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Effects of Precipitation variability on population dynamics of freshwater invertebrates in
temporary aquatic environments

A thesis submitted in partial satisfaction of the
requirements for the degree of Master of Science

in

Biology

by

Jonathan H. Bricke

Committee in charge:

Professor Jonathan Shurin, Chair
Professor Andrew Barton
Professor Elsa Cleland

2019

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The Thesis of Jonathan H. Bricke is approved and is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California San Diego

2019

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This thesis, in part is currently being prepared for publication of the material. Bricke, Jonathan; Van Allen, Benjamin; Shurin, Jonathan. Mr. Bricke was the primary investigator and author of this material.

ABSTRACT OF THE THESIS

Effects of Precipitation variability on population dynamics of freshwater invertebrates in temporary aquatic environments

by

Jonathan H. Bricke

Master of Science in Biology

University of California San Diego, 2019

Professor Jonathan Shurin, Chair

Temporary freshwater ecosystems like vernal pools sustained by precipitation are a hotbed of diversity with unique species that have evolved life-histories to cope with the impermanence of their environments. Climate change is expected to amplify interannual variation in precipitation in many regions; therefore, future vernal pool ecosystems may face greater uncertainty in hydroperiod. The impact of elevated fluctuations in water supply on aquatic communities in temporary environments is unknown. To understand the response of invertebrate communities inhabiting ephemeral freshwater pools to variation in rainfall, we conducted a mesocosm experiment manipulating rainfall to alter the amount and variability of precipitation

over 4 years.

We found that precipitation affected invertebrate abundances, but increasing variability had little effect on population densities and that past conditions are weak predictors of current population levels. The first three years of the experiment coincided with a historic drought in San Diego, CA, while the final year was the second wettest on record. Many aquatic invertebrate species that were present initially became extinct across experimental treatments during the drought, suggesting that some native species cannot survive prolonged periods of drying in small pools. Our results indicate that some vernal pool invertebrate populations are highly resilient to interannual variation in precipitation while others were unable to persist through a historic drought even when precipitation was experimentally enhanced.

Introduction

Understanding how resource availability alters ecosystems structure is an enduring ecological challenge with a growing urgency in an era of unprecedented environmental change. Water availability is a key driver in a number of terrestrial and aquatic systems (Brooks Hayashi, 2004; Bauder, 2005; Chase, 2007) (Brooks Hayashi, 2004) and can play major roles in composition and community structure (Cleland, Collins, Dickson, Farrer, Gross, Gherardi, Hallett, Hobbs, Hsu, Turnbull, Suding, 2013; Gleason Rooney, 2017; Chase, 2007). Precipitation is a defining feature of the worlds terrestrial biomes (Whittaker, 1970) and is being redistributed around the world by ongoing climate change.

Temporary waters occur on every continent and include both flowing and static forms. Temporary fresh water habitats share many physical and biological properties across regions despite differences in geology and climate. Temporary fresh water bodies can be loosely defined as ones that experience a periodical dry phase with varying length, separating them from permanent habitats that always contain water (Williams, 1997; Zedler, 2003). These habitats are heavily influenced by the timing of the wet and dry seasons and as a result can be highly influenced by climatic variations (Bauder, 2005). Species living within temporary waters have evolved a number of adaptive strategies including dormancy and migration to maintain fitness despite the periodic loss of their habitat.

Hydroperiod refers to the amount of a time that water remains in these systems, and is a key driver in many of the ecological functions (Brooks Hayashi, 2004). For aquatic species, hydroperiod represents the maximum time limit for development and is correlated with mortality and developmental rate (Wellborn, Skelly, Werner, 1996). Species richness and diversity

(Wiggins, 1980; Spencer, Blaustein, Schwartz Cohen, 1999; Brooks, 2000) (Brooks, 2000) and invertebrate abundances, biomass, and production (Leeper Taylor, 1998) have all been shown to be tightly correlated to hydroperiod in temporary habitats. Temporary water bodies often contain a unique suite of organisms with fast developmental periods that are able to complete the aquatic phase of their life cycle during the short hydroperiod (Keeley Zedler, 1998; Zedler, 2003). These species often use temporary waters as a refuge from competitors or predators that occupy permanent water bodies but are unable to tolerate even occasional drying periods (Wellborn, Skelly Werner, 1996). Despite their small area, small temporary ponds and streams contain a disproportionate share of aquatic biodiversity because the unique selective pressures they present have promoted radiation of distinct biotas (Scheffer Geest, 2006).

Variability in climate can have strong influences on the formation and duration of temporary waters. Not only has yearly precipitation been shown to effect formation and duration of pools, but within-year patterns and storm intensities also have shown strong correlation with hydroperiod (Bauder, 2005). Ponds go through multiple cycles of filling and drying within a single year. Projected changes in both yearly mean and within year storm strengths and frequency in many climate models suggest that future hydroperiods may be more variable, posing risks of extinction to some aquatic species that specialize on temporary habitats (Easterling, Meehl, Parmesan, Changnon, Karl Mearns, 2000; IPCC 2018).

San Diego is home to unique form of temporary aquatic habitat (vernal pools) that are host to many endemic specialized species (Greer Greer, 2017). Vernal pools are commonly found in Mediterranean environments, including San Diego, and are precipitation filled pools isolated from permanent water ways typically lasting between 30-90 days (Zedler, 2003). Vernal pools are highly threatened by development as they commonly occur on flat mesas where topography is particularly well suited for construction. As a result, a large fraction of vernal pools in San Diego and California have been lost to development, and several vernal pool specialist species are listed under the Endangered Species Act. These include plants such as mesa mint (*Pogogyne abramsii*), invertebrates like fairy shrimp (*Branchinecta sandiegonensis*) and amphibians such as

spadefoot toads (*Spea Hammondi*). The city of San Diego has taken steps not only to conserve but also restore vernal pool ecosystems (Black Zedler, 1998; Greer Greer, 2017) in an attempt to preserve the species that inhabit these pools. Although there have been movements toward conservation and restoration historical losses of vernal pools are still extensive (Barry, Witham, Bauder, Belk, Ferren, 1998).

