

MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*) TREE PREFERENCE AND  
INTERSITE MOVEMENT AT CALIFORNIA OVERWINTERING SITES

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## ABSTRACT

Managing Monarch butterfly overwintering groves: making room among the eucalyptus

Proper management and conservation of the coastal California overwintering sites used by western Monarch butterflies (*Danaus plexippus* L.) is critical for continued use of these sites by monarchs. Many management efforts are currently concentrating on eucalyptus-only sites because of the prevailing notion that monarchs prefer eucalyptus over native tree species. Yet, whether a preference exists or not has never been tested. Herein, we test the “eucalyptus preference” hypothesis with data from five overwintering sites comprised of blue gum eucalyptus (*Eucalyptus globulus*) and at least one other native tree species from fall 2009 to spring 2012. We found that when monarchs clustered disproportionately on a tree species relative to its availability, they clustered significantly more than expected on native trees and significantly less than expected on eucalyptus. Also, in years when the overwintering population was highest, monarchs clustered disproportionately on native conifers, and they often switched from clustering on eucalyptus in the early winter to native conifers in the middle or late winter. Our results suggest that overwintering groves should be managed to include a mixture of tree species. We cannot recommend simply planting more eucalyptus. At overwintering sites in central coastal California, native conifers such as Monterey cypress (*Hesperocyparis macrocarpa*) and pitch canker-resistant Monterey pine (*Pinus radiata*) should be planted as replacements for blue gum eucalyptus in areas where trees are likely to fall, and around the perimeter of groves.

Key words: monarch, *Danaus plexippus*, eucalyptus, habitat preference, overwintering, Monterey cypress, Monterey pine

## INTRODUCTION

Each fall, monarch butterflies (*Danaus plexippus* L.) in western North America migrate long distances to specific groves of trees on the California coast where they gather in large clusters for the winter (Williams et al. 1942, Urquhart and Urquhart 1977). Based on population estimates derived from annual surveys at these overwintering sites, it is inferred that the western population has declined by 90% since 1997 (Stevens and Frey 2004, Xerces Society 2014). The two main drivers behind this decline are hypothesized to be the loss of breeding habitat (milkweed patches) in the interior West and Southwest U.S and the loss and degradation of coastal overwintering habitat (tree groves). From 1990-1998, there was a 12% decline in available overwintering habitat for California monarchs (Meade 1999, Frey and Schaffner 2004). That trend is expected to continue, given that there has since been no management or policy put in place to stop or reverse this decline. In addition to direct loss, overwintering sites can become unsuitable for monarchs through tree cutting and removal, senescence, tree fall, and/or defoliation due to eucalyptus leaf beetle herbivory (Leong et al. 1991, Weiss et al. 1991, Fallon and Jepsen 2013).

A suitable overwintering site is comprised of a grove of trees that produce a microclimate with a narrow set of values across several parameters. Suitable grove conditions include above-freezing temperatures (Calvert et al. 1983), low light intensity and solar radiation with high water vapor pressure (Leong et al. 1991), wind speeds lower than 2 m/s (Leong 1990), and access to fresh water, typically in the form of fog drip or morning dew (Tuskes and Brower 1978). The microclimate at an overwintering grove is impacted by landscape-level factors and by the local configuration and characteristics of trees at the site. Canopy height and density, branch configuration, and type of foliage will determine the microclimate and influence if or where

monarchs cluster. These characteristics may vary considerably depending on tree species. Therefore, it is possible that monarchs may cluster on different tree species under different climatic conditions.

At California overwintering sites, monarchs have been recorded clustering on a variety of native and non-native trees, primarily blue gum eucalyptus (*Eucalyptus globulus*), Monterey pine (*Pinus radiata*), Monterey cypress (*Hesperocyparis macrocarpa*), and coast redwood (*Sequoia sempervirens*). Observations suggest that historically monarchs clustered primarily on native conifers, particularly Monterey pine (Riley and Bush 1881, 1882; Shepardson 1914). However, the introduction of eucalyptus in the mid-nineteenth century changed the landscape of coastal California. In Southern California, eucalyptus became the dominant tree on the landscape as groves were planted for lumber and shade (Santos 1997). In central California, eucalyptus was planted extensively while coastal areas forested with Monterey pine were concurrently harvested (Jones and Stokes 1994).

Today's monarchs cluster almost exclusively on eucalyptus in the southern portion of the overwintering range (i.e., Santa Barbara, Ventura, Los Angeles, Orange, and San Diego counties). Overall, monarchs use eucalyptus at 75% of California's overwintering sites (Frey and Schaffner 2004). This might lead one to conclude that monarchs prefer eucalyptus, and in fact, this has become "common knowledge." Some monarch management plans go as far as advocating for conservation and management efforts exclusively at eucalyptus-only overwintering sites (Sakai and Calvert 1991), while other management plans recommend planting more eucalyptus (Oberhauser et al. 2009).

However, monarch tree species preference has never been formally tested, partially because it is not possible to test for preference at groves comprised only of eucalyptus. It is only

possible to establish preference if alternate resources are available, and if utilization is measured relative to resource availability. By examining monarch tree use at sites with multiple available tree species, we can test the “eucalyptus preference” hypothesis and determine whether monarchs truly prefer eucalyptus, or if they merely use it in proportion to its availability. If monarchs do not prefer eucalyptus it would suggest that monarchs use eucalyptus at sites that have suitable microclimates regardless of the tree species present: if they are not preferential of the tree, then they must be preferential of the site. Such a paradigm shift would move us from managing and restoring eucalyptus towards managing and restoring overwintering sites.

## METHODS

We counted the number of clustering monarchs at five overwintering sites during the overwintering period from fall 2009 to spring 2012. Our weekly monitoring also included information on tree species used. This was compared to tree species availability.

**Study sites.** All sites were well-known climax overwintering sites. Monarchs were present throughout the entire overwintering season during every year of the study. Sites were selected from among all known climax sites because the groves contained multiple tree species.

Pacific Grove Monarch Sanctuary (36° 37' N, 121° 55' W) is located in Pacific Grove, CA, on the south edge of Monterey Bay, Monterey County. The 1.1-ha site consists of *E. globulus* on the south edge and southeast corner, *P. radiata* and *H. macrocarpa* throughout the rest of the property, with coast live oak (*Quercus agrifolia*) in the understory, and several non-native ornamental trees and shrubs.

The Big Sur private property site (36° 07' N, 121° 38' W) is located on the Big Sur coast in Monterey Co. Monarchs cluster on a 1-ha parcel containing groves of *E. globulus*, *H.*

*macrocarpa*, *P. radiata*, and *S. sempervirens*. The site also contains landscaped gardens with plantings of non-native shrubs and flower gardens.

Morro Bay Golf Course (35° 21' N, 120° 50' W) is located in Morro Bay, San Luis Obispo Co. Total area of the two overwintering groves at the site is 4 ha. These groves are mainly comprised of *E. globulus* with *P. radiata* along the outer edges, and little to no understory vegetation.

Pismo Beach Monarch Grove (35° 07' N, 120° 37' W) is located adjacent to the Pismo State Beach North Beach Campground in Pismo Beach, San Luis Obispo Co. The 1.2-ha overwintering site is comprised of a large *E. globulus* grove with some *P. radiata* and *H. macrocarpa* scattered within the northeast edge. A creek flows along the north edge of the site, which is lined with arroyo willow (*Salix lasiolepis*). There is minimal understory.

Oceano Campground (35° 07' N, 120° 37' W) is located within the Pismo State Beach Oceano Campground in Oceano, San Luis Obispo Co. The 0.7-ha overwintering site is within the 26-ha campground, and is comprised mainly of *P. radiata* with some planted Torrey pine (*P. torreyana*), and a small stand of *E. globulus*. There is extensive understory of native and non-native shrubs and forbs.

**Count surveys.** Surveys were conducted weekly at each of the five sites from mid-October through mid-March or until monarchs dispersed from the site in spring, whichever came first. In the 2009-10 season, surveys began in November at all sites except Pacific Grove, where surveys began in October. Surveys ended at all sites in February. In the 2010-11 season, all sites were surveyed from October through March. In the 2011-12 season, all sites were surveyed from October through February. The number of clustered monarchs was estimated using a standardized estimation method (described in Frey et al. 1992). In this method, multiple

observers estimate the number of monarchs present in each cluster at a site, and these values are recorded and then used to calculate average cluster size (across observers). The sum of the averages of the cluster estimates is the total site estimate. The total number of monarchs estimated clustering on each tree species during each visit was also recorded. The average number of monarchs estimated clustering on each tree species per month per site was calculated. This was used as a metric for tree utilization.

**Canopy cover measurement.** Canopy cover was measured at the five sites in summer 2012. All tree species used by monarchs at the sites were evergreen trees, and trees were therefore leafed out the same amount year-round. At each overwintering site, a polygon was mapped out which encapsulated all trees that had been used by the monarchs over the last 10 years, thereby enclosing the largest area of known use. These polygons were overlaid with a 20-m grid, and at the corners of each grid square a densiometer was used to estimate canopy cover. Only canopy above 3 m was included, as this is the lowest that monarchs have been recorded clustering at these sites. From these data, the proportion of total canopy cover of each tree species at each site was calculated. The proportion that each tree species contributed to the total canopy cover was used as a metric for tree species availability.

**Statistical analysis.** Tree use was analyzed using chi-square tests, which tested whether monarchs were using trees in proportion to their availability (Sokal and Rohlf 1995). The predicted use reflective of availability (expected cluster values referenced below), was calculated by taking the total observed counts and distributing them across tree species based on the relative canopy cover of each tree species. Three chi-square tests were run using R 2.15.3 comparing 1) the average monthly observed vs. expected monarchs clustering on eucalyptus or native conifers, 2) observed vs. expected monarch numbers during maximum occupancy (highest population

size) of that site during that season, and 3) observed vs. expected monarch numbers for mid-season (closest to December 31) surveys. A sign test of the significant chi-square results was used to determine if monarchs clustered significantly more than expected on eucalyptus or on native trees (Sokal and Rohlf 1995). Because multiple counts were done at each site in the same season, a repeated measures ANOVA (Gotelli and Ellison 2004) was run using JMP 10.0 to test for effects of tree species, site, month, and year on the number of clustering monarchs. All data were cube-root transformed for normality.

## RESULTS

Canopy cover varied greatly among sites (Table 1). Only *E. globulus* and *P. radiata* were present at every site we sampled. *E. globulus* canopy cover ranged from 15.3% at Oceano Campground to 97.4% at Morro Bay Golf Course. *P. radiata* canopy cover ranged from 1.1% canopy cover at Big Sur private property to 84.2% at Oceano Campground (Table 1).

Monarch abundance varied widely among sites and among years (Figure 1). The fewest monarchs were present in 2009-2010. At all sites except Big Sur, there were more monarchs present in 2010-2011 than in 2009-2010. There were more monarchs present at all sites during the 2011-2012 overwintering season than in any other year of our study. Likewise, the proportion of monarchs clustering on eucalyptus vs. native trees varied between sites and between years (Figure 1). In most years and at most sites, monarchs did not even cluster in the same proportions on eucalyptus throughout one season, indicating that some clusters move or all clusters switch between tree species. At three sites in at least one year monarchs clustered on eucalyptus near the beginning of the season but then switched to native conifers in the middle or at the end of the season (Figure 1).

In all three years and at all five sites, the chi-square values were significant ( $\chi^2$  values ranged from 13.27-233530.72,  $p < 0.001$  in all cases), indicating that monarchs were not clustering on tree species in proportion to canopy availability 1) over the course of a season, 2) during maximum site occupancy, nor 3) during the middle of the overwintering season. In spite of this, sign tests for each of the three years were not significant, indicating that they did not show an overall preference for any single tree species. Monarchs did cluster significantly more than expected on native trees more than 50% of the time (8 out of 15 times on a seasonal level, 8 out of 15 times at peak occupancy, and 10 out of 15 times during mid-season) (Table 2). This result forces a rejection of the “eucalyptus preference” hypothesis.

The average number of monarchs present at all sites was highest in 2011 ( $F_{2,12} = 4.73$ ,  $p = 0.013$ ), and the average number clustering on native trees was also highest in 2011 ( $F_{2,12} = 9.24$ ,  $p < 0.001$ ). These results also force us to reject the “eucalyptus preference” hypothesis in favor of a “no preference” or “preference for native trees at certain sites or years under certain conditions” hypothesis. Therefore, we are proposing a “conditional preference” hypothesis.

## DISCUSSION

On the California central coast, monarchs do not exhibit an overall preference for blue gum eucalyptus across sites and years either during the middle of the season, at maximum site occupancy, or during the overwintering season as a whole. Our results show that when significantly disproportionate use can be demonstrated, it is biased in favor of native tree species. One problem with looking at monarch tree use across the whole season is that we are averaging across all of the conditions that monarchs experience at a site within one winter and ignoring fine-scale weather variations. It is possible that monarchs only show a tree species preference

when microhabitat conditions are the least favorable. This is why we examined tree use during mid-season, when winter storms are more frequent, temperatures are lower, and wind speeds are higher. Interestingly, monarchs clustered in a different pattern during the mid-season counts than during the seasonal and maximum occupancy counts: they clustered significantly more than expected on native trees in a majority of years at all sites except one. It should be noted that this one site (Morro Bay Golf Course) is our weakest test of the preference hypothesis because it is 97% eucalyptus. The other four sites range from 15-76% eucalyptus.

Monarchs select overwintering sites based on a narrow set of microclimate parameters (Tuskes and Brower 1978, Calvert et al. 1983, Leong 1990, Leong et al. 1991, Anderson and Brower 1996). Our results show that monarchs utilize multiple tree species during a single season. We infer that different trees result in different microclimates because they have varying heights, foliage density (conifer needles are more densely packed together than blue gum eucalyptus leaves), and structure (blue gum eucalyptus often has a more open canopy structure than Monterey cypress or Monterey pine). This suggests that it is possible that monarchs may shift between tree species in response to changing weather or microclimate. When the annual overwintering population was at its highest during the three years of this study, monarchs clustered more on native tree species, and did so earlier in the season and for a greater length of time. Regardless of year, at three of the five sites monarchs switched from clustering predominantly on blue gum eucalyptus to clustering predominantly on native trees in the middle of the season. We hypothesize that when the weather is most unstable and inclement, monarchs will shift from eucalyptus to native conifers. The conditional preference hypothesis would be predicted if the microclimate in the native trees is different and at times more suitable. More study is needed to determine exactly what conditions prompt monarchs to switch tree species,

and whether there are circumstances under which they will use native trees to the exclusion of eucalyptus.

