Second Annual Survey on Narrowleaf Milkweed (*Asclepias fascicularis*) and Western Monarch Butterfly (*Danaus plexippus*) Populations in Pozo, CA

Hermann, E. R.

Abstract:

The decline in milkweed (*Asclepias* spp.) populations across the country, due to factors including agricultural development and herbicide use, has lead to a correlated reduction of monarch butterfly (*Danaus plexippus*) feeding and breeding habitat. In addition, the intentional planting of non-native milkweed in areas surrounding overwintering habitat has altered Western monarch butterfly migratory behavior, by allowing monarchs to depart from their ancestral migration pattern (timing and location), therefore decreasing the effective population size, and putting monarchs at risk of higher pathogen prevalence, due, in part, to an obligate protozoan parasite that is able to persist on the non-native, perennial species of milkweed. The presence of native milkweed plants in areas surrounding overwintering sites, such as in the Central Coast of California, provides a unique opportunity to potentially observe transitional resource utilization by monarchs that are present beyond the traditional overwintering season. If actually observed, a shift in phenology (time of resource use) shown by monarchs laying eggs on milkweed could signify a shift in population behavior. In regards to native milkweeds, we find that in the Los Padres National Forest, narrowleaf milkweed (*Asclepias fascicularis*) is locally restricted to streambeds. We come to this conclusion after mapping the distribution of milkweeds in relation to the streambed and testing hypotheses related to milkweed growth habits. Results show that there was a significant difference in the abundance and distribution of milkweed plants between the years 2016 and 2017. Stem abundance was not conclusively

correlated with environment preference for habitats inside vs. outside of the streambed. However, there was a significant increase in the number of monarch adults present in 2017. The information resulting from this study provides insight into current milkweed distribution and monarch behavior, as well as helps to inform future studies on the status of monarchs.

Keywords: monarch butterfly (*Danuas plexippus*), narrowleaf milkweed (*Asclepias fascicularis*), population distribution, environmental disturbance, population expansion

Introduction:

Monarchs, Migration and Milkweed

Monarch Butterflies (*Danaus plexippus*) lay their eggs exclusively on plants in the dogbane family (Aocynaceae), more specifically on milkweed plants in the genus *Asclepias* (L.) and other related genera of the Asclepiadoideae subfamily (Center for Biological Diversity, 2014). This co-evolutionary relationship between monarch butterflies and milkweed plants has been of interest to scientists since 1914 (Malcolm & Brower, 1989). Milkweed plants provide a source of food, nectar, and shelter for monarch larvae and adults, as well as many other insects (Allen, 2014). One of the intrinsic values of milkweed lies in the cardiac-active steroid compounds, or cardenolides, found in the plant's material. When sequestered through consumption, the cardenolides repel most predators due to their toxicity and bitter taste. Cardenolide concentrations vary across milkweed species, as well as cardenolide sequestration efficiency by monarchs, due to genetic and environmental differences, resulting in varied degrees of fitness for monarch individuals depending on the accessible milkweed species (Malcolm &

Brower, 1989). The cardenolide defense mechanism provides monarchs a means of survival and security throughout their lifecycle. Although monarchs may interact with a variety of plant species, milkweeds fulfill a unique and significant role within the lifecycle of the monarch, essentially paralleling the fates of monarchs and milkweeds.

Monarch butterflies have performed a multi-generational migration for thousands of years, a behavior that is considered a plesiomorphic trait of the subfamily, Danainae (Resh et al., 2009). However, the monarch migration is significantly longer than any other species in the taxon with an impressive multi-thousand mile journey (Resh et al., 2009; Malcolm & Brower, 1989). It is also thought that the migration cycle is evolutionarily based on the spatial and temporal diversification of milkweed plants across the United States (Resh et al., 2009; Malcolm & Brower, 1989). Within North America two monarch populations can be distinguished, an eastern and western population divided by the Rocky Mountains. There are a few non-migratory populations in the Southeastern United States, Mexico, and islands in the Pacific Ocean, but as far as is currently known these populations do not contribute to the population genetics of migratory monarchs (Center for Biological Diversity, 2014). The focus of this study is on Western monarchs, whose migration circulates from the Sierra Nevada Mountains, Nevada, Oregon, Washington, and sometimes Canada in the spring and summer down to the Central Coast of California to overwinter from October to February and back again. The length of the migration as well as the delicate morphology and noticeable aposomatic pattern of the monarch stands out to the public as a model migration organism.

Milkweed thrives as an environmental generalist across the United States, primarily filling a niche as a resilient plant in disturbed environments such as roadsides and along soybean and cornfields (Pleasants & Oberhauser, 2012). However, within the last decade and a half, a serious decline of milkweed plants in agricultural fields has been shown to correlate with a monarch population decline (Pleasants & Oberhauser, 2012). Despite the expansive range and variety of milkweed species, monarchs lay a higher density of eggs on milkweed plants found alongside agricultural fields, and are therefore at greater risk of being affected by habitat loss due to agricultural activity and glyphosate (RoundUp™) herbicide use (Pleasants & Oberhauser, 2012). The significant loss of monarch feeding and breeding habitat, decreased by nearly 60% in the Midwest alone, will yield detrimental impacts on the future of monarch population (Pleasants & Oberhauser, 2012). Therefore, understanding effective milkweed growth habits can lead to horticulturally useful information that may support monarch populations and change the tide of otherwise dismal circumstances.

Diversity of milkweed species

There are over 130 known species of milkweed in North America (Hanson et al., 2017), 27 of which provide effective breeding and feeding habitat for monarch butterflies. Within California alone, there are fifteen milkweed species. These are found in various habitats such as ruderal, oak woodland, desert, and riparian communities (Allen, 2014). There is a pattern of milkweeds being associated with continuously disturbed environments, an example of the persistence and success of milkweed plants in the face of varying conditions and competition with other species across landscapes.

In coordination with the timing of the monarch migration, California native milkweed blooms in early spring, providing habitat space for migrants, and then dies at the beginning of winter as the monarchs begin to cluster in the overwintering groves. In contrast, non-native milkweed, such as tropical milkweed (*Asclepias curassavica*) persists through the winter, allowing for monarchs to continue nectaring and reproducing on the plants into the winter. Not only does the presence of tropical milkweed plants alter the migratory behavior and breeding patterns of monarch butterflies, but also the perennial nature of the plants allows for the persistence of parasites such as O.E. (*Ophryocystis elektroscirrha*), which can be spread through a greater portion of the population when monarchs continuously use non-native milkweed plants (Altizer & Oberhauser, 1999).