To understand how hydroperiod variability and mean water availability affect communities and species in vernal pools, we conducted a mesocosm experiment manipulating mean and interannual variability in precipitation in two ways. We constructed mesocosms to mimic vernal pool habitats that were filled by rainfall and manipulated the incident precipitation with roofs. In addition to elevating and reducing precipitation over several years, we also imposed two interannual variability treatments where the amount of precipitation received by individual mesocosms varied from year to year. We sampled the experiment over four years in order to ask the following questions: 1) How do communities and populations respond to variation in mean water availability? 2) Does interannual variability affect community structure such that past conditions leave an imprint on the abundances of different invertebrate taxa that is distinguishable from the effects of hydroperiod in the present year? Answering these questions will lead to a better understanding of how communities and population will respond to predicted climate change and help in restoration and conservation efforts involving temporary waters and their unique biological diversity.

Methods

Mesocosm Treatments/Design

The precipitation experiment was initiated at the beginning of March 2014 and conducted at the University of California San Diego (UCSD) Biological Field Station (BFS) continuously run for 1054 days, concluding in January of 2017. Mesocosms consisted of 50-gallon plastic barrels, cut in half to create 50 artificial enclosures. Mesocosms received water only from natural

precipitation.

Stock Collection

Zooplankton and sediment were collected from 4 separate temporary pools along the Los Peasquitos Canyon Preserve on March 9th, 2014 in order to initiate the experimental aquatic communities in the mesocosms. 100mL of sediment were added to 50 enclosures to provide nutrients and dormant stages of crustaceans and other invertebrates. Zooplankton were collected in the field in Nalgene bottles and left overnight in a tank filled with water to acclimate to the experimental environment before being added to the enclosures. On March 10th, 2014 zooplankton stocks were mixed with zooplankton that were hatched from sediment previously collected from natural pools at Elliot Natural Reserve. Aquatic communities from two sites were used as sources to stock the experiment in order to assure a diverse assemblage of invertebrates in the experiment. Zooplankton stocks were thoroughly mixed before each enclosure received 250mL aliquot in order to assure that similar species densities were introduced into the experimental enclosures. At the time of stocking, five 250mL aliquots were collected and preserved in 70

Experimental Treatments

To assess the effects of precipitation on community structure and species abundances each enclosure was subjected to one of five precipitation treatments, three that manipulated the average level of precipitation across years and two that manipulated interannual variability in rainfall (Figure 1). The treatments consisted of five levels of precipitation identified as Ambient (A), High (H), Low (L), Variability 1 (V1), and Variability 2 (V2). Ambient treatments received only the precipitation that fell directly on the enclosure. High treatments received roughly twice the precipitation of the ambient treatments, while the low treatment received half the precipitation of the ambient treatment. Variability treatments were alternated between high and low treatment regimes, while Ambient, High, and Low remained consistent for the duration of the experiment.

The fraction of environmental precipitation received in the two variability treatments varied from year to year in opposite orders (e.g., V1 precipitation was H-L-H and V2 was L-H-L).

Rainfall was manipulated by placing angled roofs above pairs of adjacent enclosures assigned to treatments as Ambient/Ambient, High/Low, and Variability1/Variability2. Roofs were constructed from polycarbonate corrugated roof panels placed so one half would cover one enclosure while the other half would cover another enclosure. Roofs covering a High/Low treatment were angled so approximately 50% of incident precipitation would be deflected from the Low treatment enclosure and into the High treatment enclosure. Ambient/Ambient treatments had coverings with the same angular slope as High/Low treatments and holes drilled into the corrugated roof to allow water to flow evenly to each tank. Variability1/Variability2 treatments had the same roofs as High/Low treatments but the orientation of the roof was switched between years so that the tank receiving high vs. low precipitation alternated between years.

Two variability treatments were implemented to represent different scenarios of inter-annual variability given our inability to forecast precipitation during the experiment. The two treatments differed in the order of alternation between high and low precipitation (e.g., Low-High-Low-High vs. High-Low-High-Low). We implemented the two treatments because of uncertainty about the level of precipitation in any particular year. For instance, if the high water treatment coincided with a dry year and the low water treatment with a wet year, the variability manipulation would have the effect of reducing interannual variability. The two treatments therefore represent alternative variability regimes.

Sample Collection

The rainiest months in southern California are typically from December to March. Samples were collected monthly, in some cases biweekly, depending on the amount of precipitation. Drought conditions prevailed between 2014-2016 severely limited sampling of the experiment. From January 2016 to December of 2017 no samples were collected due to low water levels or enclosures being completely dry. In addition, the variability treatments were not switched

during the 2016-2017 winter. When conditions allowed, 500mL samples were collected from haphazardly selected locations in each enclosure. When water levels were high a turkey baster was used to collect sample water from various depths throughout the water column. Water was collected then filtered through a Nitex mesh filter (50 μ m) before being rinsed and preserved in 70% ethyl alcohol. Samples were stored in 50ml jars for later enumeration and identification of invertebrates. Water levels were recorded at each end of each enclosure at the time of sampling to estimate water depth. Identification and enumeration of aquatic invertebrates was conducted using a Lecia M125 stereo microscope at 8x-25x magnification. All individual invertebrates were identified and counted in each sample without subsampling. Twenty taxonomic units were identified, half of which were aquatic including cladocerans, copepods, ostracods and insects.

Statistical Analysis

Statistical analysis was performed using the statistical program R version 3.3.3 (R Development Core Team 2016). We compared species densities among treatments to test effects of mean and variability in precipitations levels on abundances of all aquatic species identified. Our data was zero-inflated as many species were frequently absent from most samples, leading to over dispersion (variance greater than the mean). We therefore analyzed models with negative binomial error terms using the package glmmadmb (Fournier Da, et al., 2012). Our model consisted of fixed effects for treatment and sampling date. Kruskal-Wallis non-parametric one-way analysis of variance (Kruskal.test, r) was used to identify significant differences among treatments on individual sample dates for species that showed significant treatment or date effects across the whole experiment. Non-parametric post-hoc tests were performed using the package dunn.test (Dinno, A., 2017) to compare differences in the 5 treatments. To test the effect of variability, we compared treatments in the H, L and A with V1 and V2 with the same water level on a given date. For instance, a difference in invertebrate abundances between the H and V1 treatments (on a date when V1 had the same water level as H) would indicate an effect of water level during past years.

Three aquatic species (*Moina*, *Ceriodaphnia*, and *Acanthocyclops*) that were analyzed occurred on only one sample date of the experiment and were excluded from the initial negative binomial model as date was not included within the model allowing for the use of just the Kruskal-Wallis test for significance, as it only allows for one response variable. There were no significance treatment effects for any of these three species so no further analysis was conducted.

