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Linking the continental migratory cycle of the monarch butterfly to understand its population decline

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Threats to several of the world's great animal migrations necessitate a research agenda focused on identifying drivers of their population dynamics. The monarch butterfly is an iconic species whose continental migratory population in eastern North America has been declining precipitously. Recent analyses have linked the monarch decline to reduced abundance of milkweed host plants in the USA caused by increased use of genetically modified herbicide-resistant crops. To identify the most sensitive stages in the monarch's annual multi-generational migration, and to test the milkweed limitation hypothesis, we analyzed 22 years of citizen science records from four monitoring programs across North America. We analyzed the relationships between butterfly population indices at successive stages of the annual migratory cycle to assess demographic connections and to address the roles of migrant population size versus temporal trends that reflect changes in habitat or resource quality. We find a sharp annual population decline in the first breeding generation in the southern USA, driven by the progressively smaller numbers of spring migrants from the overwintering grounds in Mexico. Monarch populations then build regionally during the summer generations. Contrary to the milkweed limitation hypothesis, we did not find statistically significant temporal trends in stage-to-stage population relationships in the mid-western or northeastern USA. In contrast, there are statistically significant negative temporal trends at the overwintering grounds in Mexico, suggesting that monarch success during the fall migration and re-establishment strongly contributes to the butterfly decline. Lack of milkweed, the only host plant for monarch butterfly caterpillars, is unlikely to be driving the monarch's population decline. Conservation efforts therefore require additional focus on the later phases in the monarch's annual migratory cycle. We hypothesize that lack of nectar sources, habitat fragmentation, continued degradation at the overwintering sites, or other threats to successful fall migration are critical limiting factors for declining monarchs.

Cross-continent animal migrations are some of the most spectacular ecological phenomena and are severely threatened (Wilcove and Wikelski 2008). A major hurdle in conceptualizing and conserving animal migrations is understanding the demographic connectivity and population dynamics over the migratory cycle, especially in the face of large spatial movements over long time scales. For hundreds of years the annual migration of the monarch butterfly *Danaus plexippus* from Mexico to the northern USA and Canada has captured the imagination of scientists and non-scientists alike (Gustafsson et al. 2015). Like many other migratory animals, monarch butterflies have a complex multigenerational cycle and changes in any one of the stages can affect their population dynamics (Fig. 1).

Monarchs have a history of 10-fold or larger annual population fluctuations (Swengel 1995, García-Serrano et al. 2004, Rendón-Salinas et al. 2014). Yet, a 2011 study based on 17 years of data revealed a precipitous long-term population decline at the overwintering sites in Mexico (Brower et al. 2012b, Rendón-Salinas et al. 2014) and the rate of decline may be increasing over time (Fig. 2) (Ries et al. 2015b). Nonetheless, two independent fall monitoring programs that enumerate returning monarchs from the northern USA and Canada did not show a decline over the same time period (Davis 2012, Badgett and Davis 2015). Understanding the complex population dynamics of monarchs over space and time therefore remains an important ecological as well as conservation challenge.

From a conservation perspective, it is critical to identify key stage(s) influencing population dynamics. The classic case of loggerhead sea turtle conservation exemplifies this issue, as initial efforts emphasized life stages (eggs and hatchlings) that were unlikely to substantially benefit the population (Crouse et al. 1987). The monarchs' annual cycle has several potentially critical stages (Malcolm and Zalucki 1993, Flockhart et al. 2013). In late winter, overwintering butterflies mate and fly from Mexico north to the southern USA, where most individuals lay eggs on emerging milkweeds, and die (Fig. 1). The next generation migrates

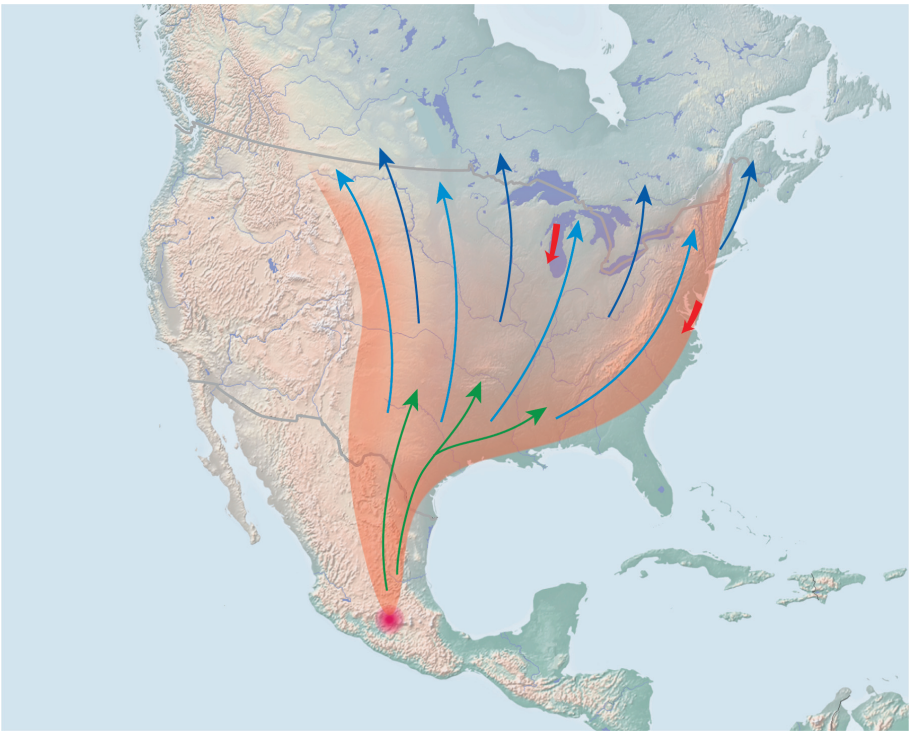

Figure 1. The annual multigenerational migratory cycle of the monarch butterfly. The southernmost red dot indicates the high elevation overwintering grounds (generation 0). North pointing arrows indicate spring and summer migration (green = generation 1, followed by 2–3 additional generations). The top of the solid red arrows indicate two funnel points of south flying monarchs for which we have count data, whereas the larger diffuse red envelope indicates the overall southern migration. There are smaller monarch populations in Mexico, California and Florida, but they are not depicted here.

north, expanding into southern Canada on both sides of the Appalachians, but east of the Rocky Mountains. Additional generations breed in these northern regions. Beginning in late August, unmated butterflies siphon through several funneling points and migrate up to 4000 km back to the overwintering grounds in Mexico. Millions of monarchs ultimately concentrate on about a dozen mountaintops, in an area less than 800 km^2 (Slayback and Brower 2007).