Asclepias fascicularis Growth Habits

In this study, we will focus on narrowleaf milkweed (*Asclepias fascicularis*), a native species to California with the widest range out of the 15 California endemic (Allen, 2014). Previous study of cultivation techniques showed that narrowleaf milkweed produces strong and withstanding root bases in soils of medium to high nutrient concentration (e.g.: Canadian peatmoss, coarse perlite, dolomitic limestone, a long-lasting wetting agent, and a proprietary blend of silicon known as RESiLIENCE® [1] (Hanson et al., 2017). Narrowleaf milkweed is known to be drought-resistant within dry, barren environments between 50 to 2,200 m in elevation (Allen, 2014; Hanson et al., 2017). Levels of water-soluble fertilizer rates did not limit Narrowleaf milkweed, yet plant height and firmness of root plug, both signs of fitness and availability for monarchs, resulted from large pot size (Hanson et al., 2017). Spatial limitation of milkweed root growth supports the hypothesis that milkweed plants perform better in environments where disturbance removes competing plants. Despite the fact that *A. fascicularis*

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[1] (Sunshine Professional Mix #4 Natural & Organic; SunGro® Horticulture, Agawam, MA). Containers were amended with controlled-release fertilizer at one of two label-recommended rates per liter of the growing medium: 2.7 $g \cdot L^{-1}$ (low) or 6.5 $g \cdot L^{-1}$(high) of 18N–2.2P–10K (Osmocote® N–P–K blended with micronutrients, Everris Nursery Mix; Everiss NA, Dublin, OH))

has been shown to be the least preferred milkweed species as compared to three of the most common native species, it is still a necessary source of nutrients and habitat for monarch butterflies (Robertson et al., 2015). More importantly, with a decline in milkweed populations across the state, native populations of milkweed are becoming increasingly important to sustain monarch populations through habitat restoration (Hanson et al., 2017). Researchers have devoted time and money to understanding effective milkweed growth habits in order to cultivate growth for the plants, which correlates to an increase in monarch population sizes and support migratory monarch populations across the United States (Allen, 2014; Hanson et al., 2017; Pleasants et al., 2017). Although not an effective measure of population size, milkweed can be used as a proxy for monarch activity (Pleasants et al., 2017). Climate change and its extenuating consequences have contributed to changes in milkweed phenology and monarch behavior, especially in regards to migratory behavior and the persistence of monarchs in Central California past overwintering (Malcolm, 2018; Wenner & Harris, 1993). Therefore, though there is information on cultivation practices, it is also important to understand the abundance and distribution of narrowleaf milkweed in natural landscapes. In addition, natural landscapes provide study sites for the evaluation of phenology and potential climate change effects on phenology.

Project Site Selection

As previously mentioned, narrowleaf milkweed grows well in a variety of disturbed environments. Streambeds provide a consistent force of disturbance that eliminates competitors, while providing an abundance of available water and nutrients. The Salinas River runs through Central California, branching into various tributary streams within the area that provide appropriate environment for native milkweed species, including narrowleaf. A portion of the

Salinas River flows through Pozo, California, approximately 30 miles east of San Luis Obispo, San Luis Obispo County. The Cal Poly San Luis Obispo Biology Department has a working relationship with the U.S. Forest Service to perform research through an internship program at the Hi Mountain Condor Lookout near Pozo. Throughout the summer of 2016, Hi Mountain interns surveyed the area surrounding the Lookout and recorded the location of narrowleaf milkweed plants along three streams connected to the Salinas River. The initial study sought to understand if there was a nonrandom pattern of narrowleaf milkweed distribution in relation to the streambed, predicting that there would be a higher density of plants within the streambed, and dissipating density as distance from the bank increased. Not only would the number of plants follow the predicted abundance pattern, it was further predicted that the number of stems per plant would show a similar pattern. In correlation, by mapping and monitoring the distribution of milkweed in a localized environment, we would be able to determine if monarch butterflies are departing from the pattern of northward migration and instead making use of milkweed plants along the Central Coast.

In the summer of 2017, the Hi Mountain interns sought to build off of the previous year's data. Repeated data collection across two years within identical sites provides an opportunity to compare the spatial and temporal changes within an environment. The intention was to determine how the pattern of milkweed distribution and abundance was affected by the difference in rainfall between the drought year of 2016, and the wet year in 2017. Furthermore, with an assumed increase in milkweed presence, we expected monarch presence to increase proportionally.

Methods:

Data Collection Procedures

We sampled narrowleaf milkweed at four different tributaries that feed into the Salinas River near Pozo, San Luis Obispo County, California (Fig. 1). The sites were initially chosen for study in 2016 and then replicated in 2017. The accessibility from San Luis Obispo, known sightings of milkweed, and consistent disturbance due to water flow in the streambed and winter precipitation provide reason as to the selection of the sites. Information about start and end coordinates of the sites, metric length, number of patches sampled, and person hours needed to sample each site from 2016 and 2017 (if available) are detailed in Tables 1 and 2, respectively. The distance monitored per site was dependent on the availability and accessibility of patches. Limitations to recording visible patches included presence of poison oak and steep terrain. The first site began at 120° 18' 8" N, 120° 22' 43" W, 30 meters southwest of the Pozo fire station gate. This road was and can be inaccessible to private vehicles due to the unsafe road conditions. Site Two began at 35°17'32"N, 120° 23' 24" W, and consisted of the upstream section, southeast of Hi Mountain Lookout Road and the downstream section northwest of the road. The intersection of the tributary to the road is 0.5 km from the Pozo station gate, by way of the main road. Site Three was a continuation from the tributary of Site Two with the starting point denoted by a massive fallen valley oak (*Quercus lobata*) tree (35°17' 19" N, 120°23' 27" W). Site Four was located 6.4 km down the road from the Pozo Station Gate at 35°16'49"N, 120° 24' 14" W, and identified by a large rocky outcrop and a metal runoff tube on the south and north sides of the road respectively. In total 7.4 km of streambed were surveyed.