Results

Mean and variability treatments effectively manipulated the water level and hydroperiod in the tanks (Figure 3). High mean water treatments (H) contained consistently more water than Ambient (A) and Low treatments. Due to high levels of precipitation during the rainy season of 2016/2017, tanks in the H and A treatments were full to capacity, leading to lower variation in water levels among treatments.

Invertebrate species composition changed markedly over the course of the experiment. Two taxa that were common during the first year (*Acanthocyclops* and *Ceriodaphnia*) were absent by the end of the experiment, likely driven to extinction by prolonged drought conditions. Other taxa (*Moina*, *Pseudacris regilla* (*pacific tree frogs*)) colonized or emerged from resting eggs when water levels became higher in 2017 (Figure 2).

Ostracods persisted throughout the experiment and were the most abundant taxon by the final sampling date. We found significant effects of the treatments on *ostracod* density in a number of time periods sampled (Figure 4), with higher densities during periods of higher water levels. Past water levels had no detectable effects on *ostracod* densities. For instance, the H and V1 treatments both had high water levels in 2017 (Figure 4F) and equivalent low densities of *ostracods* even though V1 had low water levels throughout the previous three years.

Mosquito larvae were another abundant member of the aquatic community in the experiment. *Mosquitos* exhibited no significant effects of past conditions and few differences among treatments (Figure 5). Although *mosquito* densities increased throughout the experiment, and as

water levels increased. Significant differences in the densities of *mosquito* larvae only occurred within the last sample period when water levels were at their highest and showed increased density in treatments with lower water levels.

For *Acanthocyclops* (Figure 6), *Ceriodaphnia* (Figure 7), and *Moina* (Figure 8) there were no significant effects of treatment on species densities.

Discussion

Our analysis indicates that vernal pool invertebrates are highly resilient to hydroperiod fluctuations. Their abundances are affected by present water levels but show little evidence of legacy effects of previous precipitation. These results indicate that the dominant arthropod taxa in our experiment (*ostracods and mosquitos*) have adapted to an ephemeral and unpredictable environment and are able to recover rapidly from periods of low population density.

Although the dominant taxa in our experiment were unaffected by manipulations of interannual variability in precipitation, a number of taxa that were prevalent in the first year failed to persist through three years of historic drought. Ephemeral pool ecosystems are sensitive to interannual variation in temperature and precipitation (Bauder, 2005), and as a result are likely to be greatly impacted by climate change. Our results indicate that communities may exhibit shifts in species composition due to prolonged periods of drought. While individual species display different levels of their resilience to changing water levels, all taxa had decreased numbers during drought years. The emergence of new taxa, that were not present during earlier sample dates, indicates that some taxa are either better at dealing with drought and waiting for conditions to become favorable to emerge from dormant state (Hairston, 1996; Wiggins, 1980; Brendonk Meester, 2003), or have strong dispersion ability (Vanschoenwinkel, 2008; Havel Shurin, 2004). Extinction and colonization of species highlights the variable abilities of specialist taxa living in ephemeral pool habitats to tolerate prolonged periods of drought.

Ostracods, the most common taxon throughout the experiment, showed increased abun-

dances as water availability increased. Differences found among treatments was related to the relative water that treatment was receiving within that given sample period. There was no evidence that past conditions had any detectable impact on current *Ostracod densities*. For instance, if past drought reduced the *ostracod* numbers and therefore the number of dormant eggs in the sediment pool, then we would expect to see fewer live individuals in the following year. Instead, we saw that *ostracod* numbers were equivalent between the high precipitation treatment and the variability treatment in years when it had high water levels. The increase in numbers as water availability increased indicates that *Ostracods* are highly responsive to water levels and not constrained by the previous years population. Species of *Ostracods* have been shown to have immature stages present in dry soils, coupled with non-synchronized reanimation this may give *ostracods* an increased competitive advantage over other species along with higher survival if drought resistant juveniles do not have time to develop before drought conditions return (Horne, 1993).

Opportunists taxa with flying or terrestrial adult stages such as insects of amphibians also take advantage of temporary waters. Mosquitoes are able to migrate between pools and can easily disperse to better locations with their ability of flight in their adult life-stage (Williams, 1997). *Mosquitoes* showed little variability between treatments and no consistency throughout the experiment. Opportunistic *mosquito* species have been shown to move more freely between habitats and in a wide range of habitats, in comparison with specialist *mosquito* species (Day, 2016). The only time we found significant differences was when water levels were at their highest, during the last sample period. We found that treatments that had lower water levels had higher densities of mosquito larvae, which may indicate *mosquito* preference for shallower pools. Taxa of *Ceriodaphnia* and *Acanthocyclops* occurred during the initial sample periods and were present within the initial stock collection. These taxa showed no significant differences in abundance among treatments, which may be because they became extinct in the drought years following the initial year of the experiment. Pools were populated on March 14th, 2014 and the first samples were taken March 27th which may not have allowed enough time for these taxa

to adjust to the treatment regimes. By the time of the second sample period these taxa were no longer present within samples and were never seen again in the experiment. These species may not have been able to persist in the pools even though they were collected from natural temporary pool habitats. Studies have shown direct correlation to species extinction and hydroperiod and that species richness is much higher in longer lasting pools (Spencer, Blaustein, Schwartz Cohen, 1999). These taxa may not have had the development time needed to properly withstand the severe drought conditions that followed shortly after the initial sample period.

Following the drought from 2014-2017, a number of new taxa were observed in the pools, including *Moina* (a crustacean) and *Pseudacris regilla* (Pacific Tree Frogs)). Because Pacific Tree Frogs have terrestrial adults, tree frogs immigrated into the pools when water conditions became favorable for their life-strategy. *Moina* appeared in high densities in the final sample period and showed no significant differences with treatments. Fresh water invertebrates have a number of dispersal mechanism including wind driven dispersion (Vanschoenwinkel, Gielen, Seaman Brendonck, 2007; Havel Shurin, 2004) along with various forms of animal dispersion (Havel Shurin,2004), allowing zooplankton to disperse readily to new environments. Because of their ability for dispersion, *Moina* may have been introduced from outside the experimental system. It is also possible, as a result of their life-cycle (Rottman, Graves, Watson Yanong, 2011), that they were also present within the system in lower numbers early within the experiment but did not proliferate until conditions became favorable. Within the last sample period, as a result of high water levels, differences in water levels among treatments were less than an any other sample period. This could explain, in part, why *Moina* showed no effects by treatment.

