 doi:10.20944/preprints202105.0653.v1

[1]*Article*

High survivorship of first-generation monarch butterfly eggs and larvae associated with a diverse arthropod community

Misty Stevenson [1], Kalynn L. Hudman [2], Alyx Scott [3], Kelsey Contreras [4], and Jeffrey G. Kopachena [5,*]

[1] Dallas Arboretum and Botanical Garden, 8525 Garland Road, Dallas, Texas 75218, USA; mnixon@dallasarboretum.org
[2] Department of Biological and Environmental Sciences, Texas A&M University – Commerce, Commerce, Texas 75428, USA; kalynnhudman@gmail.com
[3] Houston Zoo, 6200 Herman Park Drive, Houston, Texas 77030; ascott@houstonzoo.org
[4] Environmental Health and Safety, University of Texas at Arlington, Arlington, Texas 76019; Kelsey.Contreras@uta.edu
[5] Department of Biological and Environmental Sciences, Texas A&M University – Commerce, Commerce, Texas 75428, USA; Jeff.Kopachena@tamuc.edu
* Correspondence: Jeff.Kopachena@tamuc.edu

Simple Summary: The eastern migratory population of the monarch butterfly has declined by over 80% in recent years. However, there are gaps in our knowledge about the survival of first, or spring generation, monarchs in their core areas of Texas, Oklahoma, and Louisiana. This is important because the spring generation represents the first stage of annual population recovery from overwinter mortality. It is, therefore, an important stage for monarch conservation efforts. This study showed that, in the context of a complex arthropod community in north Texas, first generation monarch survival was high. The study found that survival was not directly related to predators on the host plant, but was higher on host plants that harbored a greater number and variety of other, non-predatory arthropods. This is likely because the presence of alternate, preferable prey enabled monarch eggs and larvae to be overlooked by predators. The implication is that, at least in the southern U.S., monarch conservation should consider strategies that promote diverse functional arthropod communities.

Abstract: The eastern migratory population of the monarch butterfly (*Danaus plexippus*) in North America has declined by over 80% in the last 20 years, prompting the implementation of numerous conservation strategies. However, there is little information on the survivorship of first-generation monarchs in the core area of occupancy in Texas, Oklahoma, and Louisiana where overwinter population recovery begins. The purpose of this study was to determine the survivorship of first-generation eggs to third instars at a site in north Texas and to evaluate host plant arthropods for their effect on survivorship. Survivorship to third instar averaged 13.4% and varied from 11.7% to 15.6% over three years. The host plants harbored 77 arthropod taxa, including 27 predatory taxa. Despite their abundance, neither predator abundance nor predator richness predicted monarch survival. However, host plants upon which monarchs survived often harbored higher numbers of non-predatory arthropod taxa and more individuals of non-predatory taxa. These results indicate that indirect top-down effects improved monarch survival in our study. The creation of diverse functional arthropod communities should be considered for effective monarch conservation, particularly in southern latitudes.

Keywords: monarch butterfly; *Danaus plexippus*; Arthropods; community structure; survivorship

1. Introduction

The monarch butterfly (*Danaus plexippus*) is an iconic North American butterfly whose seasonal distribution spans much of North America [1–6]. However, despite this large geographic distribution, there have been marked declines in populations of this species. The eastern migratory population, which occurs in much of North America east of the Rocky Mountains [3], has shown a decline of over 80% in the last 20 years [7,8]. In response to this rapid decline, the eastern migratory population of the monarch butterfly was petitioned for listing under the Endangered Species Act (ESA) in 2014 [4,9]. In the fall of 2020, the U.S. Fish and Wildlife Service (USFWS) ruled that listing the monarch butterfly under the ESA was warranted, but was precluded because limited resources had to be expended on higher-priority species [4]. Nonetheless, numerous models have estimated that under current conditions, migratory populations of monarch butterflies will reach extinction thresholds in the next few decades [4,10–13]. A summary of these models [14] shows that the causes of monarch declines are most likely due to three, non-exclusive issues; habitat loss at winter roost sites in Mexico [15,16], low population recruitment due to losses of milkweed larval host plants [11,17–20], and mortality and loss of viability due to adverse conditions during migration [21–24].

The eastern migratory population of the monarch butterfly colonizes North America each spring and summer through a series of four or five generations [3]. With the possible exception of a small population that may winter among Caribbean islands [25], the entire eastern population of monarch butterflies spend the winter in a few roosting sites in central Mexico [26]. Population size reaches its minimum in the early spring after overwinter mortality and most of these surviving individuals migrate north to lay eggs in a relatively small geographic area in Oklahoma, Texas, and western Louisiana [3,27,28]. The eggs laid by these migrants represent the first, or spring generation of the eastern population. Subsequent generations and the resulting expansion of the population through eastern North America depends on recruitment from this first generation. For this reason, productivity of first-generation monarchs in the southern U.S. has been cited as an important area for conservation efforts [11,13]. Because of this, there is a critical need for data on the survival of first-generation monarchs in order for appropriate conservation strategies to be developed [29].

