SIZE MATTERS: HOW POOL VOLUME AFFECTS THE SURVIVAL OF FAIRY SHRIMP ON THE COLORADO PLATEAU

by

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Size Matters: How Pool Volume Affects the Survival of Fairy Shrimp on the Colorado Plateau

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ABSTRACT

Branchinecta packardi is a widespread, fast-hatching species of fairy shrimp found in desert ephemeral pools on the Colorado plateau that relies on a dormant cyst stage to temporally bridge periods of pool desiccation. Determining the factors that control *B. packardi* cyst bank density in dry basins and population dynamics during periods of inundation may be a fundamental step towards predicting community composition in these temporary aquatic ecosystems. I sampled soil from 45 pools and reared individuals in mesocosms in order to track individuals through an entire inundation cycle. I found that pool characteristics such as volume, temperature, soil quality, and the presence of co-occurring species could not predict the density of cysts that were embedded in the soil. In addition, by observing individuals in a physically controlled setting, I found that population sizes of hatched individuals are most likely not controlled intrinsically by physical attributes of pools but by biotic interactions. Survivorship and body size of *B. packardi* decreased significantly with increasing population density, providing clear evidence for density dependence. The presence of a co-occurring species of fairy shrimp found in the pools appeared to further intensify resource competition. These results suggest that *B. packardi* cyst bank size is determined by stochastic fluctuations and density dependent biotic interactions.

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INTRODUCTION

Desert ephemeral pools on the Colorado Plateau are spatially isolated miniature aquatic ecosystems that are created when rock basins are filled with rainwater. Like other ephemeral aquatic systems found throughout the world, the temporary nature of pools excludes aggressive vertebrate predators such as fish, providing ideal habitat for thriving communities of zooplankton. In addition, species that inhabit these temporary aquatic pools in the desert must utilize a dormant life stage in order to persist through extreme temperatures, predictable seasonal dry periods, and unstable conditions that vary from year to year. Each occupied pool contains a deposit of sediment into which the inhabitants of the pool during its inundated phase have deposited cryptobiotic eggs, called cysts. Cryptobiosis, meaning "hidden life", is a state of suspended metabolism occupied by an organism in response to severe environmental conditions. In the temporary aquatic environments of this study, many pool inhabitants produce cryptobiotic cysts that can resist desiccation (De Stasio 1989, Clegg 2001). Using this strategy, desert ephemeral pool organisms can create temporal bridges between periods of pool inundation and potentially accumulate collections of dormant offspring in the soil. These cyst banks, or collections of cysts, bear similar properties to seed banks of annual plants and as such are essential for regional persistence in harsh, unpredictable environments (De Stasio 1989, Hairston and Caceres 1996, Brock et al 2003, Ripley et al. 2004).

Since fairy shrimp are often the earliest to hatch from deposited eggs in basin soils, they have been thought to monopolize resources (Jocque et al. 2010) and influence the community succession dynamics of zooplankton in pools (Sánchez and Angeler 2007). They also perform an ecologically important role as prey for larger tadpole shrimp and insects (Jocque et al. 2010) and have been investigated for use in potential management of mosquito populations (Blaustein and Margalit 1991). In this study, I examine the factors that are important for cyst bank density and population dynamics of a species of fairy shrimp, *Branchinecta packardi*, found in ephemeral pools on the Colorado Plateau. Widespread and fast-developing, this species has successfully colonized a broad range of ephemeral pools, from small to large and from pure-species to taxonomically rich (T. Graham, unpublished raw data). Determining the key controls on *B. packardi* population dynamics may be a fundamental step to understanding community dynamics in desert ephemeral pools.

BACKGROUND AND RESEARCH QUESTIONS

Ephemeral pools on the Colorado Plateau are formed on a layer of porous windblown sandstone that was deposited during the early Jurassic era (Loope et al. 2008, Chan et al. 2005). This unique geologic formation, which is characterized by its susceptibility to erosion by sand-carrying wind, is patterned throughout the region with numerous weathered pits that range from small, shallow depressions that may hold only a liter of water to giant wells in the bedrock that can be over fifteen meters deep and as many meters wide. When precipitation is sufficient and temperatures are suitable, fairy shrimp and other organisms quickly hatch out from cryptobiotic eggs in the soil contained each basin. Once hatched, these organisms must reproduce before the pool dries up, sometimes in a matter of days, in order to sustain future populations. The strategy of cryptobiosis allows the passive dispersal of individuals to occur between pools when wind transports cryptobiotic cysts between basins (Brendonck and Riddoch 1999, Caceres and Soluk 2002, Graham and Wirth 2008, Vanschoenwinkel 2008). Thus, soil properties may influence dispersal dynamics by affecting rates of wind transfer (Graham and Wirth 2008) since cysts in dry pools are similar to seeds in seed banks in that they must be buried or sealed beneath soil to avoid being swept by wind from a pool (Van der Valk 1986).

Inhabitants of desert ephemeral pools include algae, aquatic mites, tardigrades, rotifers, beetles (both larval and adult forms), fly larvae, and several distinctive branchiopod crustaceans: fairy shrimp, clam shrimp, tadpole shrimp, and ostracods. *Branchinecta packardi*, a species of fairy shrimp and the focal species of this study, may be the most ubiquitous branchiopod species found in the pools due to its capacity to hatch early from the soil and develop quickly to reproductive maturity. In this study I also include observations on a larger competing species of fairy shrimp (*Streptocephalus texanus*), which I found has a considerably later mean hatching time of 56 hours, and a species of tadpole shrimp (*Triops longicaudatus*) that is both a competitor and facultative predator of the cruising raptorial form (Greene 1985). Both of these co-occurring species are known to inhabit larger, more stable pools of water, possibly due to their longer maturation rates (Belk 1991, Hathaway and Simovich 1996). All branchiopods feed on organic particles and algae filtered from the water (Light 2007).

Niche theory (Gause 1934) suggests that in small volume pools the number of cysts per unit of soil should be decreased due to the low reproductive success of individuals in these fast-drying environments. Since hatching without surviving to reproduce can potentially cause strong selective pressures on life history traits when repeated generations of slow-developing individuals perish before they get a chance to reproduce (Simovich and Hathaway 1997, Reznick et al. 2002), the length of time that a pool remains inundated has been hypothesized to shape the life histories of organisms that inhabit temporary aquatic environments (Laurila and Kujasalo 2001, Wellborn et al. 1996, Johnson et al. 2005, Hulsmans et al. 2008, Vanschoenwinkel et al. 2009, Stoks and McPeek 2003, Brooks 2000, Altermatt et al. 2009, De Roeck et al. 2010). Hence, I expected that increased mortality from pool desiccation would result in distinctly lower reproductive output into cyst banks. In this study I use pool volume as an indicator of pool duration by the reasoning that the more water there is in a basin, the longer it will remain inundated (Altermatt and Ebert 2010). Similarly, in large volume, taxa-rich pools I predicted that the number of cysts per unit of soil would also be low due to increased mortality from interspecific competition and predation (Araújo and Luoto 2007, Davis 1998, Connell 1961, Gause 1934). Again, lower survivorship of individuals would lead to fewer contributions to future populations. Overall I expected to find that, when reared in a common setting, individuals from different populations would exhibit phenotypic differences that would reveal different selective pressures between ephemeral pools and that cyst bank density would be greatest at intermediate pool volumes where individuals would not experience increased mortality due to pool desiccation nor negative biotic interactions (Lamy et al 2013, Altermatt et al. 2012).

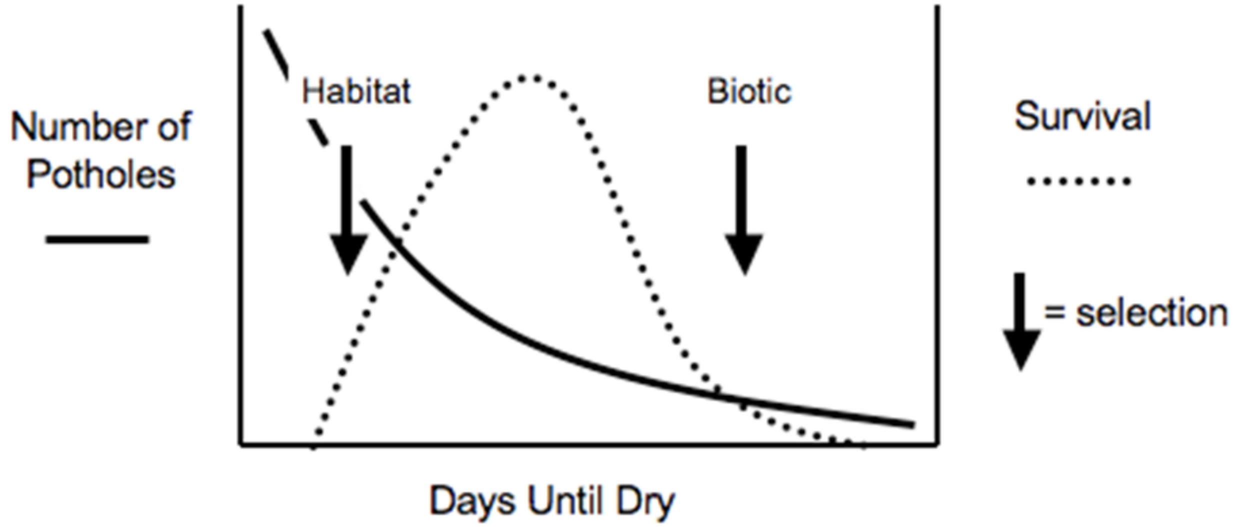

Figure 1. A schematic plot illustrating proposed selective pressures at small and large volume extremes. *B. packardi* cysts are predicted to be most dense at intermediate volumes, where survival of individuals is potentially greatest.

Question 1: What factors control the number of cysts in the cyst bank?

Cyst bank size is a measure of future security in the highly unstable environmental conditions of desert ephemeral pools. I determine which environmental correlates, if any, are related to the numbers of *B. packardi* individuals that hatch out of soil sampled from pools. Specifically, I test the variables of pool volume, relative depth, soil volume, mean temperature, and maximum temperature as important indicators of cyst habitat quality for the study species. As previously mentioned, I would expect that cyst bank density is greatest in pools that are large enough to preclude desiccation before reproduction but small enough to exclude superior competitors and predators (figure 1), because individuals in these intermediate-sized pools should exhibit the greatest reproductive success. I would expect that cyst retention would be higher in deeper pools with greater soil volume, since high walls may shelter cyst banks and larger quantities of substrate may aid in protecting cysts from wind (Graham and Wirth 2008). I would also expect that pools experiencing higher temperatures may have lower cyst density due to loss of viable cysts from damage by intense heat and solar radiation (Chan et al. 2005).

Question 2: What factors control life history traits of hatched individuals?