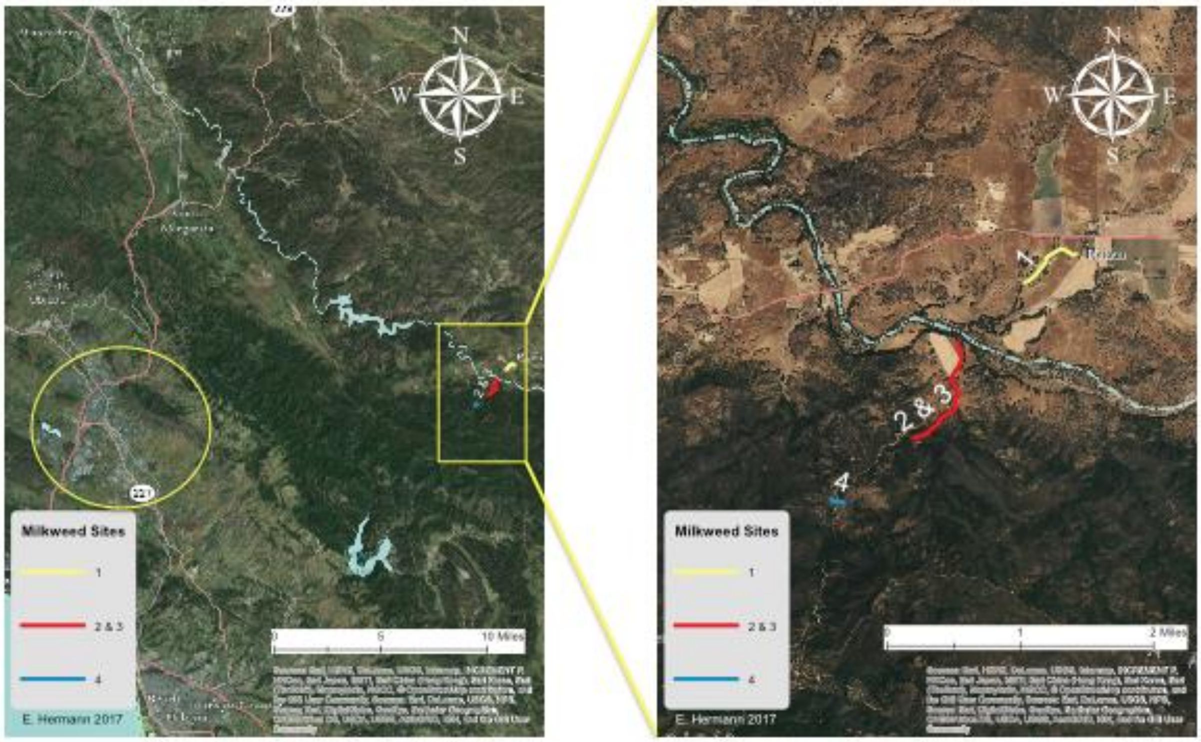

Figure 1: The map on the left displays spatial relationship between the city of San Luis Obispo in the circle, and Pozo in the square, as well as the Salinas River running NW to SE. The right map is a large-scale image of the length and distribution of the four sites within Pozo, CA.

Within each tributary, we scanned for patches and individual plants within the streambed and 20m of the center of the streambed. When we came across a plant we took note of the width of streambed (m), distance of each plant from center of streambed (m), number of stems, estimated percent (in increments of 20%) of live (healthy, green) plant tissue, presence or absence of herbivory on the leaves, and presence and classification of eggs and instars on the plant. Any sightings of adult monarch butterflies were recorded within the patch where they were identified.

As per the methods of the first year study, during the second year milkweed patches were determined by the most convenient grouping of milkweed plants within a 2 meter span based on the parameter size of GPS coordinate specificity. However, due to malfunctions of the system, we marked the GPS points through screen shots of an iPhone compass application at each 2m patch (Apple iPhone 6, iOS 9.0). The specificity of the iPhone's GPS tracking was not tested before beginning the survey, therefore it may not have been necessary to limit patch size to 2 meters (if resolution was <2m). Segmenting plants into arbitrary patches may not have been worthwhile for the time it took to count each patch and re-measure the streambed at each patch.

A useful technique when measuring plants is to look for stems angling towards each other, plants often have anywhere from 1 – 30 stems in a single plant. Be gentle when counting stems so as to not disrupt eggs and caterpillars present on the plant.

Table 1: Field site information from initial survey in 2016. See Table 2 for 2017. During collection, the data was not segregated by site so the total number of patches and the timeline of data collection are consolidated for the entire season. The start and end coordinates are approximate as the official initial GPS coordinates were irretrievable, but the survey years were intended to be identical replicates with the same start and end points. Person hours were not recorded due to lack of record keeping.

2016

Site ID	Approx. Start Coordinate	Approx. End Coordinate	Number of Patches	Date (s)
1	120° 18' 8" N 120° 22' 43" W	120° 18' 8" N 120° 22' 49" W	137	7/22/16 – 8/2/16
2	35°17'32"N 120° 23' 24" W	35°17'19"N 120° 23' 27" W		
3	35°17' 19" N 120°23' 27" W	35°17'14"N 120° 23' 38" W		
4	35°16'49"N 120° 24' 14" W	35°16' 49"N 120° 24' 10" W		

Table 2: Field site information from 2017 replicate survey. Start and end coordinates should mirror the locations surveyed from the year prior with potential variation in the end coordinates due to variation in abundance and distribution of plants, as well as accessibility within sites (i.e. fallen logs, thick vegetation, and/or poison oak (*Toxicodendron diversilobum).* Person hours were recorded throughout the course of the study period by means of the Hi Mountain journal in which each intern recorded an entry of the day's activities throughout the summer (Hermann et al., 2017).

