Immature Monarch Survival: Effects of Site Characteristics, Density, and Time

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ABSTRACT The eastern North American monarch population is declining, as evidenced by the area occupied by overwintering adults. Recently, decreasing availability of breeding habitat has been most strongly implicated in this decline. An alternative, nonexclusive explanation for the recent population decline is decreasing survival. We used 18 yr of data from the Monarch Larva Monitoring Project, a citizen science program, to determine immature monarch mortality rates over time as well as factors associated with increased mortality. Our data included field measures of mortality from egg to the final larval instar, and mortality due to parasitoids and other causes, assessed by rearing field-collected monarchs. Average egg to fifth-instar survival ranged from ∼7 to 10% across all regions. Survival from fifth instar to adult ranged from ∼60 to 90%, although this overestimates survival because monarchs are not exposed to many mortality factors when reared indoors. Both survival rates showed a great deal of temporal and spatial variation. Survival tended to be higher in sites that were planted and had more milkweed plants. There was a negative effect of per plant egg density on survival, suggesting density dependence. Survival rates appear to be declining from 1997 to 2014, and we discuss possible reasons for this pattern. Finally, we estimate that across all years in the north-central United States, where we have the most data, a minimum number of ∼29 milkweed plants are required to produce an adult monarch that will be part of the fall migratory generation.

KEY WORDS *Danaus plexippus*, monarch, Lepidoptera, habitat, survival

Eastern North American monarchs [*Danaus plexippus* L. (Lepidoptera: Nymphalidae)] are well-known for their annual fall migration, which takes them up to 4,000 km from their summer breeding grounds throughout the northern United States and southern Canada to mountainous overwintering sites in central Mexico (Solensky 2004). The generation that migrates south in the fall also begins the spring migration, laying eggs as they head northward. It is these descendants that finish the journey back to the summer breeding grounds, where an additional two to three summer generations are produced.

Recent analyses show that monarch abundance, measured at the Mexican overwintering sites, has significantly declined (Brower et al. 2012, Rendón-Salinas and Tavera-Alonso 2014, Vidal et al. 2014). It is likely that a decrease in milkweed host plant (*Asclepias* spp.) availability throughout the monarchs' breeding grounds is contributing to the decline in the overwintering monarch population. While monarchs use many *Asclepias*

species as host plants, most of the monarchs that migrate to Mexico in the fall have consumed *Asclepias syriaca* L., common milkweed (Malcolm et al. 1993). *A. syriaca* growing in agricultural fields was a key source of monarchs before the widespread use of genetically modified crops (Oberhauser et al. 2001), though it has since all but disappeared (Pleasants and Oberhauser 2013, Pleasants 2015) as adoption rates of herbicide tolerant crops have reached ∼90% (Stenoien et al. 2015). Another nonexclusive hypothesis is that overwintering abundance is declining because of increasing monarch mortality rates during the egg and larval stages. Changes in immature mortality could be due to several top-down and bottom-up forces, including 1) increased consumption of monarchs by predators or parasitoids, 2) increased larval competition (resource limitation) as females are forced to lay eggs in smaller and more dispersed habitats, or 3) increased infection rates of diseases.

The relative importance of bottom-up (resources) versus top-down (natural enemies) forces in structuring insect herbivore populations has long been debated (Hairston et al. 1960, White 1978, Oksanen et al. 1981, Hunter and Price 1992). Price et al. (1980) contended that to fully understand plant–herbivore interactions, a tri-trophic approach is required, that includes interactions between plants, herbivores, and natural enemies. For lepidopterans, bottom-up forces are likely to include the availability of larval host plants and adult nectar sources, as well as the physical and chemical

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defenses of larval host plants. Top-down forces are likely to include predators, parasitoids, and disease. It is also essential to acknowledge the importance of abiotic factors, especially weather, in determining insect–herbivore population dynamics (Birch 1957).

Several factors hypothesized to influence monarch survival or fecundity have been previously investigated. Zalucki and Lammers (2010) modeled the potential bottom-up impacts of the loss of agricultural milkweed on individual monarch fecundity, and suggested that fecundity is likely to decline with a decreasing density of host plants. Additionally, milkweed physical and chemical characteristics have been shown to influence oviposition behaviors and larval survival (Zalucki et al. 1990, 2001a,b; Malcolm 1995; Malcolm and Zalucki 1996; Zalucki and Malcolm 1999). While site characteristics, such as the availability of flowering plants and site size, have been correlated with pollinator abundance (Westphal et al. 2003, Belfrage et al. 2005), little is known about the degree to which site characteristics affect insect survival. Monarchs are also known to be affected by many top-down forces, including a large suite of egg, larval, and pupal predators and parasitoids (reviewed by Oberhauser et al. 2015). Mortality can also be caused by disease, such as that inflicted by the protozoan parasite *Ophryocystis elektroscirrha* (Altizer and Oberhauser 1999).

Survival rates of eggs and larvae have been previously documented by Borkin (1982), Oberhauser et al. (2001), Prysby (2004), Calvert (2004), and De Anda and Oberhauser (2015). All of these studies reported survival rates of ≤10% from the egg to late-instar larva stage, but they all involved limited temporal and spatial scales. All but Oberhauser et al. (2001) covered only one or a few locations, and all but De Anda and Oberhauser (2015), which was a 2-yr study, covered only a single year. Additionally, because these studies all tracked only eggs and larvae, they did not document most of the mortality caused by parasitoids, which usually occurs after pupation. In addition to resource availability and natural enemies, other factors, including temperature (Zalucki 1982, York and Oberhauser 2002, Nail et al. 2015) and insecticides (Oberhauser et al. 2006, 2009), are known to affect immature monarch survival. Because most monarch mortality occurs in the egg and larval stages, immature mortality has the potential to be an important population driver.

