

*DARLINGTONIA CALIFORNICA* PHYTOTELMA ORGANISMS:  
SPATIAL RELATIONS WITHIN THE METACOMMUNITY

By

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## ABSTRACT

### DARLINGTONIA CALIFORNICA PHYTOTELMA ORGANISMS: SPATIAL RELATIONS WITHIN THE METACOMMUNITY

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The California pitcher plant, *Darlingtonia californica*, provides a unique setting to research community ecology because it harbors small ponds of water, or phytotelmata, in its modified leaves. Each phytotelma hosts a unique community of various invertebrates who live among carcasses of drowned plant prey. These pitchers and their inquiline communities are relatively small yet abundant in the wild, resulting in an ideal natural setup for community composition comparisons. There have been no previous studies that research *D. californica* inquiline communities across multiple geographic regions concurrently. This study sought to survey phytotelma communities throughout the range of *D. californica* at three spatial scales- regions (the largest geographic scale), fens (a more localized scale), and pitchers (the smallest scale). Community composition metrics were calculated to compare richness, evenness, diversity, and abundance among the four regions. Seven taxa and 14,358 individuals were recorded in the 93 pitchers sampled in National Forests throughout southern Oregon and northern California. Non-metric multidimensional scaling ordination showed slight groupings of samples within regions. Community composition of pitchers was more similar within regions than between regions and within fens than between fens. Statistical analyses showed that region predicted variance between pitchers, and that all of

the paired combinations of regions were significantly different from one another except for the two highest elevation regions. Further tests showed some significant differences in individual species' abundance between regions, but not in univariate community composition metrics. The *D. californica* phytotelma communities were then compared to those of other pitcher plant species.

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## TABLE OF CONTENTS

ABSTRACT .....	ii
ACKNOWLEDGEMENTS.....	iv
METHODOLOGY .....	5
Sampling Design & Selection Methods.....	5
Community Assessment .....	7
Data Analysis.....	8
RESULTS AND DISCUSSION.....	11
The Inquiline Species .....	11
Community Analysis .....	15
Interpretation And Comparison .....	18
Future.....	24
LITERATURE CITED.....	27
APPENDIX .....	44
Region and Fen Descriptions.....	44

## LIST OF TABLES

Table 1. Species abundances organized by region. The last row is the proportion of total abundance found at each region. The last column represents the species' abundance out of the total abundance. ....	32
Table 2. Values organized by the four regions and each of their three fens. (Top and bottom tables have the same variables.).....	33
Table 3. <i>P</i> -values of pairwise comparisons. Each column represents the pair of regions from the first and second rows. Dashes present when both regions had a value of zero. ....	34
Table 4. Mean number of inquilines per pitcher ( $\pm$ standard error). Df=3. Chi squared and <i>p</i> -values from Kruskal-Wallis tests are listed. ....	35
Table 5. Correlation coefficients for covariates in NMDS ordination space and the two NMDS axes (see Figure 1). <i>P</i> -values are based on 999 permutations.....	36

## LIST OF FIGURES

- Figure 1. Map of study area in Northwestern California and Southwestern Oregon. Red stars represent the four regions, in order from Northwest to Southeast: Rogue River-Siskiyou National forest near Gold Beach, OR; Six Rivers National Forest near Gasquet, ..... 37
- Figure 2. A typical *Darlingtonia californica* habitat growing in and around a small stream. 38
- Figure 3. Mean heights of pitchers and depth of pitcher fluid shown proportionate to one another with standard error bars. Pitcher height correlated to elevation, which is shown in grey behind bars..... 39
- Figure 4. Total abundances of all taxa combined grouped by region and stratified by fen within regions. Although abundances were significantly different from one another, the differences did not correlate to elevation. (Sample sizes (n= number of pitchers sampled) were Siskiyou n= 23; Six Rivers n= 15; Shasta-Trinity n= 25; Plumas n= 26). ..... 40
- Figure 5. Abundances of each taxon grouped by fens, with fens color coded by region. From left to right: *S. darlingtoniae*, nematode, *M. edwardsi*, *M. orestes*, mite, *Corynoptera* sp., spider..... 41
- Figure 6. Nonmetric multidimensional scaling (NMDS) ordination of community composition data from 93 pitchers. Vector information presented in Table 1. Vector arrow length and direction correspond to the magnitude of the correlation with the two axes. .... 42
- Figure 7. A dendrogram of Bray-Curtis mean dissimilarity by region. (OR= Siskiyou; GA= Six Rivers; PL= Plumas; ST= Shasta-Trinity.) Each region's segment terminated to the within-region dissimilarity value represented on the y-axis. The closer any two are on the dendrogram, the less dissimilar they are between regions. For example, Siskiyou (OR) had more within-region dissimilarity than the group containing the other three regions. Plumas' (PL) segment is reversed because it had more dissimilarity within the region than the combination of Plumas and Shasta-Trinity (ST). ..... 43

## INTRODUCTION

The charismatic California pitcher-plant, *Darlingtonia californica* Torrey (Sarraceniaceae), is the sole carnivorous pitcher plant found west of the Rocky Mountains whose range extends from northern California to western Oregon. It is distinctly different from other carnivorous pitcher plants with its fully hooded pitcher covering, and its lack of producing digestive enzymes (Ellison & Farnsworth 2004). It is also taxonomically distinct as a monotypic species within genus *Darlingtonia* (Baldwin et al. 2012). Like all pitcher plants, the highly modified leaves function as a phytotelma, or “plant - pond” where a host of aquatic invertebrates live within the fluid of the pitcher (Fish 1985). These inquiline communities are interconnected through the trophic interactions of their food web (Kitching 2001; Beaver 1985) and share a mutualistic relationship with the host pitcher by providing the plant with nutrients through decomposition in exchange for a well-protected and resource-rich home (Adlassnig et al. 2010). The relatively undisturbed inquiline organisms inside each pitcher represents a discrete community, and because of this configuration, pitcher plants are favorable model systems for studying spatial patterns of community composition (Buckley et al. 2010). Numerous studies have examined the communities of pitcher plants but most have focused on *Sarracenia purpurea*, found in the eastern United States (Ellison et al. 2012). Of those studies conducted using *D. californica* phytotelma, none have attempted to quantify the community across the entirety of its range.



When quantifying community composition, it is important to consider the spatial scale at which communities are assembled and which abiotic and biotic factors may be influencing the dispersion of species within a scale (Buckley et. al. 2010). There are three spatial scales at which *D. californica* inquiline communities can be classified: pitchers within a fen, fens within a region, and larger scales encompassing entire geographic regions. For the purposes of this study, I characterized the local-habitat community as a fen where a population of pitchers is found and the metacommunity as the combination of all pitchers in all fens across four distinct regions. Here the metacommunity encompasses all of the biotic factors that may influence species composition among fens. By comparing communities at different spatial scales we can determine if community composition is similar within fens and regions, and then elucidate factors influencing community composition within each scale independently (Franklin and Mills 2009). If the species in a community have adapted to or originated in the same particular environment, then one would expect to find high similarity between disconnected communities. On the other hand, if small scale or localized natural selective pressures differ geographically, one might expect to find greater variability among community compositions (Lomolino et.al. 2010; Beaver 1985).

In general, community structure may be explained by “bottom-up” or “top-down” forces, each of which can be empirically examined in the field (Kitching 2001). Rather than referring strictly to trophic structure, in these microcosms “top-down” explanations include larger scale constraints like environmental conditions, natural history, and biogeographic features (such as elevation) of the location in which they occur. “Bottom-

up” explanations include intra- and interspecific interactions, and pitcher-specific resource (such as pitcher and water height) and chemical qualities (Kitching 2001). It is known that there is some degree of community structure heterogeneity (or, patchiness) between phytotelmata (Sunahara & Mogi 2004), but it is unclear if this patchiness is also characteristic of community structure at other spatial scales, as in, for example, between nearby fens or between geographical regions within the range of *D. californica*.