Despite the fact that the first generation appears to be an important bottleneck in the annual growth of eastern monarch populations, there is almost no information on the ecology and success of this generation. This gap in knowledge creates uncertainty in what measures, if any, need to be taken to increase the fecundity and survival of this generation [13]. There are only three studies that measure the survivorship of first-generation monarchs in the core areas of Texas, Oklahoma and western Louisiana [30–32]. The most recent of these studies [32] was over 20 years ago, and none of the three studies provide comprehensive data on the ecological context associated with survivorship. The age of these studies is important, because across North America monarch survival rates appear to have declined in recent years [29]. Clearly, more recent and in-depth data are needed for first-generation monarchs in the core areas of Texas, Oklahoma, and western Louisiana.

The three studies cited above all implicate arthropod predation as important factors limiting the survival of first-generation monarchs. However, these studies do not provide details on the arthropod communities associated with the host plants. In other geographic areas, and for other generations, arthropods are important correlates of monarch egg and larval mortality [33–43] and monarch eggs and larvae are subject to an extraordinary variety of arthropod predators [37,41]. Most of these studies quantify monarch mortality by looking at rates of loss to specific predators under very controlled experimental conditions. Very few studies examine survival in the context of the natural host plant arthropod community which includes non-predatory species as well as predatory species. Among the few studies that do look at community-level interactions, there is considerable variation in how host-plant arthropods affect monarch survival. In some cases, survival is higher in simple, species-poor communities, than it is in more complex communities [33,42,44]. In other cases, survival is higher in more complex arthropod communities than in less complex communities [35,45], most likely due to indirect top-down effects [34].

 doi:10.20944/preprints202105.0653.v1

Understanding the ecological context of monarch survival is important because a major component of the Monarch Conservation Implementation Plan [47], prepared by the Monarch Joint Venture, is to plant more milkweed plants throughout the species' breeding distribution. In response considerable effort has been made in planting milkweed plants in a variety of settings, including urban monarch gardens, in order to increase the availability of milkweed host plants [20,48]. However, simply planting milkweed plants ignores the potential importance that arthropod community interactions might have on monarch survival. The creation of these anthropogenic environments could, in fact, have the opposite effect by creating ecological traps [49,50].

Here we present an up-to-date assessment of first-generation monarch survival across three years at a study site in northeast Texas. Our goal was to quantify egg and larval survival, to document the arthropod community associated with monarch butterfly host plants, and to evaluate how host-plant arthropods impact monarch survival.

2. Materials and Methods

Data on monarch egg and larval survival were collected at the Cooper Lake Wildlife Management Area and adjacent portions of Cooper Lake State Park in Hopkins Co., Texas (33°18'51.09"N, 95°36'16.70"W) during the springs of 2016 through 2018. In 2016, data were collected from 28 March through 14 May, in 2017, data were collected from 21 March through 17 May, and in 2018, data were collected from 26 March through 11 May. The onset of each field season occurred when the first adults arrived and ended when eggs could no longer be found and all eggs had either reached the third instar or perished. The 2016 field study was a pilot project and, in that year, the only data that was collected was survival of eggs and larvae. More thorough studies were conducted in 2017 and 2018.

The study area contained 48 ha of old-field habitat with isolated stands of trees and woodland edges. The vegetation consisted of a diverse mixture of native and exotic grasses and forbs. The only species of milkweed present was *Asclepias viridis* and its density, measured in 2017 using thirty 50 m^2 circular plots, was 6540 plant per ha., or about 17,015 ramets per ha.

We found Monarch eggs by either by watching females oviposit or by searching individual milkweed plants. Once an egg was found, the plant was marked with a flag and the leaf containing the egg was marked with a non-toxic marker. We followed the focal animal sampling methods used by Prysby [37] and by De Anda and Oberhauser [40] to monitor each egg daily, between 10:00 h and 17:00 h, from the day it was found until it reached the third instar or the egg or larva was missing from the plant. As in other studies that used focal samples, we considered a larva to be dead if was missing from the plant [51,52]. However, early instar larvae can be difficult to find on the host plant and monarch larvae at all stages are known to temporarily leave the host plant for a variety of reasons [53,54]. Therefore, to ensure that a larva had not been overlooked or was temporarily off the host plant, we continued to monitor the plant for four days after a larva was missing from the plant. If the larva was not detected during those four days, it was considered dead and the date of its mortality was recorded as the day it was first missing from the host plant. Furthermore, during our pilot study in 2016, when some host plants were enclosed to exclude predators (data not reported here), it was found that after the larvae reached the third instar, they began to emigrate off the host plant. The tendency to leave the host plant at or after the third instar has also been observed in other studies [40,55]. This meant that once the larvae reached the third instar, we could not distinguish between emigration and mortality. For that reason, we measured survival only up to the third instar.