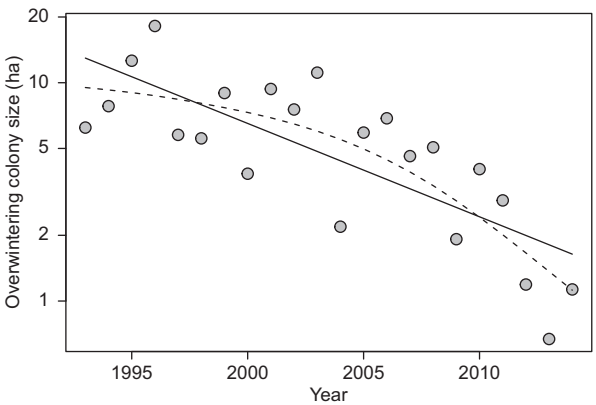

Figure 2. Temporal decline in the size of the overwintering colony in Mexico (ha). Note the logarithmic scaling of the y-axis. The fitted linear regression (solid line) is significant ($p < 0.001$) and corresponds to an average decrease of slightly over 9% per year. A fitted spline regression (dashed curve, fitted with the gam function in the mgcv library) suggests an accelerating decline; the nonlinearity is statistically significant ($p = 0.02$, F-test). Data from Rendón-Salinas et al. 2014.

Since the discovery of the overwintering grounds in Mexico, numerous threats to monarchs and their migration have been identified, most of which involve human activities such as logging and agriculture (Malcolm 1993, Oberhauser and Peterson 2003, Bradley and Altizer 2005, Brower et al. 2006). Recent analyses have specifically implicated the decline of milkweed host plants due to increased use of genetically modified herbicide-resistant crops, especially in the agricultural Midwest USA (the "milkweed limitation hypothesis") (Oberhauser et al. 2001, Brower et al. 2012a, Pleasants and Oberhauser 2013). Given that monarch caterpillars exclusively feed on milkweed, it is not surprising that milkweed decline appears to be locally impacting butterfly and caterpillar abundance (Zalucki and Lammers 2010, Pleasants and Oberhauser 2013, Stenoien et al. 2015). These local demographic effects were recently incorporated in prospective models for the monarch's migratory cycle (Flockhart et al. 2015), where a stage-structured matrix projection model for the monarch life-cycle was combined with spatial structure and migration.

Here we take an alternative approach, using multiple datasets covering 22 years of monarch monitoring programs across North America to retrospectively investigate associations between population dynamics in different regions, and to identify stages contributing to the recent population decline. Using count data reported to the North American Butterfly Association (NABA) and other citizen scientist data, we sought to follow the cycle from overwintering abundance, to spring and summer breeding populations, and finally to fall migrating butterfly counts. Our analyses

and findings overlap, in part, with other recent analyses of monarch population trends (Davis 2012, Badgett and Davis 2015, Ries et al. 2015a, b). However, our study is the first to link the entire annual migratory cycle, and the first to analyze temporal trends in population indices and stage-to-stage relationships through the migratory cycle. Thus, our analyses are uniquely positioned to address the milkweed limitation hypothesis by understanding demographic connectivity at larger temporal and spatial scales.

Material and methods

NABA citizen science data

The North American Butterfly Association (NABA) has compiled butterfly counts from participating citizens across North America since 1975. The dataset consists of thousands of observations on the number of adult monarchs, the location, number of participants, and total hours spent in the field for each census. We focus on >6000 records from 1993–2014, as each of these years had a substantial number of counts (mean of 290 counts per year) and matches the census records available from the Mexico overwintering grounds. Although census locations are not fixed, butterflies are counted across the regions of interest (Fig. 3), and should represent regional sums of adults across small scale habitat variation. We scaled each count by total group hours (Koenig 2006, Ries et al. 2015a, b). In the Supplementary material Appendix 1, we show that our results are robust to alternative ways of normalizing counts for observer effort.

To focus on the long-range migration of the eastern monarch population, we eliminated some regions from the NABA dataset. We removed counts west of 105°W corresponding to the largely separate migratory "Californian" populations (Koenig 2006) and counts from Georgia, South Carolina, and Florida, corresponding to the mostly non-migratory "Gulf Coast" populations (Brower 1995). The NABA dataset was then subdivided into three spatially distinct regions (Fig 3A): South (south of 34.5°N and west of 79°W), Northeast (north of 34.5°N and east of 79°W, corresponding to the Appalachian mountain divide), and Midwest (north of 34.5°N and west of 79°W). Northeast and Midwest include dates from 27 March to 3 October, corresponding to the entire summer breeding season. South was further temporally subdivided into two groups: Spring South (1 March through 30 June, corresponding to reproducing migrants moving north), and Fall South (1 September to 30 November, corresponding to returning migrants moving south).