Most of the previous research has not focused on the co-evolved symbiotic relationship between *D. californica* and its inquiline aquatic invertebrates, but it has been argued that their affiliation is an intimate obligate mutualism (Ellison et al. 2012). *Darlingtonia californica* does not produce its own digestive enzymes; instead, it relies on the community of organisms within the phytotelma to break down and mineralize prey while providing a habitat and food for this community of insects. Without this mineralization process, the plant would not have the means to absorb the necessary chemical components for life (Adlassnig et. al. 2010). This dependence on an aquatic community is particularly interesting because *D. californica* is the most insect-dependent carnivorous plant with 76% of its nitrogen derived from insects (Schultze et. al. 1997). This strong dependence of mutualistic activity suggests that the host plant has coevolved via reciprocal selection to some degree with the inquiline communities presented in this study.

The community composition of inquiline organisms in *D. californica* has been described at least twice in the past four decades, yet attention of the species-specific interactions between inquiline organisms and *D. californica* are poorly understood

(Aditya et.al. 2008). In 1990, Nielsen performed a study on the arthropods associated with *D. californica* which resulted in seven aquatic species and very little similarity of inquiline community compositions among 124 pitchers in three fens in Gasquet, CA (Nielsen 1988). Another study found only two species of aquatic arthropods in 119 *D. californica* pitchers in Butterfly Valley, Plumas county, CA (Naeem 1988). This inconsistency of community composition between and within regions could have been due to sampling differences, or it could have been a product of real biogeographical differences between communities.

To examine the spatial patterns of inquiline community structure, I quantified the invertebrate biodiversity within *D. californica* pitchers, along with selected elements of abiotic conditions, across three levels of space – 1) the pitcher, 2) the fen, and 3) the region – and compared the within and between levels of similarities. My objectives were to first quantify the spatial patterns of community structure among phytotelma-associated species between fens and regions, and second, determine if select abiotic and biotic factors explain community structure.

## METHODOLOGY

### Sampling Design & Selection Methods

The diversity and abundance of phytotelma species were studied on three nested spatial scales. To characterize *D. californica* communities at the largest possible scale, I sampled four regions across the range of *D. californica*: Gold Beach in the Rogue River-Siskiyou National Forest, OR; Gasquet in the Six Rivers National Forest, CA; Mount Eddy in Shasta-Trinity National Forest, CA; and Quincy in Plumas National Forest, CA (Fig. 1). (The regions will be referred to as Rogue River-Siskiyou, Shasta-Trinity, Six Rivers, and Plumas respectively, see appendix.) These four regions were chosen because they stretch across the majority of the range of *D. californica*. To see if there is variation on a more localized geographic scale, I collected pitchers from three different fens per region, totaling twelve fens. Fen elevations (m) were measured. To account for pitcher habitat variation I collected ten pitchers at each of the twelve fens, for a total of 120 pitchers. Pitcher variables included pitcher height (cm) and pitcher fluid depth (cm).

Permits for collection were required from each National Forest since *D. californica* is a protected plant, and sample size was limited by each jurisdiction's collection protocol. At each fen, ten pitchers were collected no less than one meter apart from each other. *Darlingtonia californica* forms below-ground or surface-level rhizomes that connect rosettes of pitchers together (personal observation, Fig. 2). Rosettes generally consist of a small mound of last year's browning leaves as well as the present

season's new leaves. The first leaf to emerge each year is almost always the tallest in the rosette. New leaves can continue emerging throughout the growing season but become significantly smaller towards autumn. Pitchers first fill up with sterile water (Hepburn et. al 1927) and then open up to the environment at the rim near the top of the pitcher (although there is debate as to whether or not the pitcher fluid is truly sterile before opening).

Pitchers of about the same age were collected, since abundance of aquatic insect immatures has been shown to be positively correlated with phytotelma size (Aditya et.al. 2008). I systematically moved through fen collections starting with the lowest elevation on June 30th, 2015 and finished with the highest elevation on August 19, 2015. Average height of pitchers often differed between fens, even within one region, so instead of always choosing a pitcher at one particular height (say, 70 cm), the second tallest pitcher in the rosette was the one selected, which resulted in the pitchers having a greater chance of being the same age. To qualify for selection the pitcher could not have major abnormalities such as holes in the leaf wall, damage to the opening of the pitcher, or large arachnid occupants. To minimize the fluid loss, I carefully snapped off individual pitchers at the base and placed them in a large Ziploc<sup>®</sup> bag. The bags were set in a cooler with towel-wrapped ice packs for transport, all while ensuring that the pitchers were held vertically.

## Community Assessment

No more than 18 hours after collecting pitchers from a region, the 30 pitchers were brought to the Biocore Laboratory at Humboldt State University where a dissecting microscope and camera were used to identify and count each living individual in each pitcher. Only the aquatic macroinvertebrates were counted because they were expected to be a more exclusive community due to their highly specific niche, therefore lowering my chances of counting taxa that might not be direct *D. californica* associates. Five intact pitchers were collected from each fen to rear larvae to adulthood for identification, as parasitoid hymenoptera have previously been found this way (Nielsen 1990), but all community members perished before any adults were ever collected. Since laboratory conditions differ greatly from their natural ones, there was a limited amount of time before the communities started to change, so the decision to limit the sampling to only aquatic invertebrates also opened up time that was allotted to maximizing the sample size. The entire contents of the phytotelma, including the pitcher wall and insides of decomposing carcasses, was searched for living individuals. It was important to count each living individual, as the interactions of species may play more of a role in community composition than environmental factors (Dezerald et. al. 2016). If a single taxon exceeded 1,000, I stopped counting higher and recorded the abundance as 1,000. Microorganisms (including Rotifers) were not surveyed equally among pitchers or regions; instead, I chose pitchers haphazardly based on unusually high community richness or simply because there was extra time available, therefore conclusions cannot

be drawn regarding their presence or absence in any region. Spiders inhabiting the space inside of a pitcher just above the water level, who may play a role in the community of interest, were the only non-aquatic taxon recorded. Pitcher fluids from two systematically chosen pitchers per fen were further investigated for rotifers using a compound microscope. Along with the abundances of each taxon, the pitcher's height (the lower most part of the pitcher to the top of its hood), and depth of water were measured.

### Data Analysis

This study used exploratory data analysis as well as hypothesis testing to identify patterns of community structure and potentially relevant covariates. I attempted to falsify the hypothesis that community composition is the same in all pitchers, and therefore independent of fen and region. Five community structure metrics- taxon richness, community abundance, community diversity (Brillouin index), community evenness (Camargo index), and community dominance (estimated as the proportion of community composition accounted for by the three most abundant species) - were calculated for comparison between regions. Kruskal-Wallis non-parametric tests were performed to test for any differences between regions in individual taxon abundances and community metrics. Any significant Kruskal-Wallis tests that indicated significant differences were followed by pairwise Wilcoxon rank-sum tests to determine where the differences between regions lie.

The Non-metric MultiDimensional Scaling (NMDS) axes are sometimes described as pseudo-gradients (after a necessary rotation of the ordination space) because they relate measured environmental covariates to the unknown environmental gradients presumed to structure community composition. The location of each sample in ordination space is ordered along the two NMDS axes. Correlations between covariates and the NMDS axes help us interpret the habitat conditions represented by pseudo-gradients. Greater dissimilarity indicates community assemblages that differ.

Taxa were removed from the ordination if they were present in fewer than 10% of the pitchers which is a standard for accounting for sampling effort. Species abundance, taxon richness, community diversity, community evenness, and community dominance metrics were each calculated and treated as covariates. The abiotic data included as covariates were pitcher water level (height:cm), size (height:cm) of pitcher, and elevation. Incorporating all of the previous considerations, NMDS ordination was performed using 10,000 permutations and visualized by grouping pitchers by region. In order to account for the multiple nested spatial scales I compared the Bray-Curtis dissimilarities between and within regions and fens. This allowed for an equal comparison of pitcher community variability between multiple scales of space.

Samples were analyzed for differences in community composition against multiple variables using Permutational Multivariate Analysis of Variance (perMANOVA) to assess the likelihood that observed differences among discrete groups of samples might have occurred by chance. This tested the null hypothesis that the centroids and dispersions of groups do not differ from one another. The same set of



community composition data used in the ordination was used for perMANOVA, with covariates elevation (m), region, size (height) of pitcher, pitcher water depth (height), as well as interactions between any two or more of the covariates. For this perMANOVA, 999 permutations were used. To further compare which regions differed significantly from one another, I also performed a pairwise perMANOVA with a  $p$ -value adjustment for multiple comparisons.

