SELECTION OF OVERWINTERING MICROCLIMATE BY MIGRATORY

WESTERN MONARCH BUTTERFLIES

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ABSTRACT

Selection of overwintering microclimate by migratory western monarch butterflies.

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Migratory species are expected to demonstrate habitat selection that occurs at multiple spatial and temporal scales. Western monarch butterflies migrate seasonally to overwinter in groves at geographically predictable locations along the California coast. To date, overwintering habitat selection by western monarch butterflies has been studied assuming that habitat selection occurs where overwintering aggregations form, meaning at the spatial scale where monarchs form dense overwintering aggregations within overwintering groves. We argue that since western monarch butterflies are migratory, studies of habitat selection could have commingled selection at different scales into a single spatial scale. This likely leads to ignoring some levels of habitat selection, confounding the scale of habitat selection itself, and potentially misidentifying the habitat attributes under selection. Therefore, we explore monarch overwintering habitat selection to determine whether an explicit spatial framework is necessary.

We studied nine groves on the coast of California and at each grove we collected temperature, humidity, and light data from grove edges, grove interiors, and aggregation locations over several weeks of the overwintering season. We tested the hypothesis that monarchs aggregate in locations within groves that have consistent attributes across groves. We find that locations on the outer edges of groves differed significantly in particular attributes of daily temperature and light from the interior of groves. Yet we find neither evidence supporting the hypothesis that the aggregation locations have a

unique microclimate that differs significantly from other locations inside the grove nor

that aggregation locations are uniform in their microclimatic attributes across

overwintering groves. Rather, we find that the microclimatic attributes at the aggregation

locations vary spatially with latitude. Thus, the overwintering climatic attributes that

appear to be under selection varied spatially based on locations within groves and based

on latitude of each particular grove. We conclude it will be necessary to consider spatial

effects when studying western monarch butterfly overwintering habitat selection and that

interpretations of habitat selection to date have commingled habitat selection at multiple

spatial scales.

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1. INTRODUCTION

Many species demonstrate specific habitat associations and are known to select habitat components at different spatial and temporal scales (Johnson 1980, Wiens 1989, Mayor et al. 2009). Migratory species, in particular, are expected to demonstrate habitat selection at multiple scales (Hutto 1985, Kristan 2006). Habitat selection - implying choice - is typically quantified as a disproportionate use relative to availability (Mayor et al. 2009), or as use (occupancy) and non-use (MacKenzie et al. 2017) of particular habitat elements. While recognizing that use at a specific scale does not equate with selection at that exact scale, explicit methodologies are being developed to directly quantify the hierarchical nature of habitat selection (e.g.: Bellamy et al. 2020) given that the scale of selection is not always intuitive (Mayor et al. 2009).

In addition, relevant habitat attributes, such as those under selection, can themselves be structured in hierarchical or non-hierarchical fashions (Kristan 2006). Hierarchical meaning that one attribute (and its choice) is dependent on the pre-existence of another attribute (e.g.: slow moving riverine system dependent on low topographical relief), and that habitat selection is based on threshold models, resulting in patterns of use and nonuse or presence-absence (Kristan 2006). Non-hierarchical meaning that attributes are correlated (e.g.: solar radiation and temperature) and potentially non-intuitive or non-generalizable across scales (Mayor et al 2009), and that selection may be expressed as relational, or as a gradient of use (Kristan 2006), rather than use and nonuse. For example, a migratory organism might show a hierarchical spatial selection process, where selection of specific roosting groves would be dependent on pre-existing selection of specific migratory routes (Hutto 1985). After selecting a grove, a migratory organism

might show non-hierarchical selection of roost locations constrained to a gradient of conditions within the grove. Furthermore, species can show habitat selection that is variable across temporal scales, even where temporal variation is more important than spatial variation (Weins 1989, Fahrig 1992, Mayor et al. 2009). For example, over the course of time, be it hours or days, selection could shift if the attributes under selection change, or as a function of nonlinear interactions between variables over time (Mayor et al. 2009).

When a species is shown to express specific habitat affinities and those affinities or preferences are not contextualized in space and time, then the scale of the affinity has not been addressed. If the scale has not been addressed, then even when habitat selection has been documented, it is not clear at what scale habitat selection is occurring. Selection at different scales could be confounded into a single spatially/temporally non-explicit scale. In this case the interaction of both selection and scale is obscured (Mayor et al. 2009, MacKenzie et al. 2017, Bellamy et al. 2020). Likewise, selection at a single scale might be confounded if commingled with selection that occurs at additional scale(s) (Johnson 1980). Both forms of confounding selection and scale would have the effect of adding error to an analysis, not because the variable is inherently noisy or random, but because variance would not be appropriately partitioned in space and time. Inappropriate partitioning can muddy interpretations of realized niche, while potentially misdirecting management and restoration to an incorrect scale.

One example of a species with specific habitat affinities is the monarch butterfly, or more specifically, the overwintering North American monarch butterfly (*Danaus plexippus*). The North American population is known to overwinter in groves of trees in

geographically predictable locations (Urquhart and Urquhart 1978, Brower 1995, Brower

and Missrie 1999, Martinez-Mendez et al. 2016, Ortiz-Bibian et al. 2017, Fisher et al.

2018). These groves occur in the highlands of central Mexico and along the coastline of

California. Thus, on a continental scale, the specific migratory route (Brower 1995, Pile

2014, Urquhart et al. 1970, Billings 2019) used by a monarch butterfly would define

which of these two locations it would ultimately encounter. Subsequent habitat selection

could only occur predicated on pre-existing selection of a migratory route, resulting in

habitat choices being available either in the highlands of Mexico, where monarch

butterflies shown disproportionately selection of Oyamel fir forests (Brower 1995), or

within mixed tree-species groves in coastal California (Griffiths and Villablanca 2015).

Thus, specific habitat affinities are only expressed in one spatial context or the other,

suggesting alternative realized niches, and hierarchical spatial selection.

The overwintering habitat of western overwintering monarch butterflies has been

extensively studied, but not in an explicitly spatial or temporal context. For example,

temperature, relative humidity, and solar radiation are thought to be important to

overwintering monarch butterflies (Leong et al. 1991, 2004) as these habitat attributes

appear to be selected *within groves*. But, using the conditions of these same attributes

outside of groves, Fisher et al. (2018) find the spatial probability of an occupied

overwintering grove can be reliably predicted for any location in the California

landscape. This means habitat selection could be occurring relative to habitat attributes

within and outside of groves, or that attributes grade across the grove edge to the interior,

or even from the surrounding landscape to the edge to the interior. Leong et al. (2004)

report that monarchs "choose groves that face south/west near the ocean and north/east

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near bays and inlets," and Lane (1993) concludes that overwintering sites are located in shallow canyons and gullies, or on the leeward side of hills. If these statements are correct, then habitat attributes *outside of groves* are under selection since they define the condition of the entire grove.

Habitat use by overwintering monarch butterflies also shows habitat selection at various temporal scale, ranging from the use of individual trees for a month or more (Anderson and Brower 1996), to the use of individual trees or branches for a few days or hours (Frey and Leong 1993, Leong et al. 1991, Weiss et al. 1991). In addition, Fisher et al. (2018) consider whether the habitat attributes thought to be under selection (Leong et al. 1991, 2004) are important for the duration of the five to six month overwintering season, or if attributes are more important in certain months, and they conclude the latter. So even though overwintering western monarch butterfly habitat selection appears to show a spatial and a temporal context, it has not been studied in an explicitly spatial or temporal framework.