2017

Site ID	Start Coordinate	End Coordinate	Site Length (km)	Number of Patches	Person Hours	Date(s)
1	120° 18' 8" N 120° 22' 43" W	120° 18' 8" N 120° 22' 49" W	1.6	18	17.5	7/26/17
2	35°17'32"N 120° 23' 24" W	35°17'19"N 120° 23' 27" W	3.6	156	65	7/26/17 – 8/16/17
3	35°17' 19" N 120°23' 27" W	35°17'14"N 120° 23' 38" W	1.2	34	18	8/10/17 – 8/16/17
4	35°16'49"N 120° 24' 14" W	35°16' 49"N 120° 24' 10" W	.1	23	10	8/16/17

Data Analysis Procedures

The purpose of this report is to understand the population size and distribution of milkweed plants in relation to streambeds, as the information can be used as an indicator of healthy populations and useful for milkweed population management. Therefore, it is necessary to define what it means for a plant to be either "inside" or "outside" of the streambed. The streambed itself was defined as the area between the two stream banks. We then divided the distance from the center of the streambed to the bank into 0.5-meter interval segments in order to detail the abundance of plants emanating from the center of the streambed. Then, we calculated the average width of the streambed, which resulted in 1.45 meters in 2016 and 1.7 meters in 2017.

Next we rearranged the data in order of increasing distance from the center of the streambed and separated the data so that all plants with a distance of less than 0.725m, half of the average stream width of 1.45m, would on average be inside of a streambed in 2016, and all of the plants with a distance of less than 0.85, half of 1.7m, would on average be inside of a streambed in 2017. This definition allows for a more holistic view of the plant-streambed relationship as the effect of the streambed is more aptly described through this pattern than through a distance measurement for each individual plant (as the streambed and not the distance is the variable of interest). It should be noted that a band of 20m from the streambed center was searched in each year. Thus in 2016 1.45m of streambed times 7.4 km (or 10.73 hectares) of streambed and 18.55 m times 7.4 km (or 137.27 hectares) of upland habitat were searched. Thus 12.8 times more upland habitat than stream habitat was searched. While in 2017, 1.7m of streambed times 7.4 km (or 12.58 hectares) of streambed and 18.3 m times 7.4 km (or 135.42 hectares) of upland habitat were searched. Thus 10.76 times more upland habitat than stream habitat was searched.

In order to effectively analyze the data collected, we separated the procedure into four main questions. The first question considers the relationship of plant frequency to the distance from the center of the streambed. This frequency distribution should help show the relationship of plant abundance relative to the streambed itself, and as the distance from the streambed increases.

The second question seeks to determine the relationship between stem abundance in relation to the plant's location to the center of the streambed. How does the presence of water affect the material output of the plant? T-test and Wilcoxon/ Kruskal-Wallis rank sum tests were used to compare the difference in stem count in 2016 and 2017.

The third question compares the plant distribution in relation to the streambed over the two study years. We used a contingency table to determine if the number of plants inside the streambed was significantly different from the number outside the streambed in 2016 and 2017, as well as between the years.

The fourth question categorizes the number of monarch larvae and adults present in the two years, providing insight on the usability of the milkweed patches as appropriate habitat space for monarch butterflies. We used T-test and Wilcoxon rank sum tests compare the raw numbers of monarch larvae and adults present within the sites. All tests were performed through JMP 12.2 Pro.

Results:

Plant Distribution

In 2016, there were 1,175 plants inside the average width of the stream (1.45m) and 829 plants outside the average width (up to 17 meters from the bank of the stream) (Pearson: 96.626, $p< .0001$, Figure 2). In 2017, there were 1,728 plants inside the average width of the streambed (1.7 m) and 648 plants outside (up to 15.5 m from the streambed (Pearson: 96.626, $p< .0001$, Figure 2). This was a significantly greater amount of plants inside the streambed compared to outside (Fisher's Exact test: $p<0.0001$). Overall, there were more plants in 2017 than in 2016, where the number of plants inside the stream was significantly greater in 2017 than in 2016 (Chi-Square= 96.626, DF=1, $p< 0.0001$), and there were a significantly greater number of plants outside the streambed in 2016 than in 2017 (Fisher's Exact test: $p<0.0001$). The probability of plant location in relation to the streambed was significantly different when compared between the two years (Fisher's Exact test: $p<0.0001$). In summary, during the dry year (2016) a greater proportion of the plants were outside the stream bed, while in the wetter year (2017) there was a greater number of plants over all (relative to 2016), but a smaller proportion of the wet year plants were outside the stream bed. Effectively, the wet year had more plants and more of them were in the streambed than during the dry year. Given that the area searched outside of the streambed is 10-12 times the area searched within the streambed, these results demonstrate a much greater relative abundance of narrow leaf milkweed within the stream than beyond the stream.

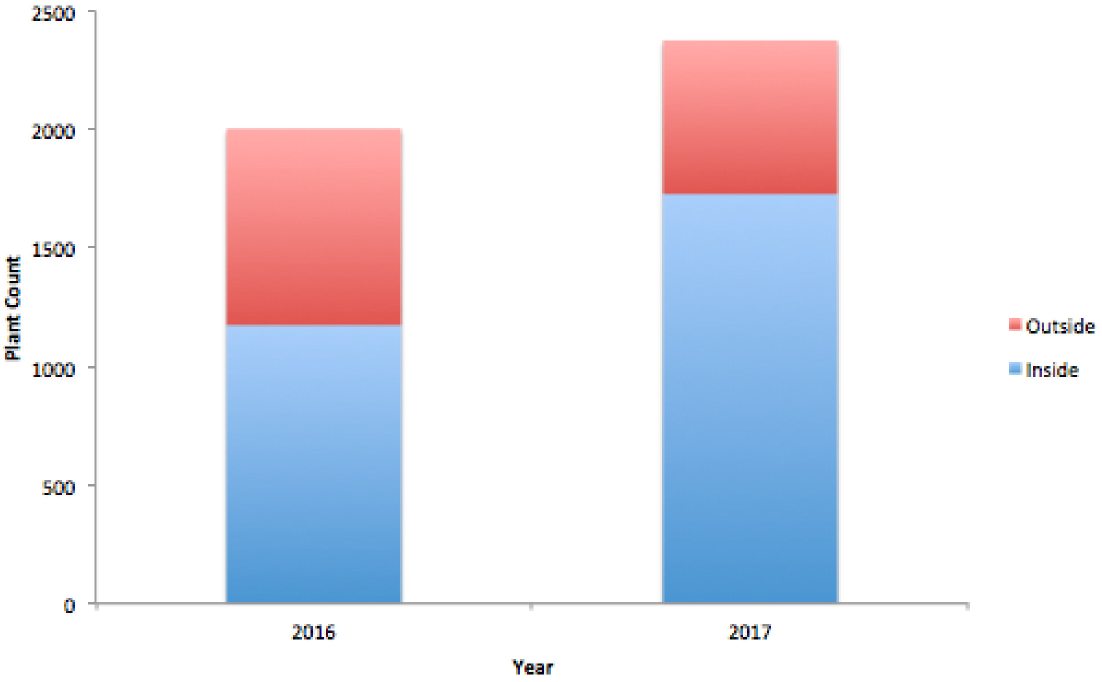

Figure 2: The number of plants inside and outside the streambed across a drought year (2016) and a wet year (2017). More plants were present in 2017, but more of the plants present in 2016 were outside the streambed. Also, plants had more stems in 2016 than in 2017, though attributing stem proportions to inside and outside across years is inconclusive.