## RESULTS AND DISCUSSION

### The Inquiline Species

Seven invertebrate taxa were observed in this study; six were found in the pitcher fluid, and one spider was non-aquatic. Even though several other taxa were found living in the pitcher above the waterline, this one non-aquatic was chosen to be included in the analysis because it was observed preying on the aquatic taxa and therefore could directly influence community dynamics. No single region had all seven taxa, and only two of the taxa, *Metriocnemus edwardsi* and *Sarraceniopus darlingtoniae*, were recorded at all four regions (Table 1). From reviewing the literature, *M. edwardsi* and *S. darlingtoniae* have been well known as *D. californica* inhabitants, so it was no surprise to record their presence in all but 10 pitchers. These two taxa have very different life cycles and utilize distinct yet overlapping niches within the pitcher habitat.

*Metriocnemus edwardsi* Jones (Diptera: Chironomidae) is a prominent member of *D. californica* phytotelmata, whose adults oviposit their eggs in the pitchers throughout the summer. Larvae of the same cohort were often found in a single pitcher, but were older larvae were sometimes found with early instars. Larvae were constantly searching for or ingesting the soft tissue of the plant's prey and found throughout all depths of fluid. This species was recorded 3,010 times in my study, and more than half of the individuals were found at Plumas. It accounted for 21% of the total community abundance, and was found in 70% of the pitchers surveyed. A study on *Sarracenia* found much of the genetic variation between *Metriocnemus* individuals (of a different species) could be explained

by landscape variables such as density of pitchers within bogs and bog size (Rasic & Keyghobadi 2011). This suggests that environmental information other than what was collected in this study could help explain the community-scale distribution patterns of *M. edwardsi*.

*Sarraceniopus darlingtoniae* Fashing & O'Connor (Arachnida: Histiostomatidae) was often found in the same pitchers as *M. edwardsi*. Unlike holometabolous insects, mites go through multiple nymphal stages before becoming adults, who resemble the largest nymphs. In most pitchers there were multiple life stages present together. Little is known about the biology or ecology of this particular species, probably in part because it is restricted to such a limited habitat. It is restricted to *D. californica* pitchers and is an obligatory inhabitant. These mites are able to move out of pitchers in certain nymphal stages and so disperse to new pitchers this way. *Sarraceniopus darlingtoniae* was found in 67% of the pitchers sampled and was present in all four regions. It was the most abundant taxon in this study at 6103 individuals, and it accounted for 42.5% of the total community abundance. It was found in the highest numbers in Six Rivers, which had an average abundance of 170 individuals in each pitcher, and found least frequently in Rogue River-Siskiyou with only 14 individuals on average per pitcher. These mites were seen all throughout the pitcher, including below the water level deep within the pitcher fluid at times.

Two additional semi-aquatic flies were far less abundant: *Corynoptera* sp. (Diptera: Sciaridae), and *Megaselia orestes* Borgmeier (Diptera: Phoridae). Both species are detritivores and were observed actively foraging on prey carcasses near the top of the

fluid layers. *Megaselia orestes* is a member of the scuttle fly family, a family with diverse feeding habits among its species including many scavengers and bacterivores. This species was found in 43% of the pitchers and was present in every region except Plumas. It accounted for 7% of the total community abundance. *Corynoptera* sp. was found in 17% of the pitchers; 79 individuals were recorded only from Rogue River-Siskiyou and Six Rivers regions, and it accounted for only 0.6% of the total community abundance. These two taxa were recorded as associating with *D. californica* nearly 30 years earlier by Nielsen in Gasquet, CA, in Six Rivers (Nielsen 1990), where they were again both present in this study.

Another mite observed in this study was a member of suborder Prostigmata. Although it was not conclusively identified to genus, it might be a member of the family Tenuipalpidae, which was found in an earlier study of *D. californica* communities (Nielsen 1988). It was only found in 5% of total pitchers and was present in only two regions, Six Rivers and Plumas. While 482 individuals were recorded in total, 420 of those were found in two pitchers at one fen in the Plumas region, and not present in two of the four regions. These mites were only found in the top-most layers of prey and the top of the fluid.

I was surprised to find a relatively prominent nematode in the *D. californica* phytotelma community because one has never previously been recorded as associated with the plant at all. There are many examples of nematode species in a variety of other pitcher plant species, including in the sister genus of *Darlingtonia*, *Sarracenia*. These nematodes were often found in high abundances; for instance, there were two pitchers

with over 1,000 nematodes, and most of the pitchers with nematodes had over 100 individuals. They were found in all regions except for Plumas, and 3,630 individuals were recorded in total among the other three regions. They accounted for 25% of the total abundance, but likely comprised a small fraction of the total biomass because of their small size. It is possible that these nematodes are endoparasites of insects and they emerge only after their host's death by pitcher carnivory. Sometimes all nematodes were found at the very base of the pitcher fluid, while other times they were found scattered throughout the fluid and carcasses.

The final taxon recorded was a spider of the family Linyphiidae who was associated with the aquatic community as a predator. These spiders were observed with a high diversity of insects captured in their webs which included at least four from this study- *S. darlingtoniae*, unknown mite, *M. edwardsi* and *M. orestes*. Spiders were found in 24% of all pitchers, but never recorded in Six Rivers. They were never recorded in Six Rivers, but it is possible that their presence may have been recorded in some pitchers that had to be removed from the study (due to lack of fluid). Though there were 42 individuals found (accounting for 0.3% of the community abundance) 22 of those individuals were very young and recorded in only two pitchers, while the rest of the pitchers had only a single older individual.

I searched for rotifers in a select few pitchers collected each region. I was told of their presence in *D. californica* (David Armitage, personal communication), and I observed their presence in nearby creek water samples. Since their excrement provides nitrogen and phosphorus, they could be important players in mineral nutrition for *D.*

*californica* (Walsh et. al. 2014). I found two individual Bdelloid rotifers in two different pitchers from the same region, Plumas. Rotifers are an interesting member of the aquatic invertebrate community who occupy a unique size niche, being an microorganism who feeds on bacteria yet could be fed on by other bacterivores. From a search of the literature, no rotifer has been found with *D. californica*. The only way they could enter the phytotelma is somehow through the opening, which is far above the water line, so maybe a splash of water or phoresis on an insect flier got them inside the pitcher.

### Community Analysis

After discarding pitchers that lost their phytotelma water, a total of 93 pitchers' community compositions were analyzed; 23 from Rogue River- Rogue River-Siskiyou National Forest, 15 from Six Rivers National Forest, 29 from Shasta-Trinity National Forest, and 26 from Plumas National Forest. Mean pitcher sizes differed significantly between regions ( $\chi^2 = 34.14$ ,  $df = 3$ ,  $p < 0.001$ , Table 2). The smallest pitcher found was 11 cm at Shasta-Trinity which was the region with the smallest average pitcher size ( $24 \text{ cm} \pm 1 \text{ cm}$ ) and at the highest elevation, while the largest pitcher found was 91 cm at Six Rivers which had the second tallest average pitcher size ( $44 \text{ cm} \pm 6 \text{ cm}$ ) and was the lowest elevation region (Fig. 3). The water depths did not differ significantly between the Rogue River-Siskiyou, Six Rivers, and Shasta-Trinity regions (16%, 29%, and 34% of pitcher heights, respectively), but Plumas had a significantly lower average water depth

of only  $2.9 \text{ cm} \pm 1.2 \text{ cm}$  which was 9% of the pitcher proportional to the average pitcher height found there.

In total, 14,358 individuals were counted in this study, with 43% found at Six Rivers fens, 26% at Plumas, 25% at Shasta-Trinity, and 7% at Rogue River-Siskiyou (Fig. 4). Mean community abundances per pitcher significantly differed between regions ( $\chi^2 = 21.01$ ,  $df = 3$ ,  $p < 0.001$ ) and for every pairwise combination of regions except Plumas-Shasta-Trinity and Plumas-Six Rivers (Table 3). Rogue River-Siskiyou had the lowest abundances per pitcher with an average of  $52 \pm 11$  individuals and Six Rivers had the highest with an average of  $409 \pm 110$  individuals per pitcher. The other community metrics calculated - community taxon richness, community diversity, and community evenness, and community dominance - did not differ significantly between the regions (Table 4). Five out of the seven taxa found had significantly different abundances across the four regions, and the other two were nearly significant (Fig. 5).