The success of hatched individuals determines how many new cysts will be contributed to following generations within a pool. Thus, it is reasonable to expect that *B. packardi* are well adapted to conditions in their pools of origin in order to be reproductively successful. I determine to what extent *B. packardi* life history traits are matched to the physical characteristics of their pools or affected by the presence of a competing species of fairy shrimp. Specifically, I test whether body size, maturation time, sex ratio, and survivorship are affected by volume and relative depth of pools, soil volume, and temperature in pools. If there is strong evidence for local adaptation, in particular I would expect that body size, maturation time, and survivorship would be lower both for individuals from smaller, hotter, fast-drying pools due to increased adult mortality by pool desiccation and that survivorship of individuals would decrease in the presence of competition for resources.

To answer these questions, I sampled cyst banks from 45 ephemeral pools and reared fairy shrimp in physically identical mesocosms. Using this method, I was able to control for the abiotic characteristics of the environment while closely observing the effects of competition from an additional species of fairy shrimp. I estimated cyst density of the original pool using the number of hatched individuals and determined important factors for population dynamics by tracking individuals in mesocosms through one cycle of inundation.

METHODS

Field study sites

The study area is located on land overseen by the Bureau of Land Management (BLM) near Moab, Utah, USA (Latitude 38.58326, Longitude -109.52482). The area encompasses 45 ephemeral pools in four clusters situated on top of four discrete "fins" of rock arranged in linear series trending southeast to northwest (Figure 2). The four fins are separated by approximately 0.5 km, 0.5 km, and 16 km. The pools vary in size and geographic isolation (Figure 3). To concentrate on pool sizes known to contain fairy shrimp, all accessible pools > 0.5 meters were included in the study.

Figure 2. Pool basins occur in a nearly linear pattern on top of Navajo sandstone "fins" found on the Colorado Plateau.

Collection of environmental data and samples

I examined an array of abiotic characteristics for each pool when the pools were dry. I measured maximum length and width of each pool. I calculated depth by taking the mean of five evenly spaced depth measurements along a string attached to both ends of the pool at its maximum center length, at the height of the visible historical water mark. Using these measures of dimension, I calculated volume by treating each pool as one half of an ellipsoid. I calculated relative depth of pools by taking maximum depth as a percentage of mean pool diameter. To calculate soil volume, I treated the body of soil as another ellipsoid with the length and width of the pool and depth of the soil, multiplied by the percent of the surface of the dry basin that was covered in soil. I also recorded hourly temperatures, since ephemeral pools on the Colorado Plateau may undergo daily and seasonal temperature fluctuations as extreme as 0 –

60 degrees Celsius (Chan et al. 2005). I placed one Thermochron iButton, coated in a clear plastic dip for waterproofing (Roznick 2012), atop the soil in each pool from May 5 to July 25, 2014, through the course of several cycles of inundation at viable hatching temperatures. To quantify general soil properties in the ephemeral pools, I calculated the relative depth of soil and soil volume in pools, and I recorded the proportion of the dry basin surface that was covered in soil.

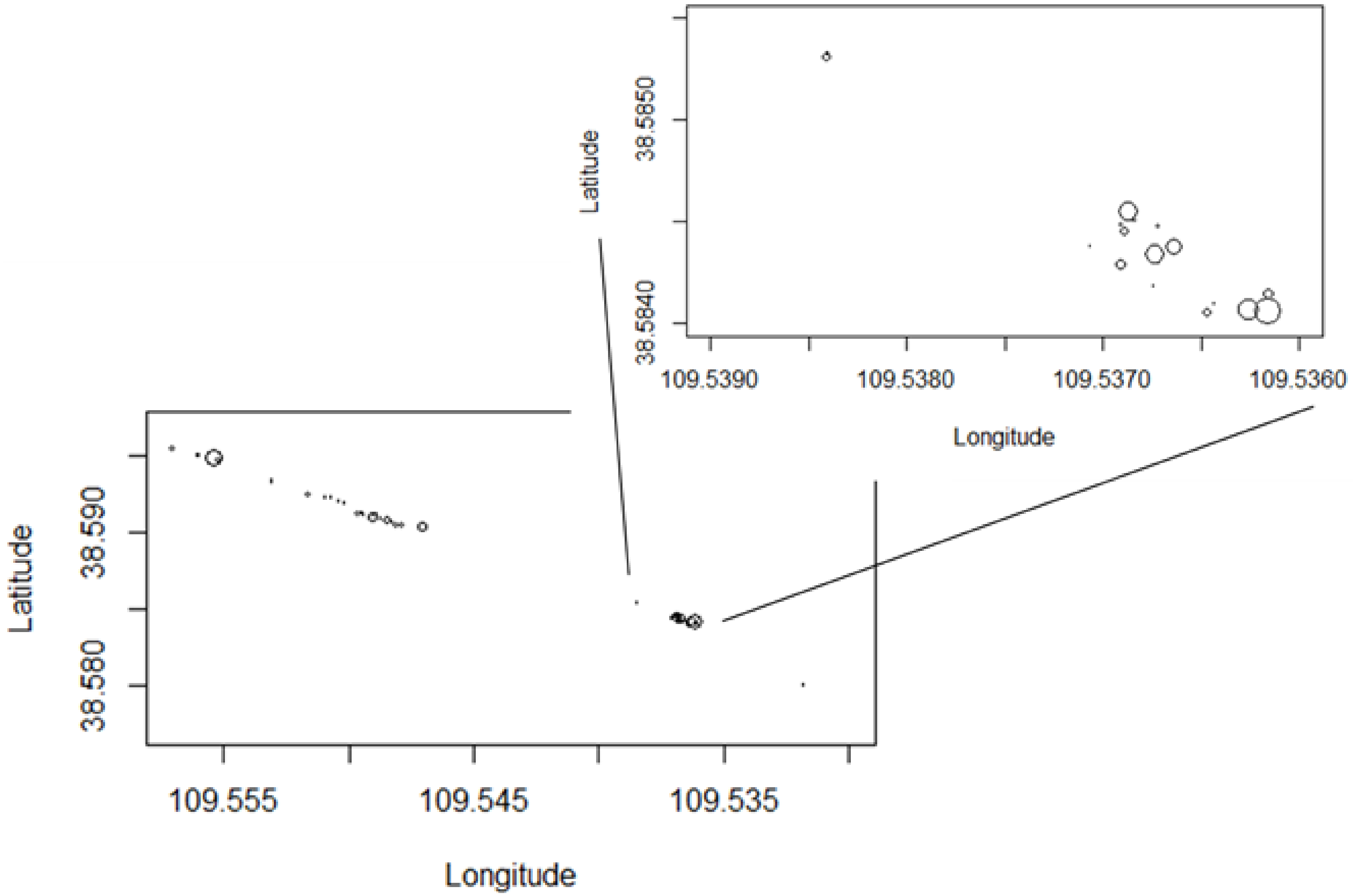

Figure 3. Locations of the 45 pools on the Colorado Plateau that were used in this study. Pools have been scaled to size to illustrate existing variation in volume and geographic isolation.

Mesocosm experiment set-up

For rearing organisms, I collected five soil samples from each pool by using a steel trowel to a depth of three centimeters, sampling in a consistent pattern across pools (Figure 6). I combined all the samples into one container per pool, and then I mixed the combined samples inside their containers in order to homogenize the soil.

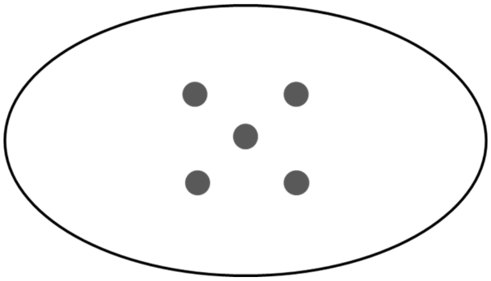

Figure 4. Soil samples were collected in a uniform pattern across pools.

To set up the laboratory experiment, I added 45 mL of the homogenized cyst-loaded soil sample from each pool to a clear, open plastic container (dimensions 16.5 cm x 14 cm, with a depth of 16.5 cm), creating an even layer of the subsample on the bottom of each mesocosm. I added one liter of microporous membrane-filtered water, which was cleared of particles and microorganisms but still retained dissolved inorganics, to each container. Component quantities were chosen to roughly mimic natural soil-water proportions at high water. The addition of water, simulating a rain event, provided organisms in the sediment with a synchronized signal to hatch. For the duration of the experiment, I kept temperature at 27 degrees Celsius, used a 12-hour light and12-hour dark diurnal cycle, and maintained daily the water level in each mesocosm. Containers were randomly arranged on three shelves, with each shelf illuminated by two linear 40-watt wide spectrum fluorescent tubes.

For *B. packardi* fairy shrimp I defined the following five progressive life history stages: *hatched* (either with embryo still enclosed in hatching membrane or with exopods visible), *early juvenile* (development of an entire set of thoracic appendages), *sexually differentiated* (visible emergence of egg sacs in females and clasping antennae in males), *adult* (possessing complete but unfilled egg sacs in females and partial clasping antennae in males), and *successfully reproduced* (exhibiting ripe eggs in females and fully functional form in males). Life stages were chosen for quick visual identification. For the *Streptocephalus texanus* fairy shrimp in mesocosms, I recorded only hatching time, since many individuals perished before reaching sexual maturity. From the time that water was added, I recorded how many fairy shrimp in each mesocosm were present at each life stage, rounding the count up to the nearest multiple of 5 if the number of live individuals was equal to or greater than 15. I took observations once every 3 hours for the first 48 hours, once every 6 hours for the subsequent 48 hours, and then finally once every 10 hours until every individual in the mesocosm had passed the final stage of successful reproduction.

To approximate body size, I removed each animal from the mesocosm immediately after the clearance of its final stage and measured maximum total body length, from the apex of the head to the posterior tip of the cercopods. I also recorded the sex of the organism, in order to calculate the ratio of females to males from the surviving individuals in each mesocosm. Hence, sex ratio was recorded as the proportion of hatched individuals that were female and that survived to reproductive maturity. All extracted organisms were preserved in 95% ethanol for future genetic analyses. I documented hatched tadpole shrimp and then removed them immediately from mesocosms to prevent consumption of fairy shrimp in the study. Other non-fairy shrimp branchiopods such as clam shrimp and ostracods, if they were present, were also removed promptly following hatching. Hence, mesocosms were designed to contain only fairy shrimp and the bacteria and algae on which they feed in their pools of origin.

In summary, for each pool I acquired hatching times, development times between the five life stages, total development time to reproductive maturity, body size measurements, a count of the total number of individuals hatched, survivorship, and sex ratio.

RESULTS

I found that the estimated volumes of pools that were surveyed ranged over 5 orders of magnitude (figure 5), from approximately 140 L to 8500 L.