Groves comprised entirely of eucalyptus may not be optimal for monarchs when compared with mixed-species groves. Single-species groves would not allow monarchs to express a choice and respond to local conditions available across different tree species. While monarchs do not exhibit an overall tree species preference, they do not use all tree species in proportion to their available canopy cover across circumstances, sites and years. Within this study, cases that show a positively disproportionate use of eucalyptus are more restrictive (or fewer) than cases where a positively disproportionate use of native conifers is evident.

We recommend that land managers in central coastal California with overwintering groves on their property manage for monarch butterflies by maintaining a diversity of tree species within the overwintering grove. If the overwintering grove is entirely blue gum eucalyptus, planting conifers native to the central California coast such as *H. macrocarpa* and pitch canker-resistant *P. radiata* (if available) would be appropriate where trees have fallen/been removed, or are likely to fall/be removed. Management must be long-term and far-sighted. Planted trees will probably not be large enough to provide clustering habitat for at least 10 years. Therefore it is best to anticipate where future trees will be needed (i.e., where they will fall or will be removed) and manage proactively rather than reactively.

Though we have not emphasized it here, the surrounding landscape is important and contributes to the overwintering grove microclimate, and this is important from a management perspective. Stands of trees immediately adjacent to overwintering groves may act as a buffer for storm winds, particularly prevailing southeast and southwest winter storm winds (Leong et al. 1991, Weiss et al. 1991). Loss of trees on the surrounding landscape may result in decreased

temperatures and increased wind speeds within a grove, leading to site abandonment and/or increased monarch mortality (Calvert et al. 1982, 1984; Weiss et al. 1991; Brower 1996; Williams et al. 2007). Native conifers can be planted around the perimeter of groves as landscape level windbreak.

We do not recommend planting more blue gum eucalyptus on the central California coast. While some biologists do, this is usually because it is very fast-growing, perceived to be useful at filling gaps in the canopy, or creating an emergency windbreak. This would be a signature of management that is reactive, and only responding to a decline in perceived habitat quality or the local number of overwintering monarchs. We would strongly disagree with planting more blue gum eucalyptus in groves that are already exclusively blue gum eucalyptus.

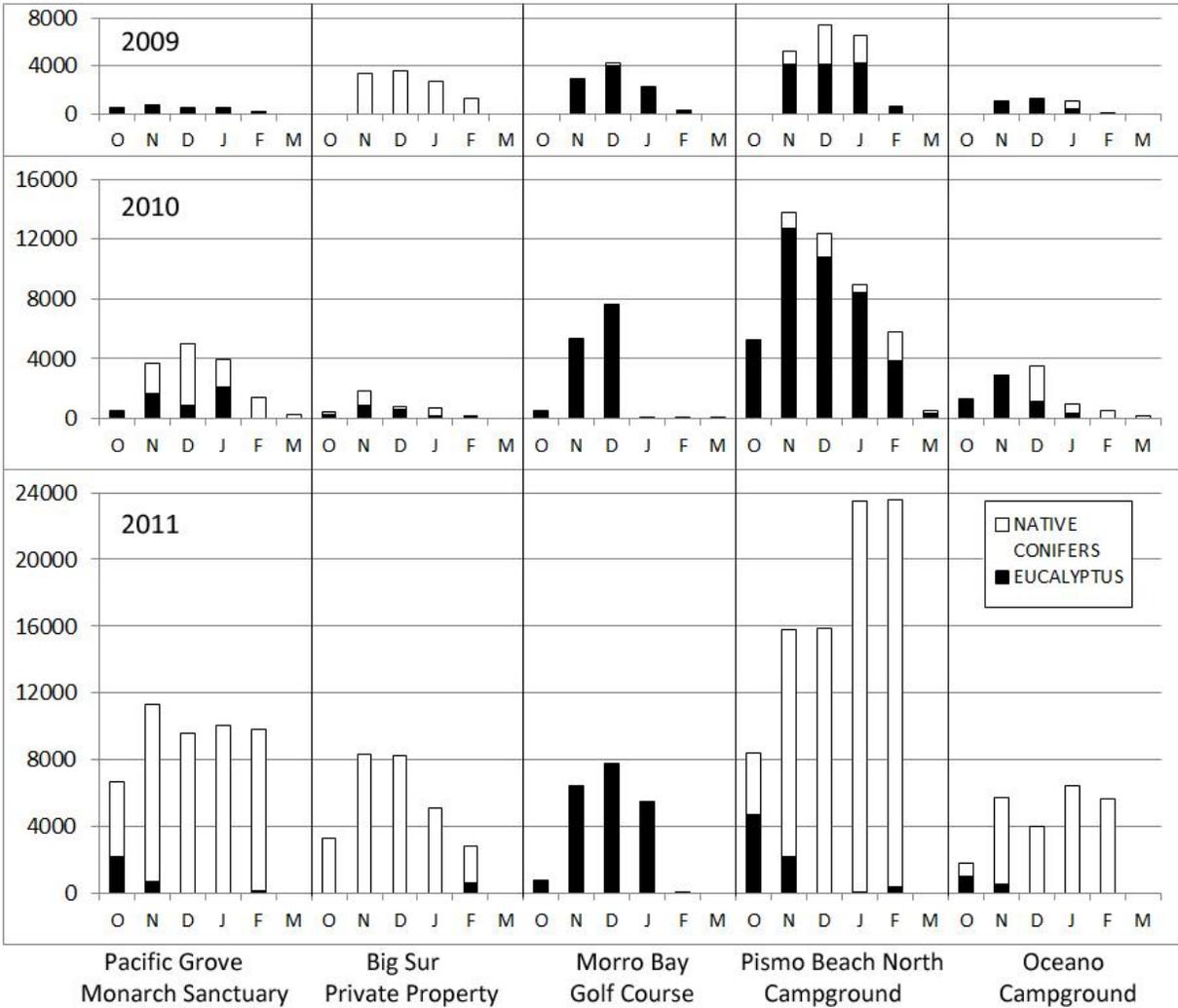
If we are to successfully manage overwintering sites, we must do so in a manner that provides the proper climactic parameters that monarchs need, such as filtered sunlight, available water, and wind speeds below 2 m/s. We must determine what the most challenging conditions are that monarchs experience while overwintering, and what microhabitats and trees they use under those circumstances. Only then can we craft management practices that will conserve and protect the habitat that is so critical to the monarch's continued survival.

**Table 1.** Canopy cover proportions by tree species at five monarch butterfly overwintering sites in Monterey and San Luis Obispo counties, California.

Site	Tree Species				
	Blue gum eucalyptus	Monterey pine	Monterey cypress	Coast redwood	Other
Pacific Grove Monarch Sanctuary	0.425	0.345	0.220	-	0.010
Big Sur private property	0.449	0.011	0.447	0.091	0.002
Morro Bay Golf Course	0.974	0.026	-	-	-
Pismo Beach Campground	0.762	0.035	0.108	-	0.095
Oceano Campground	0.153	0.842	-	-	0.005

**Table 2.** Series of sign test results for tree species utilization by monarch butterflies relative to tree species availability across three years and five California overwintering sites. Site-year cases where a chi-square test showed that monarchs clustered significantly more than expected on eucalyptus are labeled as “+”. They are labeled as “-” when a chi-square test indicated that monarchs clustered significantly less than expected on eucalyptus. One analysis was done over the course of the whole season using monthly count averages, one analysis was done on population counts at maximum seasonal occupancy, and the third was done on population counts closest to the middle of the season (December 31). P-values for all three tests were non-significant, indicating no overall tree species preference even though the chi-square test showed that trees species were not used relative to their availability.

Site	Year	Whole season	Max Occupancy	Mid-season
Pacific Grove Monarch Sanctuary	2009-10	+	+	-
	2010-11	-	-	-
	2011-12	-	-	-
Big Sur private property	2009-10	-	-	-
	2010-11	+	+	-
	2011-12	-	-	-
Morro Bay Golf Course	2009-10	-	-	+
	2010-11	+	+	+
	2011-12	+	+	+
Pismo Beach Campground	2009-10	-	-	-
	2010-11	+	+	+
	2011-12	-	-	-
Oceano Campground	2009-10	+	+	+
	2010-11	+	+	-
	2011-12	-	-	-
	Total +	7	7	5
	Total -	8	8	10
	p	1.00	1.00	0.30



**Figure 1.** Number of monarch butterflies present and tree usage at five California overwintering sites in Monterey and San Luis Obispo counties during three winters from 2009-2012. Site names are listed across the bottom. Each vertical bar represents the total number of monarchs estimated to be present at that site in that month (starting in October and ending in March of each year). Each bar is colored according to the number of monarchs clustering on blue gum eucalyptus (filled) or native conifers (un-filled) such as Monterey pine, Monterey cypress, and coast redwood.

## ABSTRACT

Testing the Monarch butterfly eucalyptus preference hypothesis at California overwintering sites

Western Monarch butterflies (*Danaus plexippus* L.) overwinter in groves of native and non-native trees along the California coast. Eucalyptus is abundant in coastal counties, and overwintering monarchs utilize this type of tree more than any other. This has led to the belief that monarchs prefer eucalyptus. Yet whether a preference exists has never been tested. We tested the “eucalyptus preference” hypothesis at five California overwintering sites with canopies comprised of eucalyptus and at least one native conifer species. We found that at no time over the course of three years did monarchs cluster on trees in proportion to their availability in the canopy. Overall, they did not cluster on one tree species significantly more frequently than another, indicating that monarchs do not prefer eucalyptus—or any tree species—all of the time. However, more often than not monarchs clustered significantly more than expected on native trees, particularly at midseason when the weather was most inclement. They also clustered disproportionately on native conifers when the overwintering population size was highest. At most sites monarchs exhibited tree switching, shifting from eucalyptus to native conifers in the middle or late winter. Based on these results, we reject the “eucalyptus preference” hypothesis. In its place, we propose the “conditional preference hypothesis”, wherein monarchs are predicted to prefer cluster trees according to microclimate conditions and prefer alternate trees within a site as climatic conditions change. Rejection of the eucalyptus preference hypothesis suggests that sites comprised exclusively of eucalyptus may not offer monarchs a suitable range of microhabitats, and further suggests we should rethink “eucalyptus-centric” management.

**Keywords:** Monarch butterfly, *Danaus plexippus*, Eucalyptus, conditional preference, habitat preference, Monterey cypress, Monterey pine, non-native species, invasive species.

## INTRODUCTION

Western monarch butterflies (*Danaus plexippus L.*) overwinter in forested groves at hundreds of sites along the California coast (Urquhart and Urquhart 1977, Leong et al. 2004). Overwintering individuals form clusters and aggregations of clusters that persist from October through late February or early March (Frey and Schaffner 2004). These clusters can be comprised of hundreds to thousands of individuals, and aggregations of clusters often contain thousands or tens of thousands of butterflies (Xerces Society 2014).

Monarchs have been recorded clustering on multiple tree species at these overwintering groves. The most commonly utilized trees include several *Eucalyptus* species (particularly *E. globulus*) and native species such as Monterey pine (*Pinus radiata*) and Monterey cypress (*Hesperocyparis macrocarpa*) (Williams et al. 1942, Sakai and Calvert 1991). The original geographic range of these two native conifers was confined to a “fog-belt” strip along the coast, and extended no further north than Marin County and no further south than Santa Barbara County (USDA, NRCS 2014). Eucalyptus was introduced from Australia in the mid-nineteenth century. It was planted extensively in every coastal California county and has since naturalized at some locations (USDA, NRCS 2014). The introduction of eucalyptus could have enabled monarch butterflies to expand their overwintering range south (beyond Santa Barbara County), since there were previously no groves of large native coastal trees in most of Southern California.

Today many of the overwintering sites are comprised solely of eucalyptus trees. Monarchs cluster almost exclusively on eucalyptus in the southern part of the overwintering range (Santa Barbara, Ventura, Los Angeles, Orange, and San Diego counties). In fact, monarchs cluster on eucalyptus at 75% of the California overwintering sites (Frey and Schaffner 2004).

Thus, despite the fact that monarchs historically clustered primarily in stands of *P. radiata* (Riley and Bush 1881, Riley and Bush 1882, Shepardson 1914), contemporary patterns of tree use have led to the belief that western monarchs both prefer and depend on eucalyptus throughout their winter range (Sakai and Calvert 1991, Oberhauser et al. 2009). For many, this “eucalyptus preference hypothesis” is so self-evident that it is neither questioned nor tested (e.g., Sakai and Calvert 1991).

Western monarch habitat preferences, cluster dynamics, and energetics have been investigated (Tuskes and Bower 1978, Leong 1990, Leong et al. 1991, Frey et al. 1992), but only at groves containing a single tree species. While these studies have established the microhabitat parameters associated with overwintering monarchs, and the precise ranges for those parameters, they have not and cannot be used to establish tree preference. Previous microclimate studies have concluded that monarchs will only cluster at a site if conditions are met for several parameters: temperatures above freezing (Calvert et al. 1983), access to fresh water (Tuskes and Brower 1978), high moisture and low light intensity and solar radiation (Leong et al. 1991), and wind speeds below 2 m/s (Leong 1990). Microclimates at overwintering sites may be the most important predictors of occupancy. But the structure and availability of different tree species at a site have never been integrated into microclimate studies. Theoretically, overwintering sites with multiple tree species could offer a diversity of microclimates for clustering monarchs. Therefore, tree preference (if any) and differences in microclimate among tree species (if any) need to be tested at overwintering sites. Such tests can only be accomplished at groves with multiple tree species.