Clustering of samples from the four regions in ordination space suggested that dissimilarities among samples within regions were less than mean dissimilarities between regions. The ordination diagram was generated solely by the counts of the common taxa. Results of the NMDS ordinations incorporating invertebrate community composition revealed overlap of all four regions near the central portion of the ordination space despite groupings of samples from each of the four regions (stress level 0.166; Fig. 6). Fen elevation ( $p < 0.001$ ) and *M. edwardsi* abundance ( $p < 0.001$ ) were inversely correlated with NMDS axis 1, while taxonomic richness ( $p = 0.18$ ), *Corynoptera* sp. abundance ( $p = 0.13$ ), and *Megaselia orestes* abundance ( $p < 0.001$ ) were positively

correlated with the first axis. Spider abundance ( $p < 0.001$ ) and pitcher size ( $p < 0.001$ ) were inversely related to the second NMDS axis, while *Sarraceniopus darlingtoniae* abundance ( $p < 0.001$ ), total inquiline invertebrate abundance ( $p < 0.001$ ), and nematode abundance ( $p = 0.003$ ) were positively correlated with the second axis (see Table 5 for complete list of vectors).

Based on the Bray-Curtis dissimilarity index, there was a greater mean dissimilarity, or distance, between regions (0.77) than within regions (0.63) when comparing community compositions of individual pitchers. This indicates that the pitchers sampled in my study have greater similarity within any single region than among all four regions (Fig. 7). Another Bray-Curtis dissimilarity index value was calculated to compare individual pitchers' community composition between fens, which resulted in greater dissimilarity between fens (0.75) than within fens (0.62).

PerMANOVA suggests that community composition differs between regions. Elevation, region, pitcher size, and pitcher water depth, along with their interactions, accounted for 55% of the total variation in community composition in the perMANOVA performed. Geographic region ( $r^2 = 0.15$ , pseudo- $F=7.61$ ,  $p = 0.001$ ) and elevation ( $r^2 = 0.12$ , pseudo- $F=2.01$ ,  $p = 0.001$ ) significantly predicted community composition variation between pitchers, while the other variables and all combinations of interactions were not significant predictors. Since the perMANOVA only indicates whether one or more groups are significantly different and not exactly which group(s) differ, the pairwise perMANOVA provided some clarity. Pairwise perMANOVA showed that five out of the six combinations of pairs of regions had significantly different community compositions



( $p = 0.001$ ), while differences among the 6th pair - Shasta-Trinity and Plumas - were not as well supported ( $p = 0.12$ ).

### Interpretation And Comparison

One of the most straightforward hypotheses regarding “top-down” community assemblage is that some biogeographical force(s) cause inherent large-scale habitat differences (Kitching 2001). The four regions studied here have very different average elevations and are subject to different climatic forces, all of which can have an effect on community composition (Hodkinson 2005). Regional elevation was correlated with variation in community composition; however, I did not find evidence that the regions differed significantly from one another with respect to community indices of taxon richness, community diversity, community evenness, or community dominance. This suggests that it is the abundance of any one (or more) taxon that is driving differences in community composition between pitchers, and that the apparent exclusion of any given species is in some way, related to elevation.

Changes in temperature, precipitation (rain and snow), the pressure of atmospheric gases, wind speed, and UV-radiation are all associated with changes in altitude (Barry 1992) and have been suggested as factors influencing the dispersion of terrestrial invertebrates (Hodkinson 2005). These differences influence the phenology of the plant itself, in addition to the life-cycles of the insects within the pitchers, potentially limiting the dispersal of species outside of their elevation thresholds within a season

(Hodkinson 2005). Although plants along elevational clines tend toward lower stature at greater elevation (Korner 1989) we did not find that pitcher size significantly influenced community structure. The highest elevation region, Shasta-Trinity, did have an average pitcher size that was significantly smaller than all of the other three regions, but it did not have the lowest inquiline species richness and had the second highest community evenness.

Another important factor that may determine community composition is the stage of disassembly, since these phytotelma are indeed ephemeral. As the pitcher ages and accumulates prey, the chemical composition and volume of the fluid and quality of resources enter a state of fluctuation (Adlassnig et. al 2011). The habitat changes to that of a harsher environment, potentially driving low-tolerant species away (O'Neill 2016). This state of flux occurs in individual pitchers as they senesce, but is also occurring among other pitchers in a fen (Adlassnig et. al 2011). When I compared the dissimilarity of communities in pitchers at the fen level, I found that communities were more similar amongst pitchers in the same fen than those of other fens, suggesting that changes in the pitcher fluid or fen water are more likely to be influenced by local environmental variation, related to permanence, rather than metacommunity spatial relationships (Urban 2004).

The climate has been warming at an unprecedented rate since the end of the last glacial period and there are numerous examples where a warming climate has led to changes in species' relative abundances and geographic ranges, caused extinctions, and created transient and novel communities dominated by generalist species and interactions

(Blois et al. 2013). In my study I re-surveyed *D. californica* fens from regions previously described in 1986 and 1988. By comparing the communities I found to the ones they described, we can make some inferences about the effect that climate change may posit for *D. californica* ecosystems. In 1988, Naeem censused 119 *D. californica* pitchers in Butterfly Valley, Plumas National Forest, for a study on the stable coexistence of the two commonly co-occurring species *M. edwardsi* and *S. darlingtoniae*. Naeem's extensive study on the community dynamics between the two species claimed that these were the only two members of the *D. californica* phytotelma community and any other taxon found would be considered a waif. My results were similar in that *M. edwardsi* and *S. darlingtoniae* were by far the most abundant taxa in Plumas. The only other two taxa found in this region in the present study were a nematode, which may not have been recorded in the 1988 study, and another mite, which was only semi-aquatic and found in relatively small numbers. David Nielson performed a comprehensive and analytical survey of the entire *D. californica* pitcher invertebrate community, aquatic and otherwise, in Gasquet, CA in Six Rivers during 1986. He found 21 total invertebrate species after a census of 124 pitchers from three fens within five kilometers of each other, and for the sake of comparison there were six aquatic invertebrate taxa found in his study. There were likewise six invertebrate inquiline taxa at my Six Rivers fens, all of them aquatic. Four of the six found in my study were also found in Nielson's survey, and the other two are the unknown mite, which very well may be one of the ones found in his study, and the nematode which may have been unrecorded in his study. All of our fens were in the same general area near the city limits of the small town of Gasquet, CA. I did not find evidence

that the fen had changed significantly throughout the decades (as seen by our similar aquatic community composition results); although we do not have empirical data to compare. It may not be surprising that such a specific and specialized microhabitat has remained mostly unchanged in this relatively short period of time.

Both rotifers and nematodes have been found in many other species of pitcher plants (Adlassnig et. al 2011) but to my knowledge, this is the first study to find nematodes and rotifers in the pitcher fluid of *D. californica*. These are interesting members of the aquatic invertebrate community who occupy a unique size niche being microorganisms who feed on bacteria yet could be fed on by other bacteriovores (Walsh et. al. 2014). Since the excrement of bdelloid rotifers provides nitrogen and phosphorus, they could be important players in mineral nutrition for *D. californica* (Walsh et. al. 2014).

The pitchers of *Darlingtonia* and *Sarracenia* sp. have similar inquiline communities and even have several members from the same genera (*Metriocnemus*, *Sarraceniopus*). However, the average number of arthropod species found in a pitcher in my study was three, whereas other pitcher plant species' averages are generally higher. Some examples of other pitcher plants include *Sarracenia purpurea*'s 165 inquiline species, *Nepenthes ampullaria*'s 59 species, and *Heliamphora nutans*' 15 species (Adlassnig et.al 2011). Interestingly, insect diversity is no lower in the range of *D. californica* than any of these other pitcher plant species' (Lomolino et. al. 2010).

Perhaps my most striking finding was the absence of mosquitoes associated with *D. californica*, despite observations of adult mosquitos at each fen I surveyed. Virtually

all phytotelmata, including entities like tree holes and leaf axils, contain at least one species of mosquito who regularly spends its larval and pupal stages as a prominent member of the aquatic community. Many phytotelmata have several mosquito species, often each specialized to a unique niche in the microhabitat. Mosquitoes have even been shown to increase the nitrogen yield of carnivorous plants by acting as keystone predators on bacterivores and increasing bacterial abundance, solidifying their mutualistic interaction with the host plant (Mouquet et.al. 2008). Pitcher plants offer an ideal space for mosquitoes to grow through the first few life cycle stages due to their limited water depth, absence of water current, lack of larger freshwater aquatic predators, and surplus of food options for specialization in feeding.

