Saniee Methods

# Study Sites

Our initial method of determining which groves to study was to choose 25 groves from the top 50 ranked groves according to Pelton et al. 2016. We wanted groves to cover a large geographic range. We also wanted them to be representative, which we defined as having 1000 or more butterflies. We were unable to use 25 groves since western monarch population sizes were at a historical low during our survey year (Pelton et al. 2019, westernmonarchcount.org) and monarchs were not present at many of the groves they had occupied in previous winters. These restrictions reduced the geographic range we could sample. For example, we planned on collecting data at groves in Marin and Monterey Counties, but groves in those areas had few, if any, overwintering monarchs in fall of 2018 and winter of 2019.

Therefore, we collected data at nine groves along the central California coast from Ventura (V), through Santa Barbara (SB) to San Luis Obispo (SLO) counties (Figure 1). The groves from south to north are: Arundell Barranca (V), Harbor Blvd (V), Tecolote Canyon (SB), Hollister Ranch (SB), Spring Canyon Vandenberg Air Force Base (SB), Black Lake (SLO), Oceano Campground (SLO), Pismo Beach State Park (SLO), and Morro Bay Golf Course (SLO). These sites were chosen because the sites had the largest populations we could locate, and we were able to obtain permission to conduct the study from all property owners.

# Duration of Study

The start and stop dates at each grove varied (Figure 2). We initially planned to start collecting data when the number of overwintering monarchs reached ³1000 butterflies per grove. But, when we realized the population was going to be historically low, thus preventing us from satisfying our condition of population size, we began collecting data in early December (start dates in Figure 2). We continued to collect data at each grove until there were no longer aggregating monarchs, which is naturally variable across location (end dates in Figure 2). In summary, though there was a clear study design, it was impossible to execute it due to the low population size and limited occupancy, therefore our sampling became adaptive.

# Sampling Design

We collected climatic data at five locations, herein referred to as sample locations, within each of the nine groves (Figure 3). At each grove one sample location was at an accessible monarch aggregation (sometimes referred to as clusters by other authors). This location (Figure 3 - aggregation) represents selected attributes (microclimate and microhabitat). A second location was inside the grove and halfway between the aggregation’s location and the edge of the grove in the southwest direction (Figure 3 SW interior). A third location was inside the grove and halfway between the aggregation’s location and the edge of the grove in the northeast direction (Figure 3 - NE interior). These second and third locations represent random sites within the grove, and 8 potentially represent either suitable but unoccupied microclimate and microhabitat or unsuitable microclimate and microhabitat. Two more sample locations were on the outer edge of the grove. Location four was on the southeast grove edge relative to the aggregation’s location and represents maximum morning sunlight and storm wind exposure (Figure 3 - SE edge). Location five was on the northwest grove edge relative to the aggregation’s location and represents the minimum light exposure and maximum prevailing wind exposure (Figure 3 - NW edge).

## Microclimatic Weather Station Instruments

To collect climatic data, we built small weather stations and placed one at each of the five sample locations (Figure 3) within each of the nine groves (Figure 1) for a total of 45 stations. Each station consisted of a light intensity (L hereafter) data logger (HOBO Pendant Temperature/Light 8K Data Logger, Part # UA-002-08) measured in lux, a humidity and temperature (H and T hereafter) data logger (Lascar EL-USB-2) measured in percent and degrees Celsius, respectively, and a wind speed and direction (WS and D hereafter) data logger (RainWise WindLog Wind Data Logger) measured in meters per second. The WS and D sensor was a propeller on a swivel arm mounted on a directional PVC support set to true north. The H and T logger was attached to this PVC with a locking collar. The L sensor was anchored onto the H and T collar using zip ties and positioned to face southeast.

