 – Kettle.1	-0.25	0.11	-2.36	0.43
Kettle.2 – Kettle.1	0.64	0.10	6.46	**<0.01**
Birch.3 – Kettle.1	1.57	0.10	15.69	**<0.01**

Ascarate.3 – Kettle.1	0.71	0.10	7.20	<**0.01**
BRT.3 – Kettle.1	1.04	0.10	10.75	<**0.01**
Kettle.3 – Kettle.1	0.99	0.10	10.09	<**0.01**
Ascarate.2 – Birch.2	-1.65	0.10	-15.97	<**0.01**
BRT.2 – Birch.2	-0.65	0.10	-16.21	<**0.01**
Kettle.2 – Birch.2	-0.77	0.09	-8.10	<**0.01**
Birch.3 – Birch.2	0.17	0.10	1.77	0.83
Ascarate.3 – Birch.2	-0.69	0.09	-7.33	<**0.01**
BRT.3 – Birch.2	-0.36	0.09	-3.91	<**0.01**
Kettle.3 – Birch.2	-0.41	0.09	-4.32	<**0.01**
BRT.2 – Ascarate.2	0.003	0.11	0.03	1.00
Kettle.2 – Ascarate.2	0.89	0.10	8.57	<**0.01**
Birch.3 – Ascarate.2	1.83	0.11	17.33	<**0.01**
Ascarate.3 – Ascarate.2	0.96	0.10	9.27	<**0.01**
BRT.3 – Ascarate.2	1.29	0.10	12.67	<**0.01**
Kettle.3 – Ascarate.2	1.25	0.10	12.03	<**0.01**
Kettle.2 – BRT.2	0.88	0.10	8.69	<**0.01**
Birch.3 – BRT.2	1.82	0.10	17.60	<**0.01**
Ascarate.3 – BRT.2	0.96	0.10	9.41	<**0.01**
BRT.3 – BRT.2	1.29	0.10	12.87	<**0.01**
Kettle.3 – BRT.2	1.24	0.10	12.21	<**0.01**
Birch.3 – Kettle.2	0.94	0.10	9.72	<**0.01**
Ascarate.3 – Kettle.2	0.07	0.09	0.77	1.00
BRT.3 – Kettle.2	0.40	0.09	4.36	<**0.01**
Kettle.3 – Kettle.2	0.36	0.09	3.79	<**0.01**
Ascarate.3 – Birch.3	-0.87	0.10	-8.97	<**0.01**
BRT.3 – Birch.3	-0.53	0.09	-5.63	<**0.01**
Kettle.3 – Birch.3	-0.58	0.10	-6.01	<**0.01**
BRT.3 – Ascarate.3	0.33	0.09	3.57	**0.02**
Kettle.3 – Ascarate.3	0.29	0.09	3.02	0.10
Kettle.3 – BRT.3	-0.05	0.09	-0.50	1.00

Appendix 5: Summary Results for Interactions between Populations & UV Exposures

Output from the *post-hoc* Tukey HSD test conducted on the GLM analysis to compare the general linear hypotheses of bdelloid mortality for the interactions between bdelloid population and UV exposures. Linear hypotheses are set up with the population first and desiccation time next, and assume that the differences between the interactions is equal to 0. UV exposure levels are represented by the following: Negative control = 0, 26 μW/cm^2 = 1, 130 μW/cm^2 = 2, and 370 μW/cm^2 = 3. Values in bold represent significant values (<0.05). S.E. = Standard error; z-value = standard score; Pr(>|z) = significance adjusted for multiple tests.

