PHYSICAL AND CHEMICAL FACTORS THAT AFFECT
DIVERSITY OF AQUATIC INVERTEBRATES IN VERNAL POOLS
IN SACRAMENTO COUNTY, CALIFORNIA

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B.S., University of California at Davis, 2005

THESIS

Submitted in partial satisfaction of
the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGICAL SCIENCES

at

CALIFORNIA STATE UNIVERSITY, SACRAMENTO

FALL
2011
Student: Angela Calderaro

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Ronald M. Coleman, Ph.D.  Date

Department of Biological Sciences
Abstract

of

PHYSICAL AND CHEMICAL FACTORS THAT AFFECT DIVERSITY
OF AQUATIC INVERTEBRATES IN VERNAL POOLS IN
SACRAMENTO COUNTY, CALIFORNIA

by

Angela Calderaro

Patterns of aquatic invertebrate diversity in vernal pools may be driven by physical
and chemical factors in conjunction with dispersal capabilities. By comparing this system
to metacommunities, the local and between community factors may predict why species
assemble or co-occur within a given space. Because the study of metacommunities
involves both the interactions within individual communities and the dispersal patterns
between communities, it allows ecologists to better understand the relative roles of local-
and regional-scale processes on diversity. This study examined in situ relative effects of
various physical and chemical factors on aquatic invertebrate communities within vernal
pools in eastern Sacramento County with particular attention on the effect of hydrologic
connectivity via overland surface flow on diversity. Light Detection and Ranging (LIDAR)
data was used in conjunction with the ArcHydro model to determine water flow and
hydrologic connectivity between pools at the study sites.
The vernal pool aquatic communities are verifiably linked within the landscape. Even though surface water connectivity was not significantly related to diversity over the four sample periods, the pools are linked by the unique abilities of these species to disperse in this temporary aquatic environment. Connectivity was significant (among many other physicochemical factors) for richness of flatworms and snails (opportunistic species) and density of crustaceans. So although it does play a small role in driving where species co-occur, future designs of vernal pool restoration and creation efforts do not need to evaluate the connectivity patterns in vernal pools to maximize taxa diversity.

Other physicochemical factors that shaped diversity include dissolved oxygen and water quality. Dissolved oxygen was positively related to overall taxa richness, as many species in vernal pools are gill-breathers. Overall taxa diversity of the pools measured by the Shannon diversity index was negatively related to total dissolved solids, a measure of water quality. For this reason, maintaining the health of these ecosystems by avoiding water quality impacts associated with development and stormwater runoff is imperative to maintain the diversity and stability of these sensitive habitats.

Jamie Kneitel, Ph.D.

Date
ACKNOWLEDGMENTS

First and foremost, I would like to thank Dr. Jamie Kneitel for his direction, assistance, and guidance. His input was invaluable in the completion of this project. I also wish to thank the other members of my supervisory committee, Dr. Ron Coleman and Dr. Michelle Stevens, for their guidance.

I especially would like to thank Mary Maret at Sacramento County Regional Parks, Aimee Rutledge at Sacramento Valley Conservancy, and Douglas Fortun at Mather Field Air Force Base for helping me obtain permissions for conducting my field work at my chosen study sites. I would also like to thank Rich Radmacher for coordinat ing access efforts.

I would like to acknowledge the help of John DeMartino, Jeannette Owen, Joyce Hunting, and Jonathan Faoro for their technical support and advice. In addition, I would like to acknowledge Mohammed Rehmatullah at UC Davis for his help on the statistical analysis.

Finally, I am forever indebted to my husband for his endless patience and encouragement in addition to his assistance conducting the field work, even though he swears he is allergic to the outdoors.
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Chapter 1

INTRODUCTION

Ecologists strive to understand the processes that determine diversity patterns within ecosystems. In aquatic communities, these patterns may be driven by local-community characteristics such as physicochemical characteristics, or larger scale processes such as dispersal. The combination of local and between community factors may predict why species assemble or co-occur within a given community (Cadotte 2006). A useful approach for understanding how these various factors determine species diversity is the study of metacommunities. A metacommunity is a set of local communities that are linked by dispersal of multiple potentially interacting species at the regional scale (Gilpin and Hanski 1991; Wilson 1992; Leibold et al. 2004). Because the study of metacommunities involves both the interactions within local communities and the dispersal patterns between communities, ecologists can identify the relative roles of local- and regional-scale processes on diversity (Kneitel and Miller 2003; Holyoak et al. 2005).

Vernal pools are ephemeral aquatic habitats within a grassland matrix that annually fill with water and dry out (Barry 1995). The natural topography of the landscape creates pools that are either hydrologically isolated or connected to other pools. This natural patchiness, therefore, provides an appropriate system to study diversity patterns and the relative importance of local versus regional factors (DeMeester et al. 2005). At the scale of an individual pool, species diversity can be examined and measured within a relatively closed system. As ephemeral aquatic habitats, vernal pools exhibit highly diverse invertebrate communities (King et al. 1996), but few ecologists have investigated factors affecting species richness in situ in vernal pools (Wilcox 2001; Urban 2004; Angeler and
Aquatic invertebrate communities in vernal pools are consistent with the metacommunity concept because they are a set of local communities linked by dispersal of multiple potentially interacting species. For these reasons, vernal pools provide the ideal system for investigating factors affecting diversity at multiple scales (Holland and Jain 1981).

Hydrologic connectivity can facilitate movement of aquatic invertebrate species from one vernal pool to the next. Much like wildlife corridors facilitate the movement of animals from patches of habitat (MacArthur and Wilson 1967), swales connecting pools would also facilitate movement of these species. For this study, hydrologic connectivity is only considered in the context of surface water connection. Hydrological connectivity between pools may increase the dispersal potential of pool inhabitants and may homogenize the physical and chemical factors between the pools (Cottenie et al. 2003). If connectivity between patches of habitat is high, then regional species richness is expected to be low, due to increased similarity between the aquatic invertebrate communities (Forbes and Chase 2002). Conversely, isolated pools may be less similar to one another given the lack of surface water connection and the increased heterogeneity of habitat characteristics. In other words, isolated pools may have greater diversity between them (β-diversity) than the pools that have a surface water connection.

The regular disturbance regime of seasonal desiccation is characteristic of vernal pools and has resulted in unique animal adaptations that allow them to disperse or remain dormant during disturbance periods (Colburn 2004; Williams 1987; Wiggins et al. 1980). The dispersal capability of the organisms in these regularly disturbed environments may overcome the beneficial dispersal effects of hydrologic connectivity, such that connectivity
might not affect species richness. Instead, environmental constraints might drive the structure of these local aquatic communities. This project examined the physical and chemical factors driving diversity patterns of aquatic invertebrates within vernal pools in eastern Sacramento County.