We regard selection of overwintering habitat by western monarch butterflies as a case where selection at different scales could be confounded into a single spatially/temporally non-explicit scale such that attribute variability at different spatial scales is not considered and therefore the scale at which selection occurs is not known. This confounding can keep us from understanding the overwintering habitat attributes under selection, and potentially lead to flawed habitat conservation and management. For example, if temperature varies at a landscape level, but is thought to be under selection within overwintering groves, then more shade plants might be planted to reduce the

temperature within a grove, but would only succeed in doing so for some increment relative to the landscape level variation.

Indeed, the hypothesis that habitat selection by overwintering western monarch butterflies could be occurring at multiple spatial scales has never been explicitly tested. Therefore, we present an analysis focused on spatial non-hierarchical variation within groves (wherein selection may be expressed as relational or gradients of use), and hierarchical variation between groves (wherein selection could be predicated on location, Kristan 2006). We analyze attributes that have been proposed to be important to habitat selection by overwintering monarch butterflies in Coastal California (Leong et al. 1991, 2004, Weiss et al. 1991, Anderson and Brower 1996). We test whether these attributes provide evidence that habitat selection is occurring, whether habitat selection is uniform across space (the among grove scales), whether habitat selection correlates with position in the landscape (the latitudinal scale), and whether the variables correlate with each other. In addition, temporal variation and associated attribute selection are currently inferred to occur (Anderson and Brower 1996), Frey and Leong 1993, Leong et al. 1991, Weiss et al 1991, Fisher et al. 2018) therefore by sampling over the overwintering season we consider temporal variation at a larger scale. Our intent is not to elucidate spatial and temporal hierarchical and non-hierarchical patterns. Instead, we test whether, in addition to the temporal habitat selection that is inferred to occur, the patterns of variation and inferred selection provide evidence of a spatial components to habitat selection. In the end, we hope to conclude whether it is or is not necessary to address microhabitat variation and selection over *space and time*.

2. METHODS

2.1 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.

2.2 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 ≥1000 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.

2.3 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

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).

2.3.1 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.

2.3.2 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 #: U-TF900) 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.

2.3.3 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 five-minute 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.

2.3.4 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.

2.3.4.1 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.

2.3.4.2 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.

2.3.4.3 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.

2.3.4.4 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.

2.3.4.5 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 straight-

line distance from the weather station to the nearest nectar source. The nearest nectar

source was also classified as either herb, shrub, or tree.

2.4 Analysis

2.4.1 Aggregation Location Effect Hypothesis

2.4.1.1 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

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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.

2.4.1.2 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 Chi-squared test comparing sample locations since we used a categorical variable (herb, shrub, or tree).

2.4.2 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

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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.

2.4.3 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.

2.4.4 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.

2.4.5 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).

3.RESULTS

3.1 Aggregation Location Effect Hypothesis

3.1.1 Temperature

We find some significant differences in temperature between aggregation locations and other locations interior and exterior to the grove (Table 1). But, at this finer spatial scale, the aggregation location does not differ significantly from all four other locations. There is evidence for a difference in average daily temperature (ADT) across locations (p = 0.0306), although the Tukey pairwise comparison showed no significant differences between pairs of locations. In order to reduce the likelihood of missing a significant result due to a conservative statistical approach, we also conducted individual t-tests between each pair of locations. We then followed with a sequential test, which gave a significant result of SE having a higher ADT than the interior of the grove (p = 0.0237, adjusted alpha = 0.025). There is no evidence of a difference in minimum daily temperature (MiDT) across sample locations, while there is strong evidence for a difference in maximum daily temperature (MaDT) across locations (p = 0.0009), with the SE sample location having a higher MaDT than all other sample locations. There is strong evidence for a difference in variance in daily temperature (VDT) across sample locations (p < 0.0001), with the SE sample location VDT being larger than all other locations. Therefore, we were unable to find evidence to support the hypothesis that monarchs cluster in parts of the grove that have unique temperature attributes relative to *all* other sample locations. The direction and magnitude of significant differences in climatic attributes across all five sample locations are shown in Figure 5.

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3.1.2 Humidity

At this finer spatial scale, we find few significant differences in humidity between aggregation locations and other locations in the groves. There is no evidence for a difference in average daily humidity (ADH), minimum daily humidity (MiDH), or maximum daily humidity (MaDH) across sample locations. There is evidence for a difference in variance in daily humidity (VDH) across sample locations ($p = 0.0093$), with the Tukey pairwise comparison indicating that SE edge had a higher VDH than NW. The aggregation location does not differ from any other sample locations for any of the humidity variables. Again, we do not find evidence that supports the hypothesis that monarchs cluster in parts of the grove that have unique humidity attributes relative to *all* other sample locations.

3.1.3 Light

At this finer spatial scale, we find few significant differences in light between aggregation locations and the other four locations of the groves. There is strong evidence for a difference in average daily light (ADL) across sample locations ($p < 0.0001$). The Tukey pairwise comparison indicated that the SE sample location is higher ADL than all other sample locations. There is also strong evidence for a difference in maximum daily light (MaDL) across sample locations ($p = 0.0002$), with the SE sample location being higher than the aggregation, NW edge, and NE interior sample locations. There is strong evidence for a difference in variance in daily light (VDL) across sample locations ($p < 0.0001$), with SE having a higher VDL than all other sample locations. Again, we are

unable to find evidence that supports the hypothesis that monarchs cluster in parts of the grove that have unique light intensity attributes relative to *all* other sample locations.

Although the aggregation location does show a few instances of being different from other locations for certain variables, overall, we do not find support for the hypothesis that the aggregation location has a distinct set of climatic variables (even when there are differences, aggregation location groups with other locations). If anything, the results at this finest scale are that measures of temperature, humidity and solar radiation set the SE location apart from all other locations combined. In cases where we observe the aggregation location to be part of a significant grouping, the group is either all the interior sites (NE, A, and SW) or all the interior sites plus the NW edge. This result does not provide evidence to support the aggregation effect hypothesis.

3.1.4 Wind

We were unable to collect enough wind data to conduct an analysis. The wind meters resulted in a file-type error and system incompatibilities. There was not enough tech support from the vendor to be able to resolve the errors and incompatibilities. Thus, we present no results for the wind data set.

3.2 Grove Effect Hypothesis

The hypothesis that monarchs overwinter in groves because the interior of groves represents suitable climatic attributes that differ from climatic attributes outside of groves

was tested by comparing climatic attributes between sample locations inside and at the edges of groves. This is the next spatial scale we tested above the "aggregation effect hypothesis" presented above. The aggregation location was pooled with the two interior locations since these three locations are not significantly different collectively or pairwise (Table 1 and above). The direction and magnitude of significant differences in climatic attributes across the interior and edges of the grove are shown in Figure 6.

3.2.1 Temperature

We find some significant differences in temperature between grove interiors and edges (Figure 4). There is evidence for a difference in ADT among the interiors and edges of groves ($p = 0.0211$), although the Tukey comparison does not show any pairwise differences. In order to reduce the likelihood of missing a significant result due to a conservative statistical approach, (Tukey's pairwise) we also conducted individual t-tests between each pair of locations. We then followed with a sequential Holm's Sequential Bonferroni test (Holm 1979), which gave a significant result of SE having a higher ADT than the interior of the grove ($p = 0.0237$, adjusted alpha = 0.025). There is evidence for a difference in MaDT among the interiors and edges of groves ($p = 0.0012$), with the NW sample location having a lower MaDT than the interior of groves and the SE sample location. There is no evidence that MiDT differs among the interiors and edges of groves. There is strong evidence that the VDT is different among the interiors and edges of groves ($p = 0.0002$), with the SE sample location higher than NW and the interiors. For all differences found, the interior nests in with one of the edges: NW MaDT differing from SE and interior, and SE VDT differing from NW and interior.