The absence of mosquito larvae in these phytotelmata provoked much speculation over an explanation. Perhaps in *D. californica*, the presence of mosquitoes may have caused a cascading negative effect in the mutualism between the plant and the phytotelmata community, indirectly negatively impacting the fitness of the plant itself. This could happen if the mosquitoes lowered the fitness of a mutualistic detritivore partner by eating it, or it could happen from something far more indirect than this. An indirect way mosquitoes could affect the plant's fitness is by preying upon any taxon who acts as a keystone species upon the microbial community altering the proportions of "good" digestive bacteria to "bad" exploiting bacteria. Mosquitoes might struggle navigating around the hood of *D. californica*. If mosquitoes ever decreased the fitness of the plant, and if an enlarged hood appeared in some plants, then it is possible that these hooded plants would have the advantage of limiting access to ovipositing mosquitoes. If

the communities were more effectively mutualistic without mosquitoes, then hooded pitchers could have increased in frequency until the trait took over the entire species. This is speculative but *something* must explain why mosquitoes are absent from these phytotelmata.

In a literature review by Adlassnig et. al. (2011), plant species that host phytotelmata were compared for diversity of inquiline species. It was shown that *D. californica* was among the three taxa with the lowest diversity, and the other two taxa, *Cephalotus follicularis* and *Sarracenia minor*, are also nearly fully hooded, like *D. californica*. Most of the other pitcher plants studied had a more ‘open-entrance’ morphology and fostered higher diversities of inquiline taxa. It is believed that the hood of at least some pitcher plants aids in reducing evaporation of pitcher fluid, but it might be possible that the hood restricts colonization of a more diverse community. In other words, the presence of a hood might be correlated to inquiline community diversity. Neither of the other two hooded species mentioned above have been found to host mosquito species in their phytotelmata communities either, an observation consistent with the hypothesis that the hood affects their ability to oviposit.

Although our study attempted to elucidate factors influencing the community assemblage of inquiline organisms across spatial scales we could not definitely assert that “top-down” or “bottom-up” forces are shaping the structure of these communities. However, my study contributes to the literature regarding the inquiline communities of *D. californica* pitchers by demonstrating that there were differences in community

composition across different regions across the range of *D. californica* and by reporting the first observation of nematodes and rotifers in the pitcher fluid.

### Future

This study could be used a launching point for a myriad of other types of research questions. The phenology of these communities could be studied by expanding the collecting process to span over many months, analyzing pitchers from the same fen at a regular time interval. This could result in a more complete picture of the *D. californica* phytotelmata community composition and dynamics by eliminating the limiting factor of chronology from the present study. Collecting GIS information at each fen would be an interesting addition to be able to quantify the distance from other *D. californica* fens for use as a proxy of degree of isolation of the metacommunity, which may have an effect on insect dispersal, and therefore abundances (LeCraw et. al. 2014). It would also be interesting to find out if there is a correlation to community composition with elemental composition and pH of the pitcher fluid, or even serpentine presence.

Another research direction that these microhabitats could be used for is to analyze the microbial ecosystem dynamics. Here, a student could use bacteriology skills to measure community dynamics of a far more complex system than metazoan invertebrates. Since the pitcher fluid is sterile before the pitcher opens, the bacterial community is seeded by the exterior surface of the first visitor to the pitcher (whether the plant's prey or pitcher occupant). One could apply a modern ecological theory to their

more robust data set such as community succession, species interactions, environmental drivers of diversity, environmental limitations on communities, metacommunity analyses, or many other theories. This may also be an ideal system to empirically evaluate the ‘Geographic Mosaic Theory of Coevolution’, which states that there is geographical variation in selective forces acting upon communities resulting in coevolutionary ‘hotspots’ and ‘coldspots’ associated with high and low biodiversity, respectively (Laine 2009). Further, when entire microbial community composition analyses become more cost effective, it will be tremendously informative to include a complete survey of all organisms in each community, including the often-overlooked species of Bacteria and Archaea. It is largely unknown whether these groups follow similar patterns of community composition and biogeographical distribution as other groups of organisms, or if their diversity is affected by the same habitat conditions as their fellow macro-community members. To properly analyze the microbial communities, it is now understood that one must use genetic techniques to eliminate any culture-dependent biases, but these studies tend to be extremely costly for such diverse communities (Siragusa et.al. 2007, Koopman et.al 2010, Koopman et.al. 2011). A single phytotelma community in *D. californica* could have as many as 200-800 or more bacterial and archaeal species per pitcher (Dr. Patricia Siering, personal communication).

Phytotelmata are particularly optimal for research because nature provides the large sample size, discreteness of variables (‘islands’), and inherent variability that all decent science needs. It is a bonus that these communities have the potential to be complex within (especially considering microbes) and have plenty of variation between



pitchers. People are well aware of this ideal scientific opportunity in other pitcher plant species and hundreds of studies have been published, but *D. californica* has received less attention in the literature than other species. This may be due to its great geographic and genetic isolation from any other pitcher plant taxon. *Darlingtonia californica* could also be used as a model system for experimental manipulation like research on species exclusion or common garden experiments. Considering the ideal nature of *D. californica* phytotelmata for use in scientific research, I would recommend this system to an aspiring or experienced researcher. Over time and with a large enough body of work, biologists might even be able to utilize the information from phytotelmata research into biodiversity conservation efforts of other types of aquatic communities, like those of ponds and lakes.

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Table 1. Species abundances organized by region. The last row is the proportion of total abundance found at each region. The last column represents the species' abundance out of the total abundance.

	Rogue River- Siskiyou	Six Rivers	Shasta- Trinity	Plumas	Total	percent of total
<i>M. edwarsi</i>	49	85	1156	1720	3010	21%
<i>S. darlingtoniae</i>	234	2549	1820	1500	6103	43%
<i>M. orestes</i>	412	479	121	0	1012	7%
Nematode	220	2970	440	0	3630	25%
<i>Corynoptera</i> sp.	63	16	0	0	79	1%
Mite	0	32	0	450	482	3%
Spider	7	0	5	30	42	0%
total abundance	985	6131	3542	3700	14358	100%
percent of total	7%	43%	25%	26%	100%	

Table 2. Values organized by the four regions and each of their three fens. (Top and bottom tables have the same variables.)

Region	Rogue River-Siskiyou			Six Rivers		
Region richness	6			6		
Region abundance	985			6131		
Number of pitchers	23			15		
Fen number	1	2	3	4	5	6
Elevation (m)	705	747	579	176	158	132
Fen richness	5	5	6	5	4	6
Fen abundance	187	219	579	1171	3150	1810
Number of pitchers	8	5	10	5	5	5

Region	Shasta-Trinity			Plumas		
Region richness	5			4		
Region abundance	3542			3700		
Number of pitchers	29			26		
Fen number	7	8	9	10	11	12
Elevation (m)	1642	1578	1525	1519	1215	1169
Fen richness	3	2	5	4	3	4
Fen abundance	1069	1026	1447	967	772	1961
Number of pitchers	10	9	10	8	9	9



Table 3. *P*-values of pairwise comparisons. Each column represents the pair of regions from the first and second rows. Dashes present when both regions had a value of zero.

	Rogue River- Siskiyou Six Rivers	Rogue River- Siskiyou Plumas	Six Rivers Shasta- Trinity	Six Rivers Plumas	Shasta- Trinity Rogue River- Siskiyou	Shasta- Trinity Plumas
perMANOVA	0.002	0.002	0.002	0.002	0.002	0.143
Pitcher height (cm)	0.54	0.02	0.001	0.37	<0.001	<0.001
<i>M. edwardsi</i> abundance	0.46	<0.001	<0.001	<0.001	<0.001	0.464
<i>S. darlingtoniae</i> abundance	<0.001	0.05	0.05	0.13	0.02	0.85
<i>M. orestes</i> abundance	0.22	<0.001	<0.001	<0.001	0.01	0.01
Nematode abundance	0.47	0.09	0.09	0.02	0.47	0.47
<i>Corynoptera</i> sp. abundance	0.76	0.09	0.02	0.06	0.06	-
Mite abundance	0.28	0.28	0.28	0.58	-	0.16
Spider abundance	0.02	0.30	0.30	0.00	0.16	0.01
community abundance	0.00	0.01	0.03	0.26	0.02	0.26
community taxon richness	0.13	0.64	0.45	0.64	0.64	0.91
community evenness	0.22	0.18	0.28	1.00	1.00	0.50

Table 4. Mean number of inquilines per pitcher ( $\pm$  standard error). Df=3. Chi squared and  $p$ -values from Kruskal-Wallis tests are listed.