In 2017 and 2018, data were collected on all other arthropods found on the host plants. To do so, each host plant was approached carefully and all arthropods on the plant were observed and recorded during this approach. Other, less mobile, arthropods were recorded upon close examination of the plant and during the course of searching for the egg or larva. This approach clearly has limitations. In order to avoid disturbing the community, none of the arthropods could be collected, whereas other arthropods would leave the host plant upon approach. As a result, though we tried to be a specific as possible, it was not possible to identify many arthropods beyond the family level. Furthermore, it is acknowledged that these observations represent only a snapshot of the arthropod community on the host plant at a given moment in time. Our interpretations of this data are made with these limitations in mind.

We also measured aspects of the size of the host plants. On the first and last days of monitoring a host plant, we measured the number of ramets, the length of each ramet, and the number of mature leaves on each ramet. For the purpose of analyses, we took the average of the two sets of measurements to quantify host plant size parameters.

Statistical analyses were conducted using SAS® Studio 3.8 software. In reporting the results of statistical tests, we focused on effect sizes. However, we used p-values of ≤ 0.05 to indicate effect sizes that were different from each other or from random values [56]. For frequency data, we used simple chi-square analyses and contingency tables. To analyze survival relative to the arthropod community we used logistic regression. In this case, a stepwise variable selection procedure was used to generate a subset of predictive models. We then used Corrected Akaike's Information Criteria scores (AICc) to select the model with the best fit (lowest AICc) [57] from among the candidate models. For simple comparisons between eggs that survived and those that died, the data were not normally distributed and Kruskal-Wallis Tests were used.

3. Results

3.1. Survival of Monarch Eggs and Larvae

The survivorship of monarch butterfly eggs to the third instar was monitored for 664 eggs; 215 in 2016, 192 in 2017, and 257 in 2018. Survivorship was rather consistent among years and varied from 11.7% in 2018 to 15.6% in 2017 (Figure 1). The overall survivorship from egg to third instar for all three years combined was 13.4%.

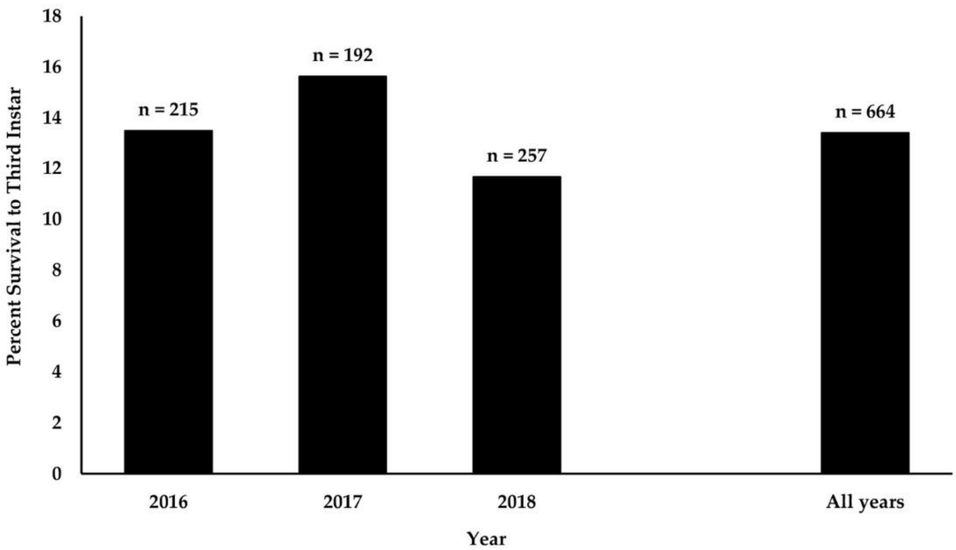

Figure 1. Percent survival of monarch eggs to third instar for each year of the study and for all years combined. Statistical analyses show that differences among years were minor. Chi-square test, 2x3 Contingency Table, Chi-square = 1.48, df = 2, p = 0.477.