While monarch fecundity and survival can be measured in laboratory studies (Svärd and Wiklund 1988, Oberhauser 1989, Oberhauser 1997), these rates are difficult to measure in the field. One source of monarch field occurrence data is a citizen science venture called the Monarch Larva Monitoring Project (MLMP). MLMP volunteers from 37 U.S. states, the District of Columbia, three Canadian provinces, and one Mexican state collected data on monarch egg and larval density at sites with milkweed (Fig. 1). These data, with records beginning in 1997, include habitat characteristics of the monitoring site and surrounding area (Prysby and Oberhauser 1999, MLMP 2015), and can thus be used to identify large- and small-scale habitat features that are associated with monarch survival.

The first goal of this study was to estimate immature survival over broad spatial and temporal scales, and to determine what local- and landscape-level site characteristics, as well as spatial and temporal factors, are correlated with egg and larval survival. Monarch survival could be influenced by several factors that vary between habitats: 1) heavily managed sites (gardens and other planted sites) might have higher survival, if weed control and watering increase host plant quality. Alternatively, 2) these planted sites might be habitat islands that attract ovipositing females that do not leave, leading to increased immature monarch densities and possible density-dependent effects (see number 7 below). 3) Survival could decrease with proximity to agricultural lands, if monarchs are affected by pesticide applications or other practices in these habitats, but 4) agricultural habitats could contain fewer predators and thus support higher survival, as suggested by Oberhauser et al. (2001) and Pleasants and Oberhauser (2013). 5) More diverse habitats could also support a more diverse and abundant suite of predators (Oberhauser et al. 2001). 6) Monarch survival might be negatively affected by egg density owing to higher disease rates (Lindsey et al. 2009, Satterfield et al. 2015) and scramble competition, defined as a situation in which the finite amount of food available for each individual decreases with a larger population size. Alternatively, 7) egg density might be an indicator of female preference for high-quality host plants and, hence, might be associated with higher survival. A second, related goal was to then determine if there are temporal trends in monarch survival, and thus if immature survival rates could be drivers of the observed decline in monarch numbers.

Finally, using monarch egg density, larval survival, and MLMP parasitism data, we calculate the approximate number of adult monarchs that come from a single milkweed plant during the summer in the north-central region of North America, the most important source of overwintering monarchs in Mexico (Wassenaar and Hobson 1998). This is particularly important for monarch conservation, as having an estimate of number of milkweeds is necessary to produce an adult monarch in the migratory generation will inform the magnitude of conservation actions required to achieve population targets.

Materials and Methods

MLMP volunteers record weekly counts of monarchs identified to instar at sites with milkweed throughout North America (Fig. 1). For a more detailed description of collection procedures and data recorded, see Prysby and Oberhauser (2004) and MLMP (2015). Briefly, data are collected as densities of eggs and each larval instar per milkweed plant observed, and several characteristics of monitoring sites are recorded each year. We used data from 1997 to 2014 for this analysis, divided into six different regions (Fig. 1) and analyzed over four different time periods defined to encompass relevant generations in regions used by monarchs throughout the year (Table 1). These time periods were determined using visualizations of the
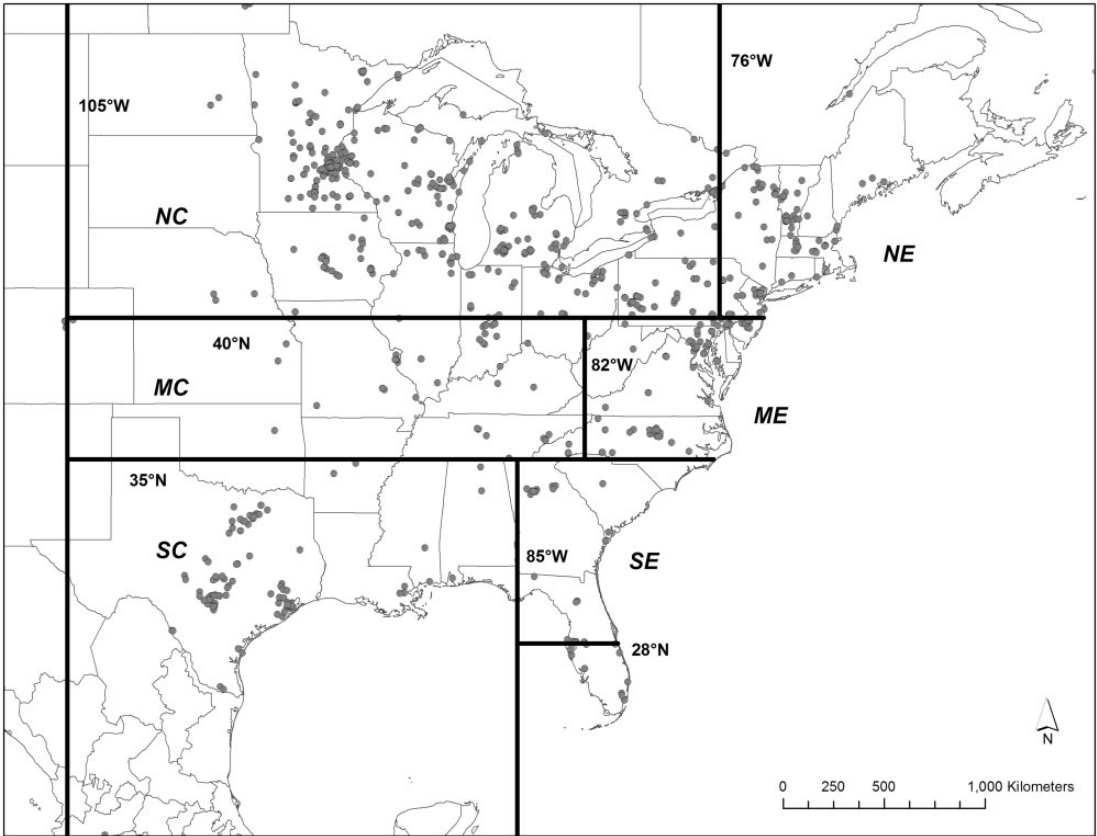
Fig. 1. Analysis regions and MLMP sites (before data cleaning). While the vertical lines dividing the east and central regions from each other are slightly different than those used in other regional analyses (e.g., Ries et al. 2015), very few sites with usable data were affected by the difference, and the data sorting was more feasible using longitude lines.