The NABA citizen science dataset has spatially and temporally variable sampling effort, and its quality has been challenged (Pleasants et al. 2016). We therefore based our population abundance indices on a temporal moving average for each region (Brown 2004). For each date, we calculated an equally weighted average of all the counts in the region falling in a seven-day window, centered around that date. This approach alleviates biases in the population index due to temporal variation in sampling intensity. Ripley's K function shows that there is no evidence for increased clustering of census points over time (Supplementary material Appendix 1). We assessed and addressed other potential biases in the NABA dataset (varying sampling intensity, missing butterfly arrivals, etc.) in multiple ways (Supplementary material Appendix 1).

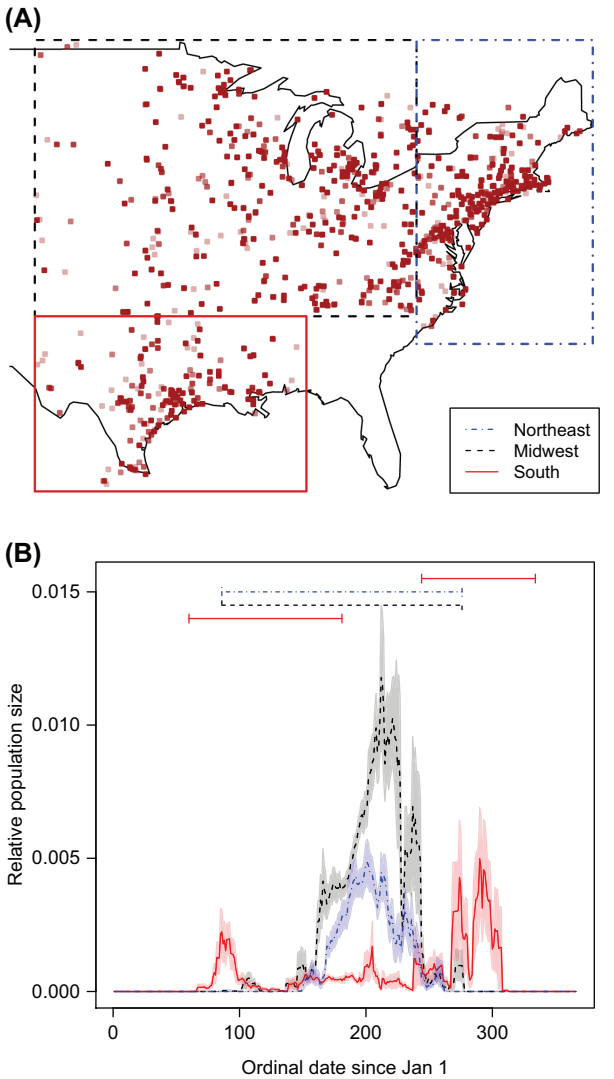

Figure 3. (A) Locations of North American Butterfly Association citizen science counts of monarch adults east of the Rocky Mountains, and separated into three regions: South (south of 34.5°N and west of Appalachians, excluding Georgia, South Carolina and Florida where monarchs are largely non-migratory), Midwest (west of Appalachians), and Northeast (east of Appalachians) (1993–2014). Red dots indicate count locations and increasing color intensity indicates overlapping count points across years. A few count points located outside of this region were included in the analyses, but are not shown on the map. (B) Mean ± SEM (across years) moving average of the relative monarch population index over days of the year in the three regions. Shown above the curves are the windows of dates for which we used data to estimate the annual population index, with colors and line types corresponding to regions. Note that South is divided into spring and fall populations. The proportional abundance reflects the regional population density, not regional total population size. The relative indices here are therefore not directly comparable.

The total monarch index for a given year was calculated by summing the population index within a region during the time periods defined above. Northern populations have

temporally overlapping and variable numbers of generations, so we calculated season-wide sums of observations in the midwest and northeast (99% of the counts taken June–August). The same number of days was used for a season each year, so sums and averages are equivalent.

Assessing butterfly phenology in NABA data across years

Seasonal population trends over the 22 years in the NABA dataset were estimated by calculating the proportional number of monarchs for each day (population index at each date / total index for that year across all regions). This value indicates the proportional abundance of butterflies seen each day. To assess whether the NABA dataset captured the known pattern of annual migratory phenology, we plotted the mean daily proportional abundance trends with the standard error calculated across the 22 years.

Additional data from repeatedly-measured sites

Cape May Point, New Jersey, is a major funneling point for southern migrating monarchs from the northeastern USA (Walton and Brower 1996, Davis 2012). Transect counts are conducted three times daily on a defined route, and the counts are normalized by hours of observation. Weekly averages are reported over nine weeks from 1 September to 31 October (1992–2014). We summed the weekly averages to get a population size index reflecting the total number of butterflies migrating south.

Peninsula Point, Michigan, is a funneling point for southern migrating monarchs from eastern and potentially midwestern Canadian populations (Meitner et al. 2004, Davis 2012). Transect counts are conducted one or two times daily on a defined route, and the counts were normalized by hours of observation. We obtained original data sheets from the Peninsula Point Monitoring Project. We averaged the daily counts for each week and summed the weekly averages to get our population index. The count period runs from early August to late September (1996–2014), so the population index for each year is based on the number of butterflies passing through over eight weeks. Two years (1996 and 1998) were missing from the original data sheets, but were presented in another study using a slightly different estimation method (Davis 2012). We used regression analysis of the data from the 17 years included in both studies to predict values for the two missing years.

Mexican overwintering sites are monitored by the World Wildlife Fund. A December estimate of total hectares occupied by roosting butterflies is reported for 1993–2014 as a proxy for population size (Rendón-Salinas et al. 2014).

Finally, United States Department of Agriculture collects data on the adoption of herbicide resistant crops and we used the mean of adoption rates for corn and soybean from 1996–2015. We expected that increase adoption of herbicide resistant crops leads to increased herbicide use and consequently, decrease in milkweed abundance. We therefore employ this dataset as a proxy for milkweed abundance in the agricultural fields (Pleasants and Oberhauser 2013). To move beyond the simply association between herbicide resistant crop adoption and monarch populations, we test whether year-to-year changes in adoption correlate with year-to-year changes in monarch population estimates.