3.2. Host Plant Arthropods and the Survival of Monarch Eggs and Larvae

Our general observations in 2016 indicated that some host plants consistently harbored more arthropods than others. We wanted to know if this variation in arthropod activity affected monarch egg and larval survival. In 2017 and 2018, data on host plant arthropods were collected for 449 eggs; 192 eggs in 2017 and 257 eggs in 2018. Of these eggs and subsequent larvae, 42 died because the plants were either trampled by wildlife, had severe stem damage from wind, or were browsed by rabbits [58]. This source of mortality represented 3% of all mortalities in 2017, 16% of all mortalities in 2018, and 11% of all mortalities for both years combined. Because this mortality did not involve arthropods, these individuals were eliminated from the analyses of the effects of arthropods on egg and larval survival.

Some eggs were infected by parasitic wasps (Hymenoptera, Apocrita, *Trichogramma*). In 2017 there were 13 parasitized eggs, accounting for 8% of overall mortality whereas in 2018 there were 5 parasitized eggs, accounting for 2% of all mortalities. Overall, *Trichogramma* parasitism was responsible for 5% of the mortalities recorded in this study. Since the source of the *Trichogramma* mortalities was known, these eggs were also removed from analyses on the effects of host plant arthropods on monarch survival.

There were 14 eggs for which the host plants had no recorded arthropods. Since these plants did not add information to the potential effects of arthropods on monarch egg and larval survival, and to avoid overdispersion in the data, these 14 eggs were also removed from the analysis.

Lastly, we had to correct the data for the inherent bias associated with arthropod counts on host plants on which eggs survived and host plants upon which arthropods died. The longer an egg or larva was monitored, the more likely it was that more kinds and greater numbers of arthropods would be associated with that individual. Since eggs that survived were often monitored for a longer period of time than eggs that died, these data would be biased in favor of detecting more arthropods associated with surviving monarch eggs and larvae. To eliminate this bias, 164 individuals that were monitored for less than 10 days were removed from the analysis. This resulted in data on 210 eggs where the mean number of days monitored and the variance in the number of days monitored was essentially the same for eggs that survived ($n = 152$) and eggs that died ($n = 58$) (T-test for mean number of days, $t = 0.30$, $p = 0.7675$; Test for equal variances, $F = 1.33$, $p = 0.1809$).

We documented 15,441 arthropods distributed among 77 different taxa on the host plants used in this analysis (Appendix A). This did not include the monarch eggs and larvae themselves. Of the 77 taxa, 27 were predatory, and three of the four most abundant taxa were predators. Six taxa were milkweed-feeding herbivores. The remaining 44 taxa were visiting the plants for nectar, harboring on the plants, or transients (Appendix A).

Though aphids were the most abundant arthropods, they were not the most frequent. Over half of the host plants had jumping spiders on them (Appendix A). Other predators that showed a high frequency on host plants were little black ants (35%) and fire ants (33%). The most frequent non-predatory arthropods were aphids (38%), leafhoppers (37%) and unknown flies (34%). However, most arthropods were uncommon and 54 of the 77 taxa (70%) occurred on less than 10% of the host plants (Appendix A).

The low frequency of many of the arthropod taxa indicated that there was considerable variation among host plants. The total number of arthropods on a host plant was highly skewed, ranging from one through 4008 (Figure 2A). As a result, though the mean number of arthropods on a host plant was 73.5, the median number was 11 and most plants held only three arthropods. Similarly, as might be expected, the taxon richness of host plant arthropods was also highly skewed and ranged from one through 20 (Figure 2B). In this case the average richness was 6.2 and varied from 1 through 20 taxa. The median richness was five, but most host plants held four or fewer arthropod taxa.

The low frequency of most arthropods made the data sparse and overdispersed. While logistic regression is robust against deviations from normality [59], sparse data can lead to inflated parameter estimates and parameters with confidence intervals that approach infinity [60]. To avoid this issue, the arthropod taxa were combined into 16 groups based on frequency, food habits (predatory or non-predatory), taxonomic affiliation, and ecological similarity (Table 1). We used logistic regression to see if these arthropod groups