**FACTORS LIMITING DIVERSITY OF AQUATIC INVERTEBRATES**

Beta diversity (diversity between habitats) is expected to be positively associated with increasing environmental heterogeneity, as more fragmented and complex landscapes typically show higher variability in their biota (Nekola and White 1999). Vernal pool systems are imbedded in a terrestrial matrix where there is typically poor surface water connectivity between pools (Wilbur 1997), and might reasonably be expected to have the high spatial variation in diversity. In temporary waters in which the dry period is cyclical and predictable (such as vernal pools), the communities will consist almost exclusively of obligate temporary water species, which are adapted to a fleeting environment (Williams 1987). If the species in the pools can disperse despite the lack of hydrologic connectivity, then the diversity of aquatic invertebrates is strongly influenced by limiting factors within individual pools. High rates of dispersal would have homogenizing effects if the patches of habitat were similar and have a negative relationship with beta diversity; passively dispersed organisms have lower beta diversity than actively mobile species (Soininen et al. 2007). Soininen et al. (2007) concluded that there is strong evidence for variation in beta diversity being driven by multiple factors related to species functional traits or characteristics, geographical gradients and general ecosystem properties.

Multiple factors may interact and contribute to diversity among vernal pools (Platenkamp 1998; Williams 1996). Physicochemical factors, including surface area, depth,
temperature, and dissolved oxygen content may affect the insect fauna in temporary pools (Williams 1996). Factors like volume, average depth, and surface area would affect the space available for different species. Pool size or permanence (hydroperiod) has a positive effect on taxa richness (Eitam et al. 2004; Brooks 2000; Batzer 2004). Higher water temperatures in the pools would encourage rapid growth of algae, which may supplement food supply for herbivores, but it may also deplete the dissolved oxygen content (Williams 1987). In addition, temperature may also affect the species that do not tolerate a wide range of temperatures (Hathaway and Simovich 1996) or species that hatch at different temperatures (Eng et al. 1990). Most studies have focused on a limited number of factors to predict diversity, whereas this study aims to evaluate several factors which may interact and provide a more complete picture of the predictors of greater diversity.

**VERNAL POOLS**

Vernal pools are unique, rare, and vulnerable communities (USFWS 2005). Vernal pools provide habitat for numerous aquatic invertebrate species, some of which are endemic to California. Since settlement of California by Europeans, vernal pool communities have been steadily declining (Holland 1998, 2009). Vernal pools are located in small depressions in annual grassland habitat, a habitat type that is easily leveled and highly desirable for urban development and agriculture. The United States Fish and Wildlife Service determined that between 60 to 90% of historic vernal pool habitat in the Central Valley was lost by 1973 (USFWS 2005). Another study estimated 13% of historic vernal pools persist in California (Holland 2009), and yet these rare habitats are still threatened by development.

Vernal pools are wetlands characterized by seasonally flooded depressions on ancient soils with an impermeable layer, such as a hardpan, claypan, or volcanic basalt.
(Barry 1995). The impermeable layer allows the pools to retain water over the winter much longer than the surrounding uplands (Barry 1995). By summer, the pools have dried up; any wildlife species that have relied on the winter rain pools must have either completed their life cycle or moved to another terrestrial habitat type. Among the many invertebrates that inhabit these pools, several species of crustaceans, branchiopods in particular, are ecologically dependent on wetlands with seasonal inundation and subsequent desiccation (Williams 1987; Eriksen and Belk 1999). Appropriate periods of desiccation are necessary for branchiopod egg dormancy (USFWS 2003). In addition, desiccation eliminates bullfrogs, fish, and other aquatic predators that depend on year-round inundation of wetland habitats to survive (USFWS 2003). Although “egg” or “cyst” have both been used in the literature to describe this life stage, for this report, the term “egg” will be used to describe this resting phase (diapause), which allows the species to persist through the dry summer or long periods of drought (Eriksen and Belk 1999). Due to the loss of this unique habitat, many of these vernal pool branchiopod species have become rare and were subsequently listed under the Federal Endangered Species Act, including the federally threatened vernal pool fairy shrimp (Branchinecta lynchi) and the federally endangered vernal pool tadpole shrimp (Lepidurus packardi).

Temporary pools represent unique challenges to species survival. Success of an organism in a temporary pool depends on the power of dispersal and plasticity of the life cycle (Williams 1987). Active dispersal of species in temporary pools generally occurs via mobile adults, including winged species (beetles, true bugs, true flies, dragonflies, etc.), and those with high dispersal ability, such as the western toad (Anaxyrus boreas), Pacific chorus frog (Pseudacris regilla), mosquitoes (Culicidae), water boatman (Corixidae), and diving beetles (Dyticidae) (Williams 1987). Passive dispersal of species relies on a resting
reproductive body that is transported by wind and other living vectors (Williams 1987). Vernal pool branchiopods have high dispersal capabilities during the resting egg phase. Branchiopod eggs are able to be dispersed widely through the guts of birds (Eriksen and Belk 1999), carried along with the wind (Brendonck and Riddock 1999), attached to mud on feathers of birds or hooves of grazing animals (Eriksen and Belk 1999), and even on human boots and car tires (Pers. comm. C. Rogers 2008). Branchiopod eggs have also been shown to pass through the digestive track of herbivores unharmed (Zedler and Black 1992). This incredibly durable resting egg phase offers the opportunity for long distance transport through various means to other aquatic environments.

Differences in life history traits and dispersal ability may be reflected in responses to hydrologic connectivity and environmental conditions, and may result in pools with differing species richness. Given their high dispersal potential and life cycle plasticity, branchiopod distribution and abundance should not be affected by hydrologic connectivity, but by limiting factors within the pool itself. According to a study by Dubbs (1987) the class Insecta was the most abundant taxa in the two vernal pools in eastern Sacramento County (66% and 64%), whereas branchiopods (Anostaca and Cladocera) accounted for less (14% and 19%). If these ratios are the norm, distribution and abundance of the class Insecta also would not be significantly affected by hydrologic connectivity as most insect species are terrestrial or winged as adults. Not all species found in vernal pools are terrestrial as adults or rely on other species to disperse their eggs, yet they are still abundant in the pools because of the plasticity of their life cycle. These include the flatworms (Turbellaria), nematode worms (Nematoda), rotifers (Rotifera), copepods (Copepoda), seed shrimp (Ostracoda), aquatic snails (Gastropoda), and water mites (Hydrachenellae) (Williams 1987). These
species may be positively affected by hydrologic connectivity; therefore, local diversity should increase within connected pools.

This study tested whether connectivity had an effect on aquatic invertebrate communities in vernal pools in eastern Sacramento County. Further, I evaluated whether physical factors (hydrologic connectivity, water temperature, depth, and surface area) and chemical factors (dissolved oxygen content, pH, electric conductivity, and total dissolved solids) were associated with diversity patterns in vernal pools.
Chapter 2

METHODS

STUDY SITE DESCRIPTIONS

Four study site locations were chosen in eastern Sacramento County (Fig. 1). Each site is dense with vernal pools (data from the draft South Sacramento Habitat Conservation Plan [Sacramento County 2008]). Figure 2 shows the outlines of the study site locations. From the southern end of Gene Andal Park to the northern end of the Kitty Hawk pools is approximately eight kilometers. The study sites are described below.

1. Kitty Hawk pools near Mather Airfield are managed by the U.S. Air Force and located northwest of Excelsior Road/Mather Boulevard. Access to this area is highly restricted.