Stem Abundance

On average, there was a marginal difference in the number of stems per plant in 2016 as compared to 2017 (2016 = 2.22, 2017 = 2.17). In total, the number of stems on plants in 2016 is significantly greater than the number of stems on plants in 2017 (ChiSquare = 8.5, DF = 1, p = 0.0035). There were a significantly greater number of stems outside the stream in 2016 (DF = 1268.026, p< 0.0001), and no significant difference between inside and outside in 2017 based on a t-test (DF = 1187.129, p = 0.1397). However, the results of the Wilcoxon test showed that there was a significant difference in stem abundance for both 2016, with more stems outside (ChiSquare = 6.2016, DF = 1, p = 0.0128), and 2017, with a greater amount inside (ChiSquare = 6.5308, DF = 1, p = 0.0106). This result is inconclusive. Therefore we cannot significantly determine if plant size (as measured by number of stems) was dependent on drought condition. If we also consider the overall number of plants in 2016 and 2017 (Figure 2), we would conclude that in the wetter year plant number was greater, but not plant size.

Larvae & Adults

The analysis used to compare the numbers of larvae and adults present in the two years resulted in a significant increase in adults in 2017 based on both the t-test and the Wilcoxon test for inside the streambed (t-ratio = 4.56, DF = 1727, t> 0.0001; Z = -3.88, p=0.0001), as well as outside (t-ratio = 2.24, DF = 647, t> 0.0126; Z = 2.53, p = 0.0113). Effectively, this could represent an increase in monarchs that corresponds with in increase in plant number.

Plant Frequency

Plant frequency in relation to distance from the streambed follows the Poisson distribution (Figures 3 & 4). As previously mentioned, the average width of the stream was calculated in order to standardize the analysis of plants inside vs. outside the streambed and provide an informative comparison of the relationship of frequency and location.

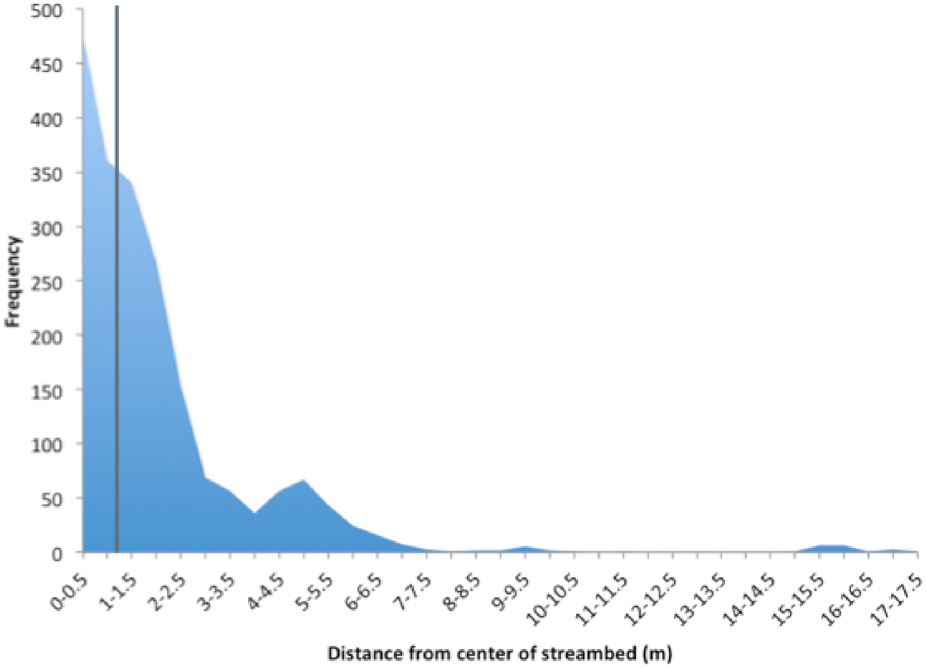

Figure 3: In 2016, the frequency of plants peaks in the center of the streambed and dissipates as the distance from the center increases. Bold vertical line demarcates the edge of the streambed. The figure plainly shows that the amount of area searched outside of the streambed is 12 times greater than the area within the streambed.

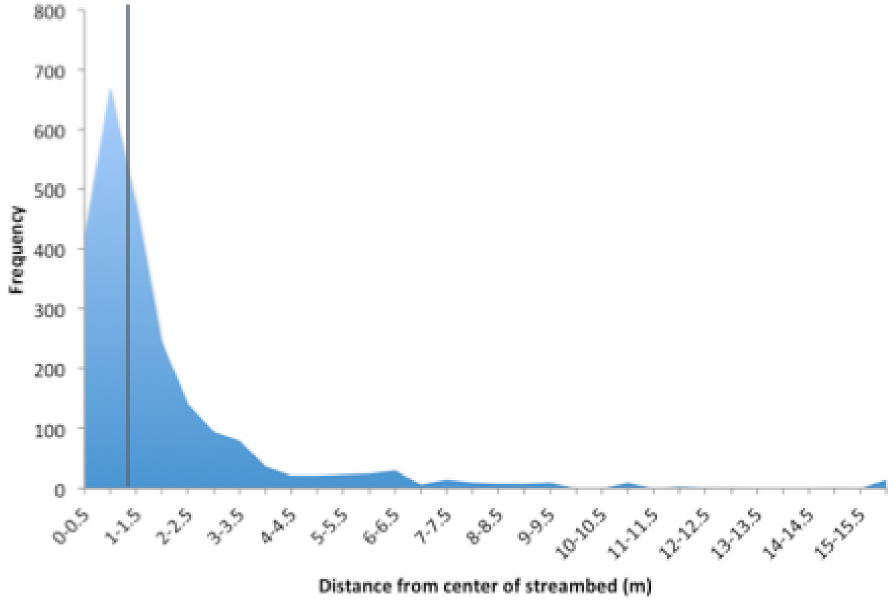

Figure 4: In 2017, the frequency of plants peaks near the center of the streambed and dissipates as the distance from the center increases. Bold vertical line demarcates the edge of the streambed. The figure plainly shows that the amount of area searched outside of the streambed is 10 times greater than the area within the streambed.