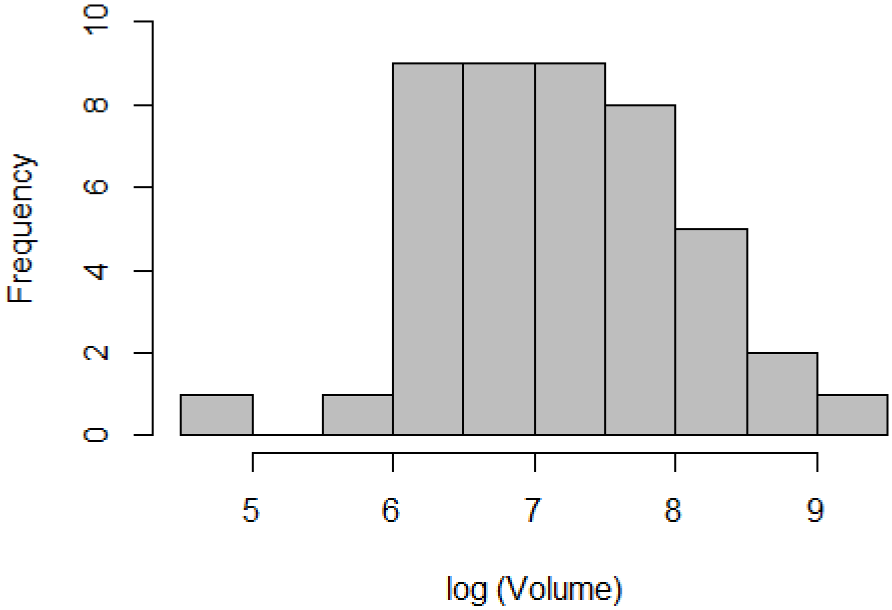

Figure 5. Distribution of pool volumes. In this study, volume is used as a proxy for pool desiccation rates.

I found that 4 pools out of 45 in the study system are currently unoccupied by *B. packardi* individuals. From these pools I observed that no individuals hatched out of three repeated attempts of three different soil samples, indicating that there are no *B. packardi* cysts in the soil. However, these pools did not differ in volume, relative depth, soil characteristics, vegetation, or isolation from occupied pools (Figure 6; other results not shown). Though there is some suggestion that they are smaller on average than other pools, this difference was not statistically significant. Furthermore, the absence of *B. packardi* did not preclude the presences of other branchiopod species, so there is no evidence that these 4 pools are exceptionally unfavorable for habitation.

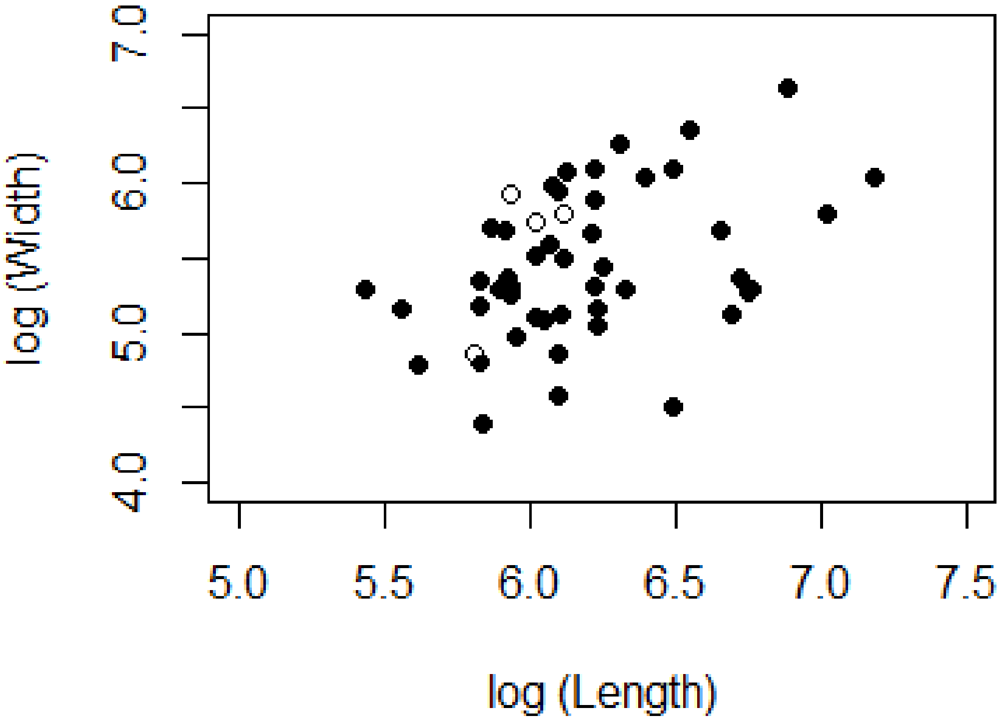

Figure 6. Pool occupancy. The four open circles represent pools that are currently unoccupied by Branchinecta packardi. Though there is some suggestion that the pools are smaller on average, the difference was not significant (t = -2.2, p = 0.08).

Mean hatching time of the focal species (*Branchinecta packardi*) was 13 hours, while mean hatching time of the competing species of fairy shrimp (*Streptocephalus texanus*) was significantly later at 56 hours following pool inundation (figure 7).

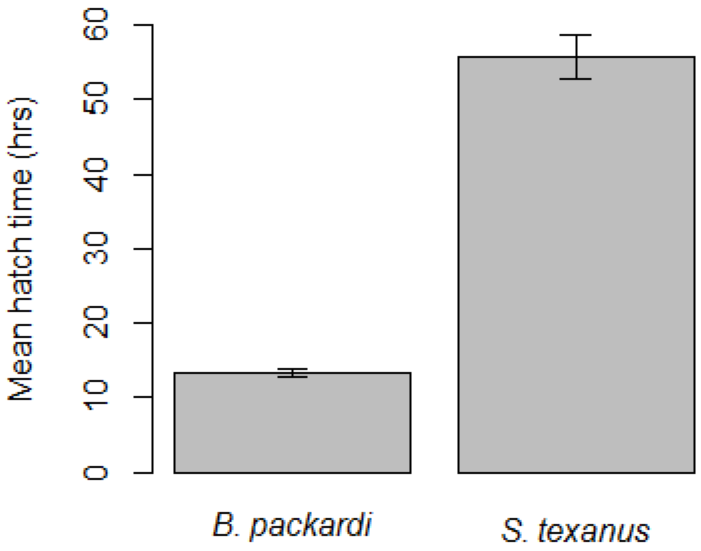

Figure 7. A comparison of mean hatching times in hours following inundation for *B. packardi* and *S. texanus* ($t = -14.26$, $p < 0.0001$).

Calculated soil volumes generally increased in a linear fashion with calculated pool volumes (figure 8), but it should be noted that much of the variation remained unexplained by pool volume.

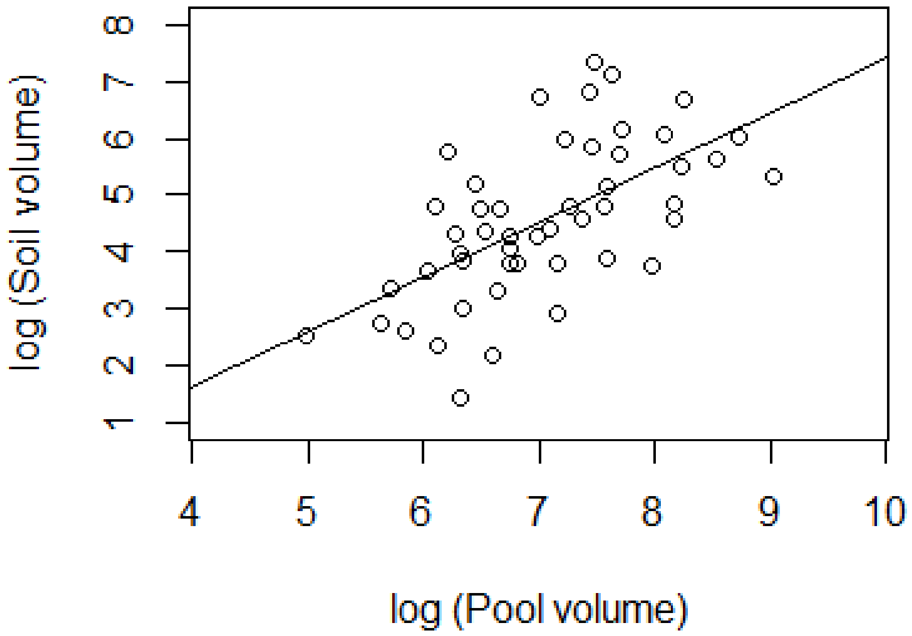

Figure 8. A significant positive linear relationship between calculated soil volume and pool volume confirms that larger pools have more soil ($R^2 = 0.35$, $p < 0.001$).

Mean pool temperatures (figure 9) had a negative linear relationship with volume (figure 10). Maximum pool temperatures (figure 11) followed the same general trend with pool volume but with higher variation (figure 12).

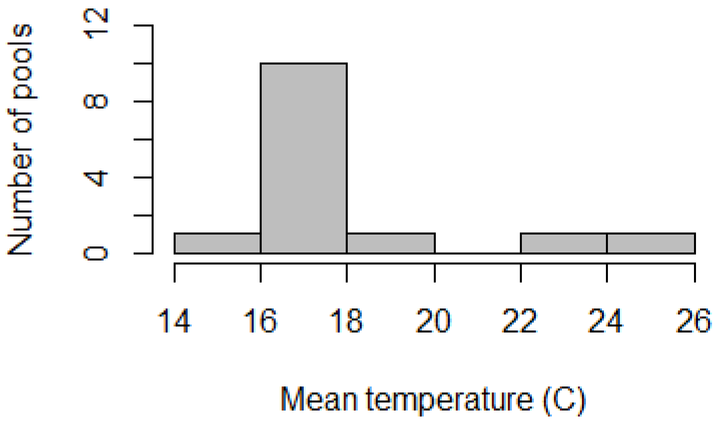

Figure 9. Distribution of mean temperatures in pools, recorded hourly from May 5, 2014 to July 25, 2014.