Analyzing relationships between regions

We used regression analysis to test for relationships between annual population indices at successive stages in the migratory cycle (from 'donor' to 'recipient', respectively): Mexico to Spring South (overwintering butterflies migrating north); Spring South to Northeast and Spring South to Midwest (butterflies migrating further north, while population size builds up over 2–3 generations); Northeast to Cape May and Midwest to Peninsula Point (fall migration south); Cape May to Fall South, Peninsula Point to Fall South, and Fall South to Mexico (migration further south); Midwest to Mexico, and Northeast to Mexico (bypassing Fall South). All analyses were performed in R ver. 2.15.1. (<www.r-project.org>).

The one NABA census that limited sampling was Fall South (we only have counts beginning in 2002 and 2014 was the single year with a count in November. The mean number of counts from 2002 to 2014 is 8.85). When we summarized the NABA regions, between-year variability was substantially higher for Fall South than for the Midwest and Northeast summer indices (Fig. 3B). These patterns call into question the quality of the fall South index perhaps due to much-reduced sampling. Nonetheless, we include the fall South index in analyses, but we do not base conclusions strictly on those results. In addition, we include regressions between the northern censuses and Mexico.

Given our knowledge of the migratory cycle, finding the expected positive relationship between successive pairwise stages indicates reasonable data quality and provides a basis for further analyses of the demographic links between the indices (WWF Mexico, NABA, Cape May, Peninsula Point) that were collected independently using different protocols. We tested for the link between overwintering and the spring migration by regressing the NABA Spring South population index against the Mexico population index. Spring South counts did not include March and April for 1993–2004, thereby missing the crucial first-generation migrants from Mexico (Supplementary material Appendix 1). Accordingly, for this link we focus on 2005–2014. We determined the link between summer breeding and the fall migration by regressing the Cape May index against the NABA Northeast index, and the Peninsula Point index against the NABA Midwest index. Our strong a priori expectation was to find a positive relationship between population indices of the 'donor' and 'recipient' regions based on the monarch's known annual cycle. Therefore, although we present two-tailed p-values, we consider p-values below 0.1 to be significant for these tests. If a data point had a studentized residual over 3 in magnitude, it was considered a possible outlier (Lund 1975). In such cases, results are presented with and without the possible outlier.

Testing for temporal trends in population relationships

We conducted forward and backward model selection and used F-tests to address whether the cause of any observed

population decline at a 'recipient' stage in the cycle was due to declining inputs (population index at the 'donor' stage), or due to a decline in the relationship between population indices at these two stages. We used 'donor', 'year' and 'donor-by-year' interaction, as predictors. We performed model selection based on AIC, and used F-tests to assess whether a potential predictor significantly improves the model's fit. Additional details of the analysis and complete output of the model selection procedure are presented in the Supplementary material Appendix 1. In four such analyses we detected a single outlier, and present results with and without the outlier.

Results

Citizen science data captures the annual migratory cycle

Figure 3B shows a moving-average index of relative population size across each year, based on the 6376 records in the NABA dataset, separated into three geographical regions. The daily population indices clearly captured the monarch's continental migration: a spring wave of population increase and decrease in the southern USA, followed by a similar but extended summer pattern in the Midwest and Northeast as the butterflies move north, and then a fall wave of returning butterflies in the south flying to Mexico. The trend varies somewhat (standard errors indicated by shaded regions) but the major features are consistent across years.

Next we assessed whether the population indices reflect the known links in the migratory cycle. Despite using distinct monitoring methods, the overwintering population index linearly predicted the Spring South population index based on NABA counts ($p < 0.001$, adjusted $R^2 = 0.753$; Table 1, Fig. 4A), reflecting the first breeding generation in the southern USA. The annual Spring South index predicted the subsequent Midwest and Northeast NABA-based indices ($p = 0.04$, adjusted $R^2 = 0.346$ and $p = 0.06$, adjusted $R^2 = 0.291$, respectively; Table 1, Fig. 4B, 4D). Although there may be spatial biases in these NABA counts (e.g. we lack data on whether surveys were conducted in crop fields), the high mobility of monarchs and the large geographic area of sampling in the Midwest and Northeast (Fig. 3) are likely to alleviate these issues. The Midwest and Northeast indices are also correlated with each other ($n = 22$, $r = 0.682$, $p < 0.001$), suggesting that variation in spatial sampling among NABA volunteers has not greatly biased these indices.

Links between summer indices and the single-location counts of fall southward flying migrants again involve distinct data sets. The NABA-based summer Northeast index predicted the Cape May, NJ fall migration count ($p < 0.01$, adjusted $R^2 = 0.283$; Table 1, Fig. 4E). The Midwest index predicted, although less strongly, the fall migration through Peninsula Point, MI ($p = 0.06$, adjusted $R^2 = 0.15$ on log-transformed data; $p = 0.05$, adjusted $R^2 = 0.17$ with one potential outlier (2014) removed; Fig. 4C). These results are what we would expect if the fall migration counts at Cape May and Peninsula Point are representative of the total adult butterfly source populations in the Northeast and Midwest regions, respectively. Overall, these statistical linkages (from Mexico through the fall migration counts) are strong evidence for data quality and expected demographic links between Mexico, spring and summer breeding generations, and fall migration indices.

In contrast, our Fall South index (2002–2014), representing butterflies returning to Mexico through the southern USA, showed no relationship with the end of summer indices (Cape May and Peninsula Point) or with the overwintering population in Mexico (Table 1, Fig. 4F–H). Given the lack of relationship with fall South, we also regressed the Mexico overwintering population index against summer indices (Midwest, Northeast, Peninsula Point and Cape May) (e.g. Fig. 4I; all analyses in Table 1). We used all four indices to independently test the relationship, yet the only significant

Table 1. Regression analyses between stages of the annual monarch migratory cycle. Statistical significance is indicated by $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, and $^{+}p < 0.1$ (two-tailed tests).