Linear Hypotheses	Coefficient Estimate	S.E.	z-value	Pr(>|z)
Ascarate.0 – Birch.0	-0.98	0.15	-6.75	**<0.01**
BRT.0 – Birch.0	-0.87	0.14	-6.16	**<0.01**
Kettle.0 – Birch.0	-0.56	0.13	-4.22	**<0.01**
Birch.1 – Birch.0	0.78	0.11	6.79	**<0.01**
Ascarate.1 – Birch.0	-0.32	0.13	-2.53	0.44
BRT.1 – Birch.0	-0.02	0.12	-0.18	1.00
Kettle.1 – Birch.0	0.27	0.12	2.33	0.59
Birch.2 – Birch.0	1.58	0.12	13.71	**<0.01**
Ascarate.2 – Birch.0	0.09	0.12	0.78	1.00
BRT.2 – Birch.0	0.47	0.12	4.11	**<0.01**
Kettle.2 – Birch.0	0.78	0.12	6.71	**<0.01**
Birch.3 – Birch.0	2.92	0.14	20.71	**<0.01**
Ascarate.3 – Birch.0	1.72	0.12	14.41	**<0.01**
BRT.3 – Birch.0	1.46	0.11	12.72	**<0.01**
Kettle.3 – Birch.0	1.98	0.12	16.28	**<0.01**
BRT.0 – Ascarate.0	0.11	0.16	0.71	1.00
Kettle.0 – Ascarate.0	0.43	0.15	2.76	0.29
Birch.1 – Ascarate.0	1.76	0.14	12.51	**<0.01**
Ascarate.1 – Ascarate.0	0.66	0.15	4.39	**<0.01**
BRT.1 – Ascarate.0	0.96	0.15	6.58	**<0.01**
Kettle.1 – Ascarate.0	1.25	0.14	8.80	**<0.01**
Birch.2 – Ascarate.0	2.56	0.14	18.14	**<0.01**
Ascarate.2 – Ascarate.0	1.08	0.15	7.41	**<0.01**
BRT.2 – Ascarate.0	1.46	0.14	10.30	**<0.01**
Kettle.2 – Ascarate.0	1.76	0.14	12.42	**<0.01**
Birch.3 – Ascarate.0	3.90	0.16	23.95	**<0.01**
Ascarate.3 – Ascarate.0	2.71	0.14	18.68	**<0.01**
BRT.3 – Ascarate.0	2.44	0.14	17.33	**<0.01**
Kettle.3 – Ascarate.0	2.96	0.15	20.22	**<0.01**
Kettle.0 – BRT.0	0.31	0.15	2.07	0.78
Birch.1– BRT.0	1.64	0.14	12.12	**<0.01**
Ascarate.1 – BRT.0	0.55	0.15	3.74	**0.02**
BRT.1 – BRT.0	0.85	0.14	5.99	**<0.01**
Kettle.1 – BRT.0	1.14	0.14	8.28	**<0.01**
Birch.2 – BRT.0	2.45	0.14	17.95	**<0.01**
Ascarate.2 – BRT.0	0.96	0.14	6.85	**<0.01**
BRT.2 – BRT.0	1.34	0.14	9.83	**<0.01**
Kettle.2 – BRT.0	1.64	0.14	12.02	**<0.01**