Table 1. Date ranges for different seasons and the monarch stage and regions analyzed

Season	Monarch stage	Dates	Regions* analyzed
Southern Spring (first generation)	Eggs	1 Mar.–31 May 31	ME, MC, SE, and SC
	Fifth instars	15 Mar.–14 June	
Northern Spring (second generation)	Eggs	1 April–30 June	NE, NC, ME, and MC
	Fifth instars	15 April–14 July	
Northern Summer (third and fourth generations)	Eggs	1 July–30 Sept.	NE, NC, ME, and MC
	Fifth instars	15 July–14 Oct.	
Southern Summer (final generation produced by a portion of the population)	Eggs	1 Aug.–31 Oct.	ME, MC, SE, and SC
	Fifth instars	15 Aug.–14 Nov.	

See text for explanation of criteria used to select date ranges.
*See Fig. 1 for delineation of regions.

monitoring data from the 18 project years. We chose time periods that were long enough to encompass year-to-year variation in monarch phenology and include all of the eggs and larvae that were produced during a single generation in most years (with the exception of the northern summer period, which covers two overlapping generations). For each time period, the monitoring events used to obtain the number of fifth instars were shifted 2 wk later than those used to obtain eggs numbers. This allows us to account for monarch development time and the fact that MLMP volunteers monitor weekly. While generations sometimes overlap between these time periods, our visualizations of region-wide data suggested that this overlap is usually not relevant within in any given region, as the vast majority of the population moves in a predictable way from one region to another during the year (Batalden et al. 2007). An alternative would have been to measure survival over the entire year, but we wanted to capture differences from one generation to the next.

Our units of analysis for egg to fifth-instar larva survival were individual monitoring sites in a given time

period and year. Data were excluded for site–time period combinations if there were ≤10 milkweeds monitored per monitoring event, or if the site was monitored fewer than five times over most multimonth time periods. However, we included sites that were monitored four or more times for the northern spring season in the north-east and north-central regions, as volunteers often did not monitor five times in April–June owing to the late appearance of milkweed (truncating the data on the early side of the time period), and because monarchs stay in these regions to produce succeeding generations (so if we extended the interval to later, data would overlap with the next generation). These monitoring frequency requirements needed to be met during both the egg and fifth-instar time periods (see Table 1). Data were also removed if volunteers recorded more larvae of a single instar than eggs, as this indicates an inability to distinguish monarch eggs or selective observation of plants with caterpillars. Finally, because we were evaluating monarch survival through the fifth instar, we also removed data from observers who collected most or all of the eggs or first through third-instar larvae that they observed, as collecting these would lead to a lack of late-instar larvae in the following weeks (collecting later instars, however, does not affect our survival estimates because of the weekly monitoring intervals).

Once the data were cleaned, we determined survival from egg through the larval stage by dividing the number of fifth instars observed at a site by the number of eggs seen at the same site in the same time period during the same year, but shifted 2 wk earlier (equation 1).

$$\text{Larval Survival}_{i,t,y} =$$

$$\frac{\text{Number of fifth instars from time period}_{i,t,y}}{\left(\begin{array}{c} \text{Number of eggs from same time} \\ \text{period two weeks earlier}_{i,t,y} \end{array} \right)} \quad (1)$$

where i=site, t=time period, and y=year

We then compared mean survival between different regions and time periods and across years. To understand survival drivers, we used a mixed-effects model with binomial error structure in R 3.1.2 (R Core Team 2014, Vienna, Austria) with package lme4 (Bates et al. 2015). As many sites are monitored for multiple years, site ID was used as a random effect (Table 2). Year was also treated as a random effect to account for stochastic variation over time. Fixed effects included time period (season), egg density (measured as total number eggs/total number of plants observed during the time period at a given site), mean number of milkweeds monitored per week, whether a site was planted, and site type (garden, natural area, crop-based agricultural area, noncrop-based agricultural area, roadside, or other [Stenoien et al. 2015 includes details on the assignment to these categories based on volunteer descriptions]). The average number of milkweeds per monitoring event was a proxy for the number of plants at a site, as these are correlated (see Stenoien et al. 2015). We

Table 2. Fixed effect variables in the best supported binomial mixed-effects models for monarch survival