	Independent	Dependent	N (years)	Slope	Adj R^2
Spring migration	Mexico[2]	Spring South	10	**10.465*** **	**0.753**
Summer breeding	Spring South	Northeast	10	**1.855+**	**0.291**
(first generation)	Spring South	Midwest	10	**2.403***	**0.346**
Summer breeding	Northeast	Cape May	22	**5.713** **	**0.283**
(up to 3 generations)	Midwest	Peninsula Point	19	*0.598*[1]	*0.018*
Fall migration	Midwest	Fall South	13	0.195	0.018
	Northeast	Fall South	13	0.195	< 0.001
	Peninsula Point	Fall South	13	–0.059	< 0.001
	Cape May	Fall South	13	0.034	0.010
	Fall South	Mexico	13	0.004	< 0.001
	Midwest	Mexico	22	0.003	< 0.001
	Northeast	Mexico	22	–0.010	< 0.001
	Cape May	Mexico	22	0.001	< 0.001
	Peninsula Point	Mexico	19	0.001	< 0.001

[1]With a single outlying year (2014) removed, this relationship was significant at $p = 0.050$ (Adj $R^2 = 0.171$). The 2014 census for PP was a statistically significant outlier (studentized residual > 3.1), see Lund 1975.

[2]Although this is the most direct test of a relationship between overwintering monarch numbers in Mexico and the spring populations in the USA, it is limited by 10 years of data. If we skip this first breeding generation (Spring South) and examine the 22 year relationship between Mexico and either the Midwest or Northeast, there is no significant relationship in either case ($p > 0.4$). The visual suggestion (not shown) of a hump-shaped relationship in scatterplots of the data is not statistically supported ($p > 0.35$ in all cases, for quadratic regression or nonparametric spline regression of Midwest or Northeast on the prior Mexico population).

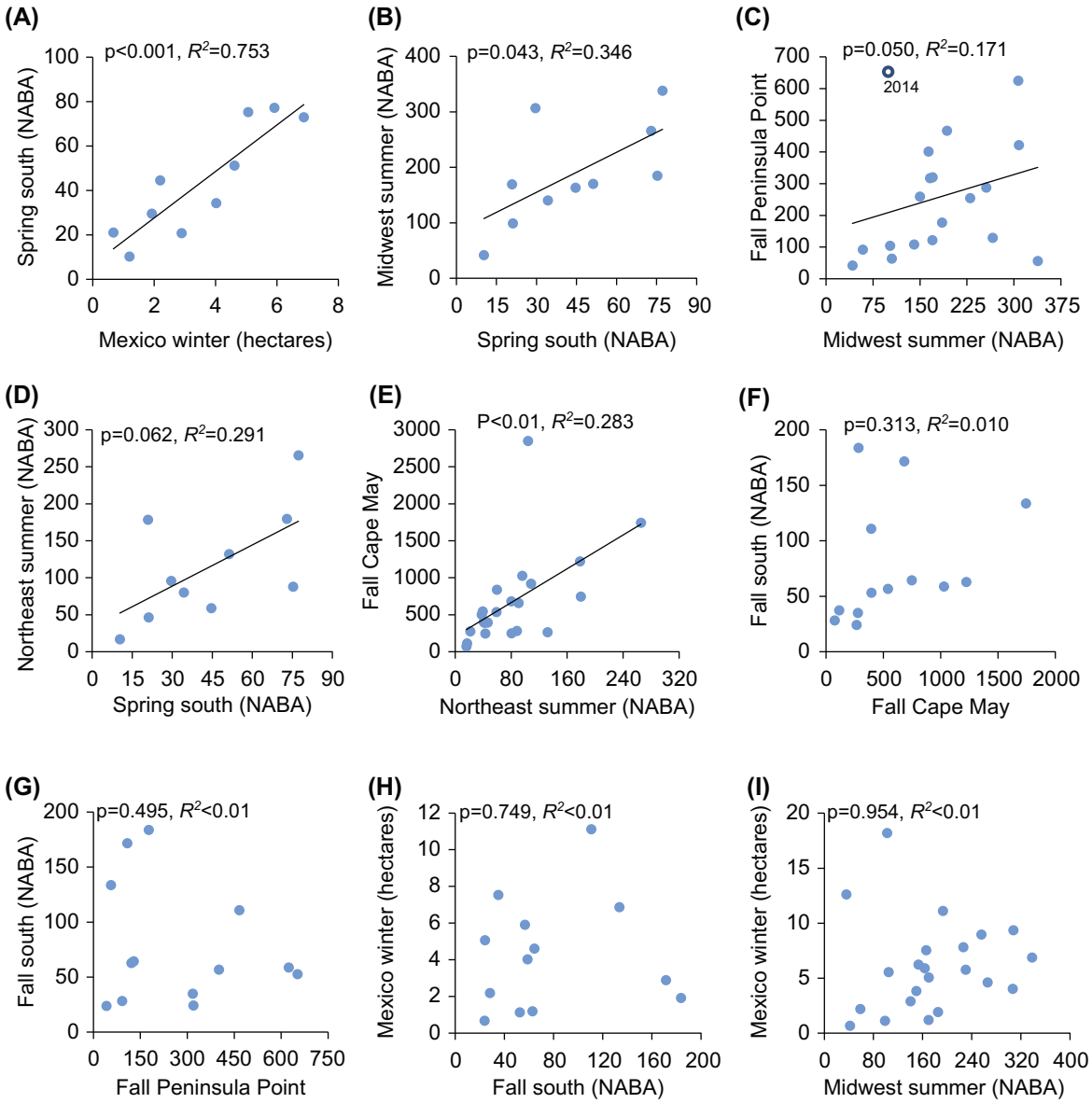

Figure 4. Continent-wide population links in the annual migratory cycle of the eastern monarch butterfly. Representative links are shown here, but others are detailed in Table 1. Regression lines are only shown for statistically significant slopes (in panel C, the outlying year 2014 is indicated and was excluded, see Table 1 for statistical justification of outlier removal). Both NABA and Fall Peninsula Point counts were normalized by sampling effort. Units for NABA counts are the sum of daily estimates from a moving average, while Fall Peninsula Point units are the sum of weekly average counts (all are normalized by effort, see Methods).

relationship was between Peninsula Point and Mexico when an outlier was removed, with a slope that decreased over time ($p < 0.01$, adjusted $R^2 = 0.62$; Supplementary material Appendix 2).