3.2.2 Humidity

We find some significant differences in humidity between grove interiors and edges. There is no evidence that ADH or MaDH differs among the edges and interior of the groves. While there is evidence that MiDH differs between the interior and edges of the groves collectively ($p = 0.0168$), the Tukey comparison does not show any pairwise differences. Therefore, if there are differences, we cannot determine what the nature of these differences are between the interior and exterior of the grove. There is evidence that VDH is different among the grove edges and interior ($p = 0.0158$), with the SE edge having a higher VDH than the NW edge.

3.2.3 Light

We find some significant differences in light between grove interiors and edges (Figure 4). There is strong evidence that ADL is different among the interior and edges of groves ($p < 0.0001$), with SE having a higher ADL than the interior and NW edge. There is also evidence that MaDL is different among the interior and edges of the groves ($p < 0.0001$), with NW having a lower MaDL than both the interior and SE edge. There is evidence that the VDL is different among the interior and edges of the grove ($p < 0.0001$), with SE having a higher VDL than both interior and NW edge. Finally, there is also evidence that NW and the interior of the groves are different in VDL, with the interior of the grove having a larger VDL than the NW edge ($p = 0.0482$).

Overall, we find qualified support for the hypothesis that the interior and exterior of the overwintering groves are significantly different in terms of temperature, humidity

and solar radiation (Table 2). The prediction from this hypothesis is observed for ADT, MaDT, VDT, VDH, and the VDL. Thus, value and variability seem important (as shown in Figure 4). In several cases we observe that one edge is different from the combined interior plus the other edge (Table 2) such that "grove effect" may actually be an "edge plus interior versus other edge" effect. This result suggests that this hypothesis may be correct, but not at the scale of the entire grove, and instead at the scale of a portion of the grove. Finally, to explore the significant variance in daily light and temperature, we find an interaction between maximum and average daily light (MDaL and ADL) and maximum daily temperature (MaDT) as seen in Figure 7, whereby grove interiors are characterized by high MaDL and low ADL and low ADT, which is possible if the maximum light is of short duration.

3.3 Aggregation Location Effect

The hypothesis that monarchs cluster in parts of the grove that have unique climatic attributes created by unique microhabitat attributes was tested by testing the prediction that aggregation locations would have different microhabitat attributes from all other (interior and edge) locations. We find few significant differences in vegetative obstruction (overhead or horizontal) between aggregation locations and other locations interior and exterior to the grove. We find no evidence that percent canopy cover differs across the five sample locations when accounting for grove differences. There is evidence of a difference in the horizontal component to the NW across sample locations, with SE location having more obstruction to the NW direction than NW location (documenting

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the edge of the grove). There is strong evidence of a difference in the horizontal

component to the SE across sample locations (p < 0.0001), with SE location having less

obstruction to the SE direction than all other locations (documenting edge of grove), and

NE having more obstruction than NW. There is also some evidence that the horizontal

component to the SE has less obstruction at the aggregation location than the NW sample

location (p = 0.0855), meaning the aggregation locations are somewhat more open to the

SE exposure than might be expected due to its location within the grove. There is no

evidence of differences for horizontal components to the SW or NE across sample

locations.

Overall, we find no evidence to support the hypothesis that the aggregation

location is significantly different in overhead or horizontal vegetative obstruction from

the four other sampling locations in the overwintering groves. We only find the

aggregation location is different from NW on the horizontal component to the SE

direction, by having less obstruction. Also, the aggregation location (pooled with all

others except SE) is significantly different from SE on the horizontal component to the

SE. This result suggests that this hypothesis is not correct, at least not at a scale that

includes multiple locations within the interior of the grove, or that at best it is only

correct for horizontal exposure to the SE.

3.4 Ground Cover

We find some significant differences in ground cover between aggregation

locations and other locations in the interior and exterior across groves. There is evidence

that percent live ground cover is different across sample locations (p = 0.0029), where aggregation locations have more live ground cover than NE and NW sample locations. There is also evidence of a difference in dead ground cover (p = 0.0055), where the aggregation location has less cover than the NE and NW sample locations. Therefore, one interior (NE) and one edge (NW) are significantly different from the aggregation location in an exchange between dead and live ground cover. There is no evidence of a difference in percent bare ground cover across sample locations.

Overall, we find no evidence that supports the hypothesis that the aggregation location is significantly different in ground cover from *all* other sampling locations in the overwintering groves. With regard to three categories of ground cover, we only find the aggregation location is significantly different from NW and NE in terms of percent live and percent dead ground cover. This result suggests that this hypothesis is not correct, at least not at a scale that includes multiple locations within the interior of the grove, or that at best it is only correct for live and dead cover relative to parts of the grove.

3.5 Litter Depth/Nectar

Overall, we find no evidence that supports the hypothesis that the aggregation locations are significantly different in litter depth or distance to nectar from all other sampling locations in the overwintering groves. There is no evidence that litter depth is different across sample locations. There is no evidence that distance to nectar or type of nectar is different across sample locations. This result suggests that this hypothesis is not

at all correct, or at least not at a scale that includes multiple locations within the interior of the grove.

3.6 Test of Uniformity of Aggregation Microclimate Across Overwintering Range

At the next larger scale, that portion of the overwintering range we sampled, we tested the hypothesis that monarchs have a uniform set of suitable overwintering climatic attributes (i.e. within grove overwintering climatic niche) via the prediction that climatic attributes at the aggregation location would not be significantly different across overwintering groves. In contrast to the prediction, we find that there is strong evidence that aggregation locations across groves are different in daily minimum (MiD), average (AD), and maximum (MaD) values of temperature (T), humidity (H), and light (L) (all p values < 0.0001).

Indeed, aggregation locations seem to simply mirror the variability in climatic conditions seen across groves (Figure 8) rather than a unique and consistent set of conditions. We further tested this hypothesis via the prediction that climatic attributes at aggregations location would be less variable across groves than the climatic attributes at other sample locations. There is no evidence that sample locations differ in their uniformity across groves in average daily temperature (p = 0.2295), average daily light (p = 0.4518), and average daily humidity (p = 0.1204). This does not support the hypothesis that monarch butterfly aggregation locations represent a variation constrained, unique, or uniform climatic niche.

Overall, we find no evidence that supports the hypothesis that the aggregation locations represent a variation constrained, unique, or uniform set of climatic attributes across overwintering groves. Likewise, other sample locations did not represent a unique or uniform set of attributes across groves. Neither are constant across the range sampled. This result suggests that this hypothesis is not correct. Instead, it suggests that the attributes at the aggregation location are a function of the collective attributes of the individual or local grove. This could be a hierarchical relationship, given that the local attribute values are predicated on the specific grove. Therefore, if a climatic niche does exist, it is a geographically variable realized niche, and thus broader than what can be discovered at a single grove, or what has been recognized to date.

3.7 Test of Correlation in Climatic Variables

We predicted correlations among variables and discovered correlations consistent with the predictions. There is evidence of a positive linear relationship between ADL and ADT ($p < 0.0001$, R squared = 0.3717). There is evidence of a negative linear relationship between ADT and ADH ($p < 0.0001$, R squared = 0.2337). There is evidence of a negative relationship between ADL and ADH ($p < 0.0001$, R squared = 0.1784). The results support our hypothesis that there are linear correlations between temperature, humidity, and light. Given these correlations, it is possible that darker and cooler grove interiors could be more humid.

3.8 Spatial Autocorrelation

As an alternative to the hypothesis that there is a unique and uniform climatic niche at aggregation locations, we tested whether there is a latitudinally variable climatic niche by considering the aggregation locations sampled at nine overwintering groves. We find there is strong evidence of a latitudinal correlation in ADL at aggregation locations ($p < 0.0001$), and no evidence of a latitudinal correlation in ADT or ADH. We find there is strong evidence of a latitudinal correlation in MaDT ($p < 0.0001$) and MaDL at aggregation locations ($p < 0.0001$), but no evidence of a latitudinal correlation in MaDH. We find no evidence of a latitudinal correlation for MiDT or MiDH. Finally, considering variances, we find strong evidence of a latitudinal correlation in VDT ($p < 0.0001$) and VDL at aggregation locations ($p < 0.0001$), while there is no evidence of a latitudinal correlation in VDH. To summarize, aggregation locations show a significant latitude correlation in ADL, MaDL, VDL, MaDT and VDT.