In order to test whether monarchs prefer eucalyptus, it is necessary to study monarch tree utilization relative to availability at overwintering sites that contain both eucalyptus and non-

eucalyptus trees suitable for clustering. It is also important that these overwintering sites are climax sites occupied by monarchs for the entire overwintering season. Some overwintering sites are transitional and are occupied only in October and November before being abandoned in midwinter. Monarchs that occupy transitional sites in the fall move to climax sites for the rest of the winter. Lastly, it is necessary to test tree utilization at different times of the overwintering season, as the weather on the central California coast is colder, windier, and wetter in December and January than in October and November (NCDC 2012). Only once all these conditions are met can we test for either a preference for eucalyptus, a preference for non-eucalyptus, or no preference.

If a test of the eucalyptus preference hypothesis shows that monarchs do prefer a particular tree species, this could provide insight into several areas of monarch biology, including: 1) how and why monarchs cluster on exactly the same tree(s) year after year, 2) whether monarchs might have either expanded their range or switched tree species in southern California, and 3) how to best proceed with the preservation and management of overwintering sites, which are under threat from development, tree cutting and removal, and senescence (Meade 1999, Frey and Schaffner 2004, Fallon and Jepsen 2013). New information on tree preference may force a reconsideration of the current eucalyptus-based management strategies.

## METHODS

**Study sites.** We surveyed monarchs at five climax overwintering sites in Monterey and San Luis Obispo Counties during three overwintering seasons (fall 2009 through spring 2012). At all sites monarchs clustered for the entire overwintering season in all three years. Sites were

selected because the grove contained eucalyptus and at least one native conifer species. General site characteristics are described below.

Pacific Grove Monarch Sanctuary (36° 37' N, 121° 55' W) is located in Pacific Grove, which is on the southern margin of Monterey Bay, Monterey County, CA. The 1.1-ha site contains *E. globulus*, *P. radiata*, and *H. macrocarpa*, with scattered coast live oak (*Quercus agrifolia*). The understory is landscaped with non-native grass, nectar plants, and shrubs.

The Big Sur private property site (36° 07' N, 121° 38' W) is located on the Big Sur coast in Monterey Co, CA. Monarchs cluster on a 1-ha parcel containing groves of *E. globulus*, *H. macrocarpa*, *P. radiata*, and *S. sempervirens*. The understory is primarily manicured lawns, non-native shrubs and flower gardens.

The Morro Bay Golf Course (35° 21' N, 120° 50' W) is located in Morro Bay, San Luis Obispo Co., CA. Monarchs cluster in two groves with a total area of 4 ha. These groves are comprised of *E. globulus* with an occasional *P. radiata* along the edges, and little to no understory vegetation.

The Pismo Beach Monarch Grove (35° 07' N, 120° 37' W) is located adjacent to the Pismo State Beach North Beach Campground in Pismo Beach, San Luis Obispo Co., CA. The 1.2-ha overwintering site contains *E. globulus*, *P. radiata*, and *H. macrocarpa*, with arroyo willow (*Salix lasiolepis*) bordering a creek. Otherwise, there is minimal understory.

Oceano Campground (35° 07' N, 120° 37' W) is located within the Pismo State Beach Oceano Campground in Oceano, San Luis Obispo Co., CA. Monarchs cluster in a 0.7-ha area within the 26-ha campground. The site is comprised of *P. radiata* and *E. globulus* with scattered Torrey pine (*Pinus torreyana*). There is extensive understory of native and non-native shrubs and forbs.

Sites that lack understory vegetation likely lack it because of shading and the allelopathic effects of eucalyptus leaf litter and roots on native plant growth (El-Khawas and Shehata 2005, Duarte et al. 2006, Zhang and Fu 2009).

**Canopy cover measurement.** Measurements were taken at all sites in summer 2012. All cluster tree species were evergreen, so canopy cover in summer was identical to that in winter when monarchs were present. At each site, we mapped out a polygon that enclosed all trees utilized by overwintering monarchs over the last 10 years. This encapsulated the largest area of known use within each grove, but did not always include the entire site. Within the polygon, we overlaid a 20x20 meter grid, and at each grid line intersection we used a densiometer to measure canopy cover by species. Only canopy above 3 meters was included, as this is the lowest that monarchs have been recorded clustering at any of these sites. We took separate densiometer readings for each tree species present. The proportion of canopy cover for each tree species was calculated as the number of densiometer squares filled by that species divided by the total number of densiometer squares filled by any canopy above 3 meters. We used the proportion contributed to canopy cover by each tree species as a metric for tree species availability.

**Count surveys.** For all three seasons, we conducted site surveys weekly from mid-October or early November (whenever the monarchs arrived) through mid-March or until monarchs left the site, whichever came first. We counted monarchs using standardized methods (Frey et al. 1992). In this method, multiple observers estimated the number of monarchs present in each cluster at a site, and these values were recorded and then used to calculate average cluster size (across observers). The sum of the averages of the cluster estimates was the total site estimate. We also recorded the tree species occupied by each cluster. We used the average

number of monarchs clustering on each tree species per month per site as a metric for tree utilization.

**Data analysis.** Three chi-square tests were run in R 2.15.3 to test whether monarchs utilized eucalyptus in proportion to its availability (Sokal and Rohlf 1995). To test whether monarchs clustered disproportionately on any tree species over the course of an entire season, we compared the average monthly number observed on each tree species (count data) vs. the expected number per tree species (counts subdivided among tree species in proportion to canopy cover availability) for each site and each year. To test for monarch preferences at more specific points during the overwintering season, we also compared observed vs. expected monarch numbers (vis a vis tree species) for each site and year during 1) the time of maximum occupancy and 2) the survey closest to the middle of the season (31 December). Given that some  $\chi^2$  values were significant, we used sign tests of the chi-square results of all sites and all years to determine if monarchs were clustering significantly more than expected on eucalyptus over the course of a whole season, during the time of maximum occupancy, or at mid-season (Sokal and Rohlf 1995). Finally, because multiple counts were done at each site in the same season and the same sites were counted across years, we ran a repeated measures ANOVA (Gotelli and Ellison 2004) using JMP 10.0 to test for effects of tree species, site, month, and year on the monthly average number of clustering monarchs per tree species.

## RESULTS

**Site specific results.** The canopy of the Pacific Grove Monarch Sanctuary was comprised primarily of *E. globulus* (42.6%) and *P. radiata* (34.5%), with some *H. macrocarpa* (22%) and a small amount of non-native ornamental trees (1%). Monarchs did not cluster on trees in

proportion to availability over the course of a whole season, during the time of maximum occupancy, or at mid-season ( $\chi^2$  values ranged from 111.12-27332.03, all  $p < 0.001$ ). Dates of maximum occupancy each season ranged from 16 November to 7 December. Monarchs clustered significantly more on native conifers during the seasonal midpoint in all three seasons and during maximum occupancy in two seasons (Figure 2). In two of the three (2010-11 and 2011-12) overwintering seasons, the monarchs moved from clustering primarily on eucalyptus at the beginning of the season to clustering primarily or exclusively on native trees in the middle and end of the season.

The canopy at the Big Sur private property site was comprised of nearly equal amounts of *E. globulus* (44.9%) and *H. macrocarpa* (44.7%). This was the site with the least amount of *P. radiata* (1.1%), and the only site where *S. sempervirens* was present (9.1%). A tiny percent of the canopy cover was also comprised of non-native ornamental trees (0.2%). Monarchs did not cluster on trees in proportion to availability over the course of a whole season, during the time of maximum occupancy, or at mid-season ( $\chi^2$  values ranged from 301.51-20935.01, all  $p < 0.001$ ). Dates of maximum occupancy each season ranged from 22 November to 6 December. The only time that monarchs clustered more than expected on eucalyptus at this site was October through December of 2010, which included the week of maximum occupancy (Figure 3). For the remainder of that season, monarchs clustered more than expected on native conifers. For the entirety of the 2009-10 and 2011-12 overwintering seasons, including midseason and at times of maximum occupancy, monarchs clustered significantly more than expected on native conifers.

The Morro Bay Golf Course had the highest percentage of eucalyptus canopy cover and the least amount of native conifer canopy cover out of all five sites. The canopy was comprised almost exclusively of *E. globulus* (97.4%), with only a small amount of *P. radiata* (2.6%).

Monarchs did not cluster on trees in proportion to availability over the course of a whole season, during the time of maximum occupancy, or at mid-season ( $\chi^2$  values ranged from 13.27-550.48, all  $p < 0.001$ ). Dates of maximum occupancy each season ranged from 29 November to 21 December. This site was unique in that during the entirety of the 2010-11 and 2011-12 seasons, monarchs clustered exclusively on eucalyptus trees (Figure 4). In 2009-10, the butterflies clustered significantly more than expected on native conifer (*P. radiata*) only in December and January, and at the time of maximum occupancy.

The Pismo Beach Monarch Grove canopy was comprised mostly of *E. globulus* (76.2%). *H. macrocarpa* made up 10.8% of the canopy, with *P. radiata* contributing only 3.5%. The rest of the canopy was comprised of native understory species such as arroyo willow (*Salix lasiolepis*) (9.5%). Monarchs did not cluster on trees in proportion to availability over the course of a whole season, during the time of maximum occupancy, or at mid-season ( $\chi^2$  values ranged from 396.80-233530.70, all  $p < 0.001$ ). Dates of maximum occupancy were the latest and most wide-ranging of any site, spanning from 22 November to 19 January. Monarchs clustered significantly more than expected on native conifers throughout the 2009-10 and 2011-12 overwintering seasons (Figure 5). During the 2011-12 season, the monarchs clustered somewhat on eucalyptus in October and November, but by December they cluster almost exclusively on native conifers. In contrast, during the 2010-11 season monarchs clustered significantly more than expected on eucalyptus in every month except February, including at midseason and at time of maximum occupancy.

Oceano Campground had the highest percentage of native canopy cover (which was composed entirely of *P. radiata*) (84.2%), and the lowest percentage of *E. globulus* cover (15.3%). A tiny percent of canopy was comprised of native understory species (0.5%). Monarchs

did not cluster on trees in proportion to availability over the course of a whole season, during the time of maximum occupancy, or at mid-season ( $\chi^2$  values ranged from 324.06-23569.17, all  $p < 0.001$ ). Dates of maximum occupancy each season ranged from 18 November to 21 December. Monarchs exhibited the same pattern of movement in all three seasons: they clustered significantly more than expected on eucalyptus at the beginning of the season, and then clustered significantly more than expected on pine at the middle or end of the season (Figure 6). They clustered significantly more on native trees during the seasonal midpoint and week of maximum occupancy in 2010-11 and in 2011-12.

**Multi-year and multi-site analyses.** The sign tests indicated that monarchs were not clustering on any tree species significantly more often over the course of the season ( $p = 1.0$ ), during maximum occupancy ( $p = 1.0$ ), or in midwinter ( $p = 0.3$ ). In other words, there is no clear preference for overwintering substrate across the entire season, across all sites, or all years. Monarchs clustered significantly more than expected on native trees more than 50% of the time (8 out of 15 times on a seasonal level, 8 out of 15 times at peak occupancy, and 10 out of 15 times during mid-season), and on eucalyptus less than 50% of the time (7 out of 15 times on a seasonal level, 7 out of 15 times at peak occupancy, and 5 out of 15 times during mid-season).

Monarch abundance varied widely between sites and between years. At four out of five sites the fewest monarchs were present during the 2009-10 overwintering season. There were significantly more monarchs present at all sites during the 2011-12 overwintering season ( $F_{2,12} = 4.73$ ,  $p = 0.013$ ) (Figure 7). In the 2011-12 season when more monarchs were present, the monarchs clustered significantly more than expected on native trees at four of the five sites during the season as a whole, during maximum occupancy, and at mid-season. The average

number clustering on native trees was also significantly higher across all sites in 2011-12 than in other years ( $F_{2,12} = 9.24$ ,  $p < 0.001$ ).

The results show a clear rejection of the eucalyptus preference hypothesis.

## DISCUSSION

Overwintering monarchs did not favor eucalyptus significantly more often than they favored native trees, nor did they favor eucalyptus overall. This means they did not express a preference for eucalyptus or a preference for non-eucalyptus. Based on these results, we must reject the eucalyptus preference hypothesis. We likewise reject a random utilization hypothesis because at any given time monarchs clustered significantly more than expected on either eucalyptus or on native trees. We offer the conditional preference hypothesis as an alternative explanation for the spatial and temporal clustering patterns we document.

The conditional preference hypothesis states that monarchs will preferentially cluster in the tree species that provides them with the most suitable microclimate within an occupied overwintering site. The most important prediction that follows from this hypothesis is that monarchs will shift among tree species as the weather changes at a site over the course of the overwintering season. In a general sense, we do have evidence of monarchs switching among tree species at Pacific Grove, Big Sur, Pismo Beach, and Oceano. At those sites, in at least one year, the majority of monarchs clustered on eucalyptus at the beginning of the season, but by the end of the season they were clustering significantly more on native conifers. Furthermore, this shift typically took place in the middle of the season around December or January, when the weather usually becomes most unstable. Temperatures are lower, storm events are more frequent, and wind speeds are higher than at other times of year (NCDC 2012). It would be

important to move beyond observing this general pattern of movement and to test the correlate predictions: movement between tree species should correspond with shifts in weather conditions, and movement should correspond with tracking of specific microhabitat parameters and their values.

The exception to the general pattern of midseason tree-switching noted above was the Morro Bay Golf Course. This site is comprised of 97% eucalyptus. The conditional preference hypothesis leads us to predict that during weather conditions under which monarchs would choose to cluster on conifers, fewer monarchs should cluster at the Morro Bay Golf Course because it does not offer enough tree species diversity (i.e., conifers). We have some general support for this prediction. In 2011-12 significantly more monarchs clustered on native trees at all sites except Morro Bay Golf Course, and the number of overwintering monarchs present increased by at least 95% from the previous year at all sites except Morro Bay Golf Course. The Morro Bay Golf Course saw an increase of only 25% over the previous year. Perhaps the lack of an option to cluster on alternative types of trees makes this site less suitable (or even unsuitable) under extreme conditions. A more specific test would involve tagging overwintering butterflies and determining whether the emigration rate from a site like the Morro Bay Golf Course exceeds the immigration rate, during inclement weather.