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

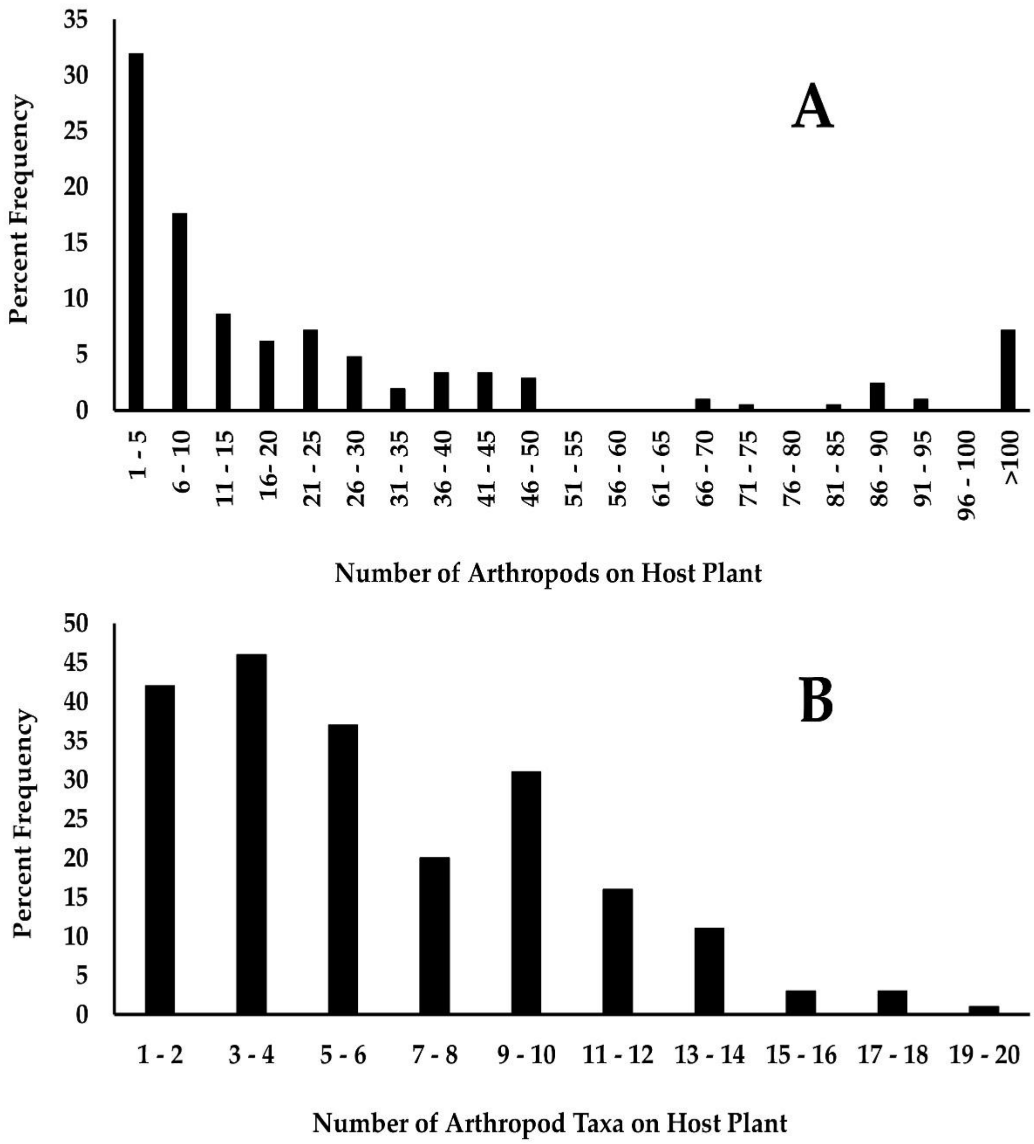

Figure 2. The number of arthropods and the number of arthropod taxa found on monarch host plants. A. Total number of arthropods on monarch host plants grouped into intervals of five. B. The number of taxa (taxon richness) found on monarch host plants grouped into intervals of two.

predicted monarch egg and larval survival. In addition, since survival varied relative to date and since the number of arthropods is likely a product of plant size, we also included date, the number of ramets on the host plant, the total length of ramets on the host plant, and the total number of leaves of the host plant as candidate variables for the stepwise variable selection procedure used to identify the best predictive models.

The results of this analysis did not identify any specific type of arthropod as having a large impact on monarch survival (Table 2). None of the predatory taxa or groups were included in any of the models, and the most important two groups were "Other Non-predatory Arthropods" and "Mites", of which only "Other Non-predatory Arthropods" significantly predicted monarch survival. Monarch survival was highest on plants that held a larger number of "Other Non-predatory Arthropods" (Table 2). Kruskal-Wallis Tests corroborated this association between survival and non-predatory arthropods. When all of the non-predatory arthropods were combined into a single group, it was

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

Table 1. Arthropod taxa associated with 210 eggs used for logistic regression analysis of monarch egg survivorship. Arthropod groups highlighted in yellow are predatory taxa. Percent frequency refers to the percent of monarch eggs or larvae that each taxon was associated with.