The tests of uniform microclimate, greater uniformity in microclimate, and spatial autocorrelation all combine to reject the existence of a singular climatic niche. Instead these results suggest that if a climatic niche does exist it is geographically variable and potentially defined by latitude. A possible driver of these correlations could be the correlation between day length and latitude, if one were to consider the role that day length/latitude would have on the observed values of maximum daily temperature, maximum daily light, and average daily light.

4. DISCUSSION

4.1 Aggregation Sites Reflect Grove Locations

We fail to find evidence that supports many of the hypotheses we tested. Specifically, there appears to not be the expected "aggregation effect," meaning the aggregation location is not climatically different relative to all other sample locations across groves. Instead, the aggregation location generally groups with other interior and edge locations in an apparent non-hierarchical (or graded) pattern of variation (Table 1). Contrary to our prediction, there does not appear to be the expected "grove effect," meaning locations within groves are not climatically distinct from both NW and SE locations for all variables. Instead, the interior locations generally group with one edge or the other, in an apparently non-hierarchical pattern of variation. Contrary to our predictions, there does not appear to be a variation constrained, unique or uniform set of climatic conditions at aggregation locations across the nine groves we sampled. Instead, aggregation locations seem to represent a subset of the climatic conditions at each of the respective groves, thereby showing a hierarchical (preconditioned on grove location) pattern. Finally, we find a correlation among latitude and climatic variables across the aggregation locations in the groves. This further supports the lack of evidence for a variation constrained, unique or uniform set of climatic conditions at aggregation locations.

4.2 A Mechanistic Model for Habitat Selection in Grove Interiors

Our analysis did not identify the expected "aggregation effect." Significant climatic attributes at interior grove locations (including the aggregation location) (Table

2) did show that across the continuum of possible values the maximum daily light is high, while average daily light and average daily temperature are low (Figure 7a). Such a pattern could develop if light was bright (high MaDL) but of short duration (low ADL). In addition, the values of temperature and light are correlated (Figure 7b). This pattern of short duration bright light (MaDL) plus low average light (ADL) and temperature (ADT) is consistent with the preservation of lipids, which are known to be associated with overwintering success (Chaplin and Wells 1982, Masters et al. 1988). Specifically, overwintering monarch butterflies exposed to energy fluxes from light can passively raise their body temperature, avoid energetically expensive shivering, and thereby exceed the body temperature required for flight with minimal energy expenditure (Masters et al. 1988, Alonso-Mejia et al. 2004). At the same time, a short duration energy flux would result in low average daily light which would correlate with low average daily temperature. Low ADT allows monarchs to maintain low internal body temperature (Masters et al. 1988), which is important to survival given that oxygen consumption (associated with lipid metabolism) increases exponentially with temperature (Chaplin and Wells 1982). Thus, we cannot assert a narrow conclusion that aggregation locations show favorable overwintering conditions, but we can support the broader conclusion that grove interiors are energetically favorable for overwintering monarchs and likely increase overwinter survival.

4.3 Managing Overwintering Habitat

We find there was significantly more live ground cover under aggregation locations than under other locations. This could be consistent with Alonso-Mejia and

Arellano-Guillermo (1992) who found that monarchs elevated even just 30cm above the ground surface had lower mortality due to freezing than monarchs on the ground. This suggests live ground cover may provide necessary thermal refugia or structure for climbing. We find that aggregation locations were significantly different from NW locations in regards to horizontal obstruction towards the SE direction. Aggregation locations had less obstruction in the SE direction. This pattern could be consistent with exposure to short duration bright light (see above) and the associated energetically favorable habitat, because less obstruction would let in more light. Or, it could be consistent with Leong et al. (2004) who found 70% of overwintering sites face south or southwest (i.e.: have that exposure), though Leong et al. (2004) infer that clusters tend to face south to reduce exposure to wind. We were unable to test Leong's inference due to our lack of wind data.

In terms of variables that are relevant to habitat selection by overwintering western monarch butterflies, wind has the potential to be the most confounded if spatial and temporal hierarchical and non-hierarchical variation are underappreciated. In the long term (weeks to months), the prevailing winds in coastal California are from the west or northwest (Greely et al. 1996 and Western Regional Climate Center-Climate of California (WRCC) https://wrcc.dri.edu/). An overwintering grove located on a flat marine terrace exposed to the West or Northwest will experience all of the prevailing (NW) wind events. An overwintering grove located on a south facing coastline, on the leeward side of an east-west or southwest-northeast oriented ridgeline (see Lane 1993), would be significantly buffered from prevailing wind events (WRCC). In addition to wind, fog, solar radiation, and temperature could be significantly different between these

two grove locations (WRCC), even if they are less than a mile or two apart. In the short term (hours to days), damaging storm winds blow from the south or southeast (Leong et al. 2004, WRCC). These are episodic winds. Even though there is agreement in the literature that wind is important and that SE storm winds can reduce monarch survival, the literature is mixed as to whether it addresses prevailing winds from the NW (Weiss et al. 1991, Leong et al. 2004), or whether it addresses episodic winds from the SE (Leong et al. 1991), or both (Leong et al. 2004). If both are relevant, then not only do they need to be explicitly addressed, but their effects and contributions to habitat selection would need to be partitioned in space and time with a non-hierarchical and a hierarchical perspective.

4.4 Scale and Hierarchical vs. Nonhierarchical Structure

We find that habitat selection is not uniform across the range. There does not appear to be a variance constrained, unique or uniform set of climatic variables at overwintering aggregation locations, although the grove exteriors tend to have more variability in daily climatic conditions than the interior of the grove. Instead, we find a previously unreported latitudinal associated pattern to climatic habitat attributes (ADL, MADT, MADL, VDT and VDL) and habitat selection at aggregation locations and grove interiors. Instead of being uniform, the conditions at the aggregation location are predicated on the grove's specific location. This is potentially influenced by day length, as there is an increase in day length with decreasing latitude (Hooker et al. 2018). Thus, habitat selection by overwintering western monarch butterflies seems to be structured over at least two spatial scales. There appear to be non-hierarchical gradients within

groves, from the edges to the interiors (with the interior often grouping with one edge), and hierarchies between groves predicated on the specific grove and its latitude.

The potential for a latitudinal dependent pattern seems to have been recognized by Chaplin and Wells (1982) who state "Some of the monarch butterfly aggregations in central California, such as the ones at Santa Cruz, persist somewhat longer than those in southern California (Tuskes and Brower, 1978). Progressive tightening of the energy balance due to higher nocturnal temperatures as one moves down the California coast may limit the southerly distribution and duration of aggregations." Thus, Chaplin and Wells recognize latitudinal variation in the available climatic conditions at overwintering groves.

Our results, combined with a mechanistic hypothesis from the work of Chaplin and Wells (1982), and its latitudinal correlates (Tuskes and Brower, 1978), lead us to infer that a spatially explicit framework will be meaningful for understanding the habitat selection of overwintering western monarch butterflies. While grove attributes are significantly different and dependent on latitude, high maximum daily light (MDL), and low average daily light (ADL) and temperature (ADT), seem under selection across all groves. Therefore, the specific level of light and temperature under selection seems to likewise depend on latitude in a manner that could positively impact lipid reserves. Thus, if correct, a mechanistic energetics hypothesis in the context of variation in climatic attributes within and between groves could inform our understanding of range limits, phenology, responses to climate change, and fitness (Chaplin and Wells 1982, Tuskes and Brower. 1978, Masters et al. 1988, Fisher et al 2018).