Birch.3 – BRT.0	3.78	0.16	23.86	<**0.01**
Ascarate.3 – BRT.0	2.59	0.14	18.50	<**0.01**
BRT.3 – BRT.0	2.33	0.14	17.11	<**0.01**
Kettle.3 – BRT.0	2.85	0.14	20.09	<**0.01**
Birch.1 – Kettle.0	1.33	0.13	10.57	<**0.01**
Ascarate.1 – Kettle.0	0.23	0.14	1.71	0.94
BRT.1 – Kettle.0	0.53	0.13	4.04	<**0.01**
Kettle.1 – Kettle.0	0.83	0.13	6.46	<**0.01**
Birch.2 – Kettle.0	2.13	0.13	16.84	<**0.01**
Ascarate.2 – Kettle.0	0.65	0.13	4.95	<**0.01**
BRT.2 – Kettle.0	1.03	0.13	8.11	<**0.01**
Kettle.2 – Kettle.0	1.33	0.13	10.47	<**0.01**
Birch.3 – Kettle.0	3.47	0.15	23.08	<**0.01**
Ascarate.3 – Kettle.0	2.28	0.13	17.42	<**0.01**
BRT.3 – Kettle.0	2.02	0.13	15.94	<**0.01**
Kettle.3 – Kettle.0	2.53	0.13	19.12	<**0.01**
Ascarate.1 – Birch.1	-1.10	0.12	-9.07	<**0.01**
BRT.1 – Birch.1	-0.80	0.11	-6.96	<**0.01**
Kettle.1 – Birch.1	-0.50	0.11	-4.56	<**0.01**
Birch.2 – Birch.1	0.80	0.11	7.39	<**0.01**
Ascarate.2 – Birch.1	-0.68	0.11	-6.01	<**0.01**
BRT.2 – Birch.1	-0.30	0.11	-2.76	0.29
Kettle.2 – Birch.1	-0.0002	0.11	-0.002	1.00
Birch.3 – Birch.1	2.14	0.14	15.80	<**0.01**
Ascarate.3 – Birch.1	0.95	0.14	8.36	<**0.01**
BRT.3 – Birch.1	0.69	0.11	6.33	<**0.01**
Kettle.3 – Birch.1	1.20	0.12	10.43	<**0.01**
BRT.1 – Ascarate.1	0.30	0.13	2.35	0.58
Kettle.1 – Ascarate.1	0.59	0.12	4.81	<**0.01**
Birch.2 – Ascarate.1	1.90	0.12	15.61	<**0.01**
Ascarate.2 – Ascarate.1	0.41	0.13	3.28	0.08
BRT.2 – Ascarate.1	0.80	0.12	6.53	<**0.01**
Kettle.2 – Ascarate.1	1.10	0.12	8.98	<**0.01**
Birch.3 – Ascarate.1	3.24	0.15	22.15	<**0.01**
Ascarate.3 – Ascarate.1	2.04	0.13	16.42	<**0.01**
BRT.3 – Ascarate.1	1.78	0.13	14.68	<**0.01**
Kettle.3 – Ascarate.1	2.29	0.13	18.01	<**0.01**
Kettle.1 – BRT.1	0.29	0.12	2.51	0.46
Birch.2 – BRT.1	1.60	0.12	13.86	<**0.01**
Ascarate.2 – BRT.1	0.12	0.12	0.96	1.00
BRT.2 – BRT.1	0.50	0.12	4.29	<**0.01**
Kettle.2 – BRT.1	0.80	0.12	6.88	<**0.01**
Birch.3 – BRT.1	2.94	0.14	20.82	<**0.01**
Ascarate.3 – BRT.1	1.74	0.12	14.55	<**0.01**
BRT.3 – BRT.1	1.48	0.12	12.87	<**0.01**
Kettle.3 – BRT.1	2.00	0.12	16.42	<**0.01**
Birch.2 – Kettle.1	1.31	0.11	11.75	<**0.01**
Ascarate.2 – Kettle.1	-0.18	0.12	-1.54	0.98
BRT.2 – Kettle.1	0.20	0.11	1.82	0.91
Kettle.2 – Kettle.1	0.50	0.11	4.51	<**0.01**
Birch.3 – Kettle.1	2.65	0.14	19.22	<**0.01**

Ascarate.3 – Kettle.1	1.45	0.12	12.53	<**0.01**
BRT.3 – Kettle.1	1.19	0.11	10.72	<**0.01**
Kettle.3 – Kettle.1	1.71	0.12	14.49	<**0.01**
Ascarate.2 – Birch.2	-1.49	0.11	-12.98	<**0.01**
BRT.2 – Birch.2	-1.10	0.11	-10.05	<**0.01**
Kettle.2 – Birch.2	-0.80	0.11	-7.30	<**0.01**
Birch.3 – Birch.2	1.34	0.14	9.83	<**0.01**
Ascarate.3 – Birch.2	0.14	0.11	1.27	1.00
BRT.3 – Birch.2	-0.12	0.11	-1.07	1.00
Kettle.3 – Birch.2	0.40	0.12	3.45	**0.05**
BRT.2 – Ascarate.2	0.38	0.11	3.33	0.07
Kettle.2 – Ascarate.2	0.68	0.11	5.94	<**0.01**
Birch.3 – Ascarate.2	2.82	0.14	20.13	<**0.01**
Ascarate.3 – Ascarate.2	1.63	0.12	13.71	<**0.01**
BRT.3 – Ascarate.2	1.37	0.11	11.98	<**0.01**
Kettle.3 – Ascarate.2	1.89	0.12	15.60	<**0.01**
Kettle.2 – BRT.2	0.30	0.11	2.72	0.31
Birch.3 – BRT.2	2.44	0.14	17.89	<**0.01**
Ascarate.3 – BRT.2	1.25	0.11	10.90	<**0.01**
BRT.3 – BRT.2	0.99	0.11	9.00	<**0.01**
Kettle.3 – BRT.2	1.50	0.12	12.91	<**0.01**
Birch.3 – Kettle.2	2.14	0.14	15.67	<**0.01**
Ascarate.3 – Kettle.2	0.95	0.11	8.27	<**0.01**
BRT.3 – Kettle.2	0.69	0.11	6.25	<**0.01**
Kettle.3 – Kettle.2	1.20	0.12	10.31	<**0.01**
Ascarate.3 – Birch.3	-1.19	0.14	-8.53	<**0.01**
BRT.3 – Birch.3	-1.46	0.14	-10.70	<**0.01**
Kettle.3 – Birch.3	-0.94	0.14	-6.63	<**0.01**
BRT.3 – Ascarate.3	-0.26	0.11	-2.30	0.62
Kettle.3 – Ascarate.3	0.26	0.12	2.12	0.75
Kettle.3 – BRT.3	0.52	0.12	4.45	<**0.01**