2. Mather Field Regional Park is managed by Sacramento County and located approximately northwest of the corner at Eagles Nest Road and Kiefer Boulevard. This area is a regional park open to the public.

3. Gene Andal Park is managed by Sacramento County and located near south of Florin Road in between Excelsior and Eagles Nest Roads. There is limited access. It is one of very few vernal pool complexes in the region that have a woodland component.

4. The Sylva property is managed by Sacramento Valley Conservancy and located north of Florin Road in between Excelsior and Eagles Nest Roads. This area is actively grazed by cattle.
Figure 1 – Regional Location of the Study Sites
Figure 2 – Location of the Study Sites in Eastern Sacramento County
The study design included a minimum of one block of 2 connected pools and 2 isolated pools selected from each study site for a total of 4 blocks (sites) and 16 pools. The pairs of isolated and connected pools were within close proximity to one another (maximum distance: 200 feet). Mather Regional Park, Gene Andal Park and the Sylva Property (study sites 2, 3, and 4) are located on the Laguna Formation, whereas Kitty Hawk (study site 1) is located on the Riverbank Formation (NRCS 2011). The Laguna Formation consists of a high terrace and the oldest alluvial substrate on the east side of the Central Valley (Smith and Verrill 1996). Most vernal pools in the Central Valley are found on this formation. The Riverbank Formation underlies the major extent of low terrace landform. This mid-Pleistocene alluvial terrace contains soils with claypans and duripans that support vernal pools (Smith and Verrill 1996).

HYDROLOGY

The draft South Sacramento Habitat Conservation Plan habitat layer (Sacramento County 2008) in Geographic Information System (GIS) was used to initially identify vernal pools at the study sites. Light Detection and Ranging (LIDAR) data was used in conjunction with ArcHydro model to determine water flow and subbasins at the study sites. LIDAR is a remote sensing technique that uses a laser mounted to an aircraft to measure vertical height of a land surface (elevation). The LIDAR data used for the study sites was flown in January 2007 by Merrick & Company. LIDAR accuracy meets or exceeds 0.21 meter vertical and +/- 0.37 meter horizontal at 95% confidence level. Contour accuracy meets or exceeds +/- 0.30 meter vertical and +/- 0.50 meter horizontal at 90% confidence level. A total of 22 surveyed ground control points were used in the production of this data (Merrick & Company 2008).
ArcHydro Tools (ESRI, version 2.0) is a geospatial and temporal data model for water resources that operate within ArcGIS (ESRI, version 10.0). ArcHydro has an associated set of tools that populate the attributes of the features in the data framework, interconnect features in different data layers, and support hydrologic analysis. Although originally ArcHydro was used to map and simulate hydrology at a watershed scale, because of the accuracy of LIDAR data, this tool can be used to determine hydrologic connectivity at a much smaller scale (i.e. a vernal pool complex). The ArcHydro model generated catchment areas and flow lines for the study sites. Using the maps produced from this data, isolated and connected vernal pools were identified based on sub-basins and flow lines generated by the program.

In addition, historical aerial imagery from May 30, 2002 (Google 2009) confirmed connectivity and isolation among pools. The total precipitation for May of 2002 (when the aerial imagery was taken) was 1.81 inches when the normal (determined between 1971 and 2000) is only 0.60 inch (Cline and Garcia 2007). Pools sampled were also evaluated by sight for surface connection throughout the 2010 sampling season. These additional methods to determine surface water connectivity were implemented to verify that the ArcHydro model was accurate.

The output from the ArcHydro model in combination with a visual site assessment and comparison of aerial imagery (i.e., for signature of wetland features) was used to identify the connected and isolated pools at each study site. Pools were chosen based on evident connectivity patterns that were confirmed during the course of the study and proximity to one another. Figures 3, 4, 5, and 6 show the connected and isolated pools at each study site, as well as the sample location, drainage patterns, and the basin boundary.
Figure 3 – Drainage Patterns, Habitat Types, and Sample Locations at Study Site #1 – Kitty Hawk. The ArcHydro Model generated the drainage patterns and catchment areas. The cover type data is from the Sacramento County Draft Habitat Conservation Plan (Sacramento County 2008).
Figure 4 – Drainage Patterns, Habitat Types, and Sample Locations at Study Site 2 – Mather Field. The ArcHydro Model generated the drainage patterns and catchment areas. The cover type data is from the Sacramento County Draft Habitat Conservation Plan (Sacramento County 2008).
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Figure 6 – Drainage Patterns, Habitat Types, and Sample Locations at Study Site #4 – Sylva Property. The ArcHydro Model generated the drainage patterns and catchment areas. The cover type data is from the Sacramento County Draft Habitat Conservation Plan (Sacramento County 2008).
At each sample location, I recorded the location using a handheld Trimble Geo XT Global Positioning System (GPS), sampled physicochemical factors, and sampled aquatic invertebrates. The pool boundary was determined by using the “Routine Determination Method” as described in the 1987 U.S. Army Corps of Engineers (USACE) Wetland Delineation Manual (USACE 1987). The 1987 Manual was used in conjunction with the Interim Regional Supplement to the USACE Wetland Delineation Manual: Arid West Region (USACE 2006). According to this manual, three positive wetland parameters must normally be present for an area to be wetland: 1) a dominance of wetland vegetation, 2) presence of hydric soils, and 3) presence of wetland hydrology. My agreements with the landowners to conduct research at the study sites prohibited the digging of holes on the properties to determine soil type and color, so the delineated boundary was determined by presence of wetland vegetation and hydrology alone. The boundary was recorded on a Trimble Geo XT GPS with sub-meter accuracy and mapped on an aerial photograph using ArcGIS (version 10.0).

**AQUATIC TAXA SAMPLING**

Pools were sampled four times over a nine-week period from February 18 to April 25, 2010, approximately three weeks apart (February 18-21, March 12-14, April 3-4 and April 25). Five pools were dry by the third sampling period and an additional 2 pools were dry by the fourth sampling period. The sites were checked on January 15 and May 15 to confirm absence of ponding water, prior to and after data collection, respectively. Surveys were conducted in accordance with the United States Fish and Wildlife Service *Guidelines for Vernal Pool Branchiopods* (USFWS 1996) and the conditions of my federal take permit (TE 219638-0). Pools were evaluated at each site visit and sampled if they contained greater than 2 inches of water. Sampling was conducted by using a standard 8-inch circular aquatic
dip-net with volumetric sweeps of 3.5 cubic feet of water (approximately three-foot long sweeps) at a predetermined location marked by flagging and recorded on a GPS unit, so that sampling occurred at the same location during each site visit. The netted contents were transferred and examined in a 12-inch by 10-inch white sorting pan. Macro-invertebrates were examined and identified using a 20X hand lens. No specimens were sacrificed for museum vouchering; however, for species that could not be identified in the field, a representative sample was preserved in ethanol and identified under a microscope. Each taxa sampled was identified according to the categories in the attached datasheet (Appendix A) and individuals estimated. Federally listed branchiopods identified during the surveys were reported to the California Department of Fish and Game’s California Natural Diversity Database and United States Fish and Wildlife Service. Only sexually mature branchiopods were used to make positive identifications regarding presence of federally listed species.