When expanding the view to look across scales we cannot define one set of suitable overwintering attributes that apply to all groves used by the western monarch population, other than strong light of low duration, reduced horizontal obstruction towards the SE direction, and live ground cover exchanged for dead ground cover. We believe this shows that overwintering western monarchs can tolerate a larger range of climatic attributes than previously appreciated, because climatic variability between groves is more than previously appreciated (Figure 8). Exploration of beneficial attributes of groves based on grove locations, rather than on intrinsic grove characteristics, seems particularly ripe for exploration or modeling. For example, Fisher et al. (2018) show that the location of an occupied overwintering site can be predicted using climatic variables. It might be fruitful to ask whether there are other variables that are good predictors at more of a landscape scale. It might also be fruitful to consider habitat attributes associated with what Chaplin and Wells (1982) call the "Progressive tightening of the energy balance…" which "…may limit the southern distribution and duration of overwintering aggregations."

4.5 Potential for Type II Error

This work has an inherently high probability of type II error, meaning a potential to not find differences when they actually exist. Here we consider some possible sources of type II error.

Due to limitations in the capability of our equipment, it was difficult to place the sensor group directly onto the aggregation of monarch butterflies. In addition, we wanted to avoid disturbing the aggregation, and placing a wind propeller too close to the aggregation could have affected clustering behavior. Therefore, sensor groups were placed approximately 2 meters away from the actual aggregation (but at the same height). Thus, if the attribute(s) under selection varied over a horizontal distance of < 2 m, we would not have the resolution to measure them.

Another shortcoming could be our sample size of only nine groves, which was a result of both budget limitations and a historically low overwintering monarch population (Pelton et al. 2019). This could have made it difficult to find a difference among aggregation locations and other locations within groves. In particular, sampling the aggregation and only two other locations inside a grove could have presented an issue. If monarchs were not saturating the locations with suitable attributes, that means there could have been locations within the grove that were suitable but were not occupied. Lack of occupancy, or under-occupancy, was likely at play due to the 86% decrease in the western monarch overwintering population size relative to the year prior to this study (Pelton, et al. 2019). As a result, what we called "interior" locations might have been defined as "aggregation" locations in years with a larger overwintering population.

Our microhabitat data was collected at one time and toward the end of the overwintering season. The microhabitat data was a snapshot of mostly fixed attributes. But for variable attributes (i.e.: distance to and type of nectar source, and percent live cover), and to the degree that they vary, it is possible we could have gotten different results had we taken the data at a different time. In order to test the hypothesis that

monarchs prefer to cluster over areas with more live ground cover, a study could provide supplemental live ground cover in groves and see if monarchs cluster preferentially over it. There is no evidence that live ground cover in the groves was simply an effect of higher humidity, since we did not find differences in humidity between the cluster and other areas of the grove.

It is possible that there are important characteristics that we did not monitor. Wind may be an attribute that defines suitable microclimate for monarchs given the conclusion that monarch clustering behavior may be heavily dependent on avoiding wind (Leong 1991). Though this may be true, we suggest explicitly considering prevailing and episodic winds, as well as hierarchical and non-hierarchical variation. In addition, the potential for type II error is relevant to wind measurements in another way. On the one hand we might conclude that any approach that uses habitat obstruction and modeling to define the wind conditions (i.e.: Weiss et al. 1991) may first need to demonstrate significant differences in habitat obstruction. We suggest this step because we were unable to show that the aggregation location had significantly different habitat obstruction relative to other sample locations. Thus, wind would not be predicted to be significantly different across sample locations if based entirely on vegetation obstruction. But, on the other hand, if we come to our conclusion erroneously (type II error), then this modeling approach might be fully justified.

Tracking aggregations over time proved to be more problematic than anticipated, and presents a challenge to any study of habitat selection on the shortest time scale. Sensor groups need to be moved to new locations as aggregations move, but those

locations blink on or off in time, creating a dynamic system. We think that valid fine-grained (hourly) comparisons on the time axis will require real-time tracking of aggregations, which we were not able to accomplish. Instead, we were able to capture spatial differences across the grove and how they change over time (daily).

However, the high likelihood of obtaining type II error does not negate the fact that when we find differences, they are very likely to exist. We argue that this work brings forth additional spatial scales at which monarch butterflies select overwintering habitat, and importantly shows that some variables that are under selection have values that correlate with latitude. Thus, we conclude that selection may not be at the scale of the aggregation itself, but it is certainly relevant at other scales.

4.6 The Relationship between Scale and Management

Ultimately, this work may have relevance to conservation in helping define how to manage or even restore overwintering groves. We find evidence that aggregation locations have more live cover and less dead cover than NW and NW sample locations. Although live cover may be under selection, there would be a tradeoff with litter. Ground litter increases survivorship in cold conditions by allowing monarchs to climb up above ground (Alonso-Mejia and Arellano-Guillermo 1992). We find evidence that aggregation locations have less horizontal obstruction towards the SE direction than do the NW edges of the groves, which may indicate that suitable aggregation attributes favor a SE exposure. This could be due to energetic benefits of morning sun exposure (Chaplin and Wells 1982, Tuskes and Brower. 1978, Master. et al. 1988), since the winter sun rises in the SE. Inversely, aggregations might select locations obstructed from the predominant

NW wind, as has been suggested by Leong and Weiss (Weiss et al. 1991, Leong et al. 2004). However, we find no evidence of the aggregation locations having more horizontal obstruction in the NW direction than any other part of the grove (thus making the SE have relatively less), indicating that it's more likely a preference for SE exposure than to obstruction towards the NW. It could also mean that landscape features that we were unable to be captured in our vegetative/obstruction images, such as hills and trees farther away, may play a role in wind abatement. Our lack of evidence for any differences in the amount of canopy cover could mean that the amount of cover is not of prima facie importance for aggregation habitat. Or, it could mean that it's not the amount of cover that's necessarily different across various parts of the grove, but rather the positioning of the cover that's contributing to a suitable microclimate for aggregating monarchs. Based on our results, we would recommend managing for some SE horizontal exposure near monarch aggregation locations since aggregations appear to occur in areas with less vegetative obstruction towards the SE direction (p = 0.0855). It may also be important to maintain NW obstruction relative to aggregations, though there may need to be more studies to find evidence to support the hypothesis that monarchs cluster in areas to avoid predominant wind exposure from the NW.

4.7 Conclusion

Most previous studies have only looked at attributes at the aggregation location, already assuming the aggregation location represents a unique climatic niche (see Leong et al. 1991 for an important exception). Our results show that there is scaling to the habitat attributes of monarch butterflies at Western overwintering groves. The aggregation sites do not seem to be unique or uniform, since aggregation locations have

attributes that overlap with other locations of the groves (including the edges), attributes of the grove depend on where the grove is located geographically, and aggregation locations have attributes that vary latitudinally. Given that aggregations are also dynamic entities, they break up, reform, and move in space and time, it is likely that there are additional attributes under selection that vary in both space and time at a finer scale than sampled here. The study of monarch habitat selection in time proved difficult for us given the dynamic nature of aggregations, and a historically low overwintering population size. Given the lack of a diagnosable realized overwintering niche, our microhabitat analysis did not result in robust management recommendations. We would recommend exposure to the SE and some obstruction to the NW, and perhaps for greater live ground cover in support of aggregation locations. Our work may not be perfect in terms of its application to within grove management, but the conclusion that we are likely not managing for one uniform niche may allow us someday to quantify the relative value of an overwintering grove, and thus to couple management with scale.