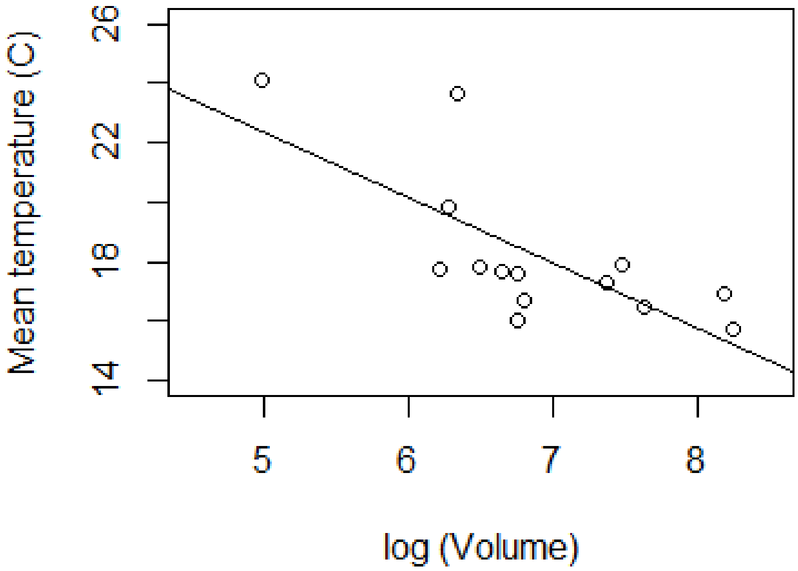

Figure 10. Negative linear relationship between mean temperature and volume of pools (R^2 = 0.53, p= 0.003). Larger pools experience lower temperatures on average.

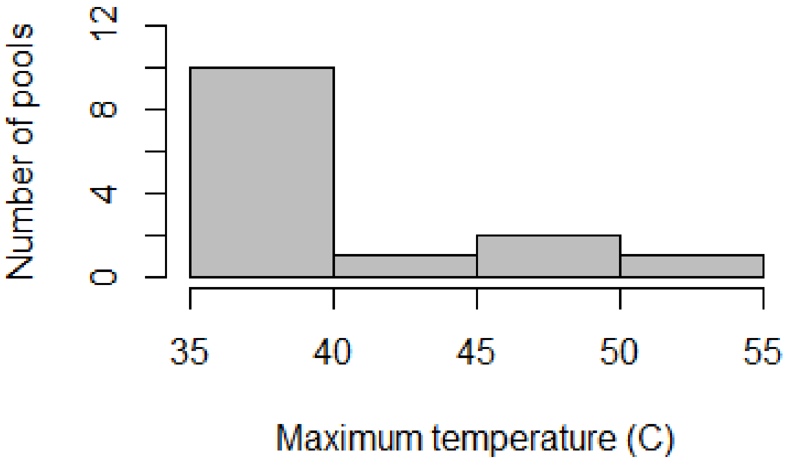

Figure 11. Distribution of maximum reached temperatures in pools, recorded hourly from May 5, 2014 to July 25, 2014.

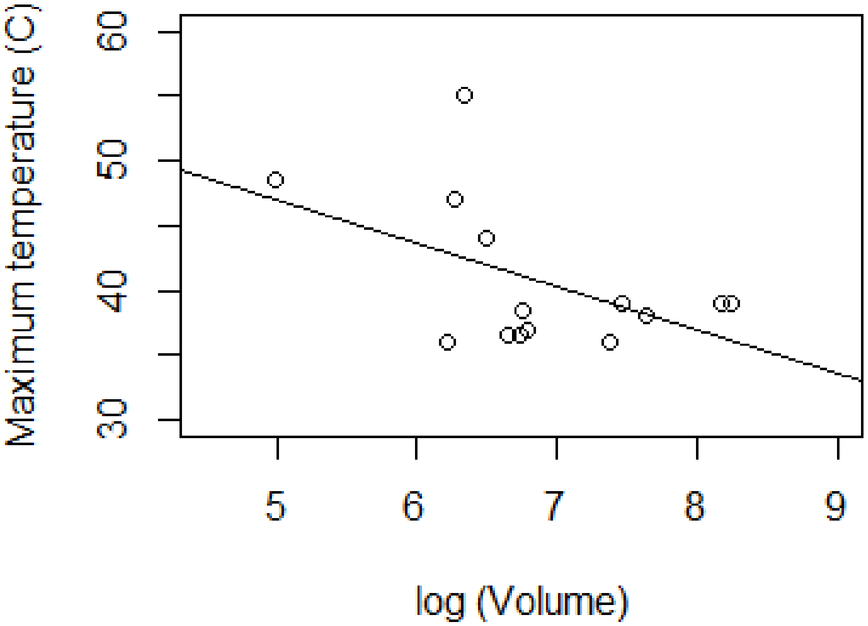

Figure 12. A negative linear trend between maximum recorded temperature and volume of pools (R^2 = 0.190, p = 0.067) indicates that smaller pools reach higher temperatures.

Factors related to cyst density in cyst banks

I used the number of *B. packardi* individuals that hatched in each mesocosm (figure 13) as an indicator of cyst bank density in the original pool. Since the same volume of soil was collected from each pool and a uniform volume of soil was then loaded into each mesocosm, differences in number of hatched individuals from mesocosms should scale to inequalities in cyst density from pools. In this study, the number of individuals hatched in mesocosms is used as a proxy for the density of cysts in pool soil.

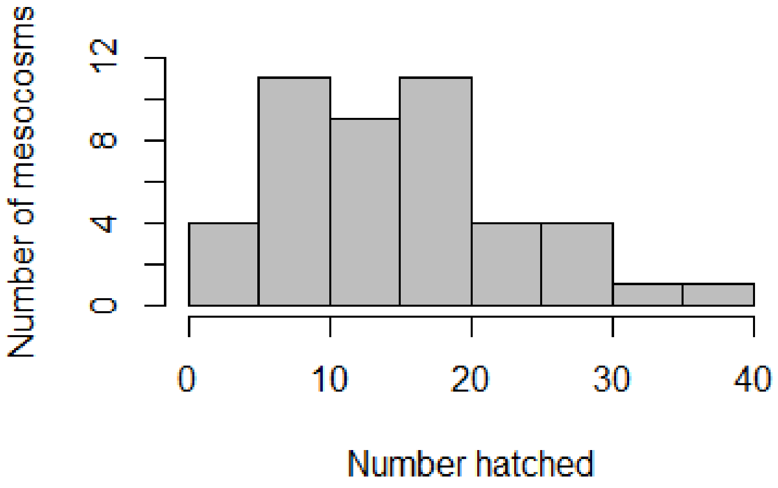

Figure 13. The approximately normal distribution of numbers of *B. packardi* individuals that hatched from mesocosms.

The number of individuals that hatched out in mesocosms was not related to the volume of the original pool (figure 14).

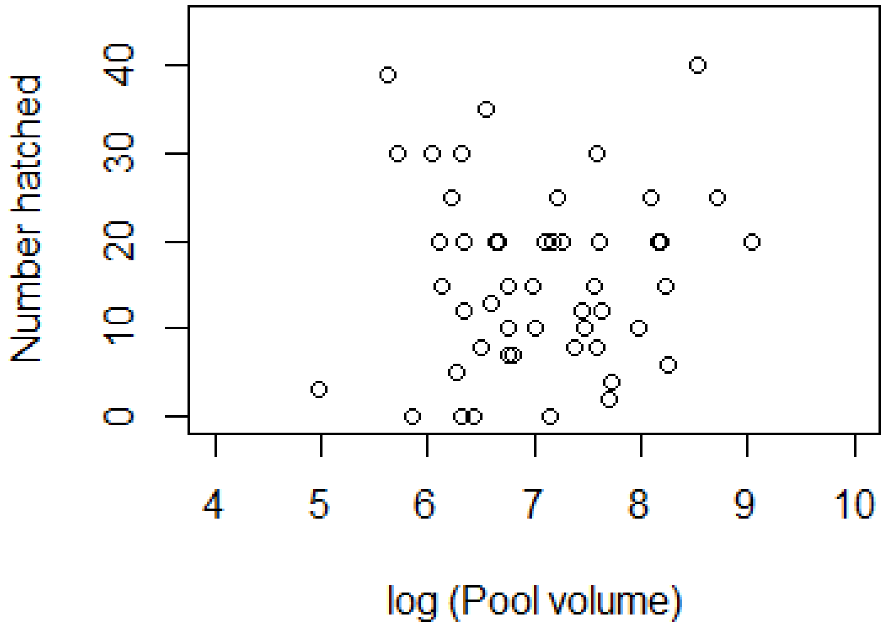

Figure 14. There was no relationship between the number of *B. packardi* individuals hatched in mesocosms and the volume of the original pool, contradicting the hypothesis that pool desiccation is a strong selective force (R^2 = -0.02, p = 0.7).

I also found that the total estimated volume of soil contained in the original pools could not predict cyst bank density (figure 15).

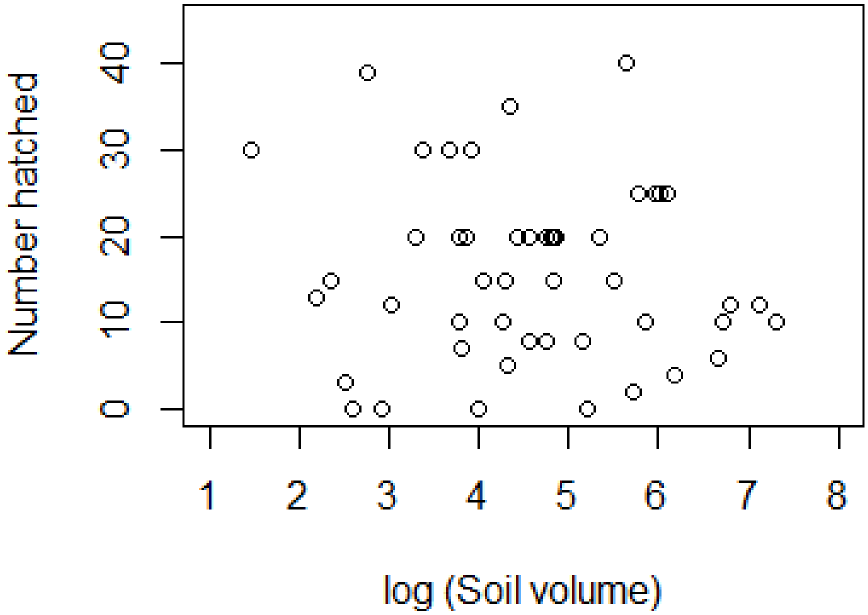

Figure 15. There was no relationship between the number of *B. packardi* individuals hatched in mesocosms and the volume of soil in the original pool, suggesting that cyst bank density does not depend on soil quantity ($R^2 = -0.0083$, $p = 0.43$)

In addition, the number of hatched individuals could not be predicted by the presence of a co-occurring competitor or predator. Estimated cyst bank density was not different in pure *B. packardi* pools compared to pools containing *S. texanus* and *T. longicaudatus* (figure 16). Both *Streptocephalus texanus* and *Triops longicaudatus* were found in larger pools on average (figure 17).