Taxon	Common Name	Total Abundance	Frequency	Percent Frequency
Hemiptera, Aphidoidea	Aphids	10792	80	38.10
Hymenoptera, Formicidae, others	Other Ants	907	37	17.62
Hymenoptera, Formicidae, *Monomorium minimum*	Little Black Ant	855	74	35.24
Hymenoptera,Formicidae, *Solenopsis invicta*	Red Imported Fire Ant	633	69	32.86
Coleoptera, Curculionidae,	Weevils	471	67	31.90
Arachnida, Acari, Mites	Mites	268	62	29.52
Arthropoda, Others	Other Non-predatory Arthropods	267	111	57.14
Araneae, Salticidae	Araneae, Salticidae	247	116	55.24
Arthropoda, Others, Predatory	Other Predatory Arthropods	227	123	60.00
Coleoptera, Chrysomelidae	Other Leaf Beetle	171	65	30.95
Coleoptera, Dermestidae	Dermestid Beetle	139	28	13.33
Diptera, unknown	Flies	138	81	38.57
Hemiptera, Cicadomorpha	Leafhopper	137	77	36.67
Hemiptera,Lygaeidae, *Oncopeltus fasciatus*	Large Milkweed Bug	108	38	18.10
Arthropoda, Others	Other Milkweed Herbivores	48	34	48.57
Coleoptera, Others	All Other Beetles	33	22	10.48

Table 2. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on arthropod groups (see Table 1) found on host plants. A stepwise selection procedure was used to generate these models with a significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Models are sorted in order of ascending AICc. Best model is based on minimum corrected AIC Score (AICc), w_i is the Akaike weight of each model.

Model	AICc	ΔAICc	w_i	Likelihood Ratio X^2	Model Probability
Other Non-predatory Arthropods, Mites	249.533	0.000	0.667	10.1993	0.0014
Other Non-predatory Arthropods	241.373	1.418	0.328	13.6759	0.0011
Intercept Only	239.955	9.579	0.006	-	-

Summary of the best fit model. Concordance of this model was 51.3%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	p-value
Intercept	1	-1.3796	0.2103	43.022	<0.0001
Other Non-predatory Arthropods	1	0.2403	0.0781	9.4557	0.0021
Mites	1	0.0989	0.1114	0.7888	0.3745

found that plants upon which eggs survived to the third instar generally had more non-predatory arthropods than did plants upon which eggs did not survive to the third instar (Survived: Median = 11, Mode = 6, Range = 1258; Died: Median = 5, Mode = 1, Range = 3918; Kruskal-Wallis Chi-square = 5.23, p = 0.0222). When all predatory arthropods were combined, no important difference in predator abundance was found between plants where eggs survived and plants where they did not survive (Survived: Median = 4, Mode = 1, Range = 815; Died: Median = 3, Mode = 1, Range = 131; Kruskal-Wallis Chi-square = 1.51, p = 0.2206).

The positive relationship between non-predatory arthropods and monarch survival suggests that indirect effects on top-down processes affect monarch survival. We wanted to see the extent to which logistic regression might predict the survival of monarch eggs and larvae based simply on four variables: the number of predatory arthropods, the number of non-predatory arthropods, the taxon richness of predatory arthropods, and the taxon richness of non-predatory arthropods. Using these four variables, the stepwise procedure identified only two potential models as predictors of monarch survival (Table 3). In this case, the best model identified the taxon richness of non-predatory arthropods as a positive predictor of monarch survival. Interestingly, the two models selected by our procedure did not differ substantially in AIC weight (w_i) and the second model indicated

Table 3. Summary of stepwise logistic regression analysis of survival of monarch eggs or larvae based on the abundance and richness of predatory and non-predatory arthropods found on host plants. A stepwise selection procedure was used to generate these models with a significance level for entry into the model set at 0.30 and significance level for removal from the model set at 0.35. Models are sorted in order of ascending AICc. Best model is based on minimum corrected AIC Score (AICc), w_i is the Akaike weight of each model.

Model	AICc	ΔAICc	w_i	Likelihood Ratio X^2	Model Probability
Number of Non-predatory Taxa	245.200	0.000	0.498	6.3725	0.0116
Number of Non-predatory Taxa, Number of Predatory Arthropods	245.427	0.227	0.445	8.2038	0.0165
Intercept Only	249.533	4.333	0.057	-	-

Summary of the best fit model. Concordance of this model was 55.5%.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	p-value
Intercept	1	-1.5094	0.2763	29.8485	<0.0001
Number of Non-predatory Taxa	1	0.1334	0.0530	6.3309	0.0119

a positive relationship between the total number of predatory arthropods and monarch survival. However, the magnitude of that effect was extremely small (MLE = 0.00357) and not statistically significant (p = 0.2648). Again, Kruskal-Wallis Tests corroborated the positive association between egg survival and the number of non-predatory arthropod taxa. Plants upon which eggs survived to the third instar generally had a greater number of non-predatory arthropod taxa than did plants upon which eggs did not survive to the third instar (Survived: Median = 4, Mode = 3, Range = 14; Died: Median = 3, Mode = 1, Range = 12; Kruskal-Wallis Chi-square = 5.91, p = 0.0151). No important difference in the number of predatory taxa was found between plants where eggs survived and plants where they did not survive (Survived: Median = 2, Mode = 1, Range = 7; Died: Median = 2, Mode = 1, Range = 7; Kruskal-Wallis Chi-square = 1.52, p = 0.2175).