The milkweed limitation hypothesis is not supported

None of the four northern indices (or the fall South index) showed a statistically significant decline across the full 22-year period covered by the NABA counts (Fig. 5). This suggests that northern populations were able to build up during the breeding generations, despite the overwinter decline. Admittedly, the Northeast and Midwest indices do show some visual indication of a decline beginning in 2005. To address this, we examined the temporal trends beginning then, but only found marginally significant declines since 2005 (Adjusted $R^2 = 0.15$, $p = 0.15$ and adjusted $R^2 = 0.31$, $p = 0.055$, for Northeast and Midwest respectively, Fig. 5). Additionally, over the same time period the declines were more severe in Mexico and in the first generation in the south (the fitted linear regressions of population index versus year correspond to decreases of 92.4% over the time period for Mexico, 78.5% for spring South, 62.1% for Midwest and 64.9% for Northeast), and there was no statistically significant trend in the Cape May, Peninsula Point or Fall South indices. The steep decline in Mexico is reflected in subsequent northern indices, as we expect from the annual cycle, but the impact is progressively attenuated at each step of the cycle (Fig. 5).

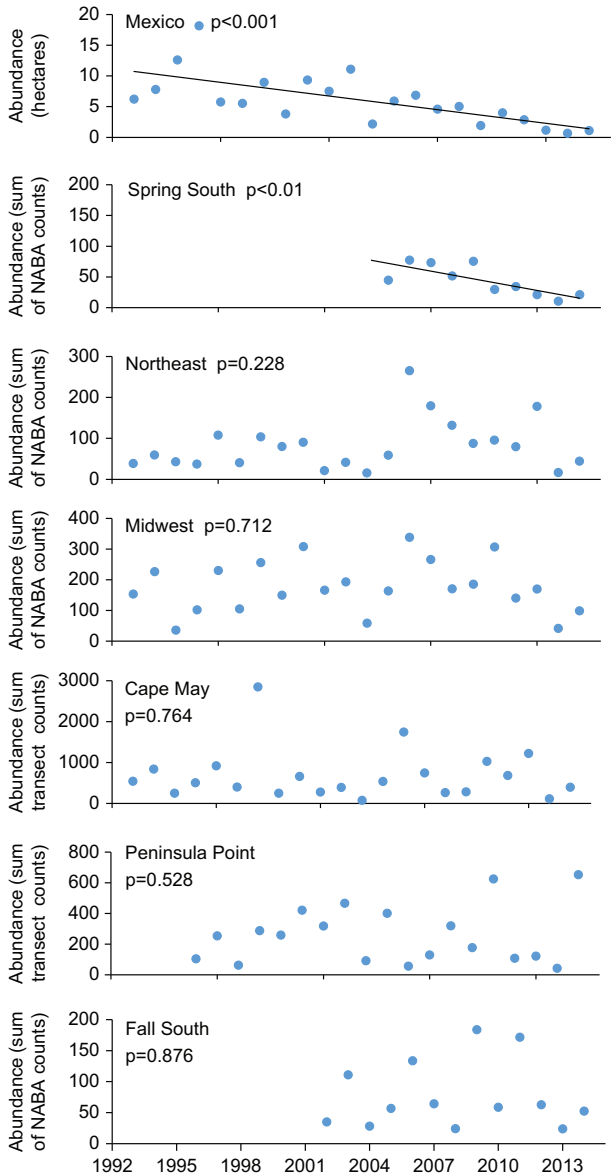

Figure 5. Temporal pattern of monarch abundances in the overwintering colonies in Mexico, southern USA, and four independent summer northern indices (1993–2014). Units for NABA counts are the sum of daily estimates from a moving average, while Fall Peninsula Point and Cape May units are the sum of average weekly transect counts (all are normalized by effort, see Methods). Only significant regression lines are shown.

We next asked if the year-to-year changes in overwintering monarch population estimates are correlated with year-to-year changes in adoption of herbicide-resistant crops. We use differenced data, rather than directly correlating monarch populations with herbicide-resistant crop adoption, because any two variables showing a trend over the same time will be correlated. If the relationship is causal, however, annual differences in adoption should correlate with annual differences in monarch populations. No such correlation is observed ($n = 21$, $r = 0.03$, $p = 0.897$, Supplementary material Appendix 1 Table A1). As a case in point, the peaks of the summer Midwest and Northeast population indices both occurred in 2006, following a 10-year period in which use of herbicide-tolerant crops rose steadily from 0 to 62.5% of corn and soybean acreage (Supplementary material Appendix 1 Table A1).

Where is the break in cycle?

We used a model selection approach to test for temporal trends in the stage-to-stage relationships between population indices (Table 2, Supplementary material Appendix S2). This procedure evaluates whether the population at 'recipient' stage (e.g. Midwest) was driven by inputs from the previous 'donor' stage (e.g. monarchs migrating from the South), or by 'year', which represents an unspecified directional effect on the stage being predicted (e.g. habitat degradation, decline of milkweed, etc.). A significant interaction between 'year' and 'donor' indicates a relationship changing over time. If milkweed reduction is the main factor in the monarch decline, we expect to see changing relationships in stages where breeding occurs. Instead, we found temporal trends at stages where the population is not building and not dependent on milkweed (Table 2).