Appendix 6: Summary Results for Interactions between Desiccation & UV Exposures

Output from the *post-hoc* Tukey HSD test conducted on the GLM analysis to compare the general linear hypotheses of bdelloid mortality for the interactions between desiccation times and level of UV exposures. Linear hypotheses are set up with the desiccation time first and UV exposure level next, and assume that the differences between the interactions is equal to 0. UV exposure levels are represented by the following: Negative control = 0, 26 μW/cm^2 = 1, 130 μW/cm^2 = 2, and 370 μW/cm^2 = 3. Values in bold represent significant values (<0.05). S.E. = Standard error; z-value = standard score; Pr(>|z) = significance adjusted for multiple tests.

Linear Hypotheses	Coefficient Estimate	S.E.	z-value	Pr(>|z)
1wk.0 – 24hr.0	0.78	0.14	5.62	**<0.01**
1mth.0 – 24hr.0	1.04	0.14	7.67	**<0.01**
24hr.1 – 24hr.0	0.57	0.14	4.05	**<0.01**
1wk.1 – 24hr.0	1.42	0.13	10.84	**<0.01**
1mth.1 – 24hr.0	2.07	0.13	16.14	**<0.01**
24hr.2 – 24hr.0	1.24	0.13	9.33	**<0.01**
1wk.2 – 24hr.0	1.90	0.13	14.88	**<0.01**
1mth.2 – 24hr.0	2.72	0.13	20.94	**<0.01**
24hr.3 – 24hr.0	3.00	0.13	22.85	**<0.01**
1wk.3 – 24hr.0	2.74	0.13	20.99	**<0.01**
1mth.3 – 24hr.0	3.85	0.14	27.42	**<0.01**
1mth.0 – 1wk.0	0.26	0.11	2.26	0.50
24hr.1 – 1wk.0	-0.21	0.12	-1.69	0.87
1wk.1 – 1wk.0	0.64	0.11	5.85	**<0.01**
1mth.1 – 1wk.0	1.29	0.11	12.14	**<0.01**
24hr.2 – 1wk.0	0.46	0.11	4.12	**<0.01**
1wk.2 – 1wk.0	1.13	0.11	10.62	**<0.01**
1mth.2 – 1wk.0	1.94	0.11	17.93	**<0.01**
24hr.3 – 1wk.0	2.22	0.11	20.20	**<0.01**
1wk.3 – 1wk.0	1.97	0.11	17.98	**<0.01**
1mth.3 – 1wk.0	3.07	0.12	25.44	**<0.01**
24hr.1 – 1mth.0	-0.46	0.12	-3.93	**<0.01**
1wk.1 – 1mth.0	0.38	0.11	3.62	**0.02**
1mth.1 – 1mth.0	1.03	0.10	10.10	**<0.01**
24hr.2 – 1mth.0	0.20	0.11	1.86	0.78
1wk.2 – 1mth.0	0.87	0.10	8.51	**<0.01**
1mth.2 – 1mth.0	1.69	0.10	16.14	**<0.01**
24hr.3 – 1mth.0	1.96	0.11	18.50	**<0.01**
1wk.3 – 1mth.0	1.71	0.11	16.20	**<0.01**
mth.3 – 1mth.0	2.81	0.12	24.00	**<0.01**
1wk.1 – 24hr.1	0.85	0.11	7.47	**<0.01**
1mth.1 – 24hr.1	1.50	0.11	13.58	**<0.01**
24hr.2 – 24hr.1	0.66	0.12	5.77	**<0.01**
1wk.2 – 24hr.1	1.33	0.11	12.12	**<0.01**
1mth.2 – 24hr.1	2.15	0.11	19.16	**<0.01**
24hr.3 – 24hr.1	2.43	0.11	21.34	**<0.01**
1wk.3 – 24hr.1	2.17	0.11	19.20	**<0.01**