	Estimate	SE	Test statistic[a]	P-value
a. Final model with year and site as random effects				
Intercept	−4.60	0.154	−29.8	<0.0001
Planted	0.748	0.196	3.82	<0.0001
Noncrop agricultural	0.626	0.208	3.01	0.00014
Natural	0.483	0.198	2.44	0.0147
Average milkweeds	0.00143	0.000133	10.7	<0.0001
Northern summer	1.16	0.0434	26.7	<0.0001
Southern fall	1.19	0.0770	15.5	<0.0001
Southern spring	1.26	0.121	10.3	<0.0001
Egg density	−1.19	0.0844	−14.1	<0.0001
b. Final model with site as random effect				
Intercept	−4.12	0.140	−29.4	<0.0001
Planted	0.794	0.196	4.04	<0.0001
Noncrop agricultural	0.670	0.210	3.19	0.00014
Natural	0.560	0.199	2.82	0.00488
Average milkweeds	0.000981	0.000117	8.39	<0.0001
Northern summer	1.22	0.0432	28.2	<0.0001
Southern fall	1.26	0.0765	16.5	<0.0001
Southern spring	1.22	0.122	10.1	<0.0001
Egg density	−1.23	0.0844	−14.61	<0.0001
Year	−0.0494	0.00469	−10.51	<0.0001

a) Site ID and year as random variables. b) Only site ID as a random variable. See text for explanation of models.
[a] test statistic = z-value.

used stepwise backward selection and AIC scores to choose the top supported model (Sakamoto et al. 1986). To determine whether immature survival rates are changing over time, we conducted a second analysis with the same predictors and site ID as a random effect, but with year as a fixed effect.

Since 1999, a subset of MLMP volunteers have collected monarch eggs and larvae to rear in their homes, and we used the outcomes of these rearings to estimate larva to adult survival. While many of these monarchs are collected from regular monitoring sites, volunteers also record data on monarchs that they rear from other locations. Thus, we do not have site characteristics for these nonsite locations, and only assigned them to region for analysis (see Fig. 1). Most volunteers collect and rear fourth and fifth instars, but our database also includes records from monarchs collected as eggs and younger larvae. Volunteers record the date, location, and larval instar at collection, as well as the outcome of each rearing (adult monarch, died of unknown cause, died accidental death, parasitized by fly, or parasitized by wasp). A notes data field allows volunteers to record additional information that they consider relevant. Volunteers only identify parasitoids to order, but the vast majority are flies, and all of the flies that we have identified to species (several dozen from throughout the United States) have been *Lespesia archippivora* (Oberhauser et al. 2007).

For this analysis, we omitted cases in which monarch death was accidental (e.g., the specimen was dropped, or crushed between the lid and rearing container), and from volunteers whose reared larvae consistently suffered rates of mortality from unknown causes at rates >40%, as our rearing experience suggests that this high mortality is likely to be due to diseases transmitted as a

result of mass rearing or poor rearing techniques, and thus may not be an accurate reflection of natural causes of mortality. We also omitted data if the monarch stage at collection was not recorded, and if 100% of the monarchs reared by the volunteer were parasitized, as it is possible that these volunteers only reported parasitized monarchs. However, because 100% parasitism could be accurate with small sample sizes, deleting only the small sample size cases in which mortality was 100% could lead to an under-representation of parasitized monarchs from small sample sizes. We thus omitted all of the cases in which the total proportion of parasitized monarchs in a given sample size category was significantly different from the overall parasitism rate (as determined by a chi-square association test). Using this criterion, we omitted all cases in which a volunteer reared fewer than four monarchs from a given site in a given year. We combined data across the entire year because previous analyses showed no consistent effect of season (Oberhauser et al. 2007).

We used MLMP egg density and survival data to estimate the number of milkweed plants needed to produce an adult monarch that will migrate to Mexico. The estimates used to calculate this value will vary across seasons, milkweed plant species, years, and regions, but the information has conservation importance that is broadly useful. We were limited in survival data (both egg to fifth instar as estimated by field observations, and fifth instar to adult as estimated by the rearing study) for all but the north-central region, but because this region produces a large portion of the monarchs that overwinter in Mexico (Wassenaar and Hobson 1998), it provides a valid starting point for estimating restoration targets. We estimated the value across all years (using our cleaned data set and only data from the late summer, which produces the migratory generation) as follows:

$$\frac{monarchs}{milkweed} = \frac{\frac{eggs}{plant}}{week} \times \frac{fifth\ instars}{eggs} \times \frac{adults}{fifth\ instar} \times weeks \tag{2}$$

where eggs per plant per week = the average of the total number of eggs/total plants observed in the north-central region per week, starting with 7–13 July (chosen because it is likely that the adults resulting from eggs laid on or after 7 July will join the migratory population); fifth instars over eggs = all of the fifth instars observed from 21 July or later/all of the eggs observed from 7 July or later; adults/fifth instar = all of adults that were reared from larvae collected as fifth instars/ the number of fifth instars collected; and weeks = the total number of weeks, starting with 7–13 July, for which we had data from the north-central region across all years of the study.

Results

Field Survival. Our analyses included 1,462 site–season–year combinations that met our inclusion criteria, representing a total of 424 sites. These sites were spread across the monarch range in approximately the same proportion, as illustrated in Fig. 1 (north-central: 1,057 site–season–year combinations from 289 sites; north-east: 86 from 40 sites; mid-central: 76 from 22 sites; mid-east: 118 from 36 sites; south-central: 144 from 36 sites; south-east: 1 from 1 site). Figure 2 illustrates mean egg to fifth instar survival rates across all years in regions for which we had >30 site–season–year observations that met our inclusion criteria. Summer survival in the north-central region was higher than spring survival in the north-central region, but there were no other significant differences between region–season combinations across all years.