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Table 1: Tests of Aggregation Location Effect

Variable	Comparison All five locations	Pairwise comparisons* NW edge	SW interior	Aggregation	NE interior	SE edge	
MiDT	p = 0.9624						MiD = minimum daily
MaDT	p = 0.0009	a	a	a	a	b (>)	
ADT	p = 0.0306*						
VDT	p < 0.0001	a	a	a	a	b (>)	
MiDH	p = 0.2694						
MaDH	p = 0.9829						
ADH	p = 0.8540						
VDH	p = 0.0093	a	ab	ab	ab	b (>)	
MaDL	p = 0.0002	a	a	a	a	b (>)	
ADL	p < 0.0001	a	a	a	a	b (>)	
VDL	p < 0.0001	a	a	a	a	b (>)	

Results from ANOVA tests comparing daily climatic conditions minimum (MiD, maximum (MaD), average (AD), and variance (VD) in daily temperature (T, °C), light (L, lux), and relative humidity (H, %) across the aggregation location, two interior locations, and two exterior locations, blocking by grove location. Empty cells are non-significant results.

* Operators are used to show relationship of variable values only when p < 0.05.

Table 2: Tests of Grove Effect

Variable	NW vs. Interior vs. SE	Pairwise comparisons* NW vs. Interior	SE vs. Interior	NW vs. SE
MiDT	p = 0.4106			
MaDT	p = 0.0012	<		<
ADT	p = 0.0211		>	
VDT	p = 0.0002		>	<
MiDH	p = 0.0168			
MaDH	p = 0.8541			
ADH	p = 0.4683			
VDH	p = 0.0158			>
MaDL	p < 0.0001	<		<
ADL	p < 0.0001		>	<
VDL	p < 0.0001	<	>	<

Results from ANOVA tests comparing daily climatic conditions minimum (MiD, maximum (MaD), average (AD), and variance (VD) in daily temperature (T, °C), light (L, lux), and relative humidity (H, %) across the interior (aggregation, SW, and NE locations) and the exterior (SE and NW) of groves. Empty cells are non-significant results.

* Operators are used to show relationship of variable values only when $p < 0.05$.

Table 3: Tests of Climatic Uniformity Across Groves

Sample Location	Temperature (C°)			Light (lux)			Humidity (%)		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
NW	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
SW	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
NE	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
SE	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.0015	p < 0.0001	p < 0.0001
Aggregation	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

Comparison of daily climatic attribute values for standardized sample locations across nine monarch butterfly overwintering groves. For standardized locations, see Figure 3.
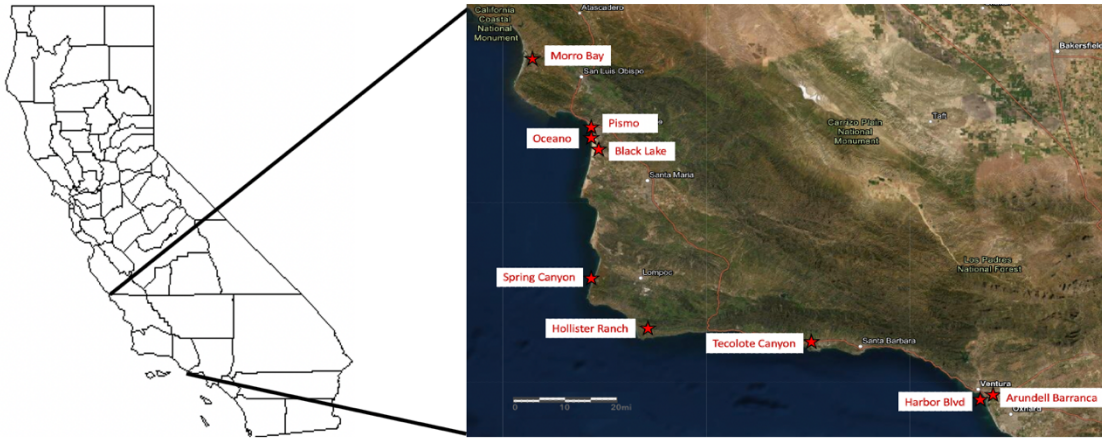
FIGURES

Map of Grove Locations

Figure 1: Nine groves sampled along the California coast in Ventura, Santa Barbara, and San Luis Obispo counties which met study design criteria (see text). 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).
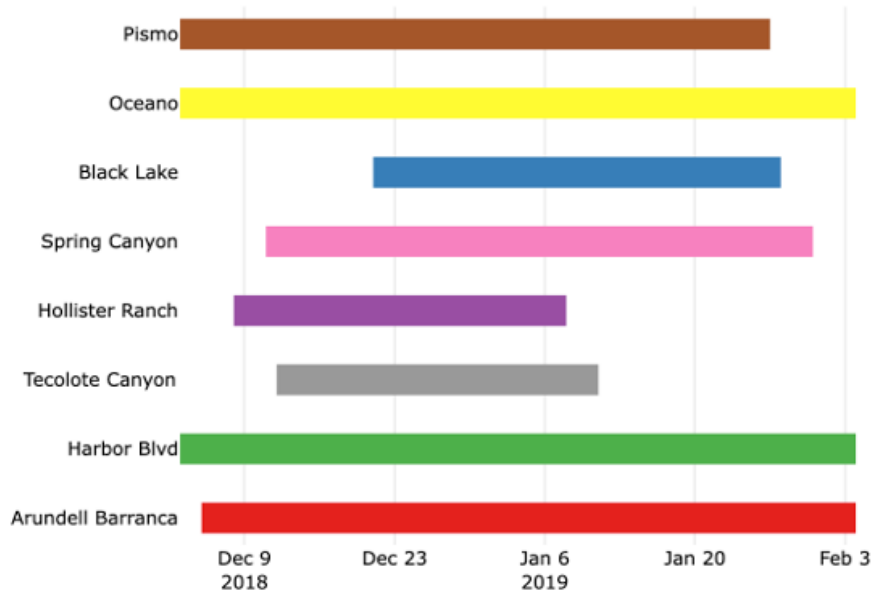
Length of Data Collection at Groves

Figure 2: Start and end dates for data collection for each grove in the study. The start dates were delayed awaiting 1000 or more monarchs per grove and varied as a function of monarch presence and counts and by access availability once monarchs were present. End dates were defined by monarchs departing entirely from a grove. The shortest sampling period was 30 days and the longest was 61 days (mean = 43 days).
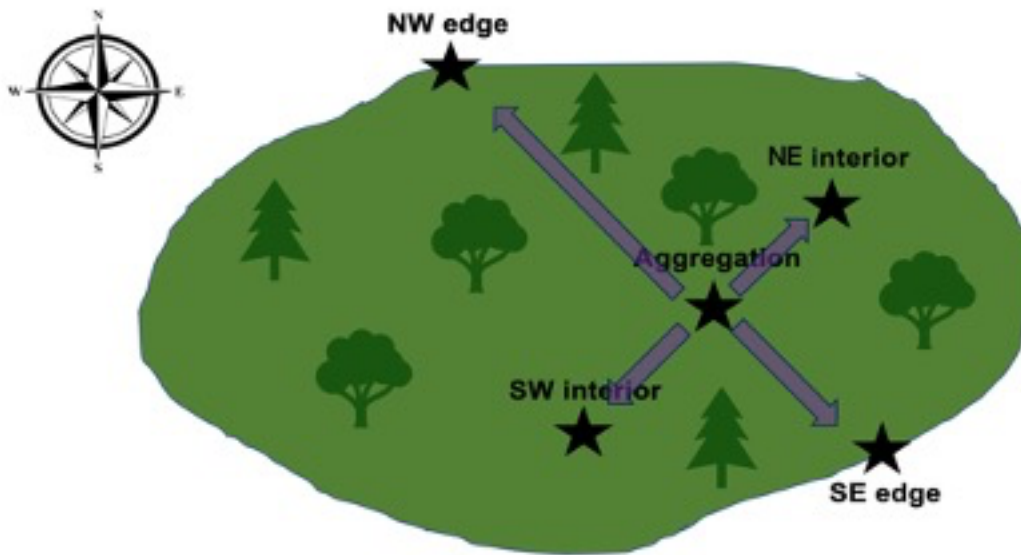
Grove Sampling Design

Figure 3: Sampling design relative to the aggregation's location within groves. The first sample location was placed in the location of an aggregation (Aggregation). Two more sample locations were placed on the SE and NW edges of each grove relative to the aggregation's location to capture morning light and prevailing winds (SE edge and NW edge respectively). Two interior sample locations were placed halfway between the aggregation's location and the grove's edge in the NE and SW directions (NE interior and SW interior respectively).