## Microclimatic Weather Station Deployment

Weather stations were hung at the aggregation’s location first and were placed at the height of and within 2 meters of the aggregation. This station (n = 1) and all others (n = 4) in each individual grove were hung at this same height (± 1m). Thus, the height of the sensors varied between groves, but not within groves. Telescoping poles that supported the weather stations were supported in place in the following manner. In each sample location, we chose a base tree with a sturdy trunk to attach the equipment base (lock box). The base of the telescoping pole was inserted into the lockbox attached with screws (within the lockbox) or with steel cable (Pro Strand,1/8” dia., Part No.: 21005100) to the base tree. We then found a second tree that had an accessible branch at the same height or higher than the monarch aggregation in that grove. We put a steel cable (Pro Strand,1/8” dia, Part No.: 21005100) over this branch by attaching a weight at the end of the cable (spooled) and lifting the weight over the branch with an extendable pole. The cable was placed over the branch so that the weighted end of the cable was on the side of the branch facing the tree where the equipment base would be attached. By connecting this cable to the tip end of the telescoping pole (with sensors), we could lift and guide the pole into place.

The wind meter was inverted to allow an upward attachment of the PVC support (and the entire weather station) to the end of the telescoping pole in the following manner. The base of the wind meter’s PVC support was connected to (screwed) a custom-built directional attachment we call the “insert.” The insert, anchoring the entire weather station, fit into a custom-built directional sleeve that hangs down vertically from a hinge at the end of the telescoping aluminum pole. The sleeve’s direction relative to north could be adjusted. The insert rotated and locked with the sleeve resulting in directionality, so we could ensure directionality to our measurements with the “north” label on the wind meter facing north on the sleeve, and the L facing southeast.

We threaded paracord through the base of the pole, through the directional sleeve, through the insert, and tied it off so that it was locked to the tip of the insert while the insert was attached to the wind meter. The insert of the weather station could be separated from the sleeve of the aluminum pole by allowing the weight of the station to pull on the paracord. This required enough extra paracord at the base of the pole that we could feed the cord into the pole (by gravity) and drop the weather station to ground level while the aluminum pole remained in its deployed position. When finished, we pulled the sensors back up into the directional sleeve, locking the insert in the proper direction. We then recoiled the paracord and fit it into the lock box, then locked the box with a keyed padlock. Data was downloaded from the weather station onto a laptop in this manner.

We employed a custom-built aluminum lock box (approx. 3” x 3” x 9”). The box was mounted at eye level onto the base tree using wood screws and cable if extra support was needed. The extended telescoping pole (Unger 30 Foot Telescoping Pole, item #: UTF900) was placed into an aluminum socket at the top of the lock box and secured to the socket with a lock nut and bolt. The pole was then extended to the appropriate length (based on the height of the cable branch and the distance of the base tree aggregation and the height of the aggregation). We then lowered the pole, cut the weight off from the end of the cable and secured the cable to the end of the pole with crimp locks (closing a cable loop). Finally, we raised the pole into the air by pulling the cable over the branch from the spool end. Once the pole was raised to the proper height, we cut the supporting cable from the spool and anchored it onto the trunk of the base tree by threading it through holes in the metal box, wrapping it around the tree trunk, and securing it with crimp locks. The lock box would be open and paracord/insert/sleeve assembly was employed when the weather station needed to be dropped to ground level for data downloads.

## Data Collection of Climatic Variables

We set the sensors to collect data every five minutes, and all the sensors in a weather station were synchronized. We downloaded data at least every 12 days (when possible) since that was the smallest storage capacity of one sensor (L) when set to fiveminute intervals. Each of the loggers has its own software, which was used to download the data collected and store it to a laptop (as .csv files) via a USB cable. Data was then erased from each sensor to allow room for the next collection period.

## Data Collection of Physical Habitat

Microhabitat data was collected only once at each grove, giving us a snapshot of habitat attributes, representative of late February. Microhabitat was quantified through image analysis. We quantified the amount of vegetative cover in the emergent layer, canopy, understory and shrub layers, as well as the ground cover layer. Different lenses were used to capture images from different layers (details below). Habitat data were collected in order to explore the correlation between habitat attributes and microclimate attributes under habitat selection (if any). Such correlated habitat attributes might serve as tools for climatic habitat restoration or management.