**Sampling Physicochemical Factors**

Physicochemical factors measured for each pool sampled included size of pool (square feet), depth of pool at the sample location (inches), water temperature (degrees Celsius), turbidity (JTU), chloride (ppm), dissolved oxygen content (ppm), electric conductivity (mS/cm), total dissolved solids (ppt), and pH. Although originally chloride and turbidity was measured at each pool, it became apparent that the LaMOTTE® Chloride and Turbidity Test Kits used were not precise at the scale needed to measure a difference between pools. As described previously, the sample pools were delineated in the field using a GPS unit. Using ArcGIS, the surface areas of the vernal pools were generated from the delineated boundary. Only the maximum surface area was used in the statistical analysis. The depth of the sample pools was measured at the sample location by measuring from the bottom of the sediment to the top of the water column with a standard measuring tape. A
Hanna (Model #HI 991301) electronic portable meter was used to measure electric conductivity, total dissolved solids, pH and water temperature. A LaMOTTE® Individual Water Test Kit was used to measure dissolved oxygen content. Methods for conducting the tests followed standard instructions provided by the manufacturers. Hydroperiod was measured as the total number of weeks the pools were ponded during the sampling periods, since pools were sampled every three weeks, hydroperiod equals 3, 6, or 9 (all pools were wet at week 1).

**Data Analysis**

Several measures of diversity were calculated for the data set. The simplest measures are species richness and density. Species richness is the number of species in each vernal pool (some were only identified to family, see Appendix A). The total species density is the number of species divided by the total number of individuals. These measures, however, do not take into account how evenly the total number of individuals in a sample is apportioned between each species. For this reason, the Shannon diversity index was calculated to determine local diversity (α-diversity) and the Jaccard similarity coefficient was calculated to compare the similarity of pairs of connected and isolated pools (β-diversity) (see formulas below, McKillup 2006). The Shannon (aka Shannon-Weiner or Shannon-Weaver) index of diversity takes into account both species richness and evenness. The index is increased either by having additional unique species, or by having greater species evenness. The larger the index value the more diverse the community. The result does not give an absolute description of a site’s diversity, but can be used to compare similar habitats. The Jaccard similarity coefficient is a statistic used for quantifying similarity of diversity among communities. In this case, the sample sets were pairs of connected pools.
and pairs of isolated pools at each study site. The Jaccard similarity index is defined as the size of the intersection divided by the size of the union of the sample sets.

\[
H' = -\sum_{i=1}^{S} (p_i \ln p_i)
\]

Shannon Diversity Index (S is the total number of species and \( p_i \) is the frequency of the \( i \)th species (the probability that any given individual belongs to the species).

\[
J_{\text{ac}} \left( \begin{array}{c}
\text{a} \\
\text{a + b + c}
\end{array} \right)
\]

Jaccard Index: \( a \) = the total number of species in common between site \( i \) and site \( j \), \( b \) = the total number of species in site \( i \) but not site \( j \), \( c \) = the total number of species in site \( j \) but not site \( i \).

I examined the relationships between the physicochemical factors and different measures of species diversity (Shannon diversity index, Jaccard similarity index, species richness, and species density) using the statistical analyses outlined in Table 1. All statistical analyses were conducted using IBM® SPSS® Statistics 20 program.

Species composition is known to change from one sampling period to another for individual pools (Rogers 1998). To evaluate the change in species diversity over time, a repeated measures general linear model for all sample periods was conducted using the Shannon diversity index with the covariate connectivity. In addition, a paired t-test was
conducted for sample periods #1, 2, and 3. The sample size was too small (n=1) to conduct an analysis on sample period #4 when several of the pools had dried up. A paired t-test using the Jaccard similarity coefficient was conducted to specifically evaluate the effect of connectivity on diversity between paired connected and isolated pools.

A Spearman bivariate correlation was conducted on diversity using Shannon diversity index and all the physicochemical factors to check for the degree of linear association between the dependent and independent variables.

Pools that hold water longer are expected to have species that use both active and passive dispersal methods and would have greater species richness over the entire sampling period. To test this, the cumulative taxa richness for each pool was compared with the observed number of weeks that pool contained water (the hydroperiod). Cumulative taxa richness over the whole study season was counted by examining taxa inventories from all sample periods.

To avoid pseudoreplication, the multiple regression tests were analyzed for sample period #2, when diversity was determined to be the highest (sample period #2). Depth of water at the sample location was also at its highest during sample period #2. This is consistent with a study whose taxa richness was highest during the mid-season sampling event, when pools had maximum ponding (Rogers 1996). For sample period #2, I performed backward multiple linear regressions for the measures of diversity including the Shannon diversity index and taxa richness to detect any relationship of similarity of communities with the physicochemical factors. The regression models should explain which factors contribute to the prediction of diversity measures with the least amount of error using the data provided. Factors that were not statistically significant (p>0.05) were removed using the
backward selection procedure in order of whose removal causes the smallest decrease in R-squared. Dummy variables were assigned to differentiate between isolated and connected pools (1 and 2, respectively).

Schnieder and Frost (1996) determined that life histories have a major influence on community structure. Separating taxa into lifestyle groups provides greater insight into how physiochemical factors affect diversity. I performed separate backward multiple linear regressions for the taxa richness and density for each group of taxa with similar lifestyles and the physicochemical factors. The lifestyle groups included amphibians, non-arthropods, crustaceans, and insects (Table 2). The four major groups of taxa present in vernal pools were sorted by classes with similar life history characteristics and desiccation adaptations. Since amphibians were only present in the two connected pools at Gene Andal Park (Study Site #3), they were removed from this data analysis as they did not meet the test for homogeneity of variance.
Table 1 – Statistical Analysis Summary

<table>
<thead>
<tr>
<th>Statistical Test</th>
<th>Dependent Variable</th>
<th>Independent Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeated Measures Analysis of Variance (ANOVA) General Linear Model with Greenhouse-Geiser Correction</td>
<td>Shannon Diversity Index for all Sample Periods</td>
<td>Connectivity</td>
</tr>
<tr>
<td>Paired t-test</td>
<td>Jaccard similarity Index for Sample Periods #1-3</td>
<td>Connectivity</td>
</tr>
<tr>
<td>Spearman Bivariate Correlation</td>
<td>Species Diversity (Jaccard Similarity Index and Shannon Diversity Index)</td>
<td>Physicochemical Factors</td>
</tr>
<tr>
<td>Backward Multiple Regression</td>
<td>Species Diversity (Shannon Diversity Index and Taxa Richness) for Sample Period #2</td>
<td>Physicochemical Factors</td>
</tr>
<tr>
<td>Backward Multiple Regression</td>
<td>Taxa Richness and Density for Each Lifestyle Group for Sample Period #2</td>
<td>Physicochemical Factors</td>
</tr>
</tbody>
</table>
Table 2 – Taxa Found within the Vernal Pools Divided into Life Style Groups