Comparison of Significant Attributes for Grove Effect
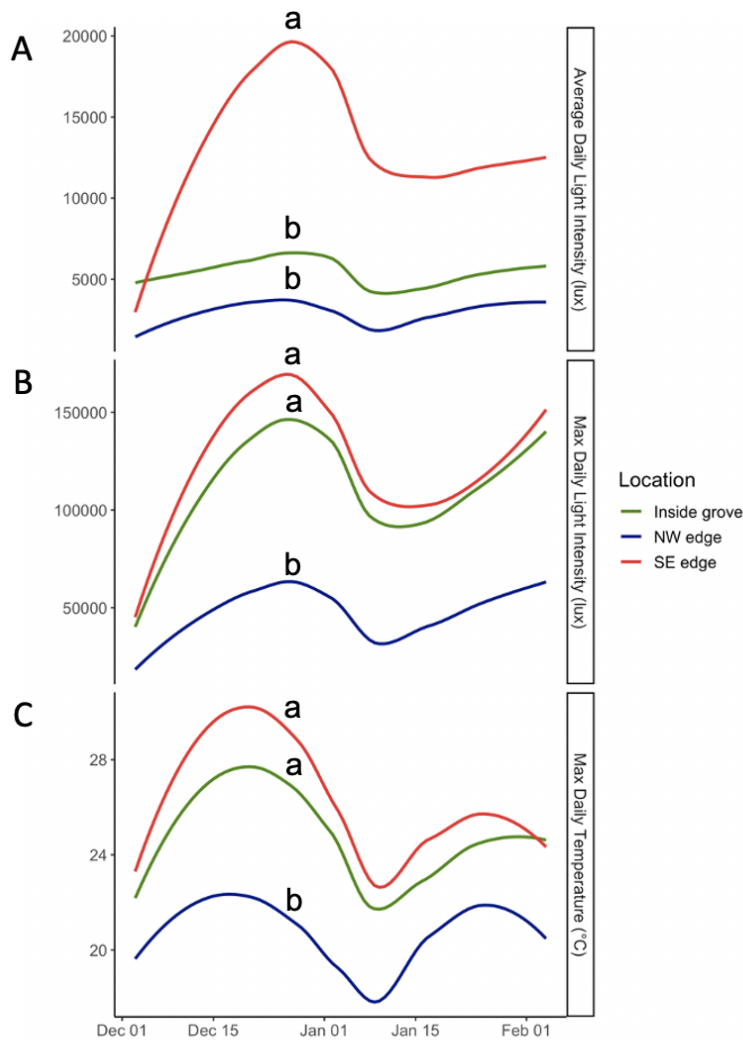
Figure 4: Climate data comparing the interior, NW edge, and SE edge of groves in

December and January of the 2018-2019 overwintering season for A) Average daily light

(ADL) intensity (lux), ($p < 0.0001$), B) Maximum daily light (MaDL) intensity (lux), ($p <$

0.0001), and C) Maximum daily temperature (MaDT) (°C), ($p < 0.0012$). Lines have

been smoothed to capture important patterns in the data and reduce noise. Within each

panel, the same letter indicates the locations are not significantly different, while different

letters indicate a difference. These are some of the significant results. All results are

shown in Table 2.

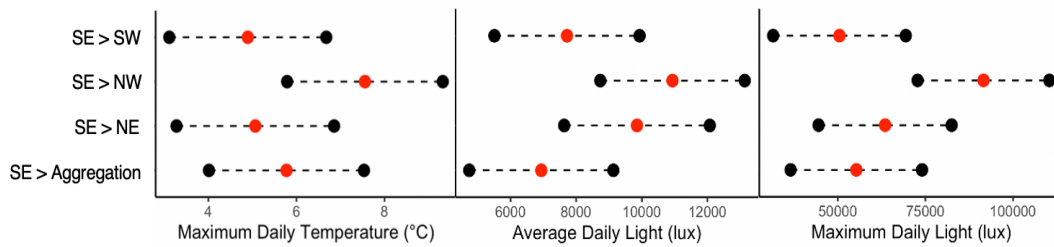**Magnitude of Differences in Significant Attributes for Aggregation Effect**

Figure 5: Magnitude of significant pairwise differences (Table 1) across all five locations

in groves. Tests account for blocking between groves and temporal autocorrelation of the

data and thus provided an estimate of the magnitude of the difference between two

groups, and a hypothetical error. All significant pairwise differences were between SE

and NW locations and between Interior and NW locations, and for Maximum Daily

Temperature, Average Daily Light, and Maximum Daily Light. The magnitude of the

difference is shown on the ordinate, which location of the pair had the greatest absolute

value is shown by the < symbol.

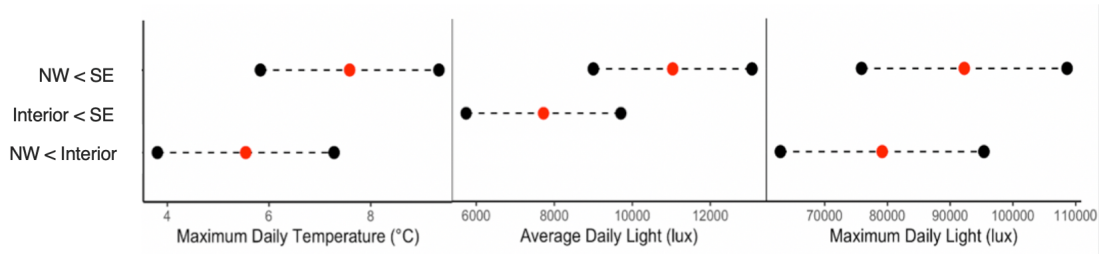**Magnitude of Differences in Significant Attributes for Grove Effect**

Figure 6: Magnitude of significant pairwise differences (Table 2) across three locations in groves, SE, NW and Interior (pooling NE and SW and Aggregation). Tests account for blocking between groves and temporal autocorrelation of the data and thus provided an estimate of the magnitude of the difference between two groups, and a hypothetical error. All significant pairwise differences were between SE and the other four locations (SW, NW, NE and aggregation), and for Maximum Daily Temperature, Average Daily Light, and Maximum Daily Light. The magnitude of the difference is shown on the ordinate, which location of the pair had the greatest absolute value is shown by the < symbol.