Figure 3 illustrates yearly survival in the south-central region in the spring, and the north-central region in the spring and summer (where sample sizes were >75 site–season–year observations). While no long-term trends are readily apparent in Fig. 3, it illustrates large year-to-year variation in survival, spanning at least an order of magnitude in each region.

In our best supported mixed-effects model with site and year as random effects (Table 2a), survival tended to be higher in sites that were planted and that had more milkweeds (e.g., larger sites), and lower in sites that had higher egg densities. Sites that were classified as noncrop agricultural (such as Conservation Reserve Program land, old pastures, and fields) or natural (such as nature reserves and state parks) had higher survival. There was lower survival during the spring season in the north (note the positive effect of all other seasons, Table 2). Surrounding area was not included in either final model. When we included year as a fixed effect, while accounting for site by keeping it as a random effect, the same terms as above were significant in the same direction. Year was also significant, with a negative effect on immature survival (Table 2b).

Reared Monarch Survival. MLMP volunteers reared 18,157 monarchs for the survival study. Of these, 16,075 met all of our inclusion criteria. The vast majority (13,931) were from the north-central region, with most of the rest (1,830) from the mid-east region. Thus, we present some summary data across all regions, but only present comparisons between the north-central and mid-east regions.

The rate of parasitism increased (and, concomitantly, adult survival decreased) with the stage at collection (Oberhauser 2012), so we only used data from monarchs collected as fifth instars to investigate year-to-year variation in survival rates of larvae to adults. In the north-central region, parasitism rates varied from 3.2 (in 2004) to 38.4% (in 2012), and in the mid-east region, from 0 (in 2011, 2013, and 2014) to 55.6% (in 2002). Fifth instar to adult survival of reared monarchs in the north-central region varied from 56 (in 2012) to 90% (in 2003), and in the mid-east region, from 38 (in 2002) to 100% (in 2014; Fig. 4). There are no significant trends over time in parasitism or fifth instar to adult survival in either region (Pearson correlation tests, all $P > 0.24$), nor are there differences between the two regions. However, because we could
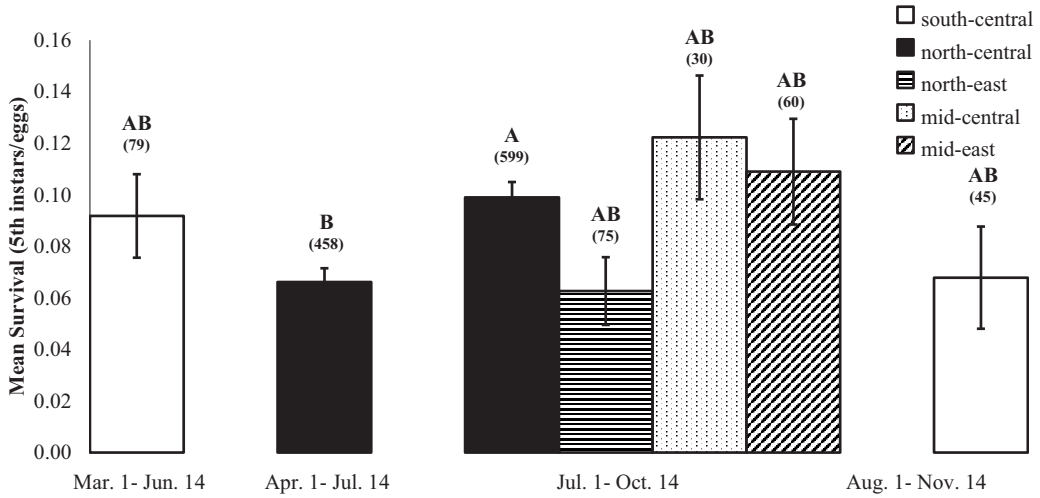
Fig. 2. Mean proportion of monarchs surviving from egg to fifth instar (as defined by equation 1 ± SE) across all years of the study for regions and time periods with a minimum sample size of 30 (sample size [number of site/time period combinations] shown above error bars). Means with different letters are significantly different (Tukey's honestly significant difference, $P < 0.05$).
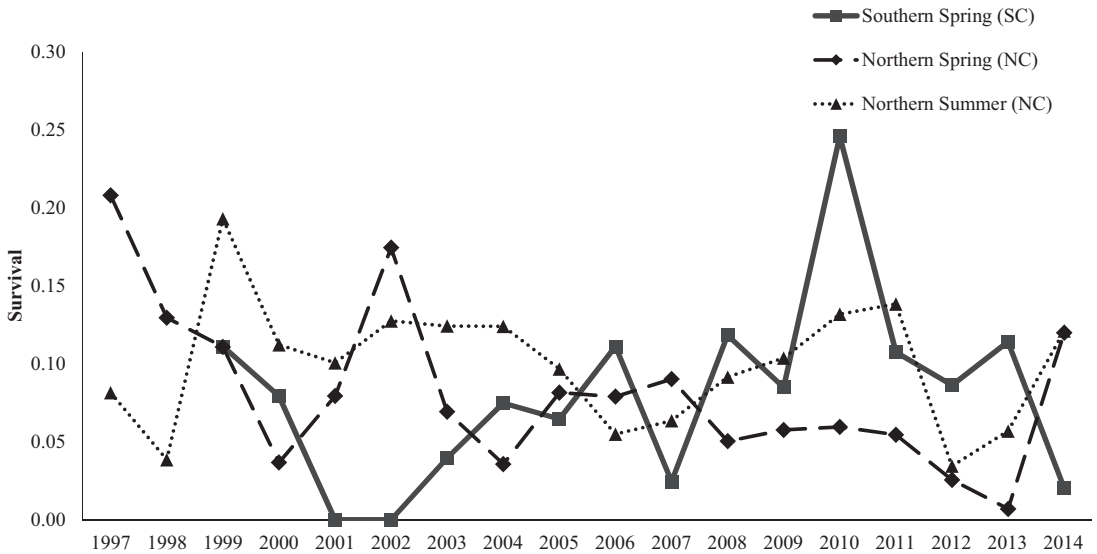