Along the northern migration, the 'donor' stage was the most important predictor of the 'recipient' stage, and the interaction term was at most marginally significant (Table 2, Supplementary material Appendix 2). For example, the Midwest summer population index is best explained by the input population size from Spring South.

Similar results hold for the beginning of the southern migration, when the butterflies fly south through funnel points such as Cape May and Peninsula Point (Supplementary material Appendix 2). However, along the southern migration, 'year' is a significant predictor of the Mexican overwintering population, indicating a decline at this stage,

Table 2. Results from model selection without an outlier (see Supplementary material Appendix 2 for full details) to address the role of migratory inputs and temporal trends in pairwise regional links of the monarch's annual migratory cycle in eastern North America.

Recipient (dependent variable)	Donor (independent variable)	Best model
Spring South	Mexico	Spring South ~ Mexico + Mexico × YEAR
Midwest	Spring South	Midwest ~ Spring South
Northeast	Spring South	Northeast ~ Spring South
Peninsula Point	Midwest	Peninsula Point ~ Midwest
Cape May	Northeast	Cape May ~ Northeast
Mexico	Midwest	Mexico ~ YEAR
Mexico	Northeast	Mexico ~ YEAR
Mexico	Peninsula Point	Mexico ~ Pen Point + Pen Point × YEAR
Mexico	Cape May	Mexico ~ YEAR

with three out of four northern USA indices failing to explain additional variation in the overwintering population index. The one exception was the relationship between migrants from Peninsula Point and Mexico with an outlier removed. Here, both the 'donor' and interaction terms were statistically significant, indicating a declining relationship over time. Importantly, Peninsula Point captures migrating butterflies and therefore a decline in the slope predicting the population size in Mexico is independent of milkweed. Lastly, our analyses show some evidence for an interaction between Fall South and 'year' in predicting monarch populations in Mexico. Again, these adult butterflies do not depend on milkweed, suggesting a temporal change in migratory success.

Ries et al. (2015b) found a significant positive correlation between summer peak populations (estimated from NABA counts) and the subsequent overwintering population in Mexico after the latter data were detrended for their annual decline. We therefore tested for associations between our summer indices and detrended Mexico data (i.e. the residuals from the nonlinear trend plotted in Fig. 2). The correlations of Mexico with our Midwest index (which is most similar to the region considered by Ries et al. 2015b) and with Peninsula Point were positive and marginally significant ($r = 0.40$, $p = 0.07$ and $r = 0.37$, $p = 0.12$ respectively). In other words, the Midwestern index weakly predicts the numbers arriving to Mexico, but only after the downward trend in Mexico is removed. We found no significant correlations between detrended Mexico data and the Northeast, Cape May or Fall South indices ($r > 0$ but $p > 0.2$ in all cases). Taken together, our results are consistent with failed migration or re-establishment at the overwintering grounds impacting the population decline in Mexico.

Discussion

The current literature on monarch population dynamics and decline is rife with inconsistent patterns and interpretations (Brower et al. 2012b, Davis 2012, Pleasants and Oberhauser 2013, Davis and Dyer 2015, Dyer and Forister 2016). While the monarch population is clearly declining in Mexico, a similar pattern is not observed in many northern regions. We have attempted to make sense of these inconsistencies by connecting the demographic dots of the annual monarch migratory cycle. Although none of the datasets employed is perfect, they represent the bulk of the available data, and we linked them in new ways. Citizen-science data allow for investigations at "large spatial scales, where important processes not detectable at local scales may dominate dynamics" (Dickinson et al. 2010).

Although limited sample size (10–22 years for population indices) and variability in the data limit the statistical power of any one test, we used several different approaches to examine population trends and the milkweed limitation hypothesis. Several trends during the years covered by our data conflict with expectations of the milkweed limitation hypothesis. The lack of an overall decline in the two NABA summer regional indices, and the two fall migratory indices covering the same years, suggest that the milkweed decline is not limiting the production of adult butterflies. Similarly, Crewe and McCracken (2015) found that the fall migrant counts at the Long Point, Ontario funneling point decreased at roughly half the rate of the decline in Mexico. Taken together these results indicate a substantial recovery, during the breeding season, from population bottlenecks. We found no correlation between annual increases in the adoption of herbicide-resistant crops (the hypothesized causal agent of milkweed declines) and annual decreases in the Mexico overwintering population. For the summer population indices, where milkweed limitation should be most evident, monarch populations actually increased substantially over the decade that included 2/3 of the total increase in herbicide-resistant crop acreage (through the mid 2000s).

Our stage-to-stage regression analyses show that monarch population changes are predictable along the annual cycle from Mexico through to the summer breeding grounds; the annual population index at each step reflects the index of the previous step. Furthermore, model selection confirmed that the regional links are sufficient to explain the population dynamics up to the fall migration. For example, the decline in the overwintering population fully accounts for the decline in the first generation in the southern USA. However, there is a break in predictability beginning with the fall migration. That three out of four northern indices fail to predict the numbers arriving in the south conflicts with our most basic expectations about monarch population dynamics and suggests an external factor, but it occurs at a stage when milkweed is not utilized. Badgett and Davis (2015) also hypothesized that diminished fall migration success is an important factor in the overwintering population decline, but this was based solely on the lack of a decline in the Peninsula Point counts, and was predicated on the assumption that Peninsula Point is representative of the total northern breeding population. Our analysis of the successive links across the entire migratory cycle more completely addresses this issue and is concordant with the hypothesis that the population decline is most strongly driven by events after monarchs rely on milkweeds.