### Vertical Vegetative Component

One set of images was taken looking up vertically from directly below each weather station. These images captured the emergent, canopy, and upper understory layers. Differences in lighting conditions were considered in the analytical approach. Functionally, we regard these as the vegetative layers that contribute to a vertical component of light and wind abatement. Standing below the weather station, the station was placed at the center of a fisheye lens (Shuttermoon, 198°) image, viewed through a camera (iPhone 8). The fisheye lens captures a circular image, encompassing 198° out of a possible 360 ° (or the top 55% of a conceptual sphere with the observer at the center and looking up). The lens was held 1.83 meters (m) above ground, resulting in an image that represents vegetation from 1.83 m and upward in all directions.

### Horizontal Vegetative Component

Another set of images was taken from directly beneath each weather station using a 0.63x wide lens with a 74° field of view in portrait format. For each sample location, a photo was taken in the NW, SW, NE, and SE direction, which results in a 360° view minus a 16° gap between images. An extension pole was used so images could be taken from 3 m above ground level. These images captured understory and shrub, and ground cover layers as well as topographical hillside obstructions in four directions. Functionally, we regard these as the vegetative layers and topographical features that contribute to a horizontal component of light and wind abatement.

### Ground Cover

We took a last set of images (using an extension pole) at 13m above ground. The camera was located directly below each weather station, but this time the lens faced directly downward in order to capture an image of the ground cover. The images were taken using the same .63x wide lens described for the horizontal images, resulting in an image covering a ground area of 4.5 m x 4.5 m.

### Litter Depth

At each weather station location, we collected five random samples of litter depth. We created two axes of 4.5 m x 4.5 m using two measuring tapes. We placed the measuring tapes on the ground so that the sensor array was located at the center of the square created by the two axes. We then used a random number generator to get two values ranging from 1-450 centimeters and used these as x and y coordinates along the measuring tapes to determine where to collect a litter depth sample. Litter depth was measured using a meter stick placed vertically on the ground until the bottom reached bare ground. We repeated these steps for five litter depths per sample location.

### Distance to Nectar Source

We recorded the distance to the nearest nectar source at each sample location using a rangefinder (Leica LRF 800 Lazer Rangemaster). This was done by measuring the horizontal distance from the observer (below the sample location) to the nearest nectar source and correcting for the height of the weather station to calculate the straightline distance from the weather station to the nearest nectar source. The nearest nectar source was also classified as either herb, shrub, or tree.

# Analysis

## Aggregation Location Effect Hypothesis

### Microclimate

It is generally hypothesized that monarchs cluster in parts of the grove that have unique climatic attributes (Leong et al. 1991, Weiss et al. 1991, Frey and Leong 1993, Anderson and Brower 1996, Leong et al. 2004). We tested this hypothesis by testing the prediction that aggregation locations would have different climatic attributes from all other (interior and edge) sample locations. Therefore, daily values for each variable at each of the five sample locations were calculated for daily minimum, average, maximum, and standard deviation. Daily values were calculated from midnight to 11:59 PM for each day. We did not analyze minimum L since all sample locations had values of zero at night. We used R version 3.6.1 to run a repeated measures ANOVA of climatic variables across sample locations, blocking by grove, and accounting for temporal autocorrelation using an autocorrelation structure of order 1 (AR(1)) for each variable. This correlation structure indicates that adjacent days are more similar to each other than non-adjacent days, which is a common structure used for time series data. This analysis partitioned variance across the five types of sample locations, while controlling for individual grove effects, and correcting for temporal autocorrelation among days. If there was a significant result (at p < 0.05), we followed up with a Tukey pairwise comparison to determine which specific differences existed between which of the five sample locations. The magnitude and direction of the difference were then plotted.