	Rogue River- Siskiyou	Six Rivers	Shasta- Trinity	Plumas	$\chi^2$	$p$
<i>M. edwarsi</i>	1.9 $\pm$ 1.1	5.7 $\pm$ 2.2	35.7 $\pm$ 4.8	82.3 $\pm$ 27.3	43.41	<0.001
<i>S. darlingtoniae</i>	13.5 $\pm$ 4.3	169.9 $\pm$ 28.9	75.8 $\pm$ 21.6	93.8 $\pm$ 28.3	19.17	<0.001
<i>M. orestes</i>	19.1 $\pm$ 7.5	31.9 $\pm$ 9.4	5.0 $\pm$ 2.1	0	29.77	<0.001
Nematode	12.9 $\pm$ 6.8	198 $\pm$ 91.4	18.3 $\pm$ 10.8	0	12.35	0.006
<i>Corynoptera</i> <i>sp.</i>	3.7 $\pm$ 2.9	1.1 $\pm$ 0.6	0	0	13.05	0.005
Mite	0	2.1 $\pm$ 1.5	0	28.1 $\pm$ 20.4	7.49	0.06
Spider	0.41 $\pm$ 0.12	0	0.21 $\pm$ 0.13	1.9 $\pm$ 0.9	1919	<0.001
Abundance	51.6 $\pm$ 11.4	408.7 $\pm$ 105.4	135.1 $\pm$ 26.8	206 $\pm$ 44.9	21.06	<0.001
Richness	2.6 $\pm$ 0.2	3.1 $\pm$ 0.3	2.6 $\pm$ 0.1	2.6 $\pm$ 0.2	2.45	0.48
Diversity	0.59 $\pm$ 0.1	0.6 $\pm$ 0.6	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	1.65	0.65
Evenness	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	7.21	0.07
Dominance	1.0 $\pm$ 0.01	1.0 $\pm$ 0.01	1.0 $\pm$ 0.01	1.0 $\pm$ 0.01	2.55	0.47

Table 5. Correlation coefficients for covariates in NMDS ordination space and the two NMDS axes (see Figure 1). P-values are based on 999 permutations.

Vectors	NMDS1	NMDS2	<i>p</i>
<i>M. edwardsi</i>	-0.99	0.05	<0.001
<i>S. darlingtoniae</i>	0.23	0.97	<0.001
<i>M. orestes</i>	0.98	-0.21	<0.001
Nematode	0.71	0.7	0.006
<i>Corynoptera</i> sp.	0.99	0.07	0.001
Mite	-0.99	0.05	0.92
Spider	-0.21	-0.98	<0.001
Abundance	0.42	0.91	<0.001
Richness	0.96	0.28	0.1
Diversity	0.97	0.24	0.27
Evenness	-0.63	-0.77	0.61
Dominance	-0.34	0.94	0.43
Pitcher size	0.62	-0.78	0.009
Water height	0.99	0.13	0.29
Elevation	-0.99	-0.13	<0.001

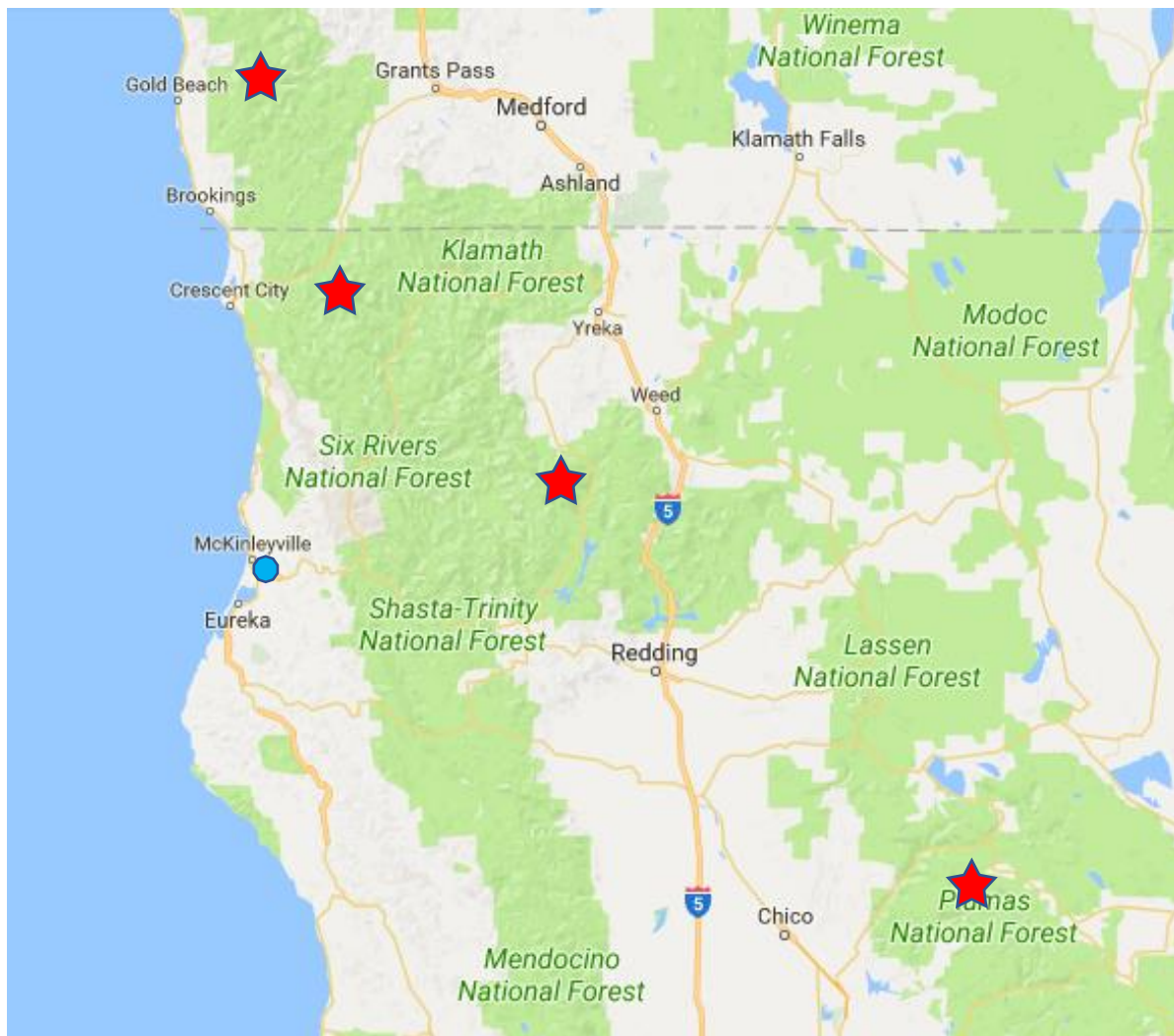


Figure 1. Map of study area in Northwestern California and Southwestern Oregon. Red stars represent the four regions, in order from Northwest to Southeast: Rogue River-Siskiyou National forest near Gold Beach, OR; Six Rivers National Forest near Gasquet,



Figure 2. A typical *Darlingtonia californica* habitat growing in and around a small stream.