Our results indicate that vernal pool invertebrates are adapted to withstand extreme levels of interannual variability in hydroperiod with prolonged drought interspersed with heavy rainfall. Precipitation and temperature are key drivers in ephemeral ecosystems (Bauder, 2005; King, 1996), but few studies have focused on the how interannual variation will affect these systems. Our results suggest that although predicted changes in storm intensity and frequency will increase environmental variability, species response in composition and abundances are

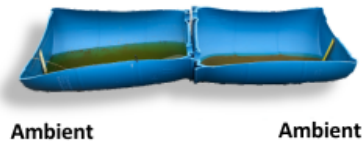
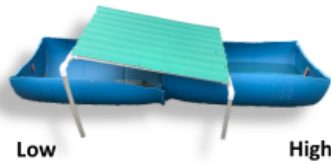
most likely predictable by changes in mean conditions alone, corresponding to similar result found in salt marsh plant communities (Noto Shurin, 2017). Heterogeneity in environmental conditions and resources are central drivers of patterns in species composition and diversity (Tilman Pacala, 1993; Chesson, 2000). Although there are inconsistencies between predictions of different models of intensity and amounts of precipitation, models consistently agree that tropospheric warming leads to increased evaporation rates resulting in increased drought severity (Weltzin, Loik, Schwinning, William, Fay, Hadda, Harte, Huxman, Knapp, Lin, Pockman, Shaw, Small, Smith, Smith, Tissue Zak, 2003). Further increases in interannual variability may reshape vernal pool communities. However, our results indicate that temporary pool organisms are well adapted to even high levels of fluctuation in hydroperiod.

This thesis, in part is currently being prepared for publication of the material. Bricke, Jonathan; Van Allen, Benjamin; Shurin, Jonathan. Mr. Bricke was the primary investigator and author of this material.

50 Artificial Pools: 5 Treatments x 10 Replicates

3 Mean Treatments:

High
Low
Ambient



2 Variability Treatments:

Variability 1
Variability 2

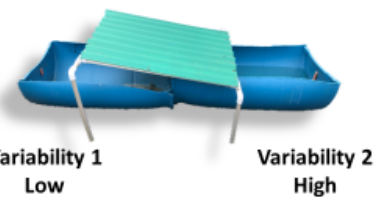
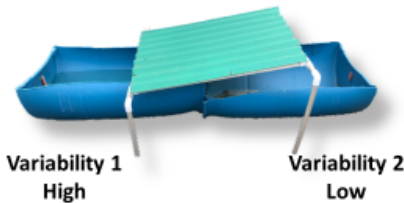


Figure 1. Mean and Variability Treatments (rooves on Ambient treatments not pictured for clarity). The 3 mean treatments remained constant throughout the duration of the experiment, while variability treatments were switched seasonally in the environmental precipitation they received (e.g. Variability 1 switched from High to Low then back to High, while Variability 2 switched from Low to High then back to Low).

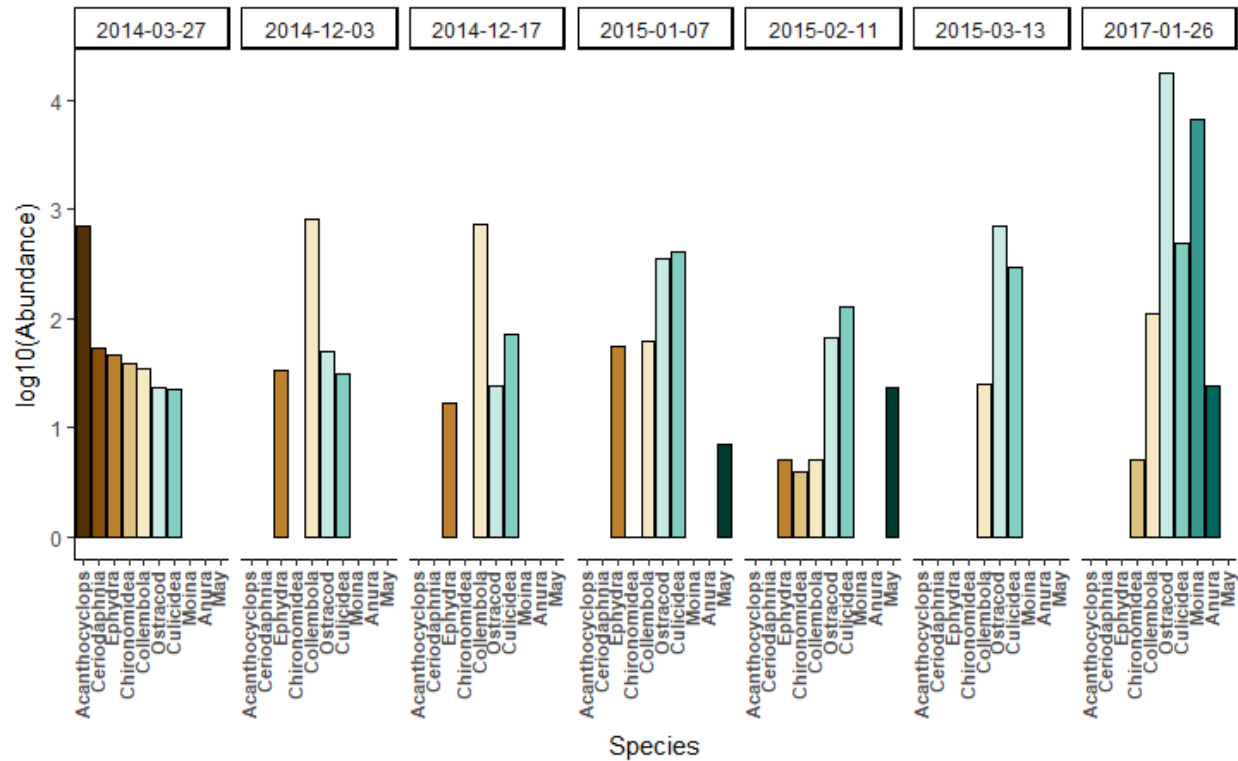


Figure 2. Rank abundance of aquatic taxonomic groups by sample date. *Acanthocyclops*, *Ceriodaphnia*, and *Ephydra* that were abundant in early sample periods became absent in later sample dates. *Moina* and *Anurans* only appeared in the last sample period. Species from left to right: *Acanthocyclops*, *Ceriodaphnia*, *Ephydra* (larvae), *Chironomidea* (larvae), *Collembola*, *Culicidea* (larvae), *Moina*, *Anura*, and *Mayfly* (larvae).