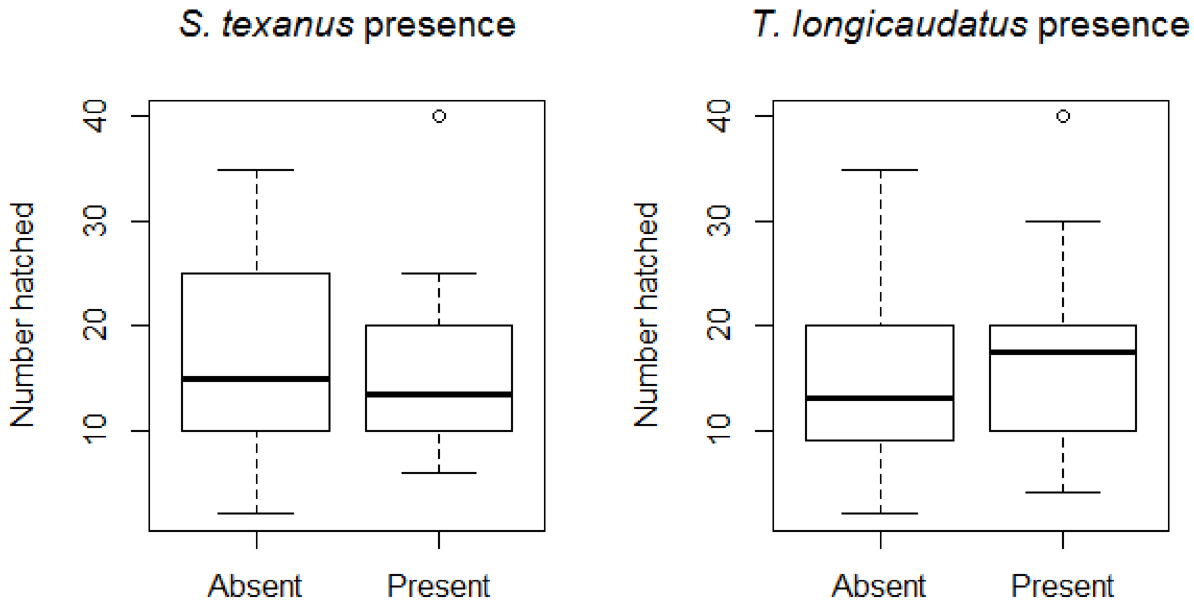

Figure 16. No discernable difference between number of hatched *Branchinecta packardi* individuals from pools with and without *S. texanus* (t = 0.34, p=0.74) and *T. longicaudatus* (t = -0.51, p=0.62), suggesting that the presences of co-occurring competitors and predators do not significantly influence cyst bank density in ephemeral pools.

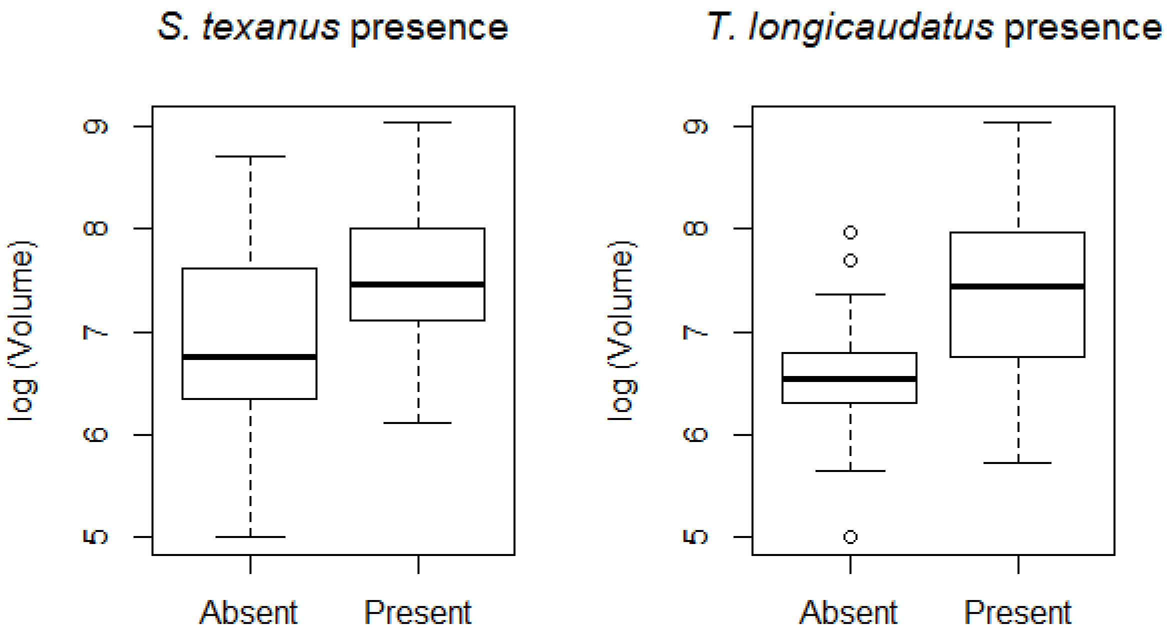

Figure 17. *S. texanus* (t = -2.57, p = 0.01) and *T. longicaudatus* (t = -3.03, p = 0.006) occur in larger volume pools on average, likely due to their requirements for longer maturation times. Larger pools have greater species richness.

To summarize the effects of pool volume, soil volume, and the presences of co-occurring species on cyst bank density, I used Akaike's Information Criterion to rank linear regression models for the number of individuals hatched in mesocosms. I found that the best supported model included only volume, but that the increases in AIC values for simple models that included the presence of either of the other species were close in AIC values to this best model (table 1).

Model	**K**	**AIC_c**	**Δ_i**	**w_i**	**R^2**
Volume	3	337.149	0.000	0.164	-0.022
Soil volume	3	337.430	0.281	0.143	-0.010
S. texanus	3	337.444	0.295	0.142	-0.016
T. longicaudatus	3	337.712	0.563	0.124	-0.023
Volume + *S. texanus*	3	337.720	0.571	0.124	-0.037
Volume + *T. longicaudatus*	4	339.400	2.251	0.053	-0.046
Volume + Soil volume	4	339.536	2.387	0.050	0.014
Soil volume + *S. texanus*	4	339.684	2.535	0.046	-0.033
Soil volume + *T. longicaudatus*	4	339.842	2.693	0.043	-0.030
S. texanus + *T. longicaudatus*	4	340.078	2.929	0.038	-0.040
Volume + *S. texanus* + *T. longicaudatus*	5	341.364	4.215	0.020	-0.062
Volume + Soil volume + *T. longicaudatus*	5	341.459	4.310	0.019	-0.042
Volume + Soil volume + *S. texanus*	5	341.910	4.761	0.015	-0.044
Soil volume + *S. texanus* + *T. longicaudatus*	5	342.217	5.068	0.013	-0.055
Volume + Soil volume + *S. texanus* + *T. longicaudatus*	6	343.986	6.837	0.005	-0.067

Table 1. Model selection statistics for the 15 linear regression models describing *B. packardi* cyst bank density in mesocosm experiment. All models are too close in ranking to choose one best. AIC_c = Akaike Information Criteria corrected for small samples, K = number of parameters, Δ_i = difference between AIC_c of model and the best fit model, and w_i = weight of evidence favoring model. R^2 = coefficient of determination.

Number of hatched individuals per mesocosm was not explained by mean temperature of the original pool ($R^2 = -0.08$, $p = 0.96$), maximum temperature of the pool ($R^2 = -0.08$, $p = 0.83$), the range of temperatures experienced by the pool ($R^2 = -0.06$, $p = 0.65$), relative soil depth of the pool ($R^2 = 0.01$, $p = 0.22$), the percent of the pool that was covered with soil ($R^2 = 0.01$, $p = 0.22$), nor elevation of the pool basin ($R^2 = -0.003$, $p = 0.35$).

Environmental factors related to individual traits

To determine which pool characteristics were potentially most important for individuals once they had hatched, I used simple linear regressions to model body size and development time, and I used beta-distributed regressions to model sex ratio (the proportion of hatched individuals that were female) and survivorship in pools, with predictor variables that included pool volume, relative depth, temperature, soil volume, and population density. Table 2 summarizes R^2 and p-values for regressions, and plots are provided for important or significant linear relationships (Figures 19, 21-22, 24, 26-31).

	Pool characteristic					
Trait	Volume	Rel. depth	Soil volume	Mean temp.	Max. temp.	Pop. density
Body size	-0.018	0.006	-0.021	-0.100	-0.078	0.110*
Maturation time	0.033	0.074*	-0.023	-0.019	-0.0005	0.057
Sex ratio ‡	0.029	0.089*	0.051	0.016	< 0.0001	0.013
Survivorship ‡	0.038***	0.002	0.032***	0.370***	0.212***	0.254***

‡ Beta-distributed regression

* significant at $p<0.05$; *** significant at $p<0.001$.

Table 2. R^2 values for linear and beta-distributed regressions between measured *B. packardi* life history traits in mesocosms and pool characteristics. Life history traits include body size, maturation time, sex ratio, and survivorship. Explanatory variables include pool volume, relative depth, soil volume, mean temperature, and maximum temperature from original pools, and density of hatched individuals in mesocosms. Statistical significance is denoted with asterisks.

Body size

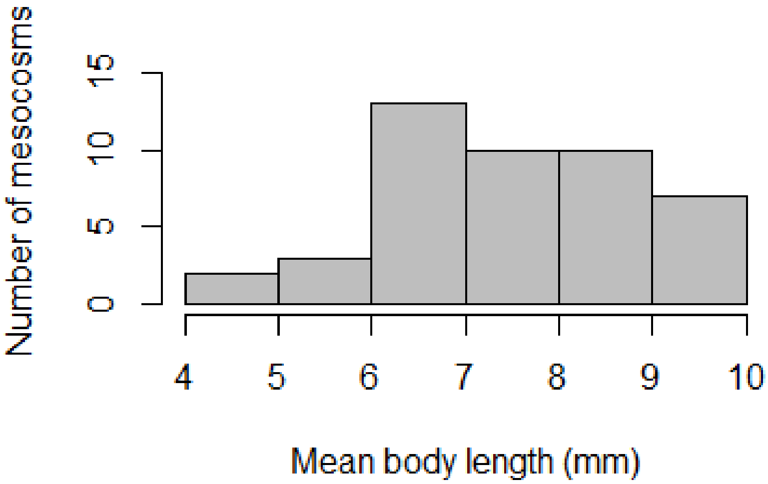

Figure 18. Distribution of *B. packardi* body sizes, measured as length from head to tail, of hatched individuals.