Fig. 3. Mean egg to fifth instar survival (across all sites that met our inclusion criteria) over time. The mean yearly sample sizes for each region are 4.9 (± 0.7 SE) for spring in the south-central region, 25.4 (± 3.1 SE) for spring in the north-central region, and 33.3 (± 3.8 SE) for summer in the north-central region.

not control for site (as a random effect) or site characteristics, these comparisons are less robust than comparisons using egg to fifth-instar survival. Note that data from the north-central region across all years are from 99 different monitoring sites (total n from sites = 3,495 plus $n = 1,689$ from nonsite locations, and the most from a single site = 458), while the data from the mid-east region are from 13 different sites, and 970 of the 1,335 are from a single site. Thus, our data from the north-central region are more likely to represent the region as a whole.

Monarchs per Milkweed Plant and Survival to Adult. Egg density in the north-central region, calculated from the total numbers of eggs and plants observed in a given week across all years, varied a great deal from July 7 to the end of the summer (Fig. 5). The mean weekly egg density, across the 14 wk illustrated in Fig. 5, was 0.043. Survival from egg to the fifth instar, calculated from survival from the northern summer period as defined in Table 1, was 0.077 (from a total of 3,912 fifth instars and 51,059 eggs). Survival from the fifth instar to adult, calculated from reared monarchs
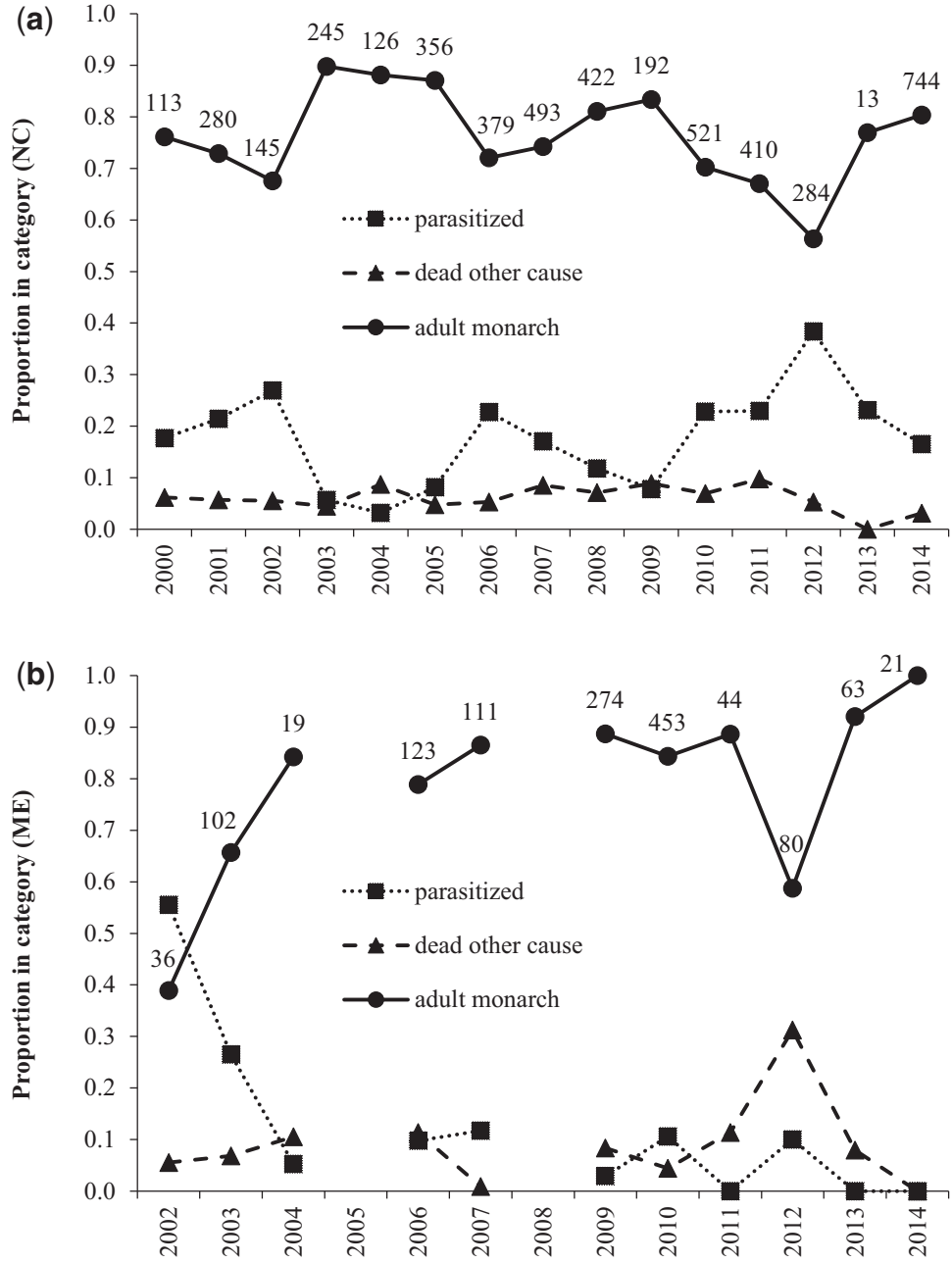
Fig. 4.   Proportion of monarchs collected as fifth-instar caterpillars that were parasitized by tachinid flies, died of other causes, and survived to the adult stage in the (a) north-central and (b) mid-east regions. Years in which < 10 fifth instars were reared are not illustrated. Sample sizes (total number of fifth instars that were collected) for each year are indicated.