<table>
<thead>
<tr>
<th>Amphibians</th>
<th>Non-Arthropods</th>
<th>Crustaceans</th>
<th>Insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western toad Bullfrog</td>
<td>Flatworm, brown</td>
<td>Clam shrimp, California</td>
<td>Backswimmer</td>
</tr>
<tr>
<td></td>
<td>Flatworm, green</td>
<td>Clam shrimp, Lentil Copepods</td>
<td>Damselfly larva</td>
</tr>
<tr>
<td></td>
<td>Flatworm, white</td>
<td>*Vernal pool fairy shrimp</td>
<td>Dragonfly larva</td>
</tr>
<tr>
<td></td>
<td>Snail, Ram’s horn</td>
<td>California fairy shrimp</td>
<td>Predaceous diving beetles (Dyticidae), Adult and Larva</td>
</tr>
<tr>
<td></td>
<td>Snail, turban</td>
<td>*Vernal pool tadpole shrimp</td>
<td>Scavenger beetles (Hydrophilidae), Adult and Larva</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seed shrimp</td>
<td>Mayfly larva</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water flea</td>
<td>Midge larva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mosquito larva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Waterboatman</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stonefly larva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ricksecker's water scavenger beetle</td>
</tr>
</tbody>
</table>

*Source: Colburn 2004.*

*Note *Species listed under the Federal Endangered Species Act.*
Chapter 3
RESULTS

PRECIPITATION

The total amount of seasonal precipitation is the most important factor affecting ponding in a vernal pool (Bauder 2005). In 2010, the year the study was conducted, the amount of precipitation was normal when compared to the historic average ($r=0.848$, $p<0.001$, Fig. 7). The historic average is estimated from a 30-year period within a 48-kilometer radius of the study sites (The Weather Channel 2011). From January to March 2010, the total precipitation was 32.3 centimeters compared to the historic average of 31.2 inches.

Figure 8 shows the incremental precipitation pattern for the 2010 sampling season as well as arrows indicating the four sample periods. Pools were ponded after a large storm in January and early February. Sample period #2 was conducted after another major rainfall event when daily precipitation surpassed one inch. Precipitation after sample period #2 consisted of short rainfall events with less incremental precipitation.

PROPERTIES OF THE POOLS

The physicochemical factors and Shannon diversity index varied across pools over the four sample periods (Table 3). Electric conductivity was relatively low, averaging 0.06 mS/cm and ranging from 0.04 to 0.13 mS/cm. These measurements are typical for pure rain water, which is typically less than 0.15 mS/cm. The correlation analysis revealed that the Shannon diversity index was negatively correlated with electric conductivity ($r(52)= -0.299$, $p=0.031$) and total dissolved solids ($r(52)= -0.312$, $p=0.024$). pH steadily increased over the four sampling periods. The Shannon diversity index varied from 0.205 to 2.048 among all
samples, surprisingly the lowest and highest scores were from the same connected pool at different sample periods (pool 3-C-1, Gene Andal). The highest mean diversity of all pools sampled occurred at sampling period #2 (Fig. 9).

The samples included a total of 25 taxa including 2 amphibians, 5 non-arthropods, 8 crustaceans, and 11 insects. The major difference between the composition of connected pools and isolated pools was the presence of amphibian species in connected pools (Fig. 10 and Fig. 11). There was no significant difference between the major groups of taxa in connected and isolated pools [amphibian – assumption of homogeneity of variance not met; non-arthropods (F(2)=0.502, p=0.482); crustacean (F(2)=2.827, p=0.099; and insects (F(2)=0.696, p=0.408]. Thus, the proportion of each group of taxa was similar between isolated and connected pools over the four sample periods.

Diversity and Hydrologic Connectivity (Surface)

The Jaccard similarity coefficients were not different in connected and isolated pools over time (t(3)=1.667, P=0.194 for sample period 1 and t(3)=1.071, P=0.363 for sample period 2). Similarly, the mean Shannon diversity index did not differ significantly between connected and isolated pools (F(3)=0.573, p=0.560) (Fig. 12). Five pools dried up by sample period #3 including all the Kitty Hawk pools (study site 1) and one of the isolated pools at the Sylva property (Pool 4-I-4). Two additional pools dried by sample period #4 (Pool 3-I-3 and 4-I-3). Some of the isolated pools dried up first resulting in a lower sample size of isolated pools by sample period #4 (n=3) compared to connected pools (n=6). Even though there was no difference in diversity between connected and isolated pools, diversity was significantly different over time (F(3)=4.031, p=0.048).
Figure 7 – The Historic Average Precipitation Estimated from a 30-year Period within a 48-kilometer Radius of the Study Sites Compared With the Actual Precipitation during the Study Period from January to July 2010 (Department of Water Resources California Data Exchange Center 2011;The Weather Channel 2011).
Figure 8 – Pattern of Incremental Precipitation from January to May 2010 Compared to the Dates of Invertebrate Sampling (Department of Water Resources California Data Exchange Center 2011). Note: Arrows indicate the sampling periods for the study. Stars indicate when pools were checked for ponding.
Table 3 – Descriptive Statistics of the Physical and Chemical Properties Measured on the Vernal Pools Studied Averaged Over the Four Sampling Periods