One possible reason that monarchs switch to conifers in midwinter is that the physical structure of those trees may allow them to cluster in a more beneficial way. At the Mexican overwintering sites, monarchs cluster almost exclusively on Oyamel fir trees where they are able to form very large, dense clusters (Urquhart and Urquhart 1976, Brower et al. 1977). Research has shown that these dense clusters provide thermoregulatory benefits to the monarchs in the center: they are warmer at night and cooler during the day (Brower et al. 2008), and they are

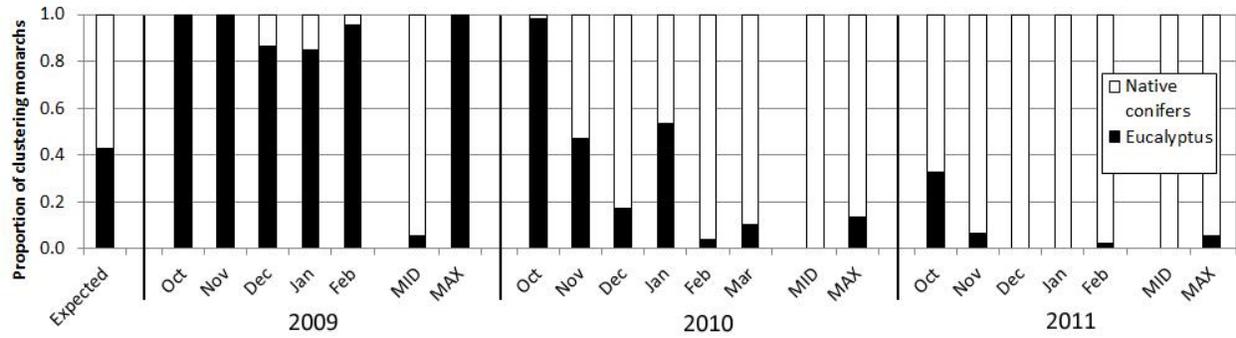
resistant to wetting thereby increasing their ability to survive freezing temperatures (Alonso-Mejia et al. 1992, Anderson and Brower 1996). It is possible that the branch structure of the native California conifers may allow the monarchs to cluster densely enough to achieve this thermal stabilization. Eucalyptus trees have a more open branch structure with widely-spaced, broad leaves that may not allow monarchs to cluster at the most beneficial density during cold, wet weather. Possibly the Eucalyptus branch and foliage structure is more suitable for clustering under the climate conditions that prevail in October and November. We offer this as a mechanistic prediction from the conditional preference hypothesis.

Population size may also play a role in clustering behavior. The eastern monarch population is much larger than the western population. Individual wintering sites in Mexico may have millions of monarchs whereas the largest California overwintering sites today only have tens of thousands (Garcia-Serrano et al. 2004, Xerces Society 2014). We observed that in 2011-12 when the overwintering population was highest, monarchs clustered disproportionately on native trees during all times of the season (overall, midpoint, and maximum occupancy) at all sites except Morro Bay Golf Course (where very few conifers were present). Perhaps there is a minimum number of monarchs that must be present at a site before they form large dense clusters as they do in Mexico. Alternatively, behavior or population size tipping points that facilitate specific behaviors may simply differ between Mexico and California overwintering populations, perhaps because freezing at coastal California overwintering sites is much less likely (NCDC 2012).

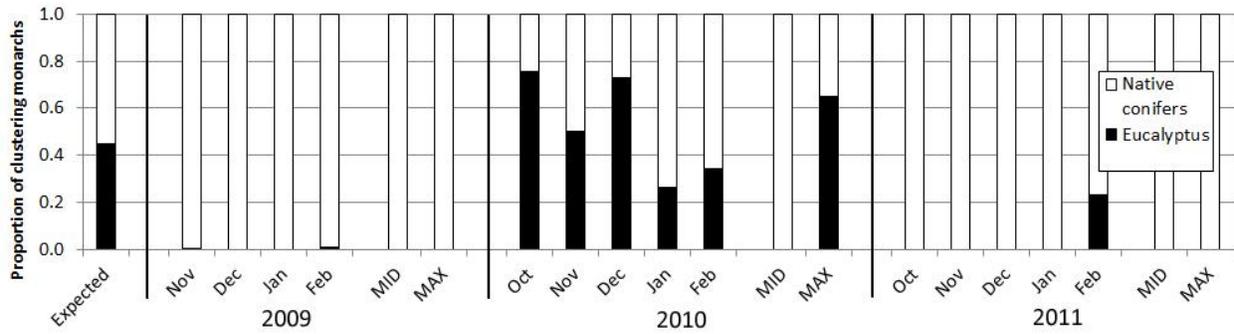
Our study is the first to examine monarch clustering behavior at California overwintering sites with multiple tree species. We suggest the possibility that monarchs will move between tree species (and not just trees) in response to microclimate conditions. Previous studies conducted

in eucalyptus-only or pine-only groves have shown that monarchs will move between trees of the same species as microclimate changes (Leong et al. 1991, Frey et al. 1992). At two California overwintering sites with only one tree species (one eucalyptus-only and one pine-only), Frey et al. (1992) found that as the overwintering season progressed monarchs used fewer cluster trees while cluster size increased. Previous studies have also shown that if a site becomes climatically unsuitable during the winter, monarchs will leave the site completely (Leong 1990, Leong et al. 1991). Tuskes and Brower (1978) showed high site fidelity at a conifer-only site and a eucalyptus-only site in northern California, but very high site movement (immigration and emigration) at a southern all-eucalyptus site. There are currently no data available on emigration rates from eucalyptus-only sites vs. mixed eucalyptus/native conifer sites, so it is unknown whether monarchs will stay longer at a site if there is a greater diversity of tree species available for use.

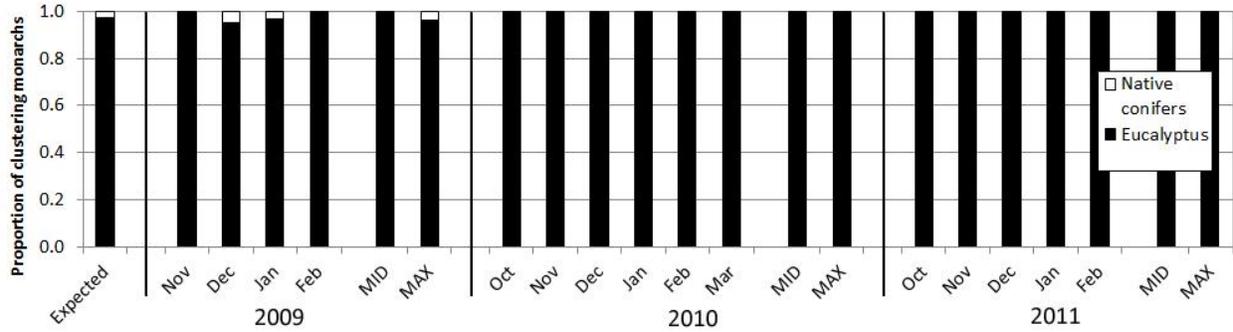
Future research should test the conditional preference hypothesis by examining monarch tree utilization when weather conditions are known to be both favorable and unfavorable. We may thus test the prediction that monarchs will shift among trees and among tree species in response to changing microclimate parameters (e.g. temperature, wind speed, and humidity), especially if microhabitat conditions are simultaneously monitored along with cluster locations. This should reveal the conditions under which monarchs switch trees or tree species, and may allow us to determine tree utilization during the most intense storms.



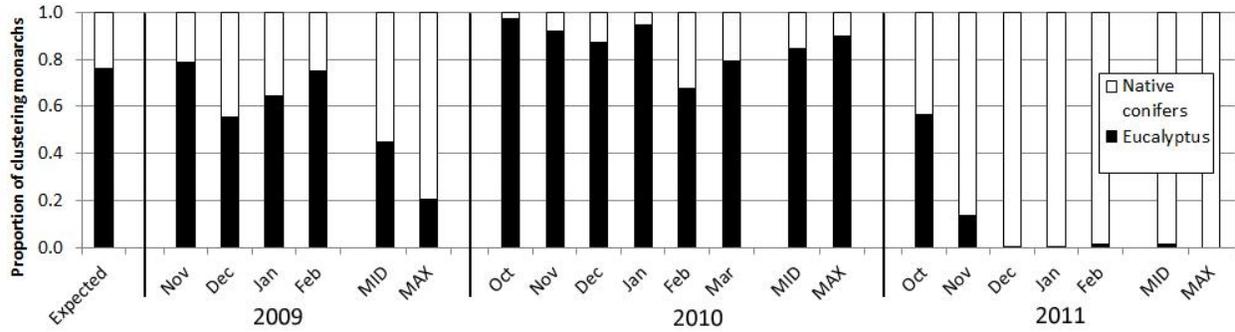
**Figure 2.** Tree use by overwintering monarch butterflies at Pacific Grove Monarch Sanctuary during three overwintering seasons. Column shading reflects the proportion of monarchs clustering on eucalyptus or native tree species. The “expected” column shows expected monarch tree use if the monarchs clustered in direct proportion to the available canopy. Monthly columns reflect average monarch tree use for that month. “Mid” refers to monarch tree use at seasonal midpoint (closest to December 31) and “max” refers to monarch tree use during the week of maximum occupancy at the site.



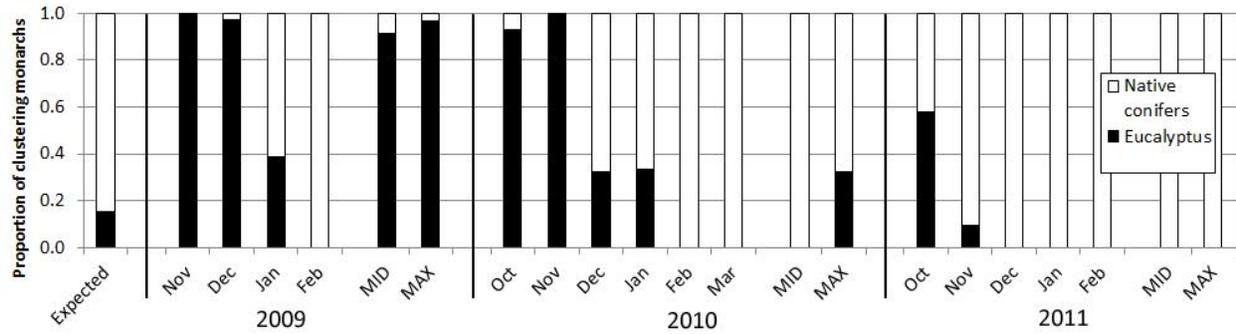
**Figure 3.** Tree use by overwintering monarch butterflies at the Big Sur private property site during three overwintering seasons. Column shading reflects the proportion of monarchs clustering on eucalyptus or native tree species. The “expected” column shows expected monarch tree use if the monarchs clustered in direct proportion to the available canopy. Monthly columns reflect average monarch tree use for that month. “Mid” refers to monarch tree use at seasonal midpoint (closest to December 31) and “max” refers to monarch tree use during the week of maximum occupancy at the site.



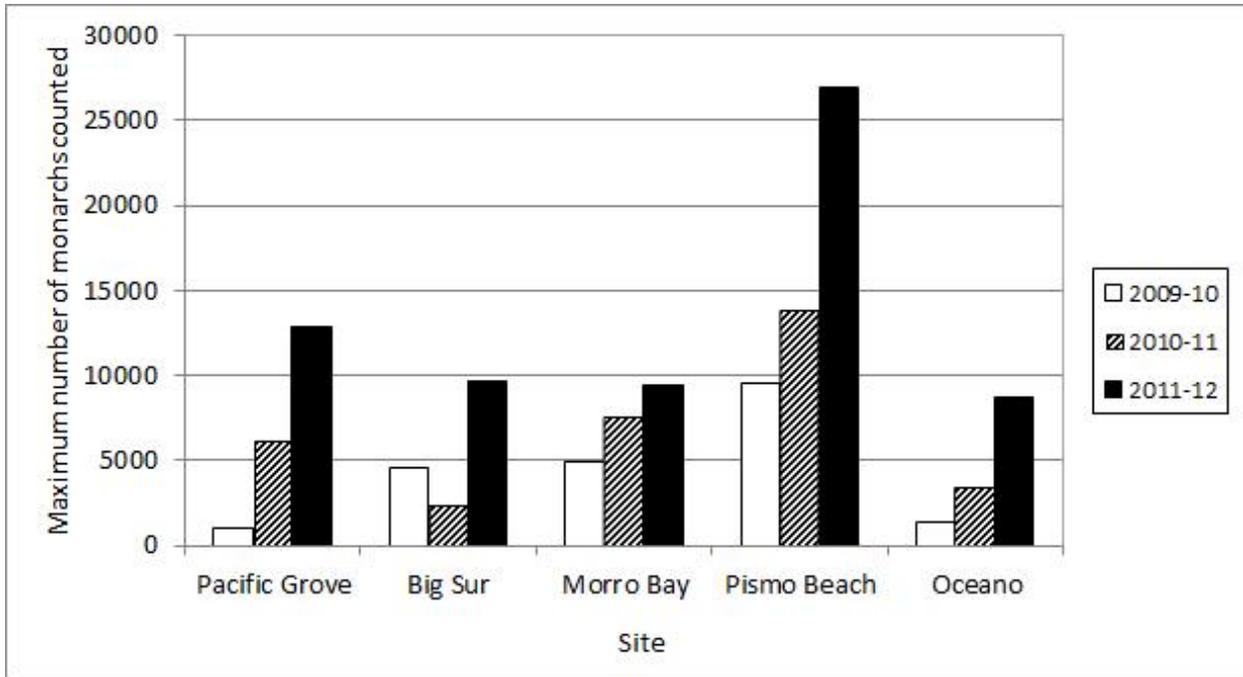
**Figure 4.** Tree use by overwintering monarch butterflies at the Morro Bay Golf Course during three overwintering seasons. Column shading reflects the proportion of monarchs clustering on eucalyptus or native tree species. The “expected” column shows expected monarch tree use if the monarchs clustered in direct proportion to the available canopy. Monthly columns reflect average monarch tree use for that month. “Mid” refers to monarch tree use at seasonal midpoint (closest to December 31) and “max” refers to monarch tree use during the week of maximum occupancy at the site.