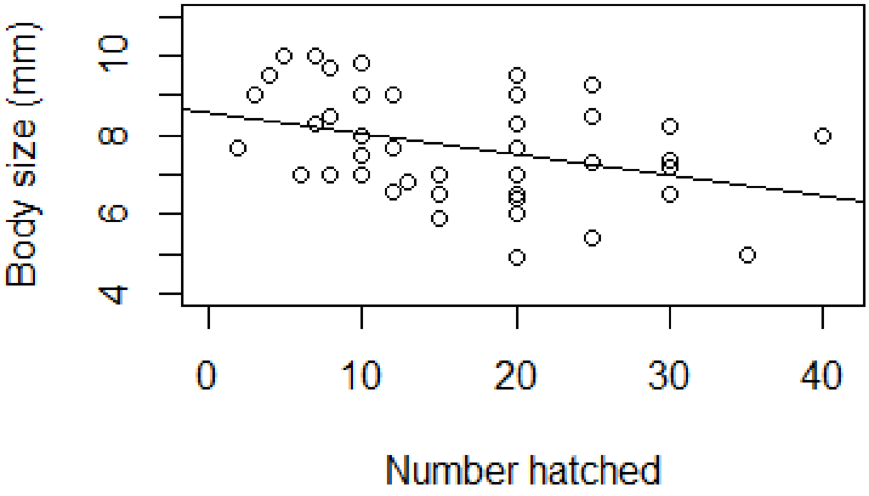

Figure 19. Significant negative linear relationship ($R^2 = 0.11$, $p = 0.015$) between *B. packardi* body size and number of individuals hatched from mesocosms.

Maturation time

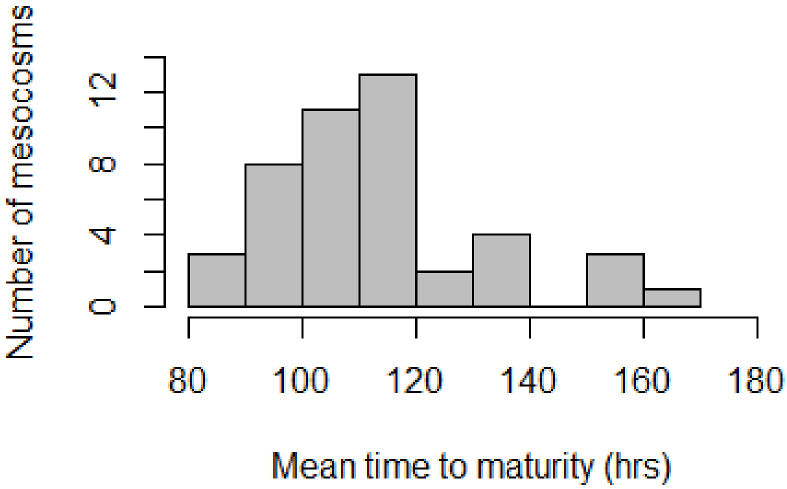

Figure 20. Distribution of *B. packardi* maturation times, measured as the time from hatch to reproductive maturity.

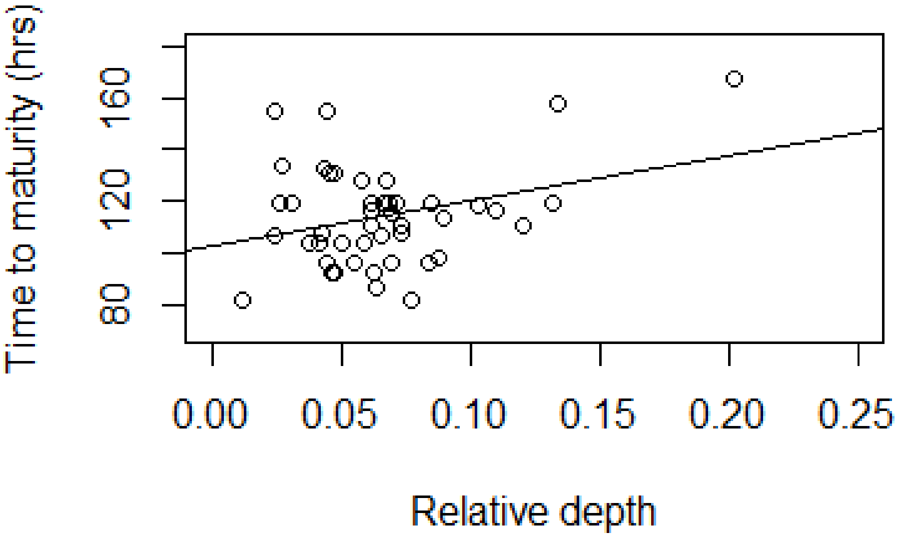

Figure 21. Weak positive trend between maturation time of hatched *B. packardi* individuals and relative depth of pools ($R^2 = 0.074$, $p = 0.04$). One possible explanation is that algal growth is limited in deeper pools due to decreased light penetration.

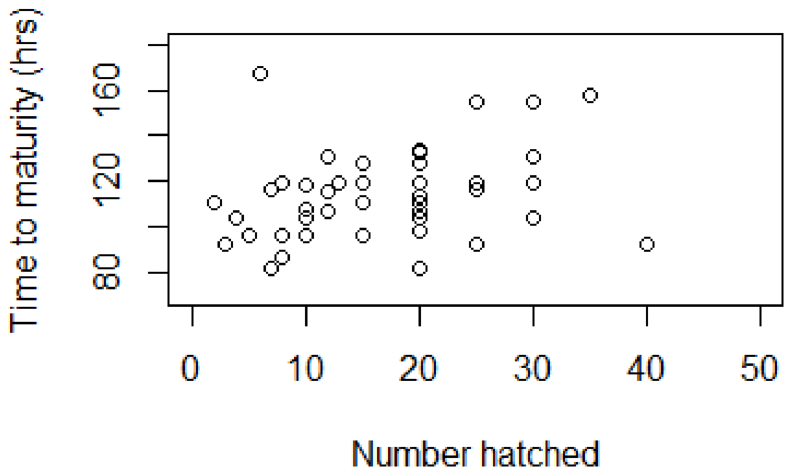

Figure 22. The trend of increasing *B. packardi* maturation times with increasing population density suggests that limited food resources in pools may cause density dependence ($R^2 = 0.057$, $p = 0.063$).

Sex ratio

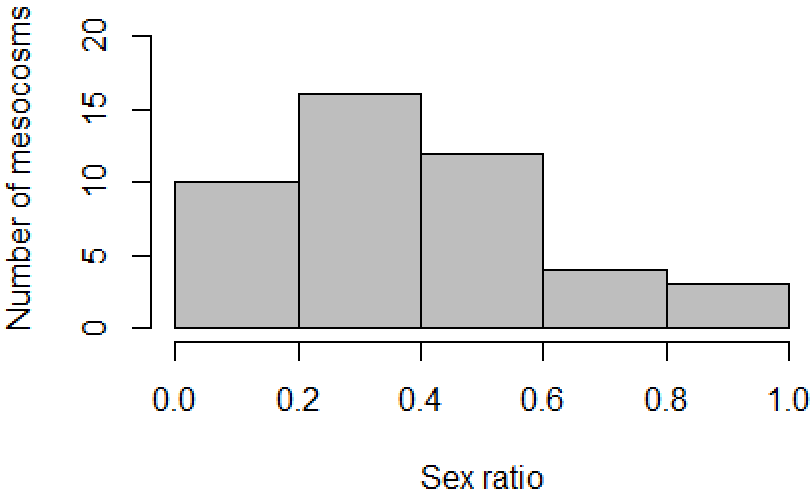

Figure 23. Distribution of sex ratios of hatched *B. packardi* individuals in mesocosms that survived to reproductive maturity. Sex ratios were measured as the proportion of individuals that are female.

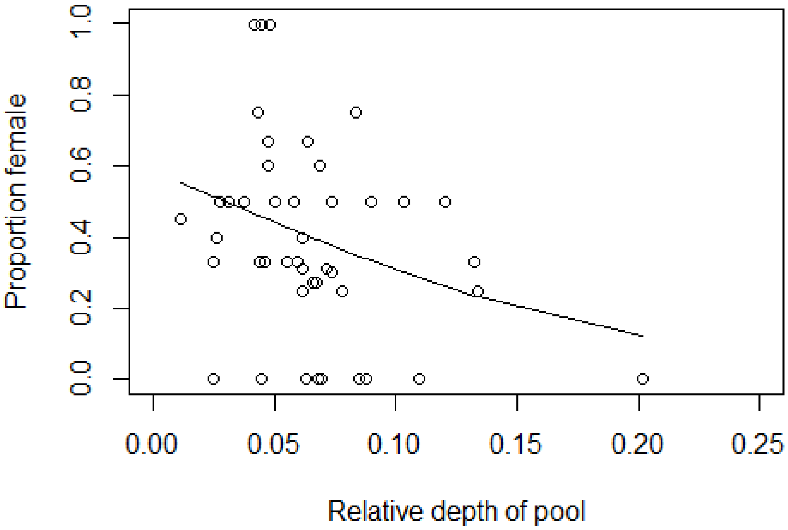

Figure 24. The trend of lower proportions of female *B. packardi* individuals in mesocosms as pools increase in depth relative to diameter could be explained by lower female survivorship due to resource competition ($R^2 = 0.089$, p = 0.046).

Survivorship

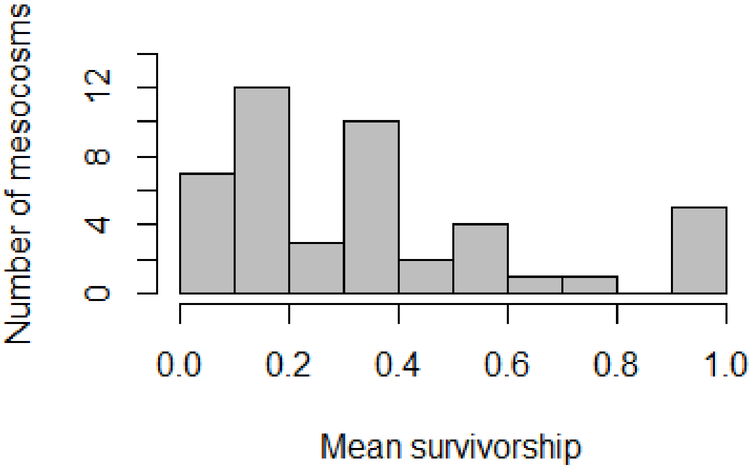

Figure 25. The non-normal distribution of survivorship values of hatched individuals supports the hypothesis that survivorship of *B. packardi* in ephemeral pools oscillates in a density dependent manner.