4. Discussion

There is tremendous variation reported in the literature regarding monarch egg and larval survival, some of which might depend on methodology [61,62]. This makes it extremely difficult to compare among studies. In an effort to rely only on comparable studies, we restrict our comparisons to studies that used the same protocols; that is, field studies using unrestricted focal individuals. By necessity, we also include comparisons with all studies providing quantitative data on first-generation monarch egg and larval survival. These comparisons are shown in Table 4.

Table 4. Comparison of monarch survival measured in previous studies to the survival measured in the current study. All of these are field studies based on eggs and larvae that were not confined to enclosures and which were in outdoor settings presumably exposed to unmanipulated arthropod communities.

Location	Measurement	Value	Equivalent Value in current Study	Citation
Florida	Survival to 3rd Instar	9.2%	13%	Brower et al. 2018 [8]
Florida	Survival to 3rd Instar	About 14%	13%	Cohen and Brower 1982 [63]
Florida	Survival to 3rd Instar	17% – 21%	13%	Zaluki and Brower 1992 [64]
Texas and Louisiana	Survival to 3rd Instar	3% (0% to 40%)	13%	Lynch and Martin 1993 [31]
Texas	Survival to 3rd Instar	0%	13%	Calvert 1996 [30]
Texas	Survival to 3rd Instar	0.24%	13%	Calvert 2004 [32]
Minnesota	Daily survival rate, survival to third instar	0.56, 1.7%	0.896, 13%	De Anda and Oberhauser 2015 [40]
Wisconsin	Survival to hatching	35%	63.3%	Borkin 1982 [53]
Wisconsin	Seven-day survival rate	18%	46%	Prysby 2004 [37]
Michigan	48-hour survival rate of first instars	15% to 40%	80%	Haan and Landis 2019 [65]

In our study 13% of monarch eggs survived to the third instar and this varied only slightly between the three years of study. This value is comparable to values reported from several studies conducted in Florida; it is slightly higher than the long-term average recorded by Brower [8], similar to survival reported by Cohen and Brower [63], but lower than the survival observed by Zaluki and Brower [64] (Table 4). On the other hand, our survival was much higher than any of the three studies that included Texas (Table 4). Lynch and Martin [31] found low monarch survivorship in north Texas and northwest Louisiana in the mid-1980's. However, in that study there was extensive variation among sites and, at one site in north Texas, monarchs utilizing *A. viridis* had an estimated survivorship to the third instar of 38% [31]. Some of the variation observed by Lynch and Martin [31] may be due to differences in site characteristics or, alternatively, sample sizes and methodology. The monarch survival that we recorded was also much higher than that recorded in the two other studies conducted in Texas. Calvert found 0% survival to third instar of monarch larvae in a pasture in central Texas in 1995 [30] and an average survival to third instar of 0.24% in three pastures in 1997 and 1998, also in central Texas [32]. As mentioned above, some of these differences could be due to site characteristics. However, there are also methodological considerations that need to be addressed. The first study by

Calvert [30] is based on only 61 eggs. It is possible that the small sample size led to an erroneously low estimate of survival. Furthermore, all three of the studies that included Texas are based on counts at single points in time, thereby creating stage-structured data [62,66]. In these studies, survivorship to the third instar is calculated by dividing the number of third instars found by the number of eggs found. There are problems with this approach (e.g. [61,62,67]). First, it does not provide an accurate estimate of the number of eggs that were laid to produce the instars observed during that survey date. This creates a source of error in the survivorship estimate. Second, stage-structured data do not account for how long the third instars detected during the survey had already been alive. As a result, these data do not account for individuals that reached the third instar but subsequently perished prior to the survey date. This omission would inflate estimates of mortality up to the third instar [61]. Lastly, detection probability can be a problem with monarch surveys as young instars are difficult to detect [62,68]. Lack of detection will also inflate estimates of mortality [55,61]. In our study, we followed individuals until they reached the third instar and revisited the plants to ensure that small instars were not simply overlooked or were not temporarily off the host plant. Consequently, our data is less likely to underestimate survival.