**Figure 5.** Tree use by overwintering monarch butterflies at Pismo Beach Monarch Grove during three overwintering seasons. Column shading reflects the proportion of monarchs clustering on eucalyptus or native tree species. The “expected” column shows expected monarch tree use if the monarchs clustered in direct proportion to the available canopy. Monthly columns reflect average monarch tree use for that month. “Mid” refers to monarch tree use at seasonal midpoint (closest to December 31) and “max” refers to monarch tree use during the week of maximum occupancy at the site.



**Figure 6.** Tree use by overwintering monarch butterflies at Oceano Campground during three overwintering seasons. Column shading reflects the proportion of monarchs clustering on eucalyptus or native tree species. The “expected” column shows expected monarch tree use if the monarchs clustered in direct proportion to the available canopy. Monthly columns reflect average monarch tree use for that month. “Mid” refers to monarch tree use at seasonal midpoint (closest to December 31) and “max” refers to monarch tree use during the week of maximum occupancy at the site.



**Figure 7.** The maximum number of clustering monarchs counted at five overwintering sites in California in three overwintering seasons from 2009-2012.

## ABSTRACT

Monarch butterflies overwintering in coastal California: low site fidelity and high intersite movement

Western monarch butterflies (*Danaus plexippus L.*) overwinter in large aggregations at hundreds of sites along the California coast. Management plans and census methods are both founded on the assumption that individual monarchs arrive at an overwintering site in the fall and stay at that site for the winter. Though populations potentially coalesce en masse from autumnal sites onto climax overwintering sites, very little individual movement between sites is inferred. Monarch movement is therefore thought to be primarily into sites (as opposed to out of or among them). We refer to this assumption and inference as the accrual hypothesis. In light of previous studies that provide evidence for movement among sites, we propose that overwintering monarchs may belong to a superpopulation. The existence of a superpopulation comprised of individuals moving in and out of sites would force us to rethink our ideas of landscape-level resource use by monarchs, our site-centric (rather than landscape-level) management strategies, and our abundance estimation techniques, which employ closed population models. We tested the closed population model, the accrual hypothesis, and the superpopulation model at three California overwintering sites using a mark-resight study design. We found that a large proportion of the monarchs at a site moved among (into and out of) monitored sites, both while the population size increased in October and November, and while the population appeared to exhibit an equilibrium winter maximum. The pattern of abundance of both tagged and untagged monarchs at monitored sites leads us to reject the closed population model and the accrual hypothesis. We found that monarchs at all three study sites are part of a larger superpopulation,

though the sites do not contribute to the superpopulation equally. We determined that mark-resight is a viable alternative to existing population estimation techniques, though mark-resight methods would need to be explored further before being applied routinely. Our results suggest we need to move away from site-based management and manage instead for landscape-level overwintering (superpopulation) dynamics.

**Keywords:** Monarch butterfly, *Danaus plexippus*, superpopulation, site fidelity, site movement, open population, overwintering population, mark-resight, detection probability.

## INTRODUCTION

Abundance is a critical parameter in population monitoring studies, especially for species in decline. Data on abundance are necessary to gauge the effectiveness of recovery efforts (e.g., Campbell et al. 2002, Lotze et al. 2011), understand the impacts of habitat modification, restoration, or loss (e.g., Parker and MacNally 2002, Munday 2004, Golet et al. 2008), measure the effects of disease and the spread of parasites (e.g., Vazquez et al. 2005), elucidate the impacts of stochastic events such as large storms or predation (e.g., Nielsen and Kiorboe 1990, Faccio 2003, Festa-Bianchet et al. 2006), and fuel interest in the undertaking of conservation efforts. Abundance estimates have been used in all of these ways in the conservation and management of western monarch butterflies (*Danaus plexippus* L.). Western monarchs migrate from breeding grounds across the western U.S. to overwinter in forested groves at multiple sites located along the California coast (Leong et al. 2004). At the overwintering groves, monarchs form large clusters of hundreds or thousands of individuals on individual trees. These overwintering aggregations of monarchs persist from October through February or March (Frey and Schaffner 2004). Because nearly the entire western monarch population gathers at these overwintering sites, it is an opportune time to estimate the annual population size. The putative efficacy of site management strategies, the local and regional role of conservation threats, and the public's interest, are gauged by the estimate the annual population size.

Abundance is measured annually via the Western Monarch Thanksgiving Count (WMTC). This survey was developed in 1997 by The Monarch Program (<http://www.monarchprogram.org>) and is now administered by The Xerces Society (<http://www.xerces.org>). During the three-week survey period (the last two weeks of November and the first week of December) volunteers visit as many overwintering sites as possible and use

a standard protocol (Frey et al. 1992; detailed in Methods below) to estimate the number of overwintering monarchs per site. These data are used to track overall monarch abundance and to evaluate habitat management outcomes at individual overwintering sites. WMTC data reveal that western monarchs have declined by 90% over the last 15 years (Xerces Society 2014). This alarming decrease in abundance underscores the necessity of accurate population estimates, and how such estimates can be (or are) used to infer causality of the decline or causality of annual fluctuations (Stevens and Frey 2010).

Movement is important because it can reveal behavioral choice on the part of monarchs, and landscape-level connections among occupied sites. For example, overwintering sites are classified as either climax or transitional. Transitional sites are occupied by monarch butterflies only during the early part of the season (October and November) whereas climax sites are occupied by clustering monarchs for the entire season (Leong et al. 2004). The monarchs that cluster at transitional sites only use them in the fall and early winter, and by mid-winter they leave transitional sites and move to cluster at climax sites. It is inferred that transitional sites provide suitable overwintering habitat until winter conditions become severe, and then transitional sites become unsuitable. At a landscape level, overwintering therefore happens in areas with climax sites, though other sites are occupied and used. Movement (or lack of movement) among sites reveals whether sites (and the populations that occupy those sites) are connected or isolated, and whether they are independent or correlated.

Movement is also important because it can potentially affect abundance estimation. If one estimates population size using a technique that assumes geographic closure and that assumption is violated by individuals moving into and/or out of the study area, the population estimators may be biased (Kendall 1999). Several tagging studies in California have

demonstrated that monarchs move among overwintering sites during the winter. It is known that movement occurs all winter and is not limited to arrival in the fall, dispersal in the spring, or coalescence onto climax sites when transitional sites are abandoned (Urquhart et al. 1965, Frey et al. 2003, Griffiths and Thorngate 2008, Griffiths 2009). While movement of monarchs among overwintering sites has been shown, the extent of this movement is not known. We therefore ask what proportion of a site's population moves on a daily basis, monthly basis, and seasonal basis, is this movement sufficient to affect population estimation methods, and is it at all meaningful to derive abundance estimates using techniques that assume a closed population? For instance, Tuskes and Brower (1978) calculated monarch abundance at overwintering sites using mark-recapture, but we question the population estimates they generated because they used a model that assumed geographic closure. Likewise, the WMTC abundance estimates are calculated without taking movement into consideration. More specifically, the WMTC estimate is based on estimates at sites visited over a three week sampling period, with no control or analysis for movement or precautions against double counting of individuals that move between or among sites. Instead the WMTC assumes static abundance across a network of occupied sites.

When estimating abundance in a system such as this one, where individuals may be moving in and out of sites or using multiple sites on the landscape, a superpopulation approach may be more appropriate (Schwarz and Arnason 1996, Kendall 1999). In general, a superpopulation is defined as the total number of animals that enter into a sampled area (or population) during the entire sampling period. Members of a superpopulation may be breeding or non-breeding, and may be separated in time but not space (for instance, as in an asynchronous breeding colony of birds where some individuals arrive and breed early, leaving as later breeders arrive). This is in contrast with a metapopulation, which consists of spatially separated sub-

populations of breeding individuals which may or may not interact with each other. The superpopulation approach has been used to derive population estimates for asynchronously breeding colonial waterbirds (Williams et al. 2011), amphibians with prolonged breeding seasons (Wagner et al. 2011), and would be applicable to birds at migration stopover sites (Williams et al. 2002). Most relevant to this study, Gould et al. (2005) used this approach to estimate the superpopulation size of elk traveling among wintering sites.

With regards to overwintering monarch butterflies, the superpopulation would be comprised of individuals that reside in a geographic area encompassing several overwintering sites, and that may enter or leave any of those sites, or the geographic area, at any time. This is distinct from a local population consisting of monarchs occupying one site at a single point in time. It is straightforward to test for the presence of a superpopulation: if the individuals at a site are not part of a superpopulation, then the local population and superpopulation estimates will be identical. Identical estimates would be predicted because all individuals would be resident at that site and only at that site. However, if the individuals at a site are part of a superpopulation, then the abundance at the local site would partially contribute to the abundance in the superpopulation, and the superpopulation estimate would be larger than the local population estimate. The existence of a monarch superpopulation has never been tested, and could have important management and conservation implications. For instance, a land manager might make the assumption that because an overwintering site has more clustering monarchs than another nearby site, the site with more monarchs is more worthy of management or protection. In reality, individuals may be moving between the two sites, or even using resources (such as nectar sources) located between the two sites, such that both sites could be important to overwintering survival.

Traditional mark-recapture techniques can be used to study a superpopulation system, but may not be suitable for overwintering monarchs because multiple recapture events would require repeatedly disturbing overwintering clusters. Monarchs minimize energy expenditure and conserve fat reserves in order to survive the winter at sites that have few to no nectar resources (Chaplin and Wells 1982, Masters et al. 1988). Activity causes them to burn reserves more quickly and can impact winter survivorship (Tuskes and Brower 1978, Alonso-Mejia et al. 1997).

Mark-resight approaches may be more appropriate for monarch abundance estimation, and for studying movement among sites, than mark-recapture approaches. In mark-resight studies, individuals are marked as a batch (i.e., different colored marks for each day and site of tagging) with highly detectable marks (bright and contrasting colors). Following the marking session, there are multiple encounter (i.e., resight) sessions where data are collected on the abundance of marked and unmarked individuals in the population. Mark-resight has been used most often to estimate population size in large mammal species that are difficult and labor-intensive to recapture, such as coyotes (Hein and Andelt 1995), bears (Miller et al. 1997), elephants (Morley and van Aarde 2006), and a variety of ungulates including elk (Bear et al. 1989, McCorquodale et al. 2013), moose (Bowden and Kufeld 1995), caribou (Mahoney et al. 1998), and others (Neal et al. 1993, McClintock and White 2007, Wingard et al. 2011). Mark-resight is faster and requires less effort than mark-recapture, and can be easily adapted for use with monarchs. Observers can scan monarch clusters with binoculars looking for tags. As a result, resighting can also be integrated into the standardized abundance estimation survey protocol: surveys require observers to scan clusters with binoculars as they estimate the number of monarchs present (see Methods below), and it is simple to simultaneously count the number of

tagged butterflies seen. Resighting is efficient if done when monarchs are clustered in overwintering aggregations because they are stationary and observers can search all individuals for marks. Lastly, unlike mark-recapture, mark-resight does not require that the marks be individually identifiable. This is beneficial because monarch tags are individually identifiable only when the monarch is in hand (which requires recapture and handling) or when the cluster is low enough that the tag can be read with a scope.

Though marking monarchs does require an initial capture, it is important to point out that studying movement of unmarked monarch is impossible. Monarchs begin arriving on the California coast in large numbers by October, and their abundance rises rapidly at sites over the course of the month. This increase in abundance continues into November, when populations at a site may increase up to tenfold (e.g., Frey et al. 2003, Frey et al. 2004). The population increase at climax sites may continue into December as monarchs move in from abandoned transitional sites. This net movement into sites obscures other movement (if it is occurring) and makes it difficult to detect movement out of sites or among sites because the only indicator of movement is abundance. Therefore, it is not surprising that it is generally assumed that after monarchs arrive at a climax site, they remain there for the remainder of the season (Koenig 2006). Based on the observed population increases at overwintering sites, one might hypothesize that monarchs are only moving into sites and not out of them or among them. We call this current view of one-way movement into sites the “accrual hypothesis”.

The accrual hypothesis predicts that during October and November (and in December at climax sites), monarch abundance at individual sites should increase from week to week. However, estimates of the number of overwintering monarchs at multiple sites in Monterey and San Luis Obispo counties show variability from week to week during this period (Hamilton et al.

2002, Frey et al. 2003, Frey et al. 2004, Griffiths and Thorngate 2008, Griffiths 2009, Villablanca 2010). This variation can be attributed to multiple factors such as sampling error, or a violation of any of the assumptions of a closed population model (e.g., immigration, emigration, mortality). If fluctuations are due to conditions consistent with an open population model, then the accrual hypothesis is incorrect. Evidence for pervasive movement out of sites such as documenting movement of tagged individuals or large changes in the proportions of tagged and untagged monarchs at a site, especially at climax sites, would be inconsistent with the accrual hypothesis.

Herein we document the use of mark-resight on an overwintering population of monarch butterflies. The goals of this study are to 1) determine if the monarchs that occupy an overwintering site are resident (a high frequency persist for multiple days in a row) 2) test the accrual hypothesis that the gradual increase in population size at a site (from early fall to early winter) is simply due to a net movement into the site, rather than the sum of movement in minus movement out, 3) test whether overwintering monarchs comprise a superpopulation by comparing the mark-resight local population estimates to the superpopulation estimate, 4) comparing the mark-resight local population estimates to the local site estimates obtained via traditional closed population survey techniques (visual cluster surveys), and 5) discuss how our views on overwintering or conservation might be modified if monarchs are overwintering as superpopulations and thus fit an open population model better than they fit a closed population model.

## METHODS

Field work was conducted from October through December 2010 at three monarch butterfly overwintering sites located in San Luis Obispo County, California. Study sites were chosen because of their proximity to each other (less than 2 miles apart) and their proximity (less than 3 miles) to a monitored overwintering site that has one of the largest local overwintering aggregations in California (Pismo Beach North Beach Campground).