Discussion:

Milkweed Distribution

We found support for our hypothesis that the abundance of milkweed plants, as well as the distribution varied from a "dry" year (2016) to a "wet" year (2017). We suppose that greater water availability due to an unprecedented level of rainfall in the winter of 2016-2017 supported a larger milkweed population size throughout the late summer of 2017. Though the greater rainfall does not support larger plants overall (as measured by number of stems). In turn, the abundance of narrowleaf milkweed seems to act as a strong attractor for monarch butterflies (as the plants visually dominate the environment, sometimes growing in thick bunches up to 4 feet tall) since monarchs were more common when milkweed was more common.

Interestingly enough, plant distribution in 2016, towards the end of the drought period, had a greater total range than in 2017, meaning that they extended further from the streambed in 2016. Although noting that the presence of milkweed is sporadic and would have relatively poor fit to the Poisson distribution after approximately 7.5m (Fig. 3). One possible interpretation of this result is that in a drought year there is less competition from other plants and thus milkweed extends further from the streambed. While in a wetter year, if there is more competition, milkweed might be restricted to the better (i.e.: more disturbed) conditions near the streambed. If this is true, then one might predict that plants should be bigger (as measured by number of stems) outside of the stream in drought years, and larger inside of the streambed during “normal” years.

Stem Abundance

The measurement of stem abundance was meant to act as a proxy for quality of environment in that a plant would be able to produce greater biomass in an environment that was not limited by nutrients or water. The results from this study show a marginal difference in the number of stems between the years. More specifically, stem count was significantly greater in 2016 outside the streambed, and inside for 2017. These results do not align with the hypothesized impact of rain (bottom up or resource driven hypothesis) on the streambed environment. But they do support a competitive release hypothesis during drought years and a disturbance or resource abundance driven hypothesis during normal years.

Monarch Presence

The widespread distribution of milkweed across the United States and its use as breeding habitat for north- and south-bound monarch migrants, as well as the summer abundance due to the perennial growth habit means that milkweed plays a significant role in the life cycle of monarch butterflies (Malcolm & Brower, 1989). Our results show that the number of monarch adults significantly increased between the years, apparently supported by the increase in milkweed individuals.

Some sources state that Western monarch populations are supported and replenished by individuals from the Eastern population (Center for Biological Diversity, 2014). Therefore, management of Western monarchs is useful, yet efforts to increase the Eastern population are of extreme importance. Though the degree of demographic connectivity between Eastern and Western populations may still need to be fully discerned.

Plant Frequency

Streams provide a source of water, nutrients, and disturbance that eliminates competition, within the area impacted by water movement. There are currently no publications available identifying the extent to which these characteristics impact plant species, especially narrowleaf milkweed, growing in and around streams. However, it is known that milkweed thrives in riparian plant communities, without limitation on space (Allen, 2014; Hanson et al., 2017). One of the most intriguing aspects to the distribution of plants in relation to the streambed is their peak in frequency at the center of the stream in 2016, and their shift in peak frequency approximately a meter from center of the streambed in 2017. This pattern potentially reveals inhibition of plant growth that could be due to the inability for seeds to germinate when fully submerged, or an overwhelming level of disturbance. When considering growth habits for narrowleaf milkweed, it may be meaningful to investigate if the level of disturbance may be of significant by removing competition from any other plants.

Implications for Future Studies

Although only some of our hypotheses were resolved through this experimental process, we were able to find out more information about monarch presence and the patterns of narrowleaf milkweed distribution in relation to streambeds, which will contribute to the bank of knowledge needed to design and readjust the experimental process for future years. In order to better capture an image of monarch habitat use in the area, it would be helpful to monitor presence in the fall and winter, as well as the spring/summer to show a difference and a potential dissipation of presence and resource utilization. Increased presence may have a correlation with the increase in plants and their spread from the streambed.

Due to the abnormalities of narrow leaf milkweed, lowest levels of cardenolides and least preference in Californian milkweed varietals, it might be prudent to expand this study to include other milkweed species in order to develop a more effective proxy to understand monarch health in future years (Robertson et al., 2015). However, the other species present in this general area is Woolypod milkweed (*A. eriocarpa*), which has been shown to detract monarch larvae due to the abundance in leaf hairs (Robertson et al., 2015). Woolypod milkweed tends to occur more on roadsides that within streambeds like narrowleaf. It would be an interesting investigation to compare the abundance of visits depending on the location and species of milkweed. Similarly, inclusion of a nearby milkweed species could provide intriguing information about milkweed use throughout the season, as well as a comparative look at the milkweed preference within.

No matter the changes to the study methods, it will be helpful to increase the amount of information in order to expand our view of this localized community interaction. Unfortunately, due to the fact that the study is based on a study across summer and fall seasons, it will be challenging to expand the number of replicates. It would also be interesting to look into other sites and determine if this is a reproducible pattern.

Acknowledgements:

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Appendix 1:

Site One: Pozo Station (Total Patches: 17)

Patch ID	Plant Count	Latitude	Longitude
1	18	35° 18' 8" N	120° 22' 43" W
2	5	35° 18' 8" N	120° 22' 43" W
3	19	35° 18' 8" N	120° 22' 43" W
4	19	35° 18' 8" N	120° 22' 43" W
5	1	35° 18' 8" N	120° 22' 43" W
6	1	35° 18' 8" N	120° 22' 43" W
7	1	35° 18' 8" N	120° 22' 42" W
8	1	35° 18' 8" N	120° 22' 42" W
9	18	35° 18' 8" N	120° 22' 42" W
10	10	35° 18' 8" N	120° 22' 42" W
11	6	35° 18' 8" N	120° 22' 42" W
12	9	35° 18' 8" N	120° 22' 42" W
13	11	35° 18' 8" N	120° 22' 42" W
14	9	35° 18' 9" N	120° 22' 43" W
15	37	35° 18' 8" N	120° 22' 43" W
16	24	35° 18' 10" N	120° 22' 43" W
17	1	35° 18' 8" N	120° 22' 49" W