1mth.3 – 24hr.1	3.28	0.12	26.93	**<0.01**
1mth.1 – 1wk.1	0.65	0.10	6.74	**<0.01**
24hr.2 – 1wk.1	-0.18	0.10	-1.79	0.82
1wk.2 – 1wk.1	0.49	0.10	5.04	**<0.01**
1mth.2 – 1wk.1	1.30	0.10	13.18	**<0.01**
24hr.3 – 1wk.1	1.58	0.10	15.71	**<0.01**
1wk.3 – 1wk.1	1.33	0.10	13.26	**<0.01**
1mth.3 – 1wk.1	2.43	0.11	21.65	**<0.01**
24hr.2 – 1mth.1	-0.83	0.10	-8.45	**<0.01**
1wk.2 – 1mth.1	-0.17	0.10	-1.78	0.83
1mth.2 – 1mth.1	0.65	0.10	6.83	**<0.01**
24hr.3 – 1mth.1	0.93	0.10	9.56	**<0.01**
1wk.3 – 1mth.1	0.67	0.10	6.99	**<0.01**
1mth.3 – 1mth.1	1.78	0.11	16.29	**<0.01**
1wk.2– 24hr.2	0.67	0.10	6.80	**<0.01**
1mth.2– 24hr.2	1.49	0.10	14.73	**<0.01**
24hr.3– 24hr.2	1.76	0.10	17.19	**<0.01**
1wk.3– 24hr.2	1.51	0.10	14.80	**<0.01**
1mth.3– 24hr.2	2.61	0.11	22.92	**<0.01**
1mth.2– 1wk.2	0.82	0.10	8.59	**<0.01**
24hr.3–1wk.2	1.09	0.10	11.29	**<0.01**
1wk.3–1wk.2	0.84	0.10	8.73	**<0.01**
1mth.3–1wk.2	1.94	0.11	17.85	**<0.01**
24hr.3–1mth.2	0.28	0.10	2.78	0.18
1wk.3–1mth.2	0.02	0.10	0.23	1.00
1mth.3–1mth.2	1.13	0.11	10.14	**<0.01**
1wk.3–24hr.3	-0.25	0.10	-2.53	0.31
1mth.3–24hr.3	0.85	0.11	7.55	**<0.01**
1wk.3–1wk.3	1.11	0.11	9.86	**<0.01**

VITA

Maite Martín was born and raised in El Paso, Texas where she graduated *summa cum laude* from the University of Texas at El Paso in spring 2014 with a Bachelor's of Science degree in Environmental Science concentrating in Biology. She continued to attend graduate school at UTEP in the Environmental Science Master's program in fall of 2014. While pursuing her graduate degree, Maite received research assistantships in the fall of 2014 and 2015 semesters, both of which were funded by NSF DEB 1257068. She served as a Teaching Assistant for the Environmental Science TIERA program (US Dept. of Ed.; P120A130103) program in spring 2015, 2016, and 2017 semesters. She also received summer assistantships through the UTEP Graduate Student RA Summer Program in 2015 and the President Diana Natalicio Environmental Internship in 2016. She presented posters for her research at the XIV International Rotifer Symposium in České Budějovice, Czech Republic (August 2015), the Association for the Sciences of Limnology and Oceanography (ASLO) Meeting in Honolulu, Hawai'i (February 2017), and the Ecological Society of America Annual Meeting (August 2017). She received a full travel award to attend the ASLO conference in 2017.

Maite graduated with her M.S. degree in May 2017 as the Graduate Student Marshal of Students.

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