**Study sites.** The 0.7-ha Oceano Campground (Oceano) site (35° 07' N, 120° 37' W) is located within the 26-ha Pismo State Beach Oceano Campground near Oceano, CA. It is comprised primarily of *Pinus radiata* and *Eucalyptus globulus*, with some Torrey pine (*Pinus torreyana*), and has an extensive understory of shrubs and forbs. The Halcyon Hill (Halcyon) site (35° 06' N, 120° 35' W) is located in Oceano, CA, and is a 3.4-ha area comprised entirely of *E. globulus* with very little understory. The Pike (Pike) site (35° 06' N, 120° 35' W) is a 0.5-ha site located on private property in Arroyo Grande, CA. The site consists of *E. globulus* with a mixed understory comprised of shrubs, forbs, and prickly pear (*Opuntia sp.*). Oceano is 2.0 miles from Halcyon and 1.8 miles from Pike, while Halcyon is 0.3 miles from Pike.

**Tagging.** Seven monarch butterfly tagging sessions took place across three sites during October, November, and December. At Oceano and Halcyon, tagging was conducted during the third week of October, the third week of November, and the second week of December. At Pike, tagging was conducted once during the third week of October. Monarchs were netted while still in clusters early in the morning when ambient temperatures were below the monarch flight threshold (55 °C). Each individual monarch was tagged with a uniquely-numbered adhesive tag 0.9 cm in diameter (Watson Label Products), and the monarch's sex and tag number were recorded. A different color tag was used for each site and for each session, such that the color of

a monarch's tag could be used to readily identify the date and location ("origin location") at which it was marked. Tags were placed on the center underside of the right hindwing (on the discal cell), so that the tag would be visible when the monarch was at rest in a cluster. Each tagging sessions terminated when there were no untagged monarchs remaining in clusters or when the temperature had risen to a point when the monarchs were leaving their clusters, whichever came first.

**Resight sessions.** Resight sessions were conducted for five consecutive mornings following the tagging session in October and for three consecutive mornings following the tagging sessions in both November and December. Observers located all clusters at each site, counted the total number of monarchs and the number of tagged monarchs, and recorded the color of all re-sighted tags.

**Standardized visual cluster abundance estimates.** The abundance of overwintering monarchs present at the three tagging locations was estimated weekly from October (when monarchs arrived and began clustering) through mid-March (when monarchs dispersed) using the standardized method described in Frey et al. (1992). In this method, multiple observers use binoculars to examine all clusters at a site. For each cluster, they estimate the number of monarchs present, and calculate the average estimated size of each cluster (across observers). The sum of the averages of the cluster estimates is the total site estimate. The number of untagged monarchs at a site was calculated by subtracting the number of tagged monarchs seen at the site from the total visual cluster estimate. Weekly visual cluster surveys were supplanted by resight sessions in the week that followed each tagging session. No visual cluster surveys were conducted during the second half of December.

Expected upper and lower bounds for abundance estimates were calculated for each visual cluster survey using the variability between observers. To quantify the amount of variability, we separated each pair (or triplet) of cluster size estimates into a lower and a higher values for each cluster, and then calculated how far these values were from their combined average. For each cluster estimate, these differences were converted into proportions, and each proportion was classified as an underestimate or overestimate. We then averaged these proportions across all sites and all surveys and generated a separate mean and 95% confidence interval for the underestimate and overestimate proportions. The lower bound of the underestimate confidence interval and the upper bound of the overestimate confidence interval were used as multipliers for lower and upper bounds (respectively) of visual cluster estimates.

**Mark-resight data analysis.** Mark-resight data analysis was conducted using Program MARK v 6.1. Because of the evidence for movement among overwintering sites discussed above, it was necessary to use a mark-resight population estimation model that allows for open populations. We used the immigration-emigration logit-normal estimator in MARK (McClintock and White 2010), which was developed from the immigration-emigration joint hypergeometric estimator in NOREMARK (Neal et al. 1993, White 1996). This model generates local population ( $N$ ) estimates and superpopulation ( $N^*$ ) estimates and does not require individually recognizable marks. We used the following data for the input variables:  $m$  (number of tagged individuals sighted during each resight day (resight occasion),  $T$  (total number of monarchs currently tagged at all sites),  $T_u$  (number of unmarked individuals sighted during each resight occasion), and  $M$  (actual number of tagged individuals present at the site during each resight occasion).

The variable  $M$  may be unknowable in our study, in part because we concede that some marked individuals may leave the sampled geographic area. The number (or proportion) that leave would depend on the amount of movement, which is exactly what we are trying to estimate. Therefore, we ran two sets of models which simultaneously varied both the amount of movement and the detection probability. The first was a naïve model, in which  $M$  was assumed to be equal to the highest number of tagged monarchs observed during the resight occasion. With this model we assume detection probability is high, such that we detected nearly every marked individual present, and therefore any undetected tagged monarchs must have left the site (i.e., the “high movement model”). In the second model,  $M$  was assumed to be equal to the number of monarchs that had been tagged at that site during the preceding tagging session(s). With this model we assume no tagged monarchs left the study area and any undetected tagged monarchs were simply undetected due to a low detection probability (i.e., the “no movement model”). Though it is likely neither of these models is totally correct, it allows us to contrast the no movement model, which is the closed population model implicitly assumed when estimating population size from traditional standardized techniques, with an appropriate open population alternative.

Because marks were not individually recognizable, individual heterogeneity in resight probabilities ( $\sigma_j$ ) was assumed to be zero. We were interested in generating  $N$  and  $N^*$  at each site for each month in order to compare these estimates to each other and to those derived from the weekly standardized visual cluster surveys (sensu Frey et al. 1992); therefore, each resight session was modeled separately. Model results were regarded as having different outcomes if their standard errors or 95% confidence intervals did not overlap.

We predict that if monarchs move between sites as part of a superpopulation,  $N^*$  will be larger than  $N$ . If individuals at a site are not part of a superpopulation, then  $N^*$  and  $N$  should be equal. Additionally, if monarchs at overwintering sites do move (an open population), then the local population estimate for each site obtained via the observer estimate method will be closer to the  $N$  value predicted by the “high movement” model than the “no movement” model. The reverse should be true if monarchs do not move (a closed population): the  $N$  value predicted by the “no movement” model will be closer to the number of monarchs observed in visual cluster surveys.

## RESULTS

**Tagging and resight evidence for movement.** A total of 5775 monarchs were tagged over three months and three sites. More monarchs were tagged at Halcyon than any other site, and more monarchs were tagged in November than any other month (Table 3). Over 40% of the monarchs at each site were tagged in October and November. In December 64% of the monarchs at Oceano were tagged, while 25% were tagged at Halcyon. The average proportion of monarchs present that were tagged per tagging session across all sites was 48%.

Tagged monarchs that did not move could be resighted at their “origin location” (the site of tagging). The proportions of tagged monarchs that were resighted at their origin location each month ranged from a low of 16% at Halcyon in December to a high of 37% at Pike in November (Table 4). The average proportion of monarchs resighted at their origin location each month was 26% across all sites and all resight occasions. If detection probabilities are high, then this suggest the majority of monarchs are not resident at their origin location, but instead move to other sites.

Tagged monarchs that did move could be resighted at a non-origin location. Tagged monarchs from all sites were resighted at all other sites. Monarchs moved the least to and from Oceano: only 1-3% of the tagged Oceano monarchs were resighted per month at the other two sites, and a maximum of 1% of tagged Halcyon and Pike monarchs were resighted each month at Oceano. There was more movement between Halcyon and Pike: up to 11% of the Halcyon origin location tags were resighted at Pike and up to 14% of the Pike origin location tags were resighted at Halcyon in one month (Table 4). The average proportion of monarchs resighted each month at non-origin locations was 4% across all sites and all resight occasions, and 8% if we exclude Oceano. The combination of low resight at origin locations and lower resight at non-origin sites suggest that monarchs lack residency and are moving, but not moving exclusively among the sites being monitored. If detection probabilities are high, then most monarchs moved to and from sites that were not monitored. These results are not consistent with an accrual hypothesis, nor with a closed population hypothesis.

**Standardized visual cluster abundance estimates.** The abundance of monarchs at all three sites increased steadily throughout October (Figure 8 and Table 5). At Oceano, monarch abundance increased in early November, decreased in late November, and then peaked in December (Figure 9). At Halcyon, monarch abundance peaked in November before declining slightly in December (Figure 10). At Pike, monarch abundance peaked in November and then decreased sharply at the beginning of December (Figure 11). This was due to substantial tree trimming, tree removal, and tree loss within the overwintering grove which took place in late November. By the beginning of January, monarchs no longer clustered at Pike, and they did not return to that site for the rest of that overwintering season.

The pattern of abundance at Halcyon and Pike was consistent with the accrual hypothesis, but the decrease in monarch abundance in late November at Oceano was not. The decrease could be due either to monarchs being present but undetected (an apparent decrease), observer bias in estimating population size (an apparent decrease), or to mortality and/or monarchs leaving the site (an actual decrease). Interestingly, we did not observe a concurrent increase in the number of tagged Oceano monarchs resighted at other sites (Table 4). This suggests that if monarchs left Oceano, they moved to an unmonitored site.

We found that the predicted variance in visual cluster abundance estimates was approximately plus or minus 11% of the empirical abundance estimate. This means that when a local population size is estimated using the standardized visual cluster survey technique, and two or more observers are present, there is a 95% probability that the empirical abundance estimate is no more than an 11% underestimate or overestimate of the expected value.

**Movement of untagged butterflies.** In addition to examining the movement of tagged butterflies, we can use the proportion of untagged monarchs at a site to infer movement of untagged individuals. For instance, at Oceano, the abundance of monarchs in the first week of December is significantly higher than the previous week (Figure 9 and Table 5), and the proportion of untagged monarchs shows a concomitant increase (Table 5). This can only be due to untagged monarchs moving in to the site. Likewise, each week that follows a tagging session (the fourth week in October and November, which follow tagging sessions in the third week) has a much larger proportion of untagged monarchs than were present the day of the tagging. For example, at Halcyon in November we tagged 44% of the monarchs present (Table 3). By the following week 92% of the monarchs at the site were untagged (Table 5), but the apparent abundance of monarchs present was unchanged from previous weeks. This is only possible if

untagged monarchs move in, tagged monarchs leave the site, and the numbers approximately equal each other. Therefore, residency appears to be very low, which does not support the accrual hypothesis. Abundance estimates appear to be fairly constant, and thus one would conclude there is little movement. Yet, in spite of the consistent abundance estimates, there are untagged monarchs moving in and tagged monarchs moving out. Therefore abundance estimates cannot be taken as evidence for static or resident populations.

**Mark-resight estimates and visual cluster estimates.** At each site, the mark-resight high movement model produced a significantly lower local population estimate (N) than the no movement model (Tables 6, 7, and 8). We compared the mark-resight N values to the visual cluster estimates done on the date closest to the resight sessions. In 6 out of 7 cases, the visual cluster estimate was closer to the population estimate from the high movement model than the no movement model. Only at Pike in October did the no movement model more closely match the visual cluster estimate. These results reject the closed population model.

At Oceano in October and at Oceano and Halcyon in December the mark-resight N value from the high movement model was significantly different than the visual cluster estimate (though still closer in value than the no movement model). At Halcyon in October and at Oceano and Halcyon in November the high movement model produced a local site estimate that was quite similar to the visual cluster estimate.

**Superpopulation estimates.** The monthly mark-resight superpopulation estimates (N\*) were significantly different from mark-resight local population estimates (N) in high movement and no movement models and across all sites and months. This provides strong evidence that monarchs at all three sites are part of a superpopulation. The N\* estimates produced by the high and no movement models did not appear to differ significantly within a site except for 1 of the 7

estimations (Halcyon in December) (Tables 6, 7, and 8). This means that in almost every site/month case, the two models converged on the size of the superpopulation. However, despite the fact that  $N^*$  was similar between models at a site, it was not necessarily similar among sites, which suggests that the three study sites do not sample the same core or peripheral portions of the superpopulation. In addition, the  $N^*$  estimates were not necessarily similar across months.  $N^*$  differed among sites in October and December, but was similar among sites in November. In October,  $N^*$  for Oceano was almost double the  $N^*$  for Halcyon and Pike, which did not differ significantly from each other (Table 6). In December the  $N^*$  for Oceano was significantly smaller than the  $N^*$  estimates for Halcyon (Table 8). These results, in conjunction with the low non-origin resights at Oceano, suggest that Oceano is either less interconnected with Pike and Halcyon than they are with each other, or that Oceano samples a different portion of the superpopulation, and that the interconnections vary both in space (location) and time (month).

**Local population estimates and superpopulation size.** The mark-resight  $N$  values generated by the high movement model for all sites each month did not sum to the high movement  $N^*$  for that month. If the high movement model is correct, then there are other overwintering sites contributing to the superpopulation(s) in the area. Conversely, the no movement model  $N$  values did sum to the no movement  $N^*$  values for each month. If the no movement model (and the  $N$  values it generates) is correct, then only Oceano and Halcyon are contributing to the superpopulation. Finally, the visual cluster abundance estimates did not sum to either the high movement or the no movement  $N^*$  values for each month. Therefore, if the visual cluster estimates are correct, then Oceano and Halcyon are not the only locations contributing to the superpopulation. Herein we reject the no movement model due to evidence of movement of tagged and untagged monarchs, and therefore accept that more sites than those

monitored contribute to the superpopulation. In addition, it is possible that Pike and Halcyon form their own superpopulation core, with movement between those two sites, and less movement to other sites.

## DISCUSSION

We documented more monarch movement among overwintering sites than has been previously acknowledged. We found direct physical evidence for movement in the form of marked individuals moving between sites. Over the course of the season, tagged monarchs from all sites of origin were resighted at all non-origin sites. Site movement occurred almost immediately. Tagged monarchs were observed at other sites within one or two days of being tagged. Site movement was pervasive. In November, it took only five days before as many Halcyon-tagged monarchs were resighted at Pike as were resighted at Halcyon. It is interesting to note that the proportion of tagged Pike monarchs resighted at Halcyon increased over the season, whereas the number of tagged Halcyon monarchs resighted at Pike also increased over time. This implies a steady movement of monarchs between the two sites. This may be one reason that the superpopulation estimates for Halcyon and Pike in October were not statistically different. It is also interesting to note that even when the number of monarchs at a site stayed fairly constant (across weeks) this was in spite of movement. These results do not support the accrual hypothesis and so we regard the accrual hypothesis as incorrect.