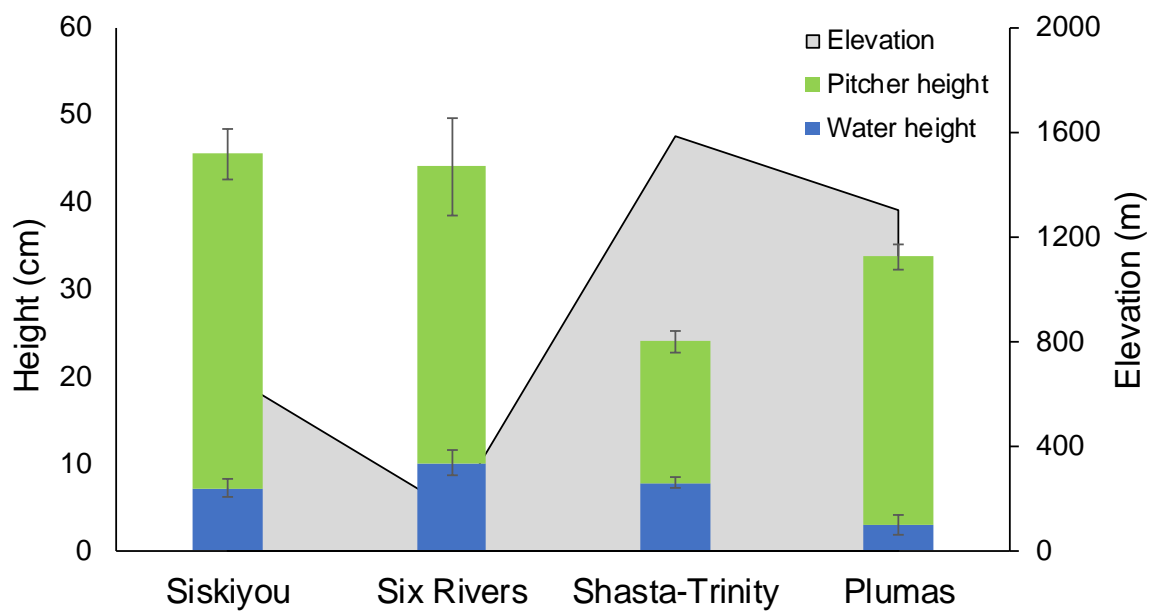


Figure 3. Mean heights of pitchers and depth of pitcher fluid shown proportionate to one another with standard error bars. Pitcher height correlated to elevation, which is shown in grey behind bars.

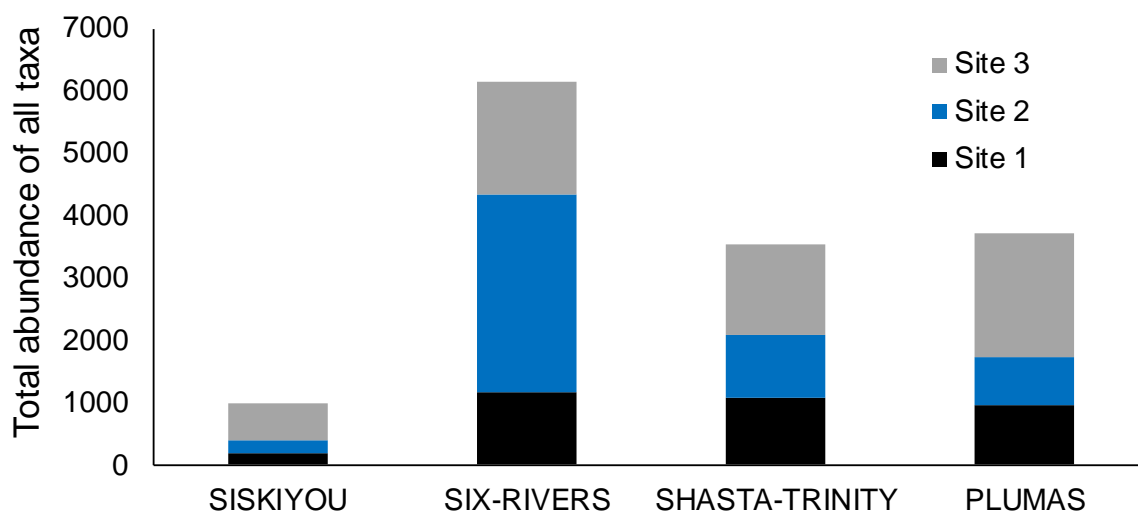


Figure 4. Total abundances of all taxa combined grouped by region and stratified by fen within regions. Although abundances were significantly different from one another, the differences did not correlate to elevation. (Sample sizes (n= number of pitchers sampled) were Siskiyou n= 23; Six Rivers n= 15; Shasta-Trinity n= 25; Plumas n= 26).

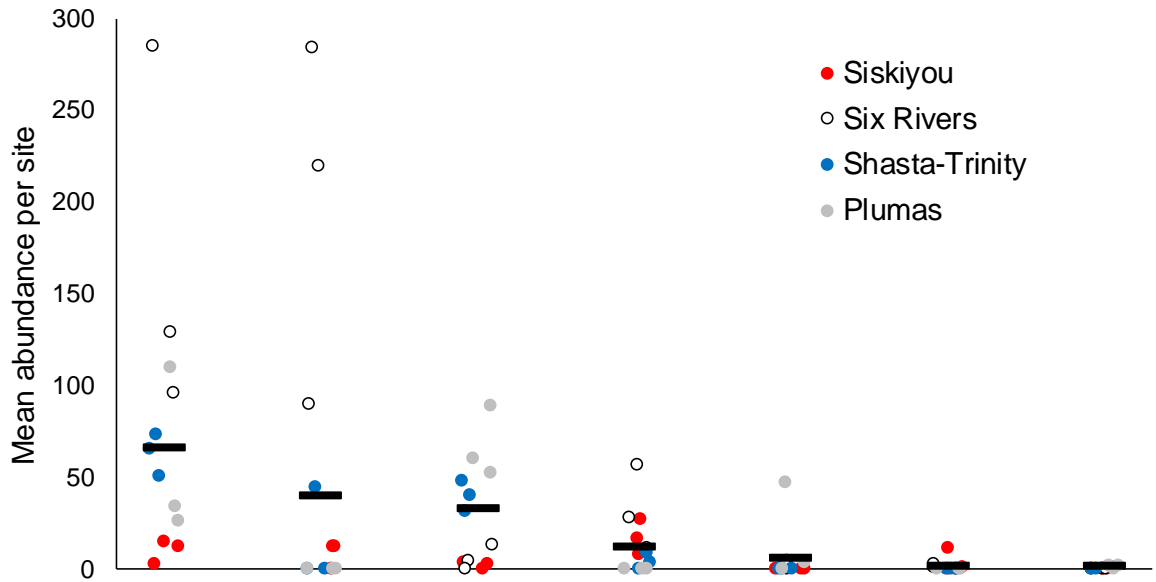


Figure 5. Abundances of each taxon grouped by fens, with fens color coded by region. From left to right: *S. darlingtoniae*, nematode, *M. edwardsi*, *M. orestes*, mite, *Corynoptera* sp., spider.



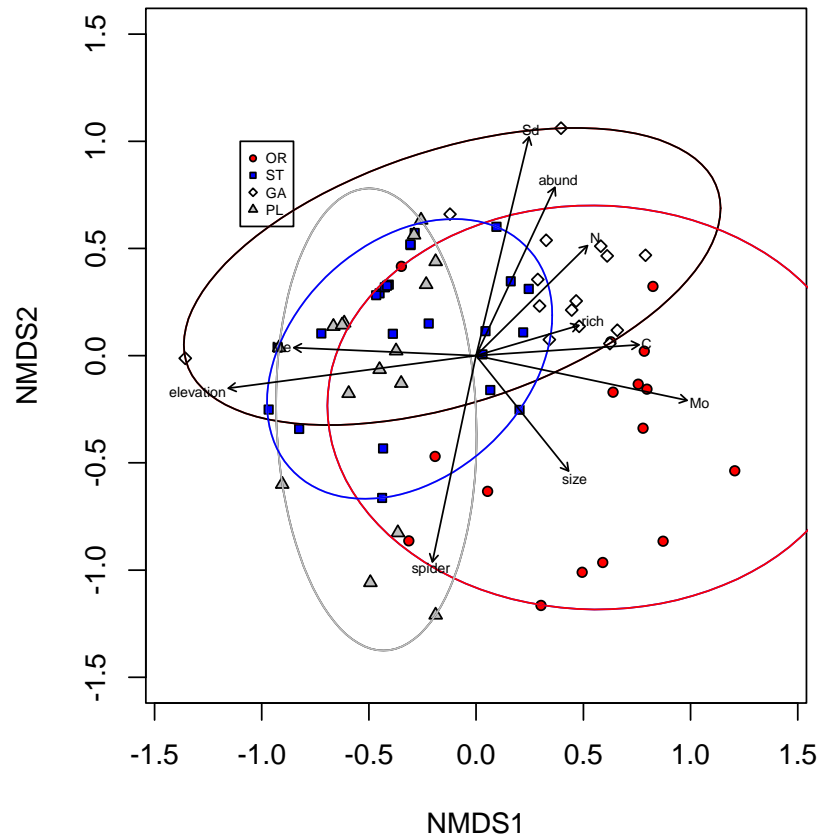


Figure 6. Nonmetric multidimensional scaling (NMDS) ordination of community composition data from 93 pitchers. Vector information presented in Table 1. Vector arrow length and direction correspond to the magnitude of the correlation with the two axes.