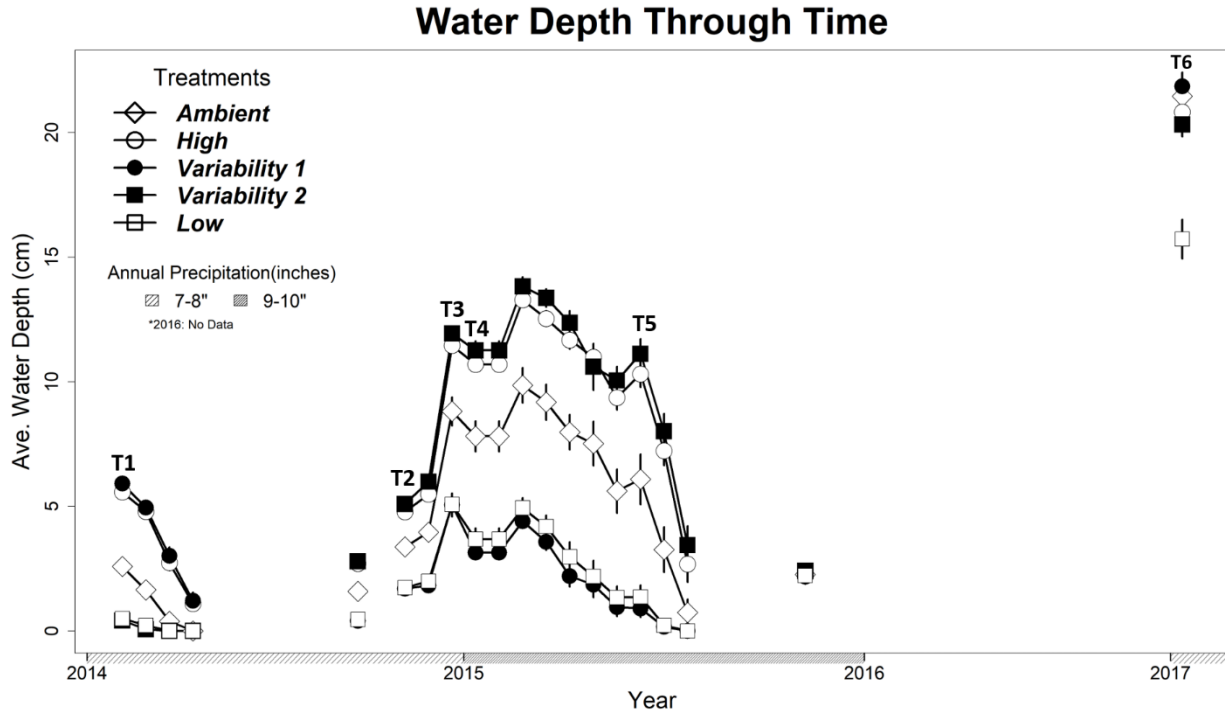


Figure 3. Water levels (cm) by treatment, where treatments are indicated by symbols as indicated in the legend. Annual precipitation for each year sample collected represented as shading on x-axis, shading indicates amount of precipitation as shown in legend. Samples were collected and analyzed indicated by T1-T6 (Time 1-6).

Table 1. Kruskal-Wallis test: shows significant treatment effects on average water level throughout the duration of the experiment.

(Kruskal-Wallis)	Average Water Level ~ Treatment		
	χ^2	df	p-value
Treatment	55.218	4	<<0.001***