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Pool #</th>
<th>Connected or Isolated</th>
<th>Area (square meter)</th>
<th>Average Depth (cm)</th>
<th>Average Water Temperature (°C)</th>
<th>Average DO (ppm)</th>
<th>Average EC (mS/cm)</th>
<th>Average TDS (ppt)</th>
<th>Average pH</th>
<th>Average Shannon Index</th>
<th>Hydroperiod (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitty Hawk</td>
<td>1-C-1</td>
<td>Connected</td>
<td>175</td>
<td>14.6</td>
<td>12.6</td>
<td>0.05</td>
<td>0.06</td>
<td>0.03</td>
<td>6.67</td>
<td>1.59</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1-C-2</td>
<td>Connected</td>
<td>208</td>
<td>16.8</td>
<td>10.8</td>
<td>6.40</td>
<td>0.04</td>
<td>0.02</td>
<td>6.22</td>
<td>1.78</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1-I-3</td>
<td>Isolated</td>
<td>133</td>
<td>15.2</td>
<td>11.9</td>
<td>6.00</td>
<td>0.07</td>
<td>0.03</td>
<td>6.54</td>
<td>1.64</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1-I-4</td>
<td>Isolated</td>
<td>144</td>
<td>9.5</td>
<td>12.3</td>
<td>3.80</td>
<td>0.13</td>
<td>0.06</td>
<td>7.05</td>
<td>1.22</td>
<td>3</td>
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<tr>
<td></td>
<td>2-C-1</td>
<td>Connected</td>
<td>281</td>
<td>15.9</td>
<td>14.0</td>
<td>5.63</td>
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<td>0.04</td>
<td>6.57</td>
<td>1.38</td>
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<td>215</td>
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<td>14.8</td>
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<td>0.07</td>
<td>0.03</td>
<td>6.71</td>
<td>1.53</td>
<td>9</td>
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<tr>
<td></td>
<td>2-I-3</td>
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<td>453</td>
<td>17.6</td>
<td>13.6</td>
<td>5.00</td>
<td>0.05</td>
<td>0.03</td>
<td>6.74</td>
<td>1.45</td>
<td>9</td>
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<tr>
<td></td>
<td>2-I-4</td>
<td>Isolated</td>
<td>642</td>
<td>25.1</td>
<td>13.5</td>
<td>4.88</td>
<td>0.05</td>
<td>0.02</td>
<td>6.87</td>
<td>1.23</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3-C-1</td>
<td>Connected</td>
<td>218</td>
<td>25.1</td>
<td>16.5</td>
<td>5.20</td>
<td>0.07</td>
<td>0.03</td>
<td>6.86</td>
<td>1.22</td>
<td>9</td>
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<tr>
<td></td>
<td>3-C-2</td>
<td>Connected</td>
<td>126</td>
<td>19.4</td>
<td>17.4</td>
<td>6.70</td>
<td>0.07</td>
<td>0.03</td>
<td>6.52</td>
<td>1.22</td>
<td>9</td>
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<tr>
<td></td>
<td>3-I-3</td>
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<td>18.6</td>
<td>13.4</td>
<td>6.13</td>
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<td>0.02</td>
<td>7.00</td>
<td>1.65</td>
<td>6</td>
</tr>
<tr>
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<td>3-I-4</td>
<td>Isolated</td>
<td>621</td>
<td>16.8</td>
<td>16.1</td>
<td>4.80</td>
<td>0.05</td>
<td>0.02</td>
<td>6.81</td>
<td>1.51</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4-C-1</td>
<td>Connected</td>
<td>232</td>
<td>22.7</td>
<td>14.5</td>
<td>5.23</td>
<td>0.06</td>
<td>0.03</td>
<td>7.01</td>
<td>1.11</td>
<td>9</td>
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<tr>
<td></td>
<td>4-C-2</td>
<td>Connected</td>
<td>829</td>
<td>17.5</td>
<td>15.1</td>
<td>4.50</td>
<td>0.07</td>
<td>0.03</td>
<td>6.93</td>
<td>1.09</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>4-I-3</td>
<td>Isolated</td>
<td>348</td>
<td>16.9</td>
<td>9.0</td>
<td>4.20</td>
<td>0.05</td>
<td>0.02</td>
<td>6.91</td>
<td>1.36</td>
<td>6</td>
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<tr>
<td></td>
<td>4-I-4</td>
<td>Isolated</td>
<td>239</td>
<td>19.4</td>
<td>9.0</td>
<td>5.90</td>
<td>0.07</td>
<td>0.04</td>
<td>6.42</td>
<td>1.20</td>
<td>3</td>
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<tr>
<td>Total Average</td>
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<td></td>
<td>330.5</td>
<td>18.88</td>
<td>13.9</td>
<td>5.11</td>
<td>0.06</td>
<td>0.03</td>
<td>6.76</td>
<td>1.36</td>
<td>6.6</td>
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<tr>
<td>Standard Deviation</td>
<td></td>
<td></td>
<td>209</td>
<td>6.29</td>
<td>5.5</td>
<td>1.76</td>
<td>0.02</td>
<td>0.01</td>
<td>0.50</td>
<td>0.53</td>
<td>2.7</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td></td>
<td>126</td>
<td>6.35</td>
<td>6.1</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>5.78</td>
<td>0.21</td>
<td>3</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td></td>
<td>829</td>
<td>31.75</td>
<td>28.5</td>
<td>10.0</td>
<td>0.13</td>
<td>0.06</td>
<td>8.18</td>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td>Sample Size (N)</td>
<td></td>
<td></td>
<td>16</td>
<td>51*</td>
<td>52</td>
<td>47*</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Note: DO=Dissolved Oxygen; EC = Electric Conductivity; TDS = total dissolved solids.

Averages were calculated over the four sample periods for each physical and chemical factor measured, except for surface area and hydroperiod.

Note: There was one missing record for a sample pool’s depth. There are less measurements for dissolved oxygen because the tests were done incorrectly early in the study.
Figure 9 – Pattern of Average Diversity Measured by the Shannon Diversity Index over the Four Sampling Periods. The box plot for each sample period shows the minimum, lower quartile, median, upper quartile, and maximum.
Figure 10 – The Proportion of Each Group of Taxa Collected in Isolated Pools

- Crustaceans: 46%
- Insects: 30%
- Non-Arthropods: 24%
Figure 11 – The Proportion of Each Group of Taxa Collected in Connected Pools

- **Amphibians**: 1%
- **Insects**: 35%
- **Crustaceans**: 42%
- **Non-Arthropods**: 22%
Figure 12 - Comparison of the Mean Shannon Diversity Index of Connected and Isolated Vernal Pools over the Four Sampling Periods
**Hydroperiod**

Cumulative taxa richness increased as the hydroperiod increased, although after 6 to 8 weeks it begins to decrease (Fig. 13); however, this pattern has not been substantiated and additional analysis is required. The Shannon diversity index was negatively correlated with hydroperiod ($r=-0.286$, $p=0.020$). In addition, the cumulative number of taxa within a pool was significantly related with hydroperiod (multiple regression: $F(15)=10.067$, $p=0.007$). Hydroperiod accounted for only 42% of the variance and therefore other factors probably contribute the pattern of diversity in the pools.

**Diversity Models**

Different physicochemical variables contributed to different measures of diversity. Total dissolved solids and pH were negatively related to the Shannon diversity index ($F(14)=5.394$, $p=0.021$), explaining 47% of the variance. Dissolved oxygen was positively related to total number of species within a pool (taxa richness) ($F(14)=4.708$, $p=0.049$) explaining only 27% of the variance, although connectivity was marginally significant (removed from model last) (Table 4).

**Species Richness and Density by Lifestyle Group**

Diversity of non-arthropods, crustaceans and insects was related to several different physicochemical factors. Area was the only factor related to all three groups (Table 5). Crustacean density was significantly related to hydroperiod, water temperature, depth, connectivity, and surface area ($F(14)=4.909$, $p=0.007$), which explained 80% of the variance (Table 6). For $p<0.01$, non-arthropod and insect densities were not significantly related to any physicochemical factors ($F(14)=3.184$, $p=0.063$ and $F(14)=6.396$, $p=0.025$,
respectively); however, for p<0.05, insect density was related to dissolved oxygen (p=0.025), which explained 33% of the variance.
Figure 13 – The Cumulative Species Richness Compared to the Length of Time the Pool was Inundated (Hydroperiod)
Table 4 – Backward Multiple Regression Test Results for The Physicochemical Variables that Affect Different Measures of Overall Diversity for Sample Period #2