Site Two: Tamarisk - Upstream (Total Patches: 124)

Patch ID	Plant Count	Latitude	Longitude
1	1	35° 17' 29" N	120° 23' 27" W
2	6	35° 17' 29" N	120° 23' 27" W
3	3	35° 17' 29" N	120° 23' 27" W
4	4	35° 17' 29" N	120° 23' 27" W
5	4	35° 17' 29" N	120° 23' 26" W
6	2	35° 17' 29" N	120° 23' 26" W
7	8	35° 17' 29" N	120° 23' 26" W
8	1	35° 17' 29" N	120° 23' 26" W
9	1	35° 17' 29" N	120° 23' 26" W
10	2	35° 17' 29" N	120° 23' 26" W
11	11	35° 17' 29" N	120° 23' 26" W
12	1	35° 17' 29" N	120° 23' 26" W
13	1	35° 17' 29" N	120° 23' 26" W
14	3	35° 17' 29" N	120° 23' 27" W
15	4	35° 17' 29" N	120° 23' 27" W
16	7	35° 17' 29" N	120° 23' 26" W
17	5	35° 17' 29" N	120° 23' 26" W
18	4	35° 17' 29" N	120° 23' 26" W
19	5	35° 17' 29" N	120° 23' 27" W
20	1	35° 17' 29" N	120° 23' 27" W
21	9	35° 17' 29" N	120° 23' 26" W

22	8	35° 17' 28" N	120° 23' 27" W
23	1	35° 17' 29" N	120° 23' 26" W
24	1	35° 17' 29" N	120° 23' 26" W
25	1	35° 17' 28" N	120° 23' 27" W
26	1	35° 17' 28" N	120° 23' 27" W
27	4	35° 17' 28" N	120° 23' 27" W
28	10	35° 17' 28" N	120° 23' 24" W
29	2	35° 17' 28" N	120° 23' 24" W
30	2	35° 17' 28" N	120° 23' 24" W
31	4	35° 17' 29" N	120° 23' 25" W
32	10	35° 17' 28" N	120° 23' 25" W
33	2	35° 17' 28" N	120° 23' 25" W
34	5	35° 17' 27" N	120° 23' 26" W
35	5	35° 17' 30" N	120° 23' 22" W
36	4	35° 17' 28" N	120° 23' 26" W
37	8	35° 17' 28" N	120° 23' 26" W
38	11	35° 17' 28" N	120° 23' 26" W
39	2	35° 17' 28" N	120° 23' 26" W
40	10	35° 17' 28" N	120° 23' 26" W
41	21	35° 17' 28" N	120° 23' 26" W
42	14	35° 17' 30" N	120° 23' 28" W
43	6	35° 17' 30" N	120° 23' 28" W
44	20	35° 17' 30" N	120° 23' 28" W

45	8	35° 17' 30" N	120° 23' 28" W
46	9	35° 17' 30" N	120° 23' 28" W
47	6	35°17'26"N	120° 23' 26" W
48	13	35°17'27"N	120° 23' 28" W
49	2	35°17'27"N	120° 23' 28" W
50	21	35°17'27"N	120° 23' 28" W
51	7	35°17'27"N	120° 23' 28" W
52	5	35°17'27"N	120° 23' 28" W
53	4	35°17'27"N	120° 23' 28" W
54	12	35°17'27"N	120° 23' 28" W
55	1	35°17'27"N	120° 23' 28" W
56	8	35°17'27"N	120° 23' 28" W
57	18	35°17'27"N	120° 23' 28" W
58	16	35°17'27"N	120° 23' 28" W
59	3	35°17'26"N	120° 23' 28" W
60	25	35°17'27"N	120° 23' 28" W
61	1	35°17'25"N	120° 23' 28" W
62	14	35°17'26"N	120° 23' 27" W
63	17	35°17'27"N	120° 23' 26" W
64	3	35°17'27"N	120° 23' 28" W
65	5	35°17'26"N	120° 23' 28" W
66	5	35°17'26"N	120° 23' 28" W
67	5	35°17'26"N	120° 23' 28" W

68	17	35°17'25"N	120° 23' 27" W
69	25	35°17'28"N	120° 23' 30" W
70	15	35°17'25"N	120° 23' 27" W
71	15	35°17'27"N	120° 23' 26" W
72	9	35°17'26"N	120° 23' 26" W
73	24	35°17'26"N	120° 23' 27" W
74	6	35°17'26"N	120° 23' 27" W
75	3	35°17'24"N	120° 23' 27" W
76	16	35°17'24"N	120° 23' 27" W
77	6	35°17'24"N	120° 23' 27" W
78	15	35°17'24"N	120° 23' 27" W
79	3	35°17'24"N	120° 23' 27" W
80	8	35°17'24"N	120° 23' 27" W
81	9	35°17'23"N	120° 23' 27" W
82	11	35°17'23"N	120° 23' 27" W
83	11	35°17'23"N	120° 23' 27" W
84	30	35°17'23"N	120° 23' 27" W
85	17	35°17'23"N	120° 23' 28" W
86	37	35°17'22"N	120° 23' 26" W
87	12	35°17'23"N	120° 23' 27" W
88	5	35°17'22"N	120° 23' 27" W
89	87	35°17'22"N	120° 23' 28" W
90	2	35°17'23"N	120° 23' 27" W

91	4	35°17'22"N	120° 23' 28" W
92	1	35°17'22"N	120° 23' 28" W
93	11	35°17'22"N	120° 23' 28" W
94	26	35°17'22"N	120° 23' 28" W
95	20	35°17'22"N	120° 23' 28" W
96	19	35°17'22"N	120° 23' 28" W
97	15	35°17'22"N	120° 23' 28" W
98	8	35°17'22"N	120° 23' 28" W
99	35	35°17'22"N	120° 23' 28" W
100	38	35°17'22"N	120° 23' 28" W
101	13	35°17'22"N	120° 23' 28" W
102	27	35°17'22"N	120° 23' 28" W
103	5	35°17'22"N	120° 23' 28" W
104	17	35°17'22"N	120° 23' 28" W
105	11	35°17'22"N	120° 23' 28" W
106	12	35°17'22"N	120° 23' 28" W
107	2	35°17'22"N	120° 23' 28" W
108	9	35°17'22"N	120° 23' 28" W
109	13	35°17'16"N	120° 23' 27" W
110	22	35°17'16"N	120° 23' 27" W
111	3	35°17'16"N	120° 23' 27" W
112	4	35°17'20"N	120° 23' 28" W
113	23	35°17'20"N	120° 23' 27" W