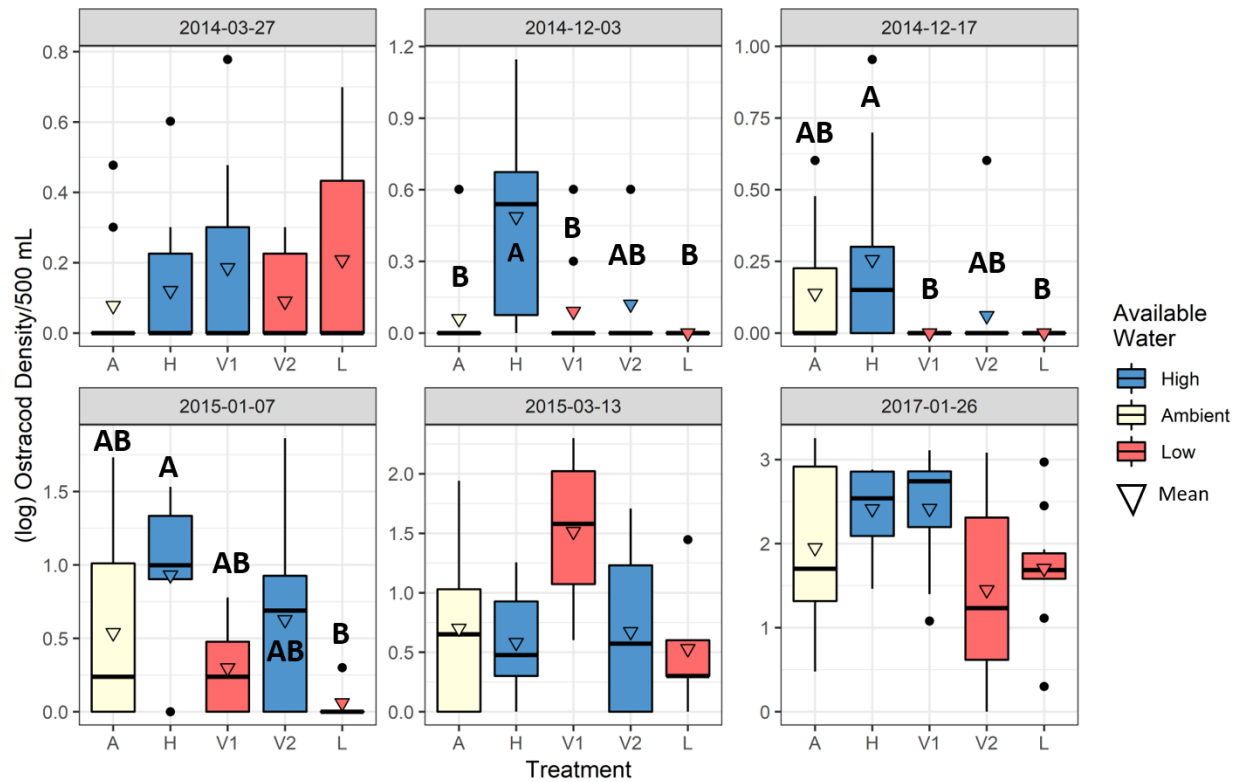


Figure 4. Boxplot of *Ostracod* densities by treatment. Each window represents a separate sample date. Boxplots represent median, upper and lower quantiles. Whiskers are variability outside upper and lower quantiles, single points represent outliers. Triangles represent mean *Ostracod* density. Color indicate available water as shown in legend. Letters above boxplot indicate treatment differences in *Ostracod* abundances as determined by dunn.test (Dinno, A., 2017).

Table 2. Negative binomial model to assess treatment and date effects on *Ostracod* abundances.

(NB) Model: Ostracod ~ Treatment + Date			
	χ^2	df	p-value
Treatment	11.964	4	0.01762*
Date	278.867	6	<<0.001***

Table 3. Kruskal-Wallis non-parametric one-way analysis of variance testing treatment effects on *Ostracod* density. Individual tests were performed on each date.

(Kruskal-Wallis)	Ostracod ~ Treatment		
Date	χ^2	df	p-value
3/27/14	1.8759	4	0.7586
12/3/14	16.445	4	0.002476**
12/17/14	12.13	4	0.01641*
1/7/15	13.241	4	0.01016*
3/13/15	5.1406	4	0.3135
1/26/17	8.1483	4	0.08629

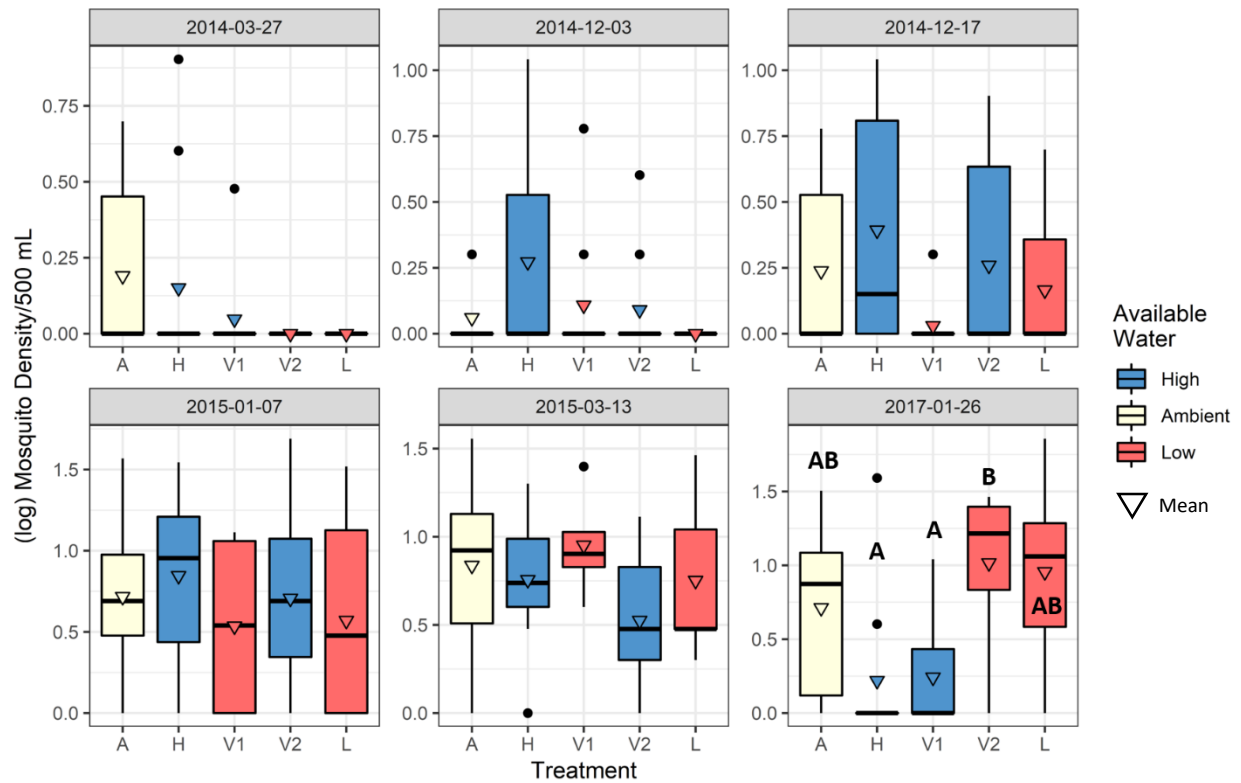


Figure 5. Boxplot of *Mosquito* densities by treatment. Each window represents a separate sample date. Boxplots represent medium, upper and lower quantiles. Whiskers are variability outside upper and lower quantiles, single dots represent outliers. Triangles represent mean *Mosquito* density. Color indicate available water as shown in legend. Letters above boxplot indicate treatment differences in *Mosquito* abundances as determined by dunn.test (Dinno, A., 2017).

Table 4. Negative binomial model to assess treatment and date effects on *Mosquito* abundances.

(NB) Model: Mosquitoes ~ Treatment + Date

	χ^2	df	p-value
Treatment	5.5399	4	0.2362
Date	157.236	6	<<0.001***

Table 5. Kruskal-Wallis non-parametric one-way analysis of variance testing treatment effects on *Mosquito* density. Individual tests were performed on each date.