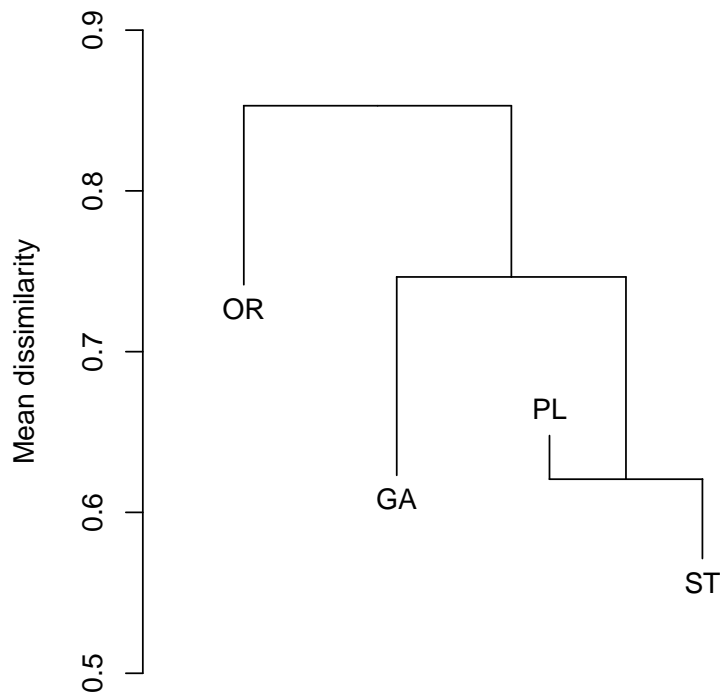


Figure 7. A dendrogram of Bray-Curtis mean dissimilarity by region. (OR= Siskiyou; GA= Six Rivers; PL= Plumas; ST= Shasta-Trinity.) Each region's segment terminated to the within-region dissimilarity value represented on the y-axis. The closer any two are on the dendrogram, the less dissimilar they are between regions. For example, Siskiyou (OR) had more within-region dissimilarity than the group containing the other three regions. Plumas' (PL) segment is reversed because it had more dissimilarity within the region than the combination of Plumas and Shasta-Trinity (ST).

## APPENDIX

### Region and Fen Descriptions

The range of *Darlingtonia californica* stretches diagonally from the northern Sierra Nevada mountains to the far northwest corner of California and continues in a narrow stretch along the coast through most of Oregon. Populations of the plant are not contiguous throughout its range, and it can be found in a variety of habitats and microhabitats. *Darlingtonia californica* is found outside of the four regions sampled, but this study was also limited by denial of permission from other US Forest Service stations. This study sampled pitchers for community composition from 12 fens, three chosen by government officials in each of the four regions, each of which is described below.

Rogue River-Siskiyou National Forest, Oregon: This was the northernmost region in the study, and the only one beyond California's border. Although populations of *D. californica* reach at least 150 miles north of here, stretching most of the Oregon coast, this region was chosen for its proximity to HSU, and because I had permission to take samples. All three fens were within the Rogue River-Siskiyou National Forest. No serpentine rock was visibly exposed, although *Chamaecyparis lawsoniana*, a serpentine affiliate, was present on one fen. *Pseudotsuga menziesii* and *Thuja plicata* were the major conifers here, and *Rhododendron occidentale* was present at all three fens. The first fen sampled in Oregon was in a fen with a couple centimeters of above-ground water, pebble-sized rocks, and some deep mud. There were many downed and mostly-soaked

logs present in the fen. Rare *Gentiana plurisetosa* was present. Peat moss covered the ground in spaces between grass clumps and other plants, so very little water was exposed. Large trees bordered the outside of the fen but caused little shadow over pitchers. The second fen had more canopy cover from alders and low branches of *Pseudotsuga menziesii*. *Darlingtonia californica* was scattered throughout a lightly flowing stream with rocks averaging around 30 cm wide throughout the growing area, with many rosettes attached to these rocks. Very little mud or peat moss occurred on the ground surface. At fen three there was a large pond adjacent to the population of *D. californica* sampled. Small *D. californica* plantlets were seen floating in 'self-made' islands with peat moss. Pitchers were collected near a foot trail in an area with deep mud where no rocks or water were present. Most of the vegetation around the pond was *Quercus vaccinifolium* and azalea, and large trees were absent from the immediate vicinity.

Six Rivers National Forest, California: This region had high levels of serpentine determined both by observations of exposed rock and presence of serpentine-indicating plants. These fens were almost directly due south of the Rogue River-Siskiyou fens and represent one of the farthest northwest populations of *D. californica* in California. The Smith River or one of its forks was never more than about 1 kilometer away, and it was the lowest elevation region at 155m above sea level. Pitchers from fen four, the first fen sampled in this region, were collected from a ditch directly adjacent to California state highway 199 with flowing water. *Chamaecyparis lawsoniana* and *Pinus jeffreyi* were present and causing about 20% canopy cover over the group of sampled pitchers. Small trees of *Chamaecyparis lawsoniana* and *Pinus jeffreyi* also caused scattered canopy cover

at fen five, and young Azalea shrubs were abundant. *Pinguicula*, another carnivorous plant was also present. Water was slightly flowing over boulders and bedrock, and peat moss covered around 30% of the ground. fen six had *Chamaecyparis lawsoniana*, *Pinus jeffreyi*, *Alnus* sp., and tree-like *Rhododendron occidentale* covering about 40% of the sampled population with their canopy. *Darlingtonia californica* plants were growing on mats of moss-covered bedrock with a very slight water flow.

Shasta-Trinity National Forest, California: Fens at this mountainous California region were the highest elevation fens in this study with an average elevation of 1582 meters above sea level. The pitchers were significantly shorter than the other four regions, with the average pitcher height being only 24cm. Fen seven was unique because *D. californica* was sparsely spread among tall grass in a large open fen/prairie containing a diverse plant community. The ground was mostly deep mud with tiny streams of water throughout, and no bedrock or stone was visible. This fen is at the highest point of California State Highway 3, where it meets the Pacific Crest Trail. At fen eight, pitchers were collected in a flowing stream running adjacent to California state highway 3, about two miles south of fen one. Large rocks averaging around 75 cm lined the stream and *D. californica* was found in fragmented patches of relatively small rosettes every 5 meters or so. There was about 20% canopy cover coming from *Pseudotsuga menziesii*, *Pinus monticola*, *Alder*, and *Rhododendron*. Fen nine was in the Trinity Alps Wilderness off of the Swift Creek trail. *Abies concolor* and *Salix* sp. Trees stretched across the small stream causing 40% canopy cover. Large boulders and various sized rocks lined the small stream, and most of the rosettes were submerged at their base by flowing water.

Plumas National Forest, California: Near the northern Sierra Nevada mountains, this was the eastern- and southern- most region sampled. Fens here were sampled near the Feather River at an average elevation of 1301 meters, and exposed serpentine rock was evident in all of the fens. At fen ten, pitchers were collected directly beside a rocky forest road with no canopy cover. Water was cascading over bedrock and *D. californica* was found growing sparsely and spread-out in an area with high diversity of habitat features. Fen eleven was in a ditch with lightly flowing water and pebble-sized rocks on the side of unpaved forest road near Butterfly Valley with about 20% canopy cover. Finally, fen twelve was on a mostly open hillside with high abundances of graminoids and grasses growing on bedrock mixed with smaller rocks. The ground was muddy with moss throughout and little-to-no flowing water.