Our model results provide evidence that monarchs may move among sites throughout the season in much larger numbers than previously thought. The mark-resight  $N$  (local population) estimates produced by the high movement model were much closer to the visual cluster survey estimates than those produced by the no movement model, particularly for Halcyon in October and Oceano and Halcyon in November. The high movement model assumed that all tagged

monarchs that were not resighted had left the site. Since a maximum of 36% of tagged monarchs were resighted at a site after a tagging session (at Pike in October), the model assumed that at every site at least two-thirds of the tagged monarchs departed within five days of tagging. This could be a function of where the sites are located on the coast, or simply attributes of these sites. Tuskes and Brower (1978) found very little immigration and emigration at overwintering sites in San Francisco and Santa Cruz and very high emigration at a site in Santa Barbara. They hypothesized that the warmer average temperatures in the more southern Santa Barbara site could lead to greater monarch activity and greater competition for resources. Our results are intermediate, and since our study sites are located between Santa Cruz and Santa Barbara, our results are entirely consistent with their hypothesis.

It is also important to consider the possibility that the act of tagging monarchs, which disturbs the clusters, could in itself cause movement. Though the data was not presented, we did find that the total number of monarchs at a site (as estimated in a visual cluster survey) decreased the day after tagging. This happened following all tagging sessions. In some cases, the number of monarchs at a site declined for two to three consecutive days after tagging. However, it is difficult to know for certain if this is an effect of tagging, since surveys were conducted on consecutive days only following tagging. We do not know the extent of day to day variations in monarch populations when tagging has not occurred. In future studies, surveys should be conducted on concurrent consecutive days at sites with and without tagging in order to further elucidate the effects of cluster disturbance on monarch movement.

The only site for which the mark-recapture high movement model did not match the visual cluster estimate was at Pike in October. At that site in that month, the no movement model generated an N that was statistically similar to the visual cluster estimate. This could

indicate that different sites are used differently by monarchs at various times of the year, and that perhaps monarchs do not move in and out of Pike during October. However, the tag resighting results do not seem to mesh with this interpretation, as 6% of the monarchs tagged at Pike moved to Halcyon within a few days of the October tagging session. Instead, we suggest the possibility that the rapid influx of monarchs to Pike during October, especially between the last day of resighting and the next visual cluster survey, impacted the effectiveness of the model comparison. At Pike, the number of monarchs more than doubled from the last day of the resight session to the day of the next visual cluster survey. At Oceano, there was only a 40% difference in the number of monarchs present between the last day of resighting and the next visual cluster survey, and at Halcyon only a 6% difference. The drastic increase in monarchs at Pike meant that the visual cluster estimate more closely matched the larger N produced by the no movement model.

The high degree of movement, and the fact that  $N^*$  estimates were much higher than N estimates at all sites in all months, supports the existence of a superpopulation. All three sites contribute to the superpopulation to some degree, as evidenced by the large difference between N and  $N^*$  (local and superpopulation estimates respectively) produced under both the high and no movement models. In 6 out of 7 site/month cases, the superpopulation estimates produced by the two models dovetailed. But in October and December, the superpopulation estimates differed among sites, and the estimates also differed between months.

Perhaps not all overwintering sites are utilized by individuals in the same way. The variability in our estimates may indicate that the superpopulation is not distributed uniformly. It could be that this superpopulation does not have a uniform and consistent rate of movement among sites, and that there is an area of higher movement among sites near the “core” and lower

movement among sites at the “periphery”, analogous to a variable (or non-symmetrical) probability of immigration and emigration. Higher immigration rates would mean that more individuals at any one site would be using alternate neighboring sites, and boosting the superpopulation size estimate. Sites closer to the core would contribute more individuals to the superpopulation.

The local population estimate returned by the high movement model is sufficiently close to our visual cluster abundance estimates to make it a meaningful metric. We propose that mark-resight is a viable option for determining the number of monarchs present at an overwintering site. However, we caution that mark-resight data from a single site cannot be used to accurately estimate the size of the superpopulation. As discussed previously,  $N^*$  differed between sites and months, except during November. Only during that month were the superpopulation estimates at Oceano and Halcyon statistically similar. Late November and early December is when the WMTC is conducted, because this is typically when populations at many overwintering sites are at their peak. Perhaps this is also when there is the highest amount of movement among sites, which could mean that both sites were contributing equally to the superpopulation.

The proportion of monarchs resighted at non-origin sites seems low (3% or less at Oceano, for instance) and there were a large number of marked individuals that were never resighted at any of the sites that we visited during the winter. It is possible that mortality contributed somewhat to the apparent loss of tagged monarchs. In California, birds (Tuskes and Brower 1978, Bell and Dayton 1986) and yellow jackets (*Vespa vulgaris*) (Leong et al. 1990) have been reported preying on monarchs at overwintering sites in several locations. Sakai (1994) estimated at least 7% mortality due to birds at a site in Southern California. However, the large proportion of monarchs that were never resighted cannot be completely explained by mortality.

Based on the evidence that we have for significant movement out of overwintering sites, the absence of the majority of tagged individuals implies that there are other unmonitored sites contributing to the superpopulation.

There were a number of nearby overwintering sites that were not monitored in this study. Tags from all three of the sites we did monitor were found at a site (Pismo Beach North Beach Campground, n=98) located less than two miles north of Oceano and at another site (Black Lake, n=13) located less than three miles south of Halcyon. In addition, within 5 miles of the study sites there are five other overwintering sites that are visited by monitors only once a year as part of the WMTC survey, and three other historic overwintering sites that are not surveyed at all. We predict that some tagged monarchs moved to these other sites and were thus not detected. This is supported by superpopulation estimates as well: neither the visual cluster survey estimates nor the high movement model local population estimates sum to the superpopulation estimates, indicating that there are other sites being used by the tagged monarchs. This suggests that we need to expand our monitoring efforts to include sites that are not often visited. Given the dynamic nature of overwintering sites and the static nature of the current monitoring in California it is possible that there are new sites (particularly transitional sites) that have gone undetected.

Our results indicate that in Central California many overwintering monarchs may use multiple sites over the course of a season. At the sites we monitored, this was not the exception, it was the norm. There also appears to be a high degree of movement between sites, and that movement seems to happen relatively quickly after tagging (within a day or two). This may be problematic because the current standardized abundance estimation methodology does not account for intersite movement. The WMTC is conducted over the course of three weeks. All

sites must be visited once within that period, but there is no constraint on the order in which sites are visited within those three weeks (i.e., nearby sites visited on the same day or within a week or two). If sites only a few miles apart are visited more than a few days apart, it is possible that individual monarchs may be counted multiple times as they move among sites.

Some California overwintering sites are monitored for abundance on a weekly basis, either for research purposes or to generate information that is then provided to the public. As seen in Table 5, there is variance in the site counts from week to week, which previously has been attributed to “noise” in the data, or sampling error. After calculating the within-day (observer-based) variance in visual cluster estimates, we are able to say that estimates that are more than 20% different from each other (11% away from their shared mean) are probably significantly different from each other. This is a small amount of variance compared to the large amount of movement we are hypothesizing (at least two-thirds of tagged monarchs leaving individual sites). Given the evidence for high monarch movement among sites, we argue that weekly population fluctuations are meaningful (i.e., somewhat reflective of actual changes in the population), indicating that local population sizes at sites are in fact changing constantly. It also suggests that even if weekly estimates are similar in number, it is likely that the individual monarchs are different. Given these results, we suggest that fluctuations of more than 10%, which are detected by experienced observers, might be useful in identifying biologically meaningful disturbances to overwintering populations. These disturbances might be natural (such as storm events) or anthropogenic.

Extensive intersite movement of overwintering monarchs and the existence of a superpopulation have important management and conservation implications. If monarchs are using multiple sites, then overwintering sites are not biologically or statistically independent.

Currently, overwintering sites are treated as “islands” of habitat on the landscape and are managed only on a site-by-site basis. This results in a patchwork of protections and no unifying management strategies. For instance, Oceano Campground is part of Pismo State Beach, and is managed by the California Department of Parks and Recreation. But both Halcyon Hill and The Pike are on private property and do not have any protection from alteration or development. This was illustrated dramatically at The Pike when tree trimming and tree removal around power lines led to the loss of cluster trees and the complete abandonment of the site by monarchs (Figure 8). Our results suggest that it is critical that we manage for monarchs on a broader landscape level, protecting and managing groups of overwintering sites (superpopulation sites).

We must also re-evaluate our monitoring protocols and implement abundance estimation techniques that account for geographically open populations. This can arguably only be done with some form of mark-resight or mark-recapture. Future studies should continue to use mark-resight to monitor movement across landscapes and quantify how movement varies across months and sites. Monarch movement patterns can be further elucidated by tagging a larger proportion of overwintering monarchs at sites, expanding resighting efforts and visiting more overwintering sites, and continuing tagging and resight sessions throughout the season. It might be particularly useful to determine if there is clinal variation in the amount of movement among sites. Management strategies may need to be implemented at the landscape level in Southern California and more at the site level in Northern California.

**Table 3.** Fall 2010 tagging effort over three tagging sessions at three overwintering sites in San Luis Obispo County, California. The unique number of individuals newly tagged during a session, and the proportion of each session’s population that was newly tagged at each site are presented. Tagging was not conducted at Pike in November or December.

	October	November	December	Total
Oceano Campground	109 (0.42)	1524 (0.46)	1086 (0.64)	2719
Halcyon Hill	75 (0.71)	1951 (0.44)	944 (0.25)	2970
The Pike	86 (0.46)	-	-	86
Total	270	3475	2030	5775

**Table 4.** Total number of monarchs tagged and proportion of tagged monarchs resighted at Oceano Campground (“Oceano”), Halcyon Hill (“Halcyon”) and The Pike (“Pike”) in San Luis Obispo County, California. Origin location identifies the site where the tag was applied. Cumulative total tagged is a sum of the total number of monarchs tagged at that site up to that session. Resight location identifies the site where tags were seen (resighted). Resight values (proportions) include all resights at a location, regardless of the date the monarch was tagged (i.e., in November, resights include monarchs tagged in October and November). Bolded values identify the proportion of tagged monarchs from an origin location that were resighted at their origin location (and did not appear to move). Five resight occasions (days) were possible in October, three in November and three in December. At Pike there was no new tagging in November or December and no resight session in December.

Month	Origin location	Cumulative total tagged	<u>Resight location</u>		
			Oceano	Halcyon	Pike
October	Oceano	109	<b>0.27</b>	0.03	0.01
	Halcyon	75	0	<b>0.32</b>	0.01
	Pike	86	0	0.06	<b>0.36</b>
November	Oceano	1633	<b>0.22</b>	0.02	0.03
	Halcyon	2026	0.01	<b>0.22</b>	0.11
	Pike	86	0	0.10	<b>0.37</b>
December	Oceano	2719	<b>0.17</b>	0.02	-
	Halcyon	2970	0.01	<b>0.16</b>	-
	Pike	86	0.01	0.14	-

**Table 5.** Abundance estimates of overwintering monarchs obtained using visual cluster survey estimation protocols at Oceano Campground (“Oceano”), Halcyon Hill (“Halcyon”) and The Pike (“Pike”) in San Luis Obispo County, California. Proportions indicate the proportion of untagged monarchs present at that site (number untagged seen out of total monarchs present) during that week’s visual cluster survey. In addition to estimating the number of overwintering monarchs, tagging and resight sessions took place during week 3 of October, week 3 of November, and week 2 of December. Bolded values are the estimates that will be compared to the estimates generated using mark-resight methods, as they are the closest in time to the tagging and resight sessions.

	October			November			December
	Wk 1	Wk 2	Week 4	Week 1	Week 2	Week 4	Week 1
Oceano	16	25	<b>934</b> (0.99)	3148 (0.99)	<b>3376</b> (0.99)	2656 (0.91)	<b>3486</b> (0.94)
Halcyon	9	148	<b>602</b> (0.98)	3109 (0.99)	<b>4595</b> (0.99)	4593 (0.92)	<b>4000</b> (0.96)
Pike	4	85	<b>1626</b> (0.99)	2642 (0.99)	3125 (0.99)	2944 (0.99)	600 (0.99)

**Table 6.** October 2010 local population size (N) and superpopulation size (N\*) estimates for overwintering monarchs occupying three sites in San Luis Obispo County, California under two mark-resight (IELNE) models. The high movement model assumes that the marks are highly detectable and that there is a great deal of movement into and out of sites, while the no movement model assumes low detectability and no movement. Estimates for N and N\* are provided under both models as are measures of confidence (standard error and 95% confidence intervals). For each of the sites we also show “Survey” data, which are population estimates (analogous to N) obtained by using a standardized protocol to visually estimate the number of monarchs present in clusters. The visual cluster survey data presented here were collected the week following the resight sessions. The visual cluster survey confidence intervals represent lower and upper empirical estimates, and are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)