<table>
<thead>
<tr>
<th>Model</th>
<th>Model R²</th>
<th>p</th>
<th>Predictor Variable</th>
<th>Slope</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Taxa Richness</td>
<td>0.27</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dissolved Oxygen</td>
<td>0.124</td>
<td>2.170</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH</td>
<td>-0.166</td>
<td>-1.682</td>
<td>0.118</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Total Dissolved Solids</td>
<td>-22.273</td>
<td>-3.045</td>
<td>0.010</td>
</tr>
<tr>
<td>Shannon Diversity Index</td>
<td>0.47</td>
<td>0.021</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Multiple regression models were chosen when p<0.05. Other variables tested but removed include surface area (log transformed), electric conductivity, depth, and hydroperiod.
Table 5 - Backward Multiple Regression Test Results for The Physicochemical Variables that Affect Taxa Richness by Lifestyle Group for Sample Period #2

<table>
<thead>
<tr>
<th>Lifestyle Group</th>
<th>Model $R^2$</th>
<th>p</th>
<th>Predictor Variable</th>
<th>Slope</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Arthropods</td>
<td>0.90</td>
<td>0.006</td>
<td>pH</td>
<td>-0.925</td>
<td>-3.882</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depth</td>
<td>-0.233</td>
<td>-3.372</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surface Area*</td>
<td>-1.470</td>
<td>-3.297</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Connectivity</td>
<td>-0.787</td>
<td>-3.331</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hydroperiod</td>
<td>0.144</td>
<td>2.719</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dissolved Oxygen</td>
<td>-0.101</td>
<td>-2.209</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Electric Conductivity</td>
<td>11.240</td>
<td>0.914</td>
<td>0.391</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>0.65</td>
<td>0.007</td>
<td>Water Temperature</td>
<td>-0.156</td>
<td>-3.894</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Dissolved Solids</td>
<td>75.198</td>
<td>-2.190</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surface Area*</td>
<td>1.371</td>
<td>2.096</td>
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</tr>
<tr>
<td>Insects</td>
<td>0.72</td>
<td>0.007</td>
<td>Electric Conductivity</td>
<td>77.298</td>
<td>4.109</td>
<td>0.002</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Water Temperature</td>
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<td>-2.546</td>
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<td></td>
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<td>Depth</td>
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<td></td>
<td></td>
<td>Surface Area*</td>
<td>-0.821</td>
<td>-1.159</td>
<td>0.274</td>
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Note: Multiple regression models were chosen when p<0.01. *Surface area was log transformed.
Table 6- Backward Multiple Regression Test Results for The Physicochemical Variables that Affect Density by Lifestyle Group for Sample Period #2

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Insects</td>
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Note: Multiple regression models were chosen when p<0.01. Other variables tested but removed include dissolved oxygen, electric conductivity, pH, and total dissolved solids. *Surface area was log transformed.
Chapter 4

DISCUSSION

CONNECTED VERSUS ISOLATED POOLS

Just as movement corridors increase dispersal, hydrologically connected pools may increase the dispersal potential of aquatic invertebrates between the pools (Cottenie et al. 2003). Conversely, life history characteristics may have more influence on community structure (Schnieder and Frost 1996) than the benefits of connectivity. There was no significant difference in the diversity of connected pools and isolated pools over the four sample periods. The special adaptations of the organisms in this fleeting environment allow for broad dispersal despite a surface water connection. They can disperse in life stages other than their aquatic stage (i.e., flying or crawling adults, transportation of dormant eggs by birds, wind, hooves, etc.). These communities do not seem to be dispersal limited and therefore conform to species sorting (with environmental gradients) and mass effects (from effective dispersal and presence of resting egg bank) perspectives of metacommunities (Holyoak et al. 2005), which requires environmental heterogeneity in the system studied. Since connectivity does not seem to play a role in overall diversity of the system then it must be the environmental heterogeneity that is structuring these communities.

The study design may have influenced the negative relationship of connectivity and species diversity. The connected and isolated pools at each study site were located in close proximity to one another (maximum distance was approximately 60 meters). Carl and Blumenshine (2005) found invertebrate assemblages among close pools were more similar because they were more likely to be transferred between nearby pools than between distant...
pools. The proximity of the pools studied may have had a greater influence on species composition than the surface water connection.

**HYDROPERIOD, DEPTH AND SURFACE AREA**

The positive relationship between hydroperiod and diversity is consistent with the results of other studies that show aquatic habitat with longer hydroperiods have disproportionately greater number of species (Ripley and Simovich 2009; Della Bella et al. 2005; Schneider and Frost 1996; Eitam et al. 2004; Batzer 2004); however, biotic interactions (i.e., competition and predation) can limit species diversity (Schneider and Frost 1996; Urban 2004). Pools that stay ponded longer allow for colonization by herbivorous and predaceous insects (Moorhead et al. 1998) and completion of life cycles.

In other studies, depth and surface area were positively correlated with species diversity in vernal pools (King et al. 1996; Simovich 1998; Eitam et al. 2004; Ripley and Simovich 2009). Even though depth was not significantly related to overall taxa richness or the Shannon diversity index in the present study (Table 5), depth was significantly related to taxa richness of non-arthropods, crustaceans, and insects (Table 6). Depth and surface area may be an indicator of pond permanence, with the assumption that larger and deeper pools stay ponded longer (see Platenkamp 1998). The relationship of non-arthropod richness and surface area was likely the result of larger surface areas supporting greater number of resting eggs (Colburn 2004; Gallagher 1993). Larger pools are more easily found by active flying dispersers (Schneider and Frost 1996; Wilcox 2001), which may explain the relationship between insect richness and surface area. Surface area was also significantly related to crustacean richness and density, which is consistent with the findings of Gallagher (1996) and Platenkamp (1998) who evaluated surface area and Branchiopoda (fairy shrimp)
richness. Hydro-regime is more important for local richness of passive dispersers like crustaceans than active dispersers that can migrate when pools dry up (Vanschoenwinkel et al. 2009). The crustacean density was related to several physical factors such as hydroperiod, depth, connectivity and surface area suggesting that the limiting factors for density of crustaceans is presence of water instead of water chemistry, whereas crustacean richness was greatly influenced by water quality evidenced by the negative relationship with total dissolved solids. So although the physical factors, such as hydro-regime, limit crustacean density and to a lesser extent richness of individual lifestyle groups, it is the water chemistry that plays a significant role in predicting taxa diversity.

**DIVERSITY MODELS**

*Shannon Diversity Index*

Although many recent papers focus on the relationship between physical factors and species diversity in vernal pools (Gamble and Mitsch 2009, Ripley and Simovich 2009), the strongest predictors seem to be chemical factors. The Shannon diversity index was negatively related to total dissolved solids and pH. As pools gradually desiccate, electric conductivity, pH, and total dissolved solids increase (Colburn 2004; Rogers 2011). On one hand, pH fluctuates widely in response to rainfall (Colburn 2004) and daily as vegetation photosynthesizes during the day and respires at night. For these reasons, pH may not be a good indicator for monitoring pools because a lower pH may be a result of daily fluctuations or a pool’s gradual desiccation. The negative correlation with electric conductivity and total dissolved solids and higher significance of total dissolved solids in the regression model indicate that this variable carries greater weight than pH in determining diversity. The measure of total dissolved solids is an indicator of water quality, as is electric conductivity. Since electric conductivity is a measure of the capacity of water to conduct electrical
current, it is directly related to the concentration of salts dissolved in water, and therefore to the total dissolved solids (USGS 2005). This study indicated that as water quality decreases so does overall diversity. Similarly, Ransom and Dorris (1972) found a high, inverse correlation between diversity and conductivity in a reservoir in Oklahoma. The thresholds at which certain species cannot tolerate higher levels of total dissolved solids would be useful for establishing monitoring objectives in conservation management.