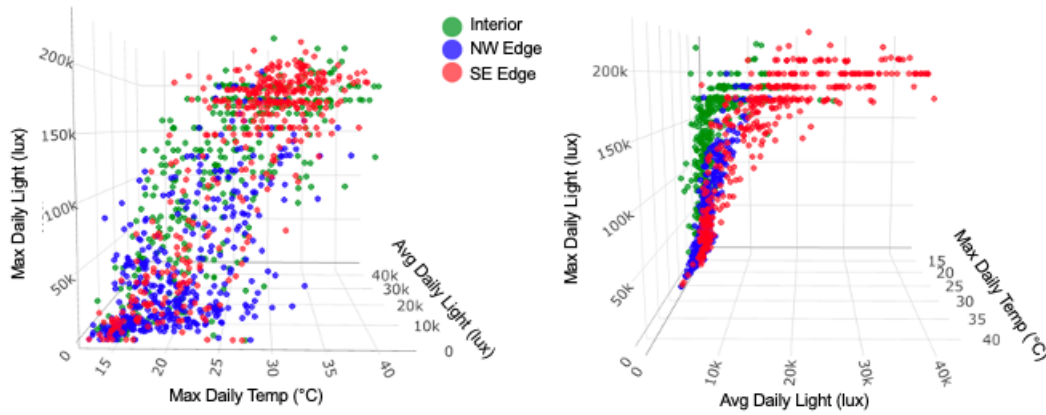**Significant Climatic Variables for Grove Effect**

Figure 7: Relationship between maximum daily light and average daily light, and between maximum daily temperature and maximum daily temperature. The two interior locations are pooled with the aggregation location since these three are not significantly different ($p > 0.6762$) collectively or pairwise. A. The grove interior has relatively low ADL in comparison to the SE (brightest) edge of the grove ($p < 0.0001$). In addition, the inside of the grove has relatively high MaDL in comparison to the NW edge of the grove ($p < 0.0001$), such that the grove interior is characterized by low ADL and high MaDL. This is possible if the maximum light is of short duration. B shows the positive correlation between MaDL and MaDT.

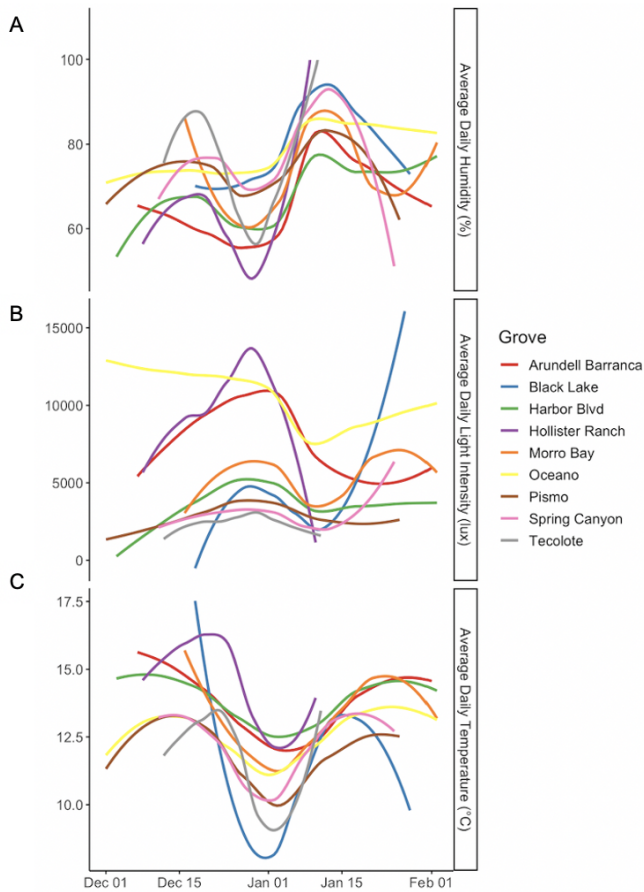**Comparing Average Daily Climate Across Groves**

Figure 8: Climate data for the interior of groves in December and January of the 2018-2019 overwintering season for A) Average daily humidity (%), B) Average daily light intensity (lux), and C) Average daily temperature (°C). Lines have been smoothed to capture important patterns in the data and reduce noise. In an attempt to define the uniform climatic attributes that occur at aggregation locations across groves, instead we find that aggregation locations are no more uniform than other parts of the grove, and that taken collectively the insides of groves are quite dissimilar.

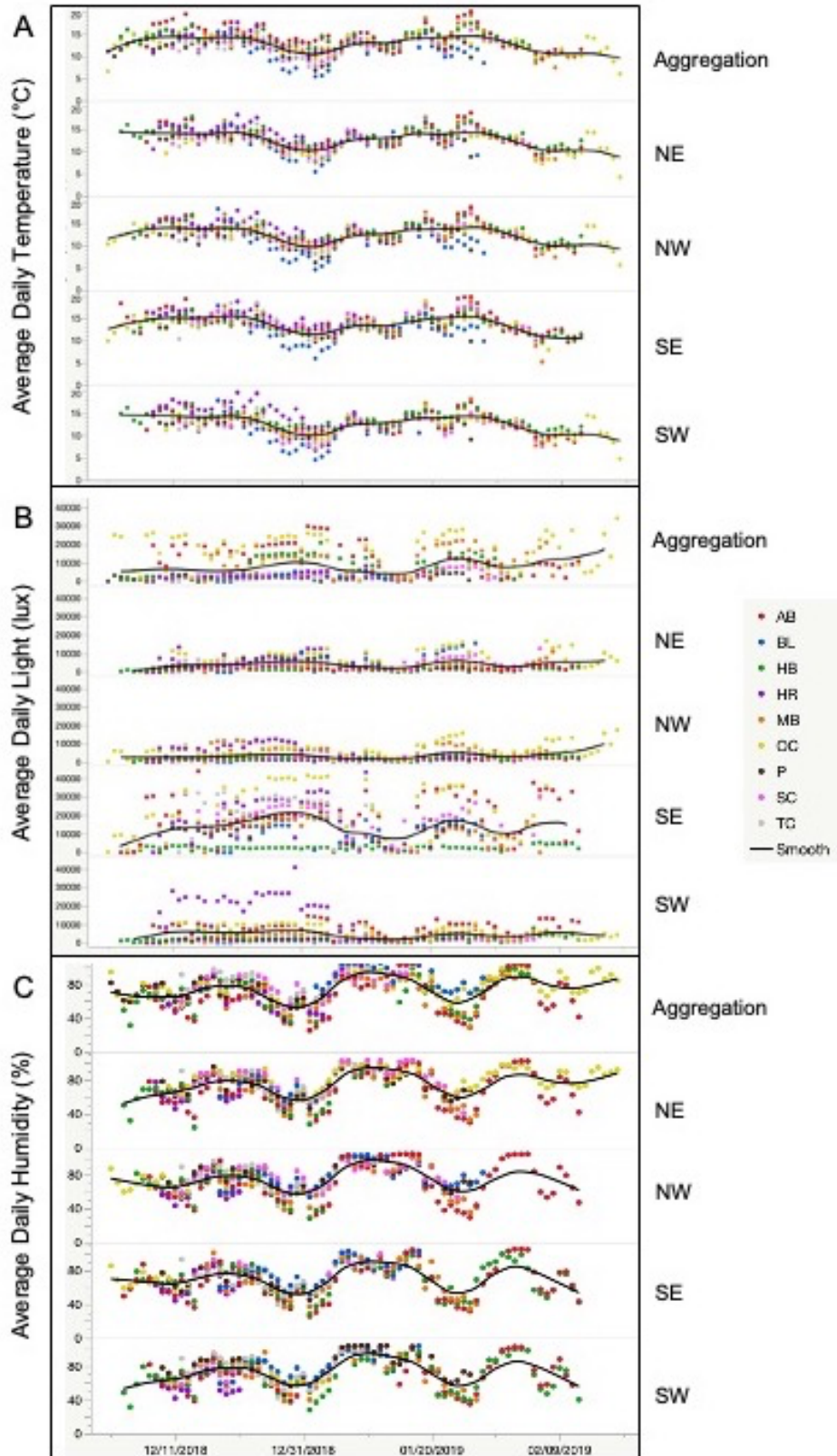**Comparing Uniformity Across Groves by Sample Location**

Figure 9: Climate data for each sample location across groves in December and January of the 2018-2019 overwintering season for A) Average daily temperature (°C), B) Average daily light intensity (lux), and C) Average daily humidity (%). When testing for uniformity across groves at each sample location, we find that the aggregation does not show less variation for any of the climatic variables across groves than other sample locations.