114	8	35°17'20"N	120° 23' 27" W
115	1	35°17'20"N	120° 23' 27" W
116	9	35°17'20"N	120° 23' 27" W
117	13	35°17'20"N	120° 23' 27" W
118	2	35°17'20"N	120° 23' 27" W
119	38	35°17'20"N	120° 23' 26" W
120	51	35°17'20"N	120° 23' 26" W
121	2	35°17'20"N	120° 23' 26" W
122	1	35°17'19"N	120° 23' 27" W
123	13	35°17'19"N	120° 23' 27" W
124	3	35°17'19"N	120° 23' 27" W

Site Two: Tamarisk - Downstream (Total Patches: 32)

Patch ID	Plant Count	Latitude	Longitude
1	15	35°17'30"N	120° 23' 27" W
2	1	35°17'30"N	120° 23' 27" W
3	14	35°17'31"N	120° 23' 26" W
4	11	35°17'31"N	120° 23' 26" W
5	5	35°17'30"N	120° 23' 27" W
6	11	35°17'30"N	120° 23' 26" W
7	32	35°17'30"N	120° 23' 26" W
8	11	35°17'30"N	120° 23' 26" W
9	8	35°17'31"N	120° 23' 26" W
10	15	35°17'31"N	120° 23' 26" W
11	6	35°17'31"N	120° 23' 26" W
12	11	35°17'31"N	120° 23' 26" W
13	5	35°17'31"N	120° 23' 26" W
14	4	35°17'31"N	120° 23' 26" W
15	6	35°17'31"N	120° 23' 25" W
16	12	35°17'31"N	120° 23' 25" W
17	3	35°17'31"N	120° 23' 25" W
18	5	35°17'31"N	120° 23' 25" W
19	6	35°17'31"N	120° 23' 25" W
20	8	35°17'31"N	120° 23' 26" W
21	12	35°17'31"N	120° 23' 26" W

22	7	35°17'32"N	120° 23' 25" W
23	3	35°17'32"N	120° 23' 25" W
24	3	35°17'32"N	120° 23' 25" W
25	7	35°17'32"N	120° 23' 25" W
26	4	35°17'32"N	120° 23' 25" W
27	3	35°17'32"N	120° 23' 25" W
28	11	35°17'32"N	120° 23' 25" W
29	8	35°17'32"N	120° 23' 24" W
30	6	35°17'32"N	120° 23' 24" W
31	7	35°17'32"N	120° 23' 24" W
32	6	35°17'32"N	120° 23' 24" W

Site Three: Log (Total Patches: 34)

Patch ID	Plant Count	Latitude	Longitude
1	11	35°17' 19" N	120°23' 27" W
2	19	35°17' 19" N	120°23' 27" W
3	21	35°17' 19" N	120°23' 27" W
4	5	35°17' 19" N	120°23' 27" W
5	13	35°17' 19" N	120°23' 27" W
6	1	35°17'19" N	120°23'28"W
7	45	35°17'19" N	120°23'28"W
8	7	35°17'19" N	120°23'28"W
9	7	35°17'18"N	120°23'28"W
10	18	35°17'18"N	120°23'28"W
11	21	35°17'18"N	120°23'28"W
12	10	35°17'19"N	120°23'29"W
13	29	35°17'18"N	120°23'29"W
14	5	35°17'18"N	120°23'29"W
15	14	35°17'18"N	120°23'29"W
16	2	35°17'18"N	120°23'29"W
17	14	35°17'18"N	120°23'29"W
18	19	35°17'18"N	120°23'29"W
19	14	35°17'18"N	120°23'29"W
20	3	35°17'18"N	120°23'30"W
21	4	35°17'17"N	120°23'32"W

22	3	35°17'17"N	120°23'32"W
23	4	35°17'17"N	120°23'32"W
24	20	35°17'16"N	120°23'34"W
25	35	35°17'16"N	120°23'34"W
26	9	35°17'17"N	120° 23' 33" W
27	23	35°17'17"N	120° 23' 33" W
28	5	35°17'15"N	120° 23' 34" W
29	8	35°17'15"N	120° 23' 37" W
30	22	35°17'15"N	120° 23' 37" W
31	23	35°17'15"N	120° 23' 37" W
32	5	35°17'15"N	120° 23' 37" W
33	2	35°17'14"N	120° 23' 38" W
34	2	35°17'14"N	120° 23' 38" W

Site Four: Oak Woodland - Upstream (Total Patches: 10)

Patch ID	Plant Count	Latitude	Longitude
1	9	35°16'48"N	120° 24' 13" W
2	10	35°16'48"N	120° 24' 13" W
3	4	35°16'48"N	120° 24' 13" W
4	6	35°16'48"N	120° 24' 13" W
5	3	35°16'48"N	120° 24' 13" W
6	7	35°16'49"N	120° 24' 14" W
7	3	35°16'49"N	120° 24' 14" W
8	4	35°16'49"N	120° 24' 14" W
9	3	35°16'49"N	120° 24' 14" W
10	2	35°16'49"N	120° 24' 14" W

Four: Oak Woodland - Downstream (Total Patches: 13)

Patch ID	Plant Count	Latitude	Longitude
1	8	35°16'48"N	120° 24' 12" W
2	9	35°16'49"N	120° 24' 12" W
3	3	35°16'49"N	120° 24' 12" W
4	24	35°16'49"N	120° 24' 12" W
5	12	35°16'48"N	120° 24' 12" W
6	5	35°16'48"N	120° 24' 11" W
7	1	35°16'49"N	120° 24' 11" W
8	3	35°16'49"N	120° 24' 11" W
9	10	35°16'49"N	120° 24' 10" W
10	1	35°16'49"N	120° 24' 10" W
11	2	35°16'49"N	120° 24' 10" W
12	21	35°16'49"N	120° 24' 10" W
13	15	35°16'49"N	120° 24' 10" W

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