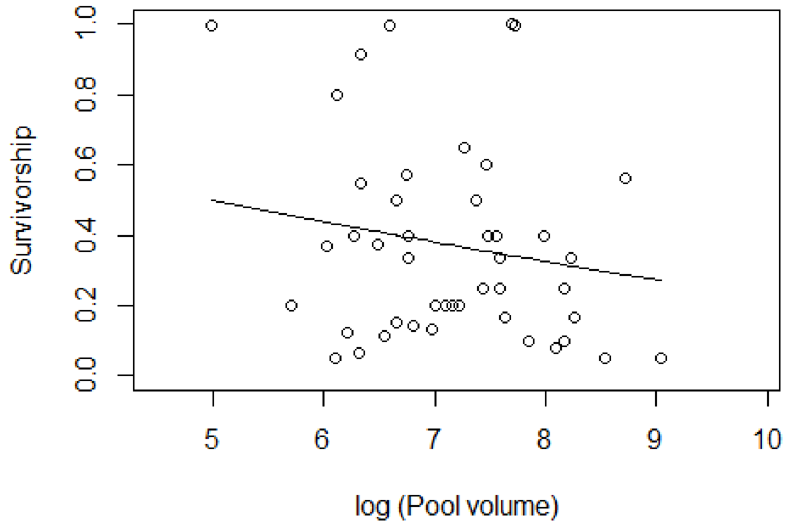

Figure 26. The trend of decreasing values of *B. packardi* survivorship in mesocosms for increasing volumes of the original pool may be an artifact of the decrease in survivorship due to the presence of the competing fairy shrimp in larger pools ($R^2 = 0.038$, $p < 0.0001$).

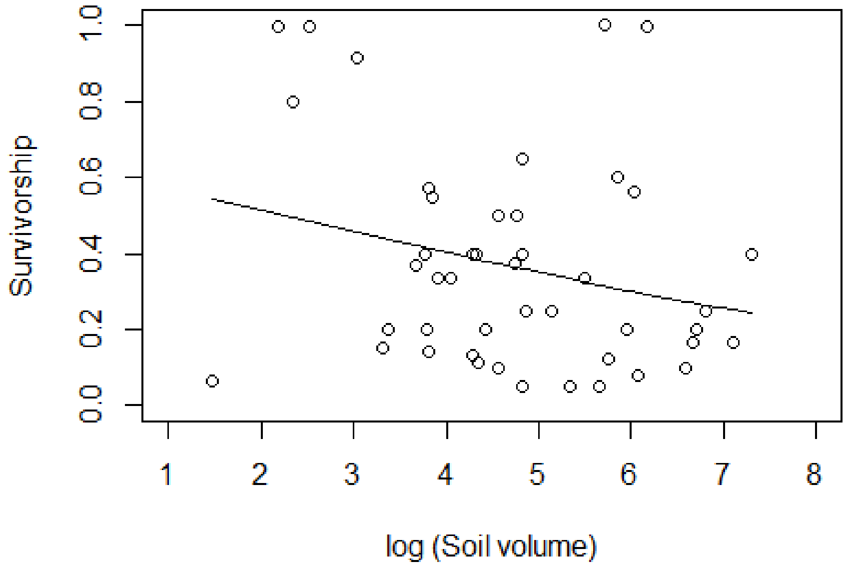

Figure 27. The weak trend of decreasing *B. packardi* survivorship in mesocosms for increasing values of soil volume from original pools could be confounded by the fact that larger volume pools contain more soil ($R^2 = 0.032$, $p < 0.0001$). This trend could also be explained by differences in soil attributes.

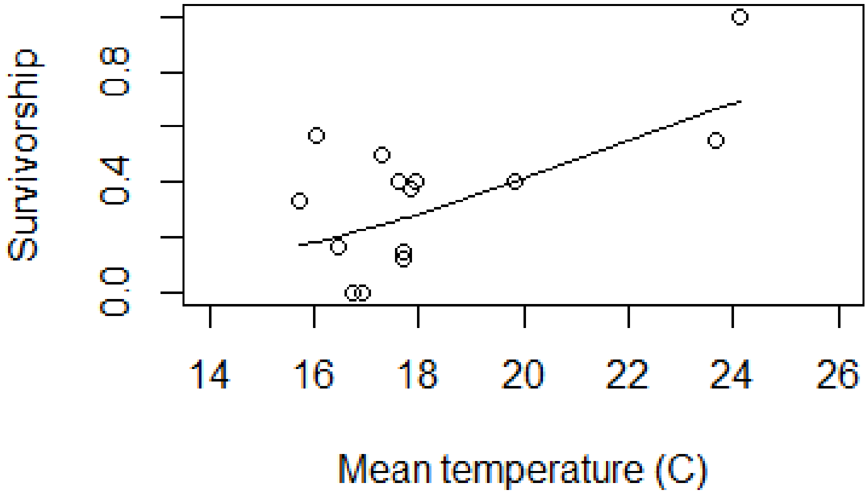

Figure 28. Survivorship of hatched *B. packardi* individuals is well modeled by a beta-distributed regression of mean temperatures experienced by pools. Survivorship is lower in cooler pools ($R^2 = 0.37$, $p < 0.0001$).

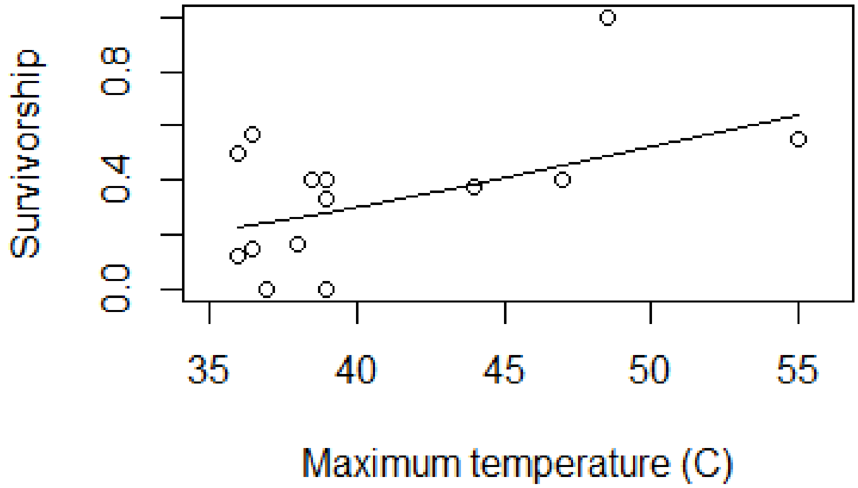

Figure 29. Survivorship of hatched *B. packardi* individuals is well modeled by a beta-distributed regression of mean temperatures experienced by pools ($R^2 = 0.21$, $p < 0.0001$). Survivorship is lower in pools that experience more extreme heat.

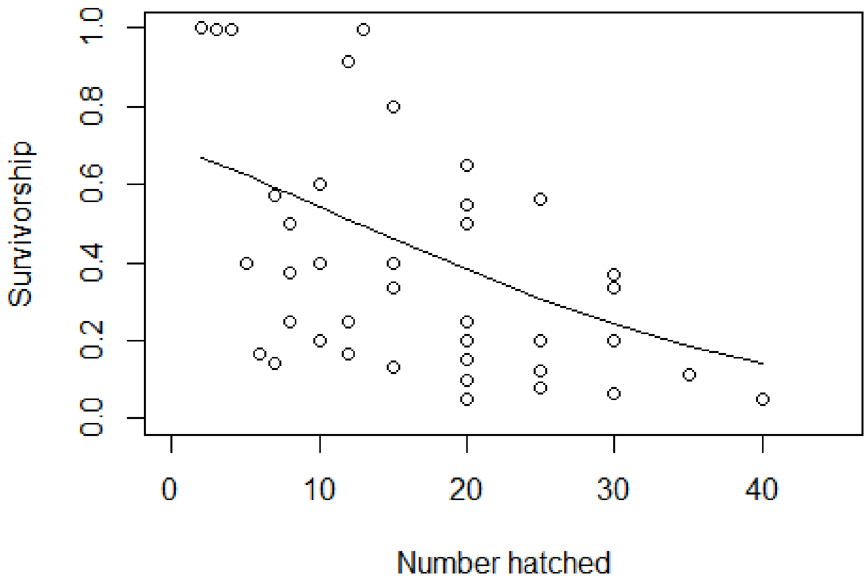

Figure 30. Survivorship of hatched *B. packardi* individuals is well modeled by a beta-distributed regression of the number of individuals hatched and coexisting in mesocosms ($R^2 = 0.25$, $p < 0.0001$). Survivorship decreases with increasing population densities in mesocosms, clearly indicating that survivorship of *B. packardi* individuals is density-dependent.

In addition to intraspecific resource competition, the presence of the competing species of fairy shrimp, *Streptocephalus texanus*, corresponded with lower survivorship in *B. packardi* (figure 31).

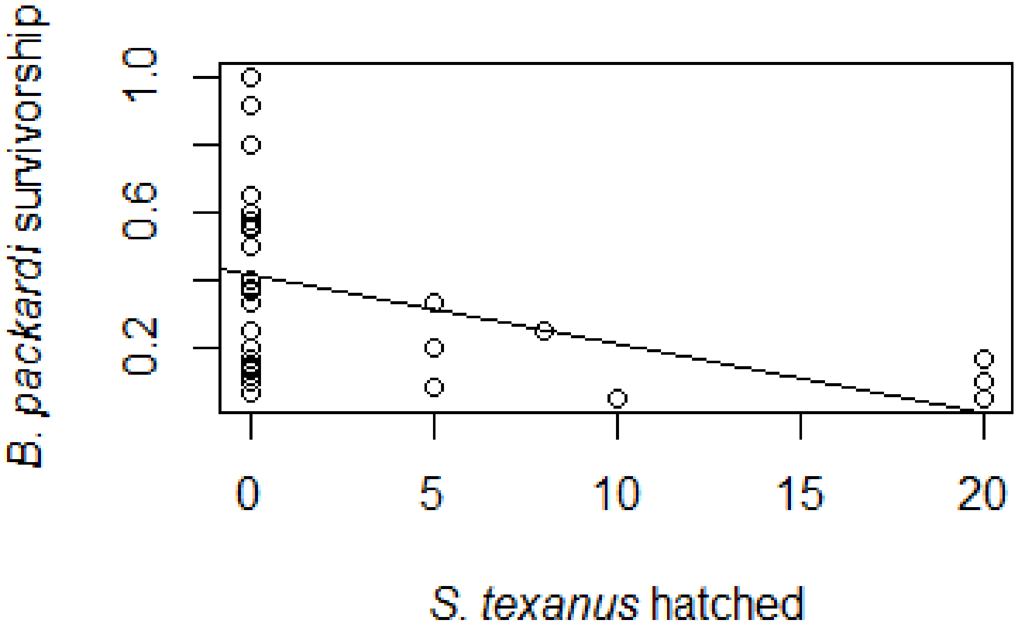

Figure 31. The survivorship of *B. packardi* in mesocosms decreased significantly with increasing numbers of a co-occurring species of competing fairy shrimp, *Streptocephalus texanus* ($R^2 = 0.13$, $p = 0.008$), providing further evidence for the importance of biotic interactions in controlling fairy shrimp survivorship.