**Species Richness**

Species richness can be partially explained (27% of variance) with a positive relationship to dissolved oxygen. Although the relationship of dissolved oxygen and diversity has been studied in rivers (Connolly et al. 2004; Jacobsen 2008; Breitburg et al. 1997) and reservoirs (Sharma and Rawat 2008), it has not been studied in depth in temporary pools. In one study on Northern California vernal pools, dissolved oxygen was always above 5 mg/ml (5,000 ppm) and therefore not biologically limiting (King et al. 1996). The pools in my study never reached such high levels of dissolved oxygen (<29 ppm).

Many variables can affect the amount of oxygen in the water including water temperature, depth, flora and fauna, wind, etc. The temperature of water influences the amount of dissolved oxygen present; less oxygen dissolves in warm water than cold water. Oxygen can also vary throughout the day with photosynthesis and respiration (Williams 1987; Scholnick 1994). Most vernal pool invertebrate species are gill-breathers and may suffocate in pools with low dissolved oxygen (Rogers 1996). Reduced levels of available oxygen may limit the species that can occur. In this study, pools with higher levels of
dissolved oxygen supported greater number of species, which is consistent with the findings of Sharma and Rawat (2008).

The R-squared values for the overall diversity analyses are low (27%, 47%) and therefore the measured variables are not adequately describing all the factors that affect diversity. Separating the taxa into lifestyle groups provides greater insight into how the variables are affecting diversity.

**Diversity Models for Lifestyle Groups**

The regression model for non-arthropod richness (flatworms and snails) showed a positive relationship with electric conductivity and hydroporid. This pattern indicates that more non-arthropods are found in pools that pond for a longer duration and have poorer water quality (high electric conductivity). Flatworms, a voracious predator (Colburn 2004), were more common in the pools than snails. Flatworms are opportunistic species exploiting the abundance of crustaceans (prey) and poor water quality. The physicochemical factors did not explain density of non-arthropods which may be controlled by other factors such as competition or predation.

Crustaceans were more abundant in the pools than another lifestyle group (greater than 40%, see Fig. 10 and Fig. 11). Similar to the regression model for the Shannon diversity index, crustacean richness was negatively related to total dissolved solids. When water quality is higher, there are more crustaceans and therefore greater overall diversity. In contrast, insects and non-arthropods show an affinity towards poorer water quality (a positive relationship with electric conductivity). Generally, as water quality decreases (shown by increases in total dissolved solids and electric conductivity), crustacean richness decreases. Since crustaceans account for over 40% of the composition of the pools, the
Shannon diversity index also decreases (Fig. 14). Non-arthropod richness increases as water quality worsens as they are opportunistic species. Similarly, insect richness also increases as water quality worsens probably as a result of late colonization by flying adults or as other opportunistic species (i.e., mosquitoes) develop.
Figure 14 – Effects of Water Quality on Diversity and Taxa Richness
Generally, rates of biological processes increase as temperature increases (Brown et al. 2004). So although water temperature is a physical parameter, it greatly affects the water chemistry of the pool. The amount of dissolved oxygen in the water decreases as temperature increases. Higher water temperatures in the pools would encourage rapid growth of algae, which may supplement food supply for herbivores, but it also depletes the dissolved oxygen content (Williams 1987). Water temperature was significantly related to insect richness, as well as crustacean richness and density. These significant relationships may be a result of the effect of water temperature on other chemical parameters. Water temperature was positively correlated to pH, which may indicate as temperature increases, pools dry up, and pH increases (Colburn 2004; Rogers 2011). Thus, water temperature is related to a multitude of factors and may not be useful as an indicator for habitat health or functionality.

Insect richness was negatively related to surface area and positively related to electric conductivity and depth. Some insects (damselflies, predaceous diving beetles, backswimmers and waterboatman) will colonize pools after they have been filled. In addition, flying adults will more easily find larger pools over smaller pools (Wilcox 2001). Another reason for the observed pattern is that larvae of insects also take longer to mature and therefore deeper pools may be preferred (Colburn 2004). Similar to total taxa richness, dissolved oxygen was marginally related to insect density, although the density of insects may be controlled by other factors such as competition or predation. However, what was most significant was electric conductivity (i.e., water quality).
Chapter 5

CONCLUSIONS

The vernal pool aquatic communities are verifiably linked within the landscape. Even though surface water connectivity was not a driving force for the majority of taxa within the pools, the pools are linked in the ability of these species to disperse whether actively or passively in this temporary aquatic environment. Although richness of non-arthropods was related to connectivity, many other physicochemical factors contributed to this diversity model. There is no difference in diversity between connected and isolated pools. As such, future designs of vernal pool restoration and creation efforts do not need to evaluate the connectivity patterns in vernal pools to maximize species diversity.

Overall taxa diversity of the pools measured by the Shannon diversity index was negatively related to total dissolved solids, a measure of water quality. For this reason, maintaining the health of these ecosystems by avoiding water quality impacts associated with development and stormwater runoff is imperative to maintain the diversity and resiliency of these sensitive habitats. The thresholds at which certain species cannot tolerate higher levels of total dissolved solids would be useful for establishing monitoring objectives in conservation management.

As expected multiple factors interact and contribute to other measures of diversity. Because of high rates of dispersal, diversity of crustaceans and insects are affected less by environmental constraints than the diversity of non-arthropods. Densities of non-arthropods and insects were not significantly related to the physicochemical factors measured suggesting that these taxa may be represented evenly throughout the metacommunity.
Crustaceans were the most abundant group. Their density was only related to physical factors, and crustacean richness was related to the physical factors including water quality.

If taxa richness is generally measured to determine the overall health of an aquatic habitat (Plafkin et al. 1989), then determining the factors contributing to taxa richness would aid the development of proper management objectives for vernal pool ecosystems. The entire health of the ecosystem must be considered to propagate the recovery of federally listed vernal pool invertebrate species and prevent the decline of other vernal pool obligate species. The sensitive nature of the pools and the species that inhabit them magnifies the importance of understanding how this habitat functions so that they may be managed better for conservation of rare species.
APPENDIX A

Blank Data Form
### Invertebrate Monitoring Data Collection

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#### General Pool Conditions

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#### Pool Number

<table>
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<tr>
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<td>Hydrophilidae adult</td>
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<td>midge larva</td>
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<td>mosquito larva</td>
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<tr>
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<tr>
<td><strong>Fish</strong></td>
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<tr>
<td>Gambusia</td>
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</tr>
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#### Additional Notes

- *clam shrimp, California*
- clam shrimp, lentil
- copepods
- *fairy shrimp (B lynchii)*
- *fairy shrimp (B mesoalennis)*
- *fairy shrimp (Linderike)*
- seed shrimp
- *tadpole shrimp (Lepidurus)*
- water flea
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