across all years in the north-central region, was 0.76 (from a total of 4,731 monarchs collected as fifth instars). The product of these three values and the number of weeks during which data were collected (14) is 0.035 monarchs per milkweed. Thus, in the region for which we have all of the data required to calculate this value, MLMP data suggest that one monarch that is likely to migrate to Mexico is produced per ∼28.5 (the inverse of 0.035) milkweed plants, across all years of this study.

Across all years and weeks, summer survival in the north-central region from egg to adult is ∼0.077 × 0.76 = 0.058 (5.8%). In the spring, egg to fifth-instar survival (from a total of 1,986 fifth instars and 34,876 eggs) in this region across all years of the study is 0.057. Assuming that fifth instar to adult survival rates are the same in the spring and summer (Oberhauser et al. 2007), egg to adult survival in the spring is 0.057 × 0.76 = 0.0431 (4.3%). Note that both
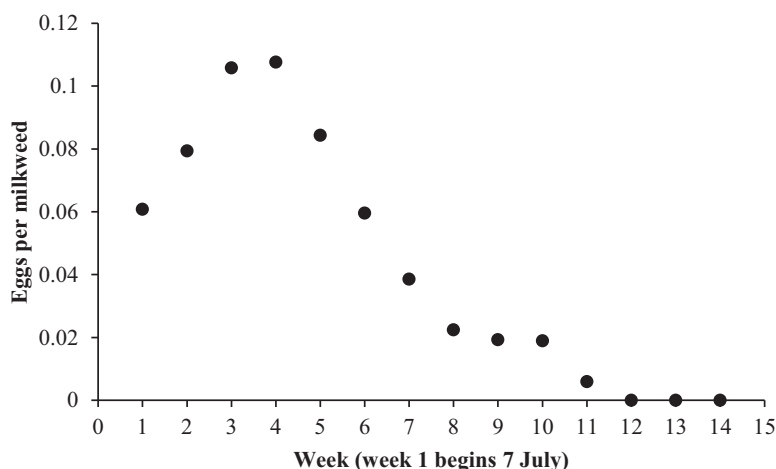
Fig. 5.   Weekly per milkweed plant egg density in the north-central region for the monarchs that are likely to migrate to Mexico. Values calculated by summing all eggs and all plants observed in a given week across all years of the study (egg $n = 61,046$ and milkweed $n = 885,188$).

of these estimates depend on reared monarchs for fifth instar to adult survival estimate.

Discussion

Here, we discuss several factors that were associated with monarch immature survival, including variation between regions and seasons, planted versus non-planted sites, and site types. We also discuss evidence for density-dependent mortality, and a decline in survival rates over the time of our study. Finally, we discuss how our findings can inform planting targets for milkweed, by estimating the number of plants needed to produce a monarch that will migrate to Mexico.

While we documented a great deal of variation in immature survival rates from year-to-year field survival rates (egg to fifth instar) across all regions and seasons, when combined across all years of the study, are similar to rates found in many other studies (Borkin 1982, Oberhauser et al. 2001, Prysby 2004), ranging from ∼7 to 10%. Our rates are higher than those reported by Calvert (2004) and De Anda and Oberhauser (2015). Both of the latter studies were conducted in one or a few sites and over only two years, and survival in our study varied a great deal across years, so this difference is not surprising.

Across all years, survival rates were lower in the spring in the north-central region than they were in the summer in the north-central region (Fig. 2). The difference between north-central spring and summer is counterintuitive, as predator abundance might be expected to increase over the course of the summer. However, slower development rates in May and June could increase exposure to predators. Other hypotheses for this difference are that cooler temperatures have direct effects on survival (Nail et al. 2015), or that higher per plant densities in the spring (Stenoien et al. 2015) lead to scramble competition. Our full model also showed lower survival during the spring season in the north (Table 2). When both egg to fifth instar and

fifth instar to adult survival (the latter is overestimated because of indoor rearing) are taken into account in the north-central region, where we have enough data to estimate both parameters, our results suggest ∼6% survival from egg to adult in the summer, and ∼4% survival in the spring.

Several site-level factors affected survival. In our best supported model, planted sites had higher survival, suggesting that site management practices could influence survival or that planted sites might be selected in areas with attributes that promote survival (e.g., sites might be chosen in areas that are ideal for milkweed growth). Two site types (natural and noncrop agricultural) were also associated with higher survival. These site types include areas such as Conservation Reserve Program land (noncrop agricultural) and nature reserves (natural) that are often intended to increase habitat for wildlife, including pollinators, but more work is needed to investigate the mechanisms for the correlation between these site types and immature monarch survival. None of the other site characteristics that we hypothesized could be important (agricultural sites or garden sites) significantly affected survival in our final model.