(Kruskal-Wallis)		Mosquitoes ~ Treatment		
Date	χ^2	df	p-value	
3/27/14	6.5183	4	0.1636	
12/3/14	5.472	4	0.2422	
12/17/14	4.9769	4	0.2897	
1/7/15	2.0277	4	0.7307	
3/13/15	4.2154	4	0.3845	
1/26/17	15.4098	4	0.0039**	

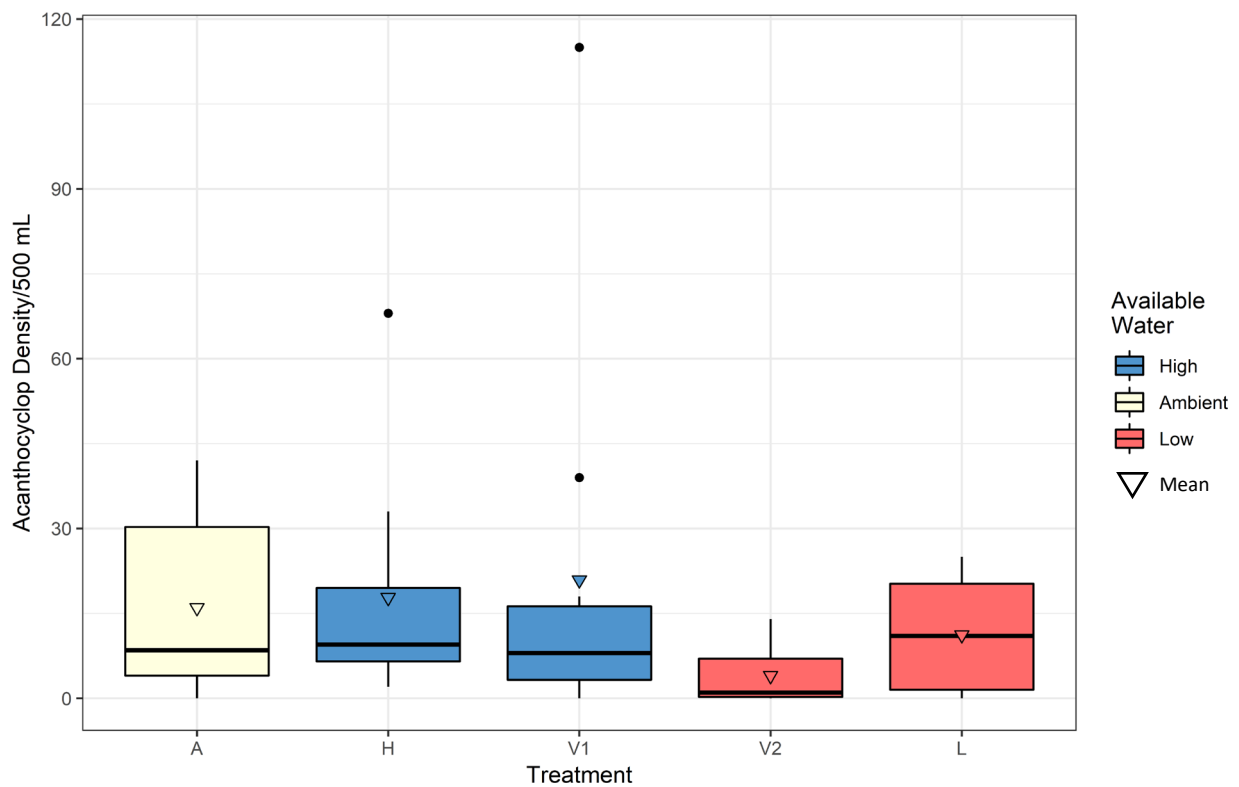


Figure 6. Boxplot of *Acanthocyclops* densities by treatment. Each window represents a separate sample date. Boxplots represent medium, upper and lower quantiles. Whiskers are variability outside upper and lower quantiles, single dots represent outliers. Triangles represent mean *Acanthocyclops* density. Color indicate available water as shown in legend. Letters above boxplot indicate treatment differences in *Acanthocyclops* abundances as determined by dunn.test (Dinno, A., 2017).

Table 6. Kruskal-Wallis test: shows no significant treatment effects on *Acanthocyclops* throughout the duration of the experiment.

(Kruskal-Wallis)	Acanthocyclops ~ Treatment		
	χ^2	df	p-value
Treatment	7.036	4	0.134