To summarize the effects of pool volume, soil volume, population density, and the presence of the competing species of fairy shrimp on *B. packardi* survivorship, I used Akaike's Information Criterion to rank beta-distributed regression models for the survivorship of individuals hatched in mesocosms. I found that the model that best described *B. packardi* survivorship was the one that incorporated parameters of soil volume, density, and *S. texanus*. This model was approximately three times more likely than the next best model, which included volume as an additional parameter (table 2).

Model	**K**	**AIC_c**	**Δ_i**	**w_i**	**R^2**
Soil + Density + *S. texanus*	5	-507.149	0.000	0.776	0.314
Volume + Soil + Density + *S. texanus*	6	-504.657	2.492	0.223	0.314
Soil + Density	4	-485.972	21.177	0.000	0.302
Volume + Soil + Density	5	-483.747	23.402	0.000	0.302
Volume + Density + *S. texanus*	5	-452.566	54.583	0.000	0.297
Density + *S. texanus*	4	-442.729	64.420	0.000	0.286
Volume + Density	4	-412.074	95.075	0.000	0.275
Density	3	-374.200	132.949	0.000	0.246
Soil + *S. texanus*	4	-283.445	223.704	0.000	0.087
Volume + Soil + *S. texanus*	5	-281.306	225.843	0.000	0.084
S. texanus	3	-273.547	233.602	0.000	0.083
Volume + *S. texanus*	4	-272.375	234.774	0.000	0.088
Volume + Soil	4	-226.099	281.050	0.000	0.039
Soil	3	-225.262	281.887	0.000	0.038
Volume	3	-205.131	302.017	0.000	0.032

Table 3. Model selection statistics for the 15 beta-distributed regression models describing *B. packardi* survivorship in mesocosm experiment. Volume = volume of original pool, Density = *B. packardi* population density in mesocosms, Soil = volume of soil in original basin, *S. texanus* = number of hatched *S. texanus* individuals in mesocosms. The best model, which includes soil volume, population density, and the competing species of fairy shrimp, is approximately three times more likely than the next best model which includes volume as a parameter. AIC_c = Akaike Information Criteria corrected for small samples, K = number of parameters, Δ_i = difference between AIC_c of model and the best fit model, and w_i = weight of evidence favoring model. R^2 = pseudo-R^2 for model.

DISCUSSION

If organisms inhabiting extreme habitats such as desert ephemeral pools are unable to migrate to suitable environment, they often must persist through periods of harsh conditions in a dormant state. Hence, dormant life stages can be an important species attribute for bridging adverse conditions, providing temporal gene flow for small populations, and promoting biodiversity by drastically increasing the chances of survival in habitat patches (Hairston et al. 1995, Hairston and De Stasio 1988, Marcus and Lutz 1998). Niche theory suggests that *Branchinecta packardi* cyst bank density should be lower in smaller pools, since drying time is most likely dictated by pool size and therefore frequent pool desiccation in smaller pools may prohibit maturation of individuals. Similarly, cyst bank density should be lower in large pools due to biotic interactions and resource limitation. Niche theory would also suggest that cyst bank density should be greatest in pools that are large enough to provide for the completion of the B. packardi life cycle but small enough to exclude slower-developing superior competitors and predators. Determining what factors influence the sizes of cyst banks in pools can provide valuable insight on community dynamics in desert ephemeral pools.

Cyst bank density

As expected, there was considerable variation in the cyst bank size among pool. However, my prediction that the volumes of ephemeral pools would predict the density of cysts in ephemeral pool cyst banks was not supported by the data. At small volumes, I did not find evidence that the density of cysts in the soil was reduced from high mortality caused by premature pool desiccation or passive wind dispersal from soil-deficient shallow pools. At large volumes, I did not find evidence that cyst density was reduced by competition and predation in taxa-rich pools. The number of individuals hatched was not different in pools with competing species and predators than in pure-species pools. Furthermore, the variation in the number of hatched individuals in mesocosms could not be explained by any of the measured biotic or abiotic characteristics of the original pools. These results indicate that the number of cysts per unit of soil is not affected by desiccation rates or other physical characteristics of ephemeral pools, and it is also not controlled by the presences of co-occurring species. Though it is possible that sampling error was underestimated because cysts are not evenly distributed in pools, that maternal effects exist, or that differences in hatching proportions are important, these data suggest that the density of *B. packardi* cysts deposited into cyst banks is stochastically determined.

Although the volume of soil in pools was related to the volume of the pool, only 35% of the variation could be explained by pool volume. Soil volume was also not explained by relative depth of pools ($R^2 = -0.0044$, $p = 0.37$), suggesting that the particular shape of the pool does not matter. Variation in soil volume could be due to differing positions atop sandstone fins. For example, sand released by the wind erosion of basin walls is less likely to accumulate when the basin is located on the windward side of the fin because wind scours the surface and carries the sediment to the leeward side (Loope et al. 2008). It has also been hypothesized that pools are lined with biofilms that catalyze the erosive processes involved in basin expansion (Chan et al. 2005), and therefore differences in the microbial communities of pools may be responsible for some of the observed variation in soil volume in ephemeral pools. Hence, data on microbial taxa abundances may help to explain the weak relationship between soil volume and pool volume. Though soil properties were not shown here to play an extensive role in cyst densities in pools, or the life history traits of hatched individuals, they may be important in buffering temperature fluctuations in dry pools. They may also affect female cyst deposition and bioturbation in pools, as studies have shown that fairy shrimp cyst viability can be affected by storage conditions (Brendonck et al. 1996, Ripley et al. 2004).

On a related note, the extreme temperatures recorded in basins may play a role in natural selection in dry ephemeral pools. I found that larger pools had lower temperatures on average and did not experience temperatures that were as high as temperatures experienced by smaller pools. It is possible that since smaller pools are not situated deeply in large basins where shade is provided by basin walls, they are more exposed to solar radiation. In the 14 pools that were monitored hourly, maximum temperatures of up to 55 degrees Celsius were reached, which may prohibitively hot to organisms even if they are in a dormant phase. The pool that reached this temperature is notably flat, exposed, and often dry while other pools remain inundated, and it has a relative depth of 0.011, approximately 5 times greater than the average relative depth of all pools. However *B. packardi* survivorship in this pool was 55% and individuals from this pool reached reproductive maturity in just 82 hours, nearly 40% faster than the average, which may be due to the absence of other competing and predatorial species. However the success of *B. packardi* in this pool may also be due to a high thermal tolerance and intrinsically fast maturation time. The population occupying this pool may face strong selective pressures to produce thick cyst shells to protect embryos from intense UV radiation and to develop to reproductive maturity in a pool where quick desiccation risk is high.

I found that surviving *B. packardi* individuals in mesocosms were disproportionately male ($t = -2.97$, $p = 0.005$), with greater proportions of males particularly in deeper pools. Males may have greater survivorship in larger, taxa-rich pools due to stronger competitive abilities, lower resource requirements, or a combination of the two. Alternately, sex allocation by females may change according to environmental conditions (West 2009). The ratio of females to males in populations is important for estimations of population growth, since males do not directly contribute to reproductive output.

Additional data is needed to determine whether this observed pattern is due to sex allocation or differential survival in mesocosms.

Life history traits of hatched individuals

Pool volume, relative depth, soil volume, and temperature of original pools did not explain the variation in number hatched, body size, sex ratio, or development time in *B. packardi,* though there was some suggestion that maturation time increased with relative depth of the original pool and, as previously mentioned, some suggestion that the proportion of females was greater in mesocosms representing shallow pools. The delay of maturation in deeper pools may be explained by food limitation. Since light is often limited in pools with greater relative depth, algal growth may be diminished (Burfurd 1997, Moheimani and Borowitzka 2007), leading to longer time required for development. This may also explain why a greater proportion of females survived to reproductive maturity when they hatched in mesocosms containing soils from shallower pools. If female fairy shrimp require greater resources than males in order to produce offspring, then they may be more likely to perish in food-limited environments. Overall, however, I did not find strong evidence that the abiotic characteristics of pools enact selective pressures on *B. packardi* life history traits, since increasing maturation times and proportions of females in deeper pools could both be explained simply by resource limitation in mesocosms.

Survivorship, a necessary component for the contributions of individuals to future population growth, was similarly linked to biotic interactions, pool volume, soil volume, and temperature. There was clear evidence that *B. packardi* population growth is density dependent again due to competition for resources. Survivorship declined dramatically and individuals developed to significantly smaller sizes in mesocosms where large numbers of conspecific individuals hatched. Additionally, there is some suggestion that it took more time for individuals to develop to reproductive maturity in mesocosms with high population densities than in those with low population densities. A longer development time could be potentially detrimental to species living in ephemeral pools, where individuals must race to reproduce before pool desiccation. Intraspecific competition in resource-limited pools can be strong, since individuals of the same species require the same resource requirements to persist (Gause 1934, Hairston et al. 1960, Clutton-Brock et al. 1987, Silva Matos et al. 1999) and since individuals in ephemeral pools have limited time in which to consume as much as possible during periods of pool inundation.

Moreover, I found that *B. packardi* survivorship decreased as the number of hatched *Streptocephalus texanus* individuals increased in the mesocosms. This strong negative association between *B. packardi* survivorship and the presence of the competing species of fairy shrimp provides further evidence of heavy resource competition in ephemeral pools. It is likely that the observed trend of decreasing *B. packardi* survivorship for increasing values of pool volume is an artifice of the fact that *S. texanus* inhabits slower-drying pools. Mesocosms representing larger pools contained more individuals of the competing fairy shrimp species, increasing the biotic interactions that resulted in decreased survivorship. Though the best model for *B. packardi* survivorship included soil volume as an important parameter, the trend of decreasing mean survivorship with increasing soil volume could have been confounded by the tendency of larger pools, which also contain larger populations of *S. texanus*, to hold more soil. Moreover, larger volumes of soil often signified a larger proportion of sand, which could contain less organic matter and thus lower quantities of algal food.

These data taken together suggest that food resources are limited in ephemeral pools and that intra- and interspecific competition is the basis for *B. packardi* population dynamics. In the case of this species in desert ephemeral pools, a greater number of hatched individuals from one generation does not lead to a greater number of cysts in the next generation because dense populations lead to low suvivorship and therefore decreased reproductive output. Though data over a span of consecutive generations is required to truly confirm the presence of density dependence, this study suggests that population size is controlled more by biotic factors than by any of the measured abiotic characteristics of the pools. The prediction for time series data of *B. packardi* populations is that every ephemeral pool is oscillating in a density dependent manner over time, with the presence of the co-occurring fairy shrimp *S. texanus* intensifying competition pressure for food resources.

CONCLUSION

This study shows *Branchinecta packardi* to be remarkably resilient species, possessing the ability to survive in a wide range of pool conditions. Rather than demonstrating that cyst bank size was deterministic and controlled by pool characteristics, the data from this study suggest that this species is not substantially affected by its abiotic surroundings and that variation in number of individuals hatched is instead explained by stochastic, density dependent survivorship.

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