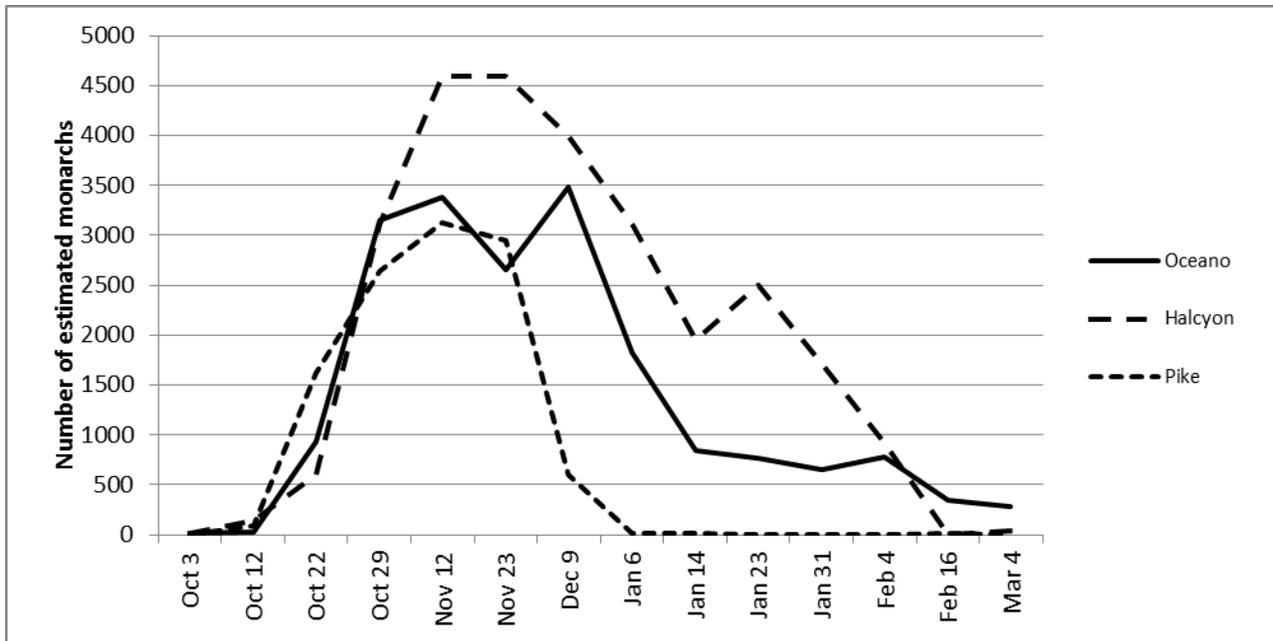
Site	Model	Parameter	Value	SE	Lower CI	Upper CI
Oceano	High movement	N	1362	4.04E-11	1362	1362
		N*	10501	733	9176	12056
	No movement	N	3395	264	2942	3984
		N*	8449	707	7197	9980
		Survey	934	-	836	1037
	Halcyon	High movement	N	641	1.E-03	641
N*			5253	383	4564	6070
No movement		N	1285	109	1106	1539
		N*	4629	441	3859	5598
		Survey	602	-	539	668
Pike		High movement	N	750	3.E-03	750
	N*		5610	385	4915	6428
	No movement	N	1468	104	1293	1704
		N*	4625	373	3965	5432
		Survey	1626	-	1455	1805

**Table 7.** November 2010 local population size (N) and superpopulation size (N\*) estimates for overwintering monarchs occupying two sites in San Luis Obispo County, California under two mark-resight (IELNE) models. The high movement model assumes that the marks are highly detectable and that there is a great deal of movement into and out of sites, while the no movement model assumes low detectability and no movement. Estimates for N and N\* are provided under both models as are measures of confidence (standard error and 95% confidence intervals). For each of the sites we also show “Survey” data, which are population estimates (analogous to N) obtained by using a standardized protocol to visually estimate the number of monarchs present in clusters. The visual cluster survey data presented here were collected the week preceding the resight sessions. The visual cluster survey confidence intervals represent lower and upper empirical estimates, and are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)

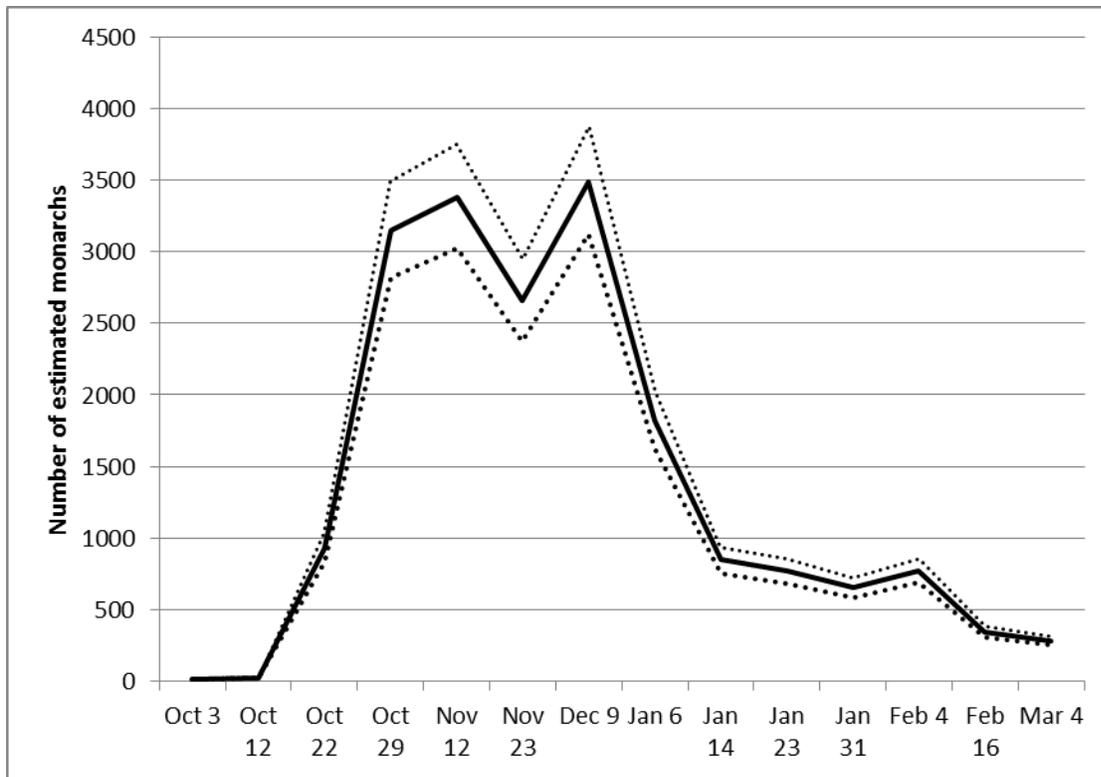
Site	Model	Parameter	Value	SE	Lower CI	Upper CI
Oceano	High movement	N	3090	29	3047	3164
		N*	29163	844	27568	30878
	No movement	N	12725	323	12115	13381
		N*	29187	803	27666	30816
		Survey	3376	-	3020	3748
Halcyon	High movement	N	4353	74	4216	4506
		N*	32790	972	30953	34764
	No movement	N	17625	477	16723	18595
		N*	32609	928	30853	34491
		Survey	4595	-	4111	5101

**Table 8.** December 2010 local population size (N) and superpopulation size (N\*) estimates for overwintering monarchs occupying two sites in San Luis Obispo County, California under two mark-resight (IELNE) models. The high movement model assumes that the marks are highly detectable and that there is a great deal of movement into and out of sites, while the no movement model assumes low detectability and no movement. Estimates for N and N\* are provided under both models as are measures of confidence (standard error and 95% confidence intervals). For each of the sites we also show “Survey” data, which are population estimates (analogous to N) obtained by using a standardized protocol to visually estimate the number of monarchs present in clusters. The visual cluster survey data presented here were collected the week preceding the resight sessions. The visual cluster survey confidence intervals represent lower and upper empirical estimates, and are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)

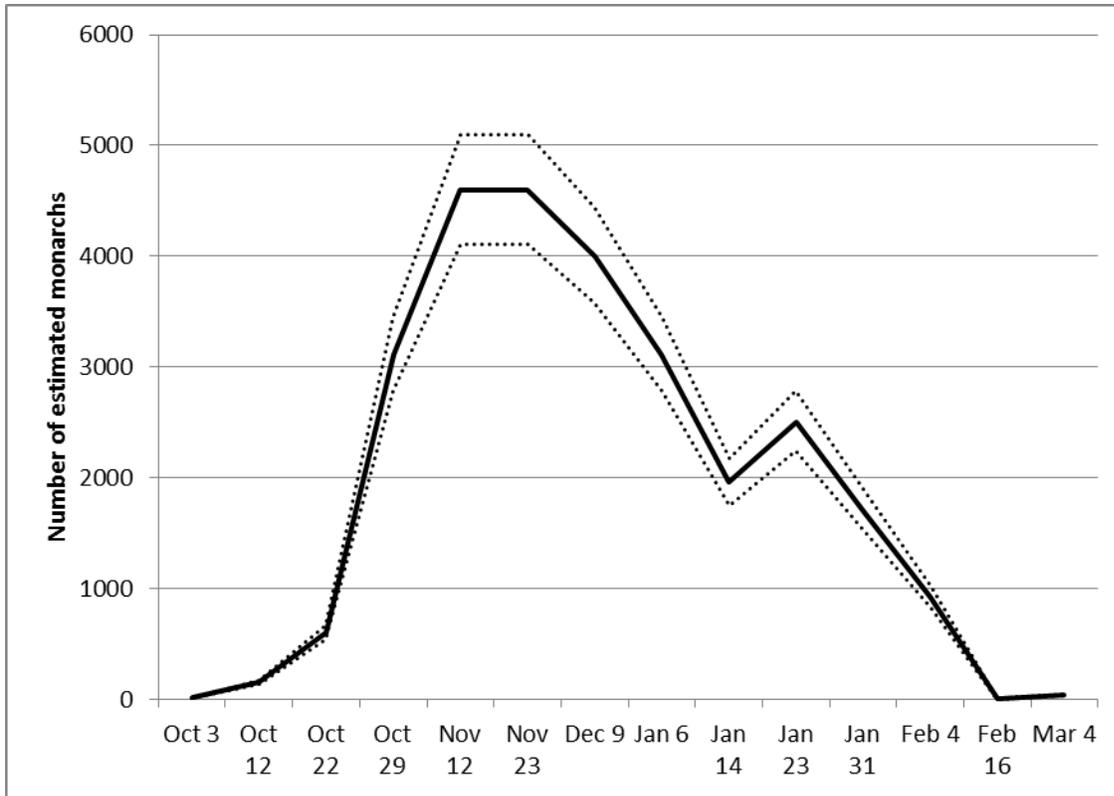
Site	Model	Parameter	Value	SE	Lower CI	Upper CI
Oceano	High movement	N	1902	22	1877	1974
		N*	22033	602	20900	23260
	No movement	N	10359	240	9907	10847
		N*	22046	540	21024	23142
		Survey	3486	-	3119	3870
Halcyon	High movement	N	3291	2.26E-22	3291	3291
		N*	33654	770	32190	35208
	No movement	N	13207	262	12711	13737
		N*	25749	543	24717	26847
		Survey	4000	-	3578	4441



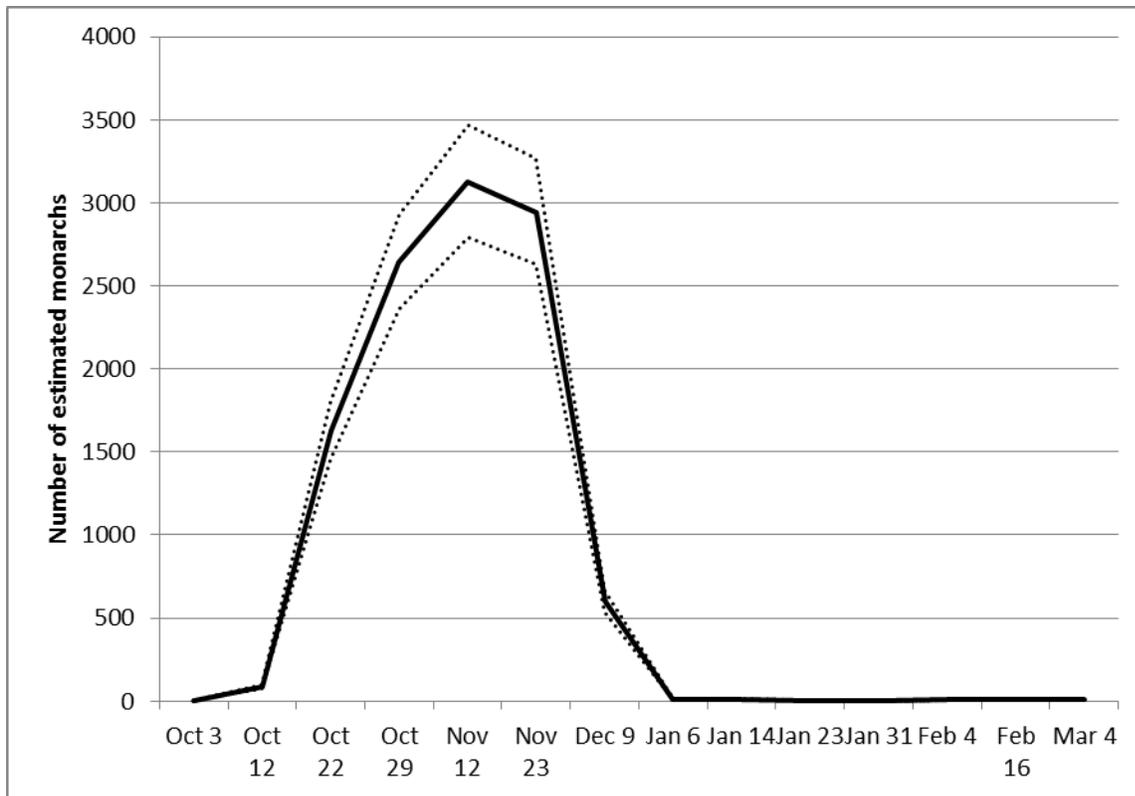
**Figure 8.** Estimated number of overwintering monarchs at Oceano Campground (“Oceano”), Halcyon Hill (“Halcyon”), and The Pike (“Pike”) in San Luis Obispo County, California from October 2010 through March 2011. Estimates are based on visual cluster survey data using a standardized methodology, and not on mark-resight data.



**Figure 9.** Weekly estimated overwintering population size based on visual cluster surveys conducted using standardized survey protocols from October 2010 through March 2011 at Oceano Campground, San Luis Obispo County, California. Solid line is the mean population estimate, dotted lines represent the upper and lower empirical estimates. Mean and upper and lower bounds are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)



**Figure 10.** Weekly estimated overwintering population size based on visual cluster surveys conducted using standardized survey protocols from October 2010 through March 2011 at Halcyon Hill, San Luis Obispo County, California. Solid line is the mean population estimate, dotted lines represent the upper and lower empirical estimates. Mean and upper and lower bounds are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)



**Figure 11.** Weekly estimated overwintering population size based on visual cluster surveys conducted using standardized survey protocols from October 2010 through March 2011 at The Pike, San Luis Obispo County, California. Solid line is the mean population estimate, dotted lines represent the upper and lower empirical estimates. Mean and upper and lower bounds are based on the variation in cluster size estimates between observers conducting the same survey (see methods.)

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