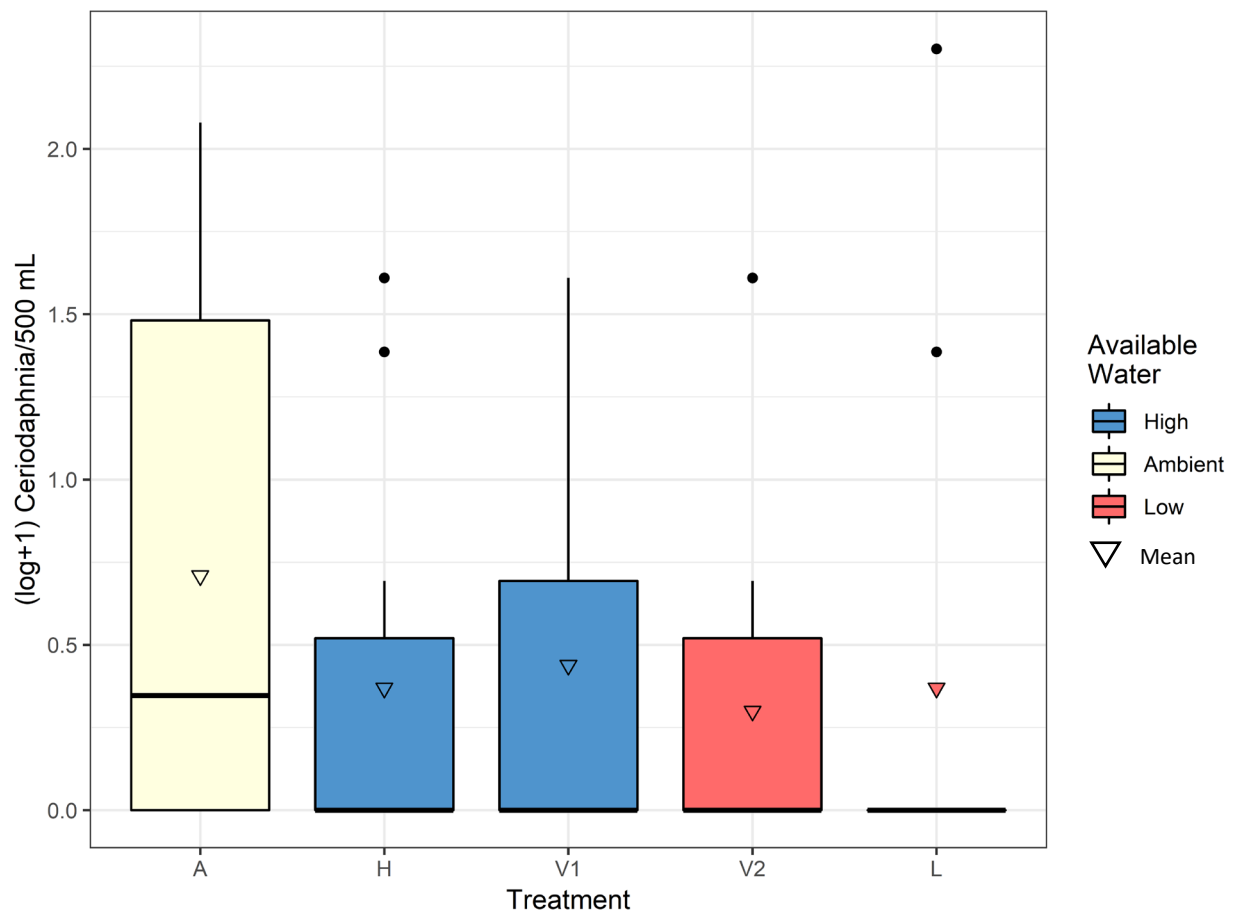


Figure 7. Boxplot of *Ceriodaphnia* densities by treatment. Each window represents a separate sample date. Boxplots represent medium, upper and lower quantiles. Whiskers are variability outside upper and lower quantiles, single dots represent outliers. Triangles represent mean *Ceriodaphnia* density. Color indicate available water as shown in legend. Letters above boxplot indicate treatment differences in *Ceriodaphnia* abundances as determined by dunn.test (Dinno, A., 2017).

Table 7. Kruskal-Wallis test: shows no significant treatment effects on *Ceriodaphnia* throughout the duration of the experiment.

(Kruskal-Wallis)	Ceriodaphnia ~ Treatment		
	χ^2	df	p-value
Treatment	2.3008	4	0.6806

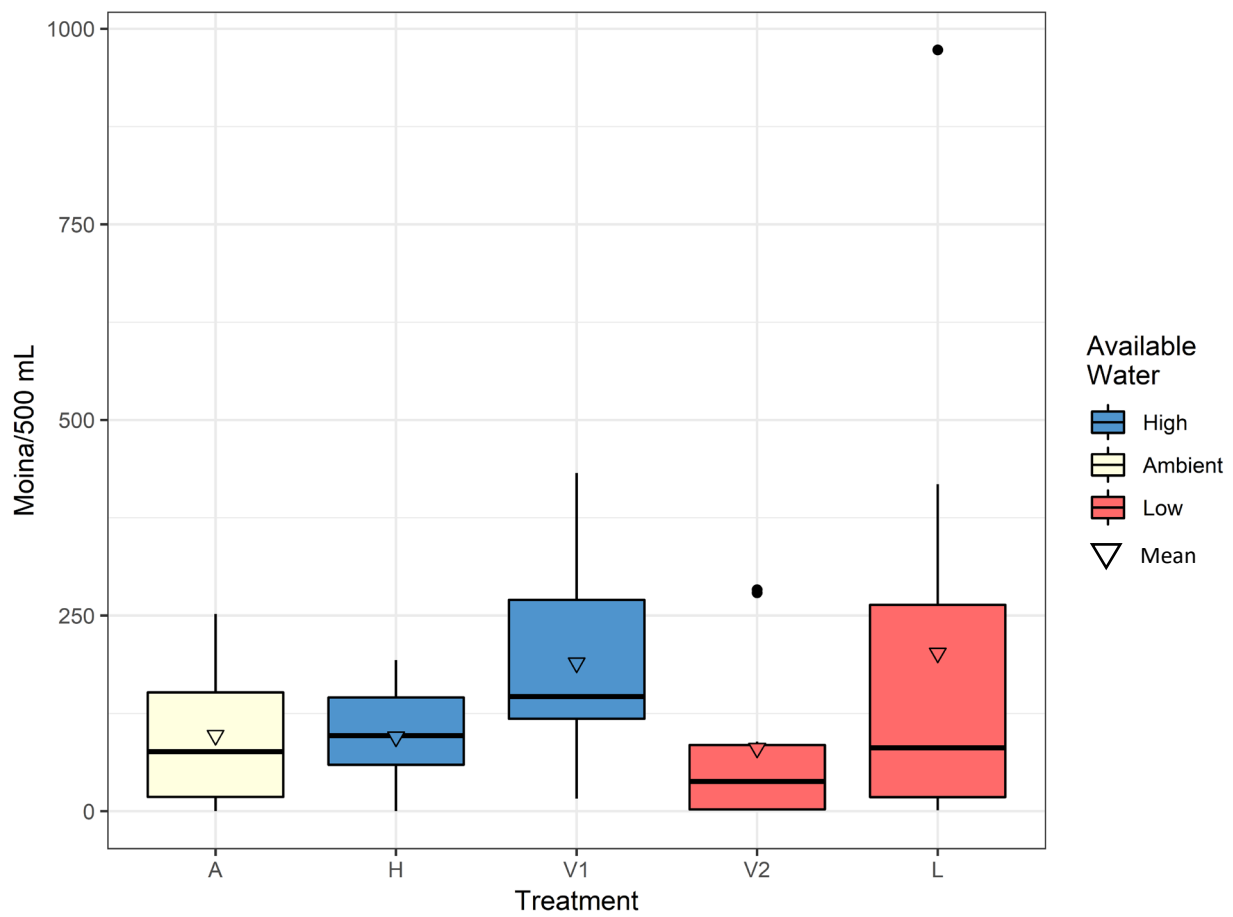


Figure 8. Boxplot of *Moina* densities by treatment. Each window represents a separate sample date. Boxplots represent medium, upper and lower quantiles. Whiskers are variability outside upper and lower quantiles, single dots represent outliers. Triangles represent mean *Moina* density. Color indicate available water as shown in legend. Letters above boxplot indicate treatment differences in *Moina* abundances as determined by dunn.test (Dinno, A., 2017).

Table 8. Kruskal-Wallis test: shows no significant treatment effects on *Moina* throughout the duration of the experiment.

(Kruskal-Wallis)	Moina ~ Treatment		
	χ^2	df	p-value
Treatment	6.3124	4	0.177

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