### Microhabitat

It is generally hypothesized that monarchs aggregate in parts of the grove that have unique climatic attributes created by unique microhabitat attributes (Leong et al. 1991, Weiss et al. 1991, Frey and Leong 1993, Anderson and Brower 1996, Leong et al. 2004). We tested this hypothesis by testing the prediction that aggregation locations would have different microhabitat attributes from all other (interior and edge) locations. To render a quantitative variable, the images representing vertical and horizontal vegetative components in the groves were uploaded into ImageJ (1.50i), where color thresholds in hue, saturation, and brightness were adjusted until all pixels representing vegetation were unselected from the photo, leaving only sky. The selected sky portions were then used to calculate the inverse, or the proportion of vegetative obstruction in each of the photos. Thus, the quantitative variable is the proportion of vegetative obstruction of sky. To render a quantitative variable from the photos on ground cover, we overlaid a 5 x 5 square grid image onto the downloaded photos. Each square in the grid was categorized as either “bare ground,” “live cover,” or “dead cover.” Thus, the quantitative variable is percent cover. The final quantitative variable was distance to the closest nectar source, in meters, from each of the sample locations. We then used a categorical variable indicating if the nectar source was an herb, shrub, or tree. Nectar type was analyzed separately from distance. To test for differences across the five types of locations, we used R to fit an ANOVA for all quantitative variables in the microhabitat data. In each analysis, we blocked by grove, which accounted for the source of variability across groves since we were only interested in the variation across sample locations. For the type of nectar source available, we pooled data across groves to increase our sample size since we had one record for each sample location within each grove. For type of nectar, we ran a Chisquared test comparing sample locations since we used a categorical variable (herb, shrub, or tree).

## Grove Effect Hypothesis

It is generally hypothesized that monarchs overwinter inside groves because the grove interior contains suitable attributes that differ from the grove exterior. We tested this hypothesis by doing an analysis that tested the prediction that climatic attributes inside the grove would be different from climatic attributes at the edges of the grove, thus allowing us to determine if there is a grove effect on microclimate. The sample locations categorized as “interior” are aggregation, SW interior, and NE interior (Figure 3). For our models, we pooled the two interior locations with the aggregation location since these three are not significantly different collectively or pairwise (see results Aggregation Effect Table 1). Sample locations SE edge and NW edge were their own categories in this analysis, to avoid pooling distinct climatic attribute’s effects while reflecting pairwise results (see Table 1). Thus, we compared differences in climatic variables across three types of sample locations: the SE edge location, the interior of the grove, and the NW edge location. For these three types of sample locations, the daily values of each variable were calculated for minimum, average, maximum, and standard deviation. Daily values were calculated from midnight to 11:59 PM for each day. We did not analyze minimum L because all sample locations had values of zero at night. With the interior data pooled with aggregation site, again we used a repeated measures ANOVA, blocked by grove, accounted for temporal autocorrelation (AR(1)), and followed up significance testing with a Tukey pairwise comparison. The magnitude and direction of the difference were then plotted.

## Uniformity Hypothesis

In order to test the hypothesis (Leong et al. 1991, Weiss et al. 1991, Frey and Leong 1993, Anderson and Brower 1996, Leong et al. 2004) that monarchs use a single overwintering realized microclimatic niche, we tested the prediction that climatic attributes at aggregation locations would be more uniform across groves than other sample locations using a two-step process. We ran a fixed effects model to test for the effect of the interaction of grove and sample location on each climatic variable, using a temporal correlation structure of AR(1). We then took the random effect estimates for the interaction of every grove and sample location combination and ran a Levene’s test for unequal variances across sample locations for each climatic variable.

## Correlation Tests: Lack of Independence Between Climatic Attributes

We hypothesized that some climatic variables would be correlated. We predicted that light and temperature would have a positive correlation. We predicted that humidity and temperature would have a negative correlation. We also predicted that daily minimums, averages, and maximums within the same variable would be highly correlated. We used a linear correlation matrix and R to identify pairwise significant correlations (p < 0.05) between daily minimums, averages, maximums, and standard deviations for all climatic variables. Significant results for the analyses presented above were interpreted more conservatively if variables were found to be correlated.

## Spatial Autocorrelation: Correlation between Latitude and Climatic Attributes

We hypothesized that there would be spatial autocorrelation in daily values of climatic data. We predicted that there would be a spatial correlation with latitude for both temperature and light due to the correlation between latitude and day length. We used data only from aggregation locations to compare climatic variables across latitude and conducted a Derbin-Watson test for spatial autocorrelation of each climatic variable for latitude. The exclusive focus on aggregation location data makes this a test for a latitudinally variable climatic niche (defined as selected habitat) rather than a singular or uniform climatic niche (as tested above).