Our model selection analysis indicates that over the years, populations of adult monarchs consistently build up during the summer, beginning in the first (southern USA) breeding generation. There is some evidence that the density of monarch eggs has declined since 2007 (Stenoien et al. 2015), and that larval survival has also declined (Nail et al. 2015); these results, however, are inconsistent with the trends in summer adult counts and initial number of fall migrants (Fig. 4). We believe that adult counts are complementary to egg and larval counts, but are more useful in analyses such as ours because the adult stage represents migrants after egg and larval mortality (which is typically very high in the field). Our analysis indicates that an unknown, annually increasing effect, is impacting the monarch population by the time they reach Mexico, producing a consistent decline over the past two decades.

One way in which lack of milkweed could drive monarch declines is if the monarchs that reach Mexico are a small geographical subset of those breeding in the USA during the summer months. It has been suggested that the midwestern USA is the critical area for monarch breeding that populates the overwintering grounds (Pleasants and Oberhauser 2013, Flockhart et al. 2015). Although NABA counts are not

Table 3. Proposed threats to the sustainability of the eastern monarch butterfly annual migration.

Proposed threat	References
Habitat destruction/logging at the overwintering sites	Malcolm 1993, Brower et al. 2012b, Vidal et al. 2014
Habitat destruction/reduced nectar availability on southern migration	Alonso-Mejía et al. 1997, Brower et al. 2006
Disease, predation, and parasitoids	Bradley and Altizer 2005, Oberhauser et al. 2015
Climate change/extreme weather	Oberhauser and Peterson 2003, Brower et al. 2012b
Herbicides/genetically modified herbicide tolerant crops (loss of milkweed)	Zalucki and Lammers 2010, Pleasants and Oberhauser 2013, Flockhart et al. 2015
Insecticides/genetically modified insecticidal crops	Krischik et al. 2014
Automobile accidents, especially during the migration	McKenna et al. 2001
Electromagnetic fields/microwave emissions	Guerra et al. 2014
Trap plants	Casagrande et al. 2014, Batalden and Oberhauser 2015

typically conducted in agricultural fields where milkweed declines are strongest (Oberhauser et al. 2001, Pleasants and Oberhauser 2013), our regional population indices sum over large areas (Fig. 3A), and predict the numbers flying south in the fall that are drawn from all habitats (Fig. 4). Results from stable isotope work and tagging are variable, but indicate that well over half of the monarchs making it to Mexico are derived from outside the agricultural Midwest (Wassenaar and Hobson 1998, Hobson 2008, Flockhart et al. 2013). Few eastern coastal migrants reach the overwintering sites, but non-coastal migrants east and west of the Appalachians are more successful (Brindza et al. 2008, Steffy 2015).

Many factors have been suggested to explain the overwintering population decline of the monarch butterfly (Table 3). Our analyses point to the fall migration and re-establishment on the wintering grounds as key issues. Uncovering the cause of the trends at these stages may be critical to understanding the decline in Mexico. The severe "100-year" drought in Texas (2010–2015) likely had a strong impact on spring and fall migrants, corresponding to the lowest monarch numbers on record (Brower et al. 2015, Zipkin et al. 2012). Factors such as sub-lethal insecticide effects in the breeding grounds (Krischik et al. 2014) or lack of nectar sources during the fall (Brower et al. 2006) may be important in driving a wedge between summer and overwintering populations. Milkweed is typically no longer flowering during the fall migration, and other plant species (many in the Asteraceae) serve as nectar sources. However, the condition of fall migrants might be affected by the environments they experience early in life, including milkweed shortage, insecticides, or other changes in habitat quality.

Other aspects of the breeding and migratory behavior of monarchs are changing, and their roles in population dynamics are unknown. The sex ratio of monarchs at the overwintering sites has changed over the past three decades from 53% female to 43% female (Davis and Rendón-Salinas 2009). As well, the pace of the fall southern migration has sped up over the past decade (Howard and Davis 2015). We currently have few estimates of the sub-lethal impacts of poor quality summer breeding habitat, insecticide residues, and intensified agriculture on the monarchs' migratory success. Predator, parasitoid, and disease impacts can also be severe (Bradley and Altizer 2005, Oberhauser et al. 2015). Determining the extent to which these and other factors contribute to the dynamics of eastern monarchs is a high priority.

Conclusion: conservation and controversy

The past two years have seen tremendous media attention and scientific discourse on the population decline of the monarch butterfly (Wagner et al. 2014, Berenbaum 2015, Rubinoff 2015, Shapiro 2015, Pleasants et al. 2016). While there has been consistency in some of the analyses, other research and interpretations has called into question the extent to which we truly understand fluctuations in monarch population sizes, especially given the complex annual migratory cycle (Dyer and Forister 2016, Espeset et al. 2016, Ries et al. 2015b). We hope that our analysis linking the annual population steps has shed light on this important conservation issue, and moreover that our approach will be useful in understanding the similar challenges faced by many long-distance migrants (Wilcove and Wikelski 2008).

The monarch butterfly is far from being threatened, but the eastern USA migration, one of the most spectacular animal migrations in the world, may be an endangered phenomenon (Brower et al. 2012b). To identify and manage the risk factors associated with its decline, deeper critical analyses of the existing data are essential. We do not dispute that milkweed is essential for larval monarchs, and might serve as a buffer against further aggravation. Yet our analyses indicate that other stages are critical, so milkweed conservation alone is unlikely to be sufficient to preserve the migration. Additional resources are necessary to study and improve the transition between summer breeding in the USA and overwintering in their highland forested habitats in Mexico.

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Supplementary material (available online as Appendix oik-03196 at <www.oikosjournal.org/appendix/oik-03196>). Summary of annual data used in analyses (Table A1), analyses examining quality and potential biases in the NABA dataset (Appendix 1), and analyses to examine temporal change in the relationship between stages of the monarch's annual migratory cycle (Appendix 2) are available online.