Egg density was negatively correlated with survival, suggesting that density-dependent factors may affect survival, although it should be noted that overall density throughout this study period has not increased (Stenoien et al. 2015). As further support for density-dependent survival, sites in which more milkweed plants were monitored had higher survival rates, and there is a negative correlation between the number of plants monitored and per plant egg densities (Stenoien et al. 2015). Density dependence could be driven by predation, parasitism, disease (Lindsey et al. 2009), or competition amongst immature monarchs for resources. Many studies suggest that immature monarch survival is largely driven by the top-down effects of predators and parasitoids (Borkin 1982, Prysby 2004, De Anda and Oberhauser 2015), and there is evidence

that natural enemies drive some species' population dynamics (e.g., larch budmoths [Turchin et al. 2003] and the southern pine beetle [Turchin et al. 1999]), although other work points to the importance of bottom-up forces on populations (e.g., White 1978). Future research should attempt to ascertain the interacting influences of natural enemies, milkweed characteristics, the potentially cascading effects of sequestered milkweed toxins on natural enemies, and abiotic factors (including temperature and precipitation) on these year-to-year and density-dependent variations in immature monarch survival.

There was a significant negative effect of year on monarch survival from egg to fifth instar, when we controlled for site, suggesting that immature survival rates declined over the time period of this study. We are not sure what is causing this decline over time; one possible explanation is that the rate of *O. elektroscirrha* infection is increasing (Satterfield et al. 2015), and monarch larvae infected with *O. elektroscirrha* show decreased survival (Altizer and Oberhauser 1999). Another possibility that cannot be tested using the data from this study is that predators are killing larval monarchs at higher rates, although we did not detect an increase in tachinid fly parasitism over the course of the study (Fig. 4). The decline does not appear to be driven by increased larval competition as females are forced to lay eggs in smaller and more dispersed habitats because overall density did not increase over the period of this study (Stenoien et al. 2015). While there was a negative trend over time when we accounted for site, neither of our survival metrics (egg to fifth instar or fifth instar to adult) improved a regression model against the area occupied by monarchs overwintering in Mexico (Stenoien et al. 2015). Thus, our calculated survival rates do not appear to be driving the fluctuations observed in monarch numbers at the overwintering sites, at least not in a simple way. However, it is possible that this analysis did not uncover complex interactions between abiotic conditions, egg densities, and the large suite of predators that attack monarchs (reviewed in Oberhauser et al. 2015).

We could not conduct the analysis of fifth instar to adult survival at the site level because few individual sites had enough data to do robust analyses of survival, and many volunteers collect larvae at a variety of sites. Thus, our analyses of fifth instar to adult survival are done at the regional level, and we did not detect any differences in this survival metric when comparing regions (we could only compare the north-central and mid-east regions) or over time. There was a fairly constant rate of pupal death from other causes across the years of the study, at least in the north-central region for which we have the most data, suggesting that tachinid fly parasitism was an important driver of survival from the fifth instar to adult, at least for reared monarchs. However, our assessment of fifth instar to adult survival is calculated based on indoor rearing, and thus does not include pupal mortality from predators such as *Polistes* wasps (Oberhauser et al. 2015) or pupal parasitoids (Oberhauser et al. 2015), or from extreme weather conditions. Addressing this data gap will be difficult because finding pupae in the wild is rare (our complete database only includes 31 monarchs that were collected as pupae). We do know that parasitism by *Pteromalus cassotis* Walker, a gregarious parasitic wasp of lepidopteran pupae, can cause high mortality (up to 100% at some sites; Oberhauser et al. 2015) that is not taken into account in our analysis.

A key finding of this study is the large variation in immature monarch survival from year to year, both from the egg to the fifth instar, as measured by field sampling, and from fifth instars to adults, as measured by reared larvae collected from the wild. While we are not accounting for mortality from pupal parasitoids or predators, or abiotic factors that could affect pupal survival, this finding demonstrates that it will be important to consider immature mortality in population models and conservation actions. Another key finding is the positive association between survival and the number of plants monitored, suggesting that conservation actions should encourage plantings with large numbers of milkweed plants, not only because more plants will support more monarchs but also because survival is likely to be higher.

The amount of milkweed required to produce an adult monarch has, to our knowledge, never been calculated using long-term field data. While ~29 milkweeds for the production of one adult monarch is a good starting point for conservation efforts, this is a conservative estimate because pupae were not exposed to many sources of mortality that are likely to be important in the wild. Additionally, this estimate is likely to only apply to the north-central region and the milkweed species common to sites in this region (*A. syriaca*). However, depending on the region and species of milkweed available for planting, this measure can be used as a minimum in monarch conservation planning.

Finally, this study documents the value of the intensive monitoring done in a program like the MLMP; none of the parameters estimated here would have been obtainable with less intense monitoring. MLMP volunteers have provided an incredible amount of data across a wide geographic range. However, there remain significant data gaps that document the need for recruitment in regions that are not currently well-represented by MLMP volunteers. Of the 16 possible region–time combinations for this analysis, only 7 had enough data for robust analyses (see Fig. 2). While we have fairly good coverage in the northern and south-central regions, more data from the mid-latitudes and the south-east would provide a more complete picture of monarch survival and population dynamics.

Acknowledgments

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