The survival rates measured in our study were also considerably higher than survival rates reported for studies using the same focal individual method and applied to later monarch generations further north (Table 4). In our study, the estimated daily survival rate across all three age classes was 0.896, which is much higher than the 0.56 recorded for monarch eggs and first through second instars in Minnesota [40]. In that study, survivorship to the third instar was estimated to be only 1.7%; over seven times lower than the survivorship we measured. In Wisconsin, two studies found survival rates to be less than half of that found in the current study (Table 4) [37,53]. A study in Michigan found that 48h survival of first instars varied from just over 15% to over 40% depending on disturbance regime [65]. In our study, the equivalent 48h survival would average 80%.

Our data indicate that spring-generation monarch survival at our site in Texas was high relative to most other studies with the exception of those conducted in Florida. It is possible that high survivorship is typical of first-generation monarchs in the southern U.S., though a broad geographic analysis based on long-term data suggests otherwise [29]. Our data also show that monarch survival was best predicted by the abundance and richness of arthropod taxa that are typically non-predatory. More non-predatory arthropods and a greater number of non-predatory arthropod taxa were associated with greater monarch survival. Despite the fact that predatory arthropods represented three of the most abundant taxa, and that jumping spiders, which are known predators of monarchs [40], were the most frequent arthropods on host plants, neither the abundance nor the number of predatory taxa provided any predictive power in explaining monarch mortality. These results suggest that top-down indirect effects may be operating in this system.

Though our data only provide a snapshot of the arthropod activity on each monarch host plant, the community that was revealed was remarkably rich and diverse. These 77 arthropod taxa occupied the host plants for a variety of reasons. Six taxa were herbivores that either tolerate milkweed plants or are milkweed specialists [69]. Over the course of the study, some host plants were in various stages of flowering. Milkweed flowers produce abundant nectar and, for that reason, milkweed plants attract many different arthropods. In Arizona, *Asclepias tuberosa* flowers are visited by over 80 different species of arthropods [70] and, in Oklahoma, *A. viridis* flowers are visited by over 23 families of insects [71]. Furthermore, milkweed plants like *A. viridis* have a stout growth form that makes the plants attractive to insects seeking physical structures on which to rest or form harborages. Spiders, for example, will select plants based on plant architecture [72] and this seemed to be true of the jumping spiders observed on more than half of the host plants in our study. Many other arthropods are simply transient, using the milkweed plant as a temporary resting place within the larger context of the surrounding plant community. In turn, all of these arthropods attract many different predators to the host plants [40,43]. In our study,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

27 of the 77 arthropod taxa observed on monarch host plants were predators and they represented three of the four most abundant arthropods.

Among arthropod predators, diet breadth is often determined by hunting method, encounter rates, infochemical cues, and size [73]. Many of the arthropods that have been cited as preying on monarch eggs and larvae [37,40,41,43,74], including all of those predatory species recorded in our study, are polyphagous. When compared to many of the other potential prey items on the host plants, monarch eggs and monarch larvae up to the third instar are small and solitary. Furthermore, monarch eggs and larvae contain cardenolides, whereas many of the other phytophagous insects on the host plant either do not sequester cardenolides or are not as efficient as monarchs in sequestering these compounds [75]. Because some polyphagous predators are averse to prey with high levels of cardenolides [76] they may avoid consuming monarch eggs and larvae in favor of consuming other prey on the host plant. For many of the host plants, there were other larger and more abundant arthropods available as prey, particularly on those plants that were flowering. For this reason, it is unlikely that the predators we observed on the host plants arrived specifically searching for monarch eggs and larvae. Consequently, the consumption of the eggs and larvae, when it occurred, was opportunistic. This, and the overall complexity of the arthropod community associated with the host plants, makes it difficult to isolate single causal agents leading to monarch mortality or survival.

Several aspects of the interactions among the arthropods on the host plant are important. In ecological communities, indirect effects occur when the impact of a species or group of species on a focal species (monarchs in this study) is altered by the presence of a third species or group of species [77,78]. Top-down indirect effects promote species richness among trophic levels and top-down regulation by predators has been shown to increase herbivore diversity and affect herbivore fitness in other invertebrate communities [79]. Specifically, preferential predation by a predator on one prey species can lead to increases in the population of less preferred prey species [80,81]. Furthermore, on plants where there are numerous arthropods, intraguild predation may also be important in reducing predator pressure [80,82]. Top-down regulation has been proposed as important for monarch survival [35,37,38,40,41,43,74]. However, indirect effects associated with top-down processes are not well documented, though they are known to occur. For example, in field and laboratory studies, consumption of monarch larvae by ladybugs (*Harmonia axyridis*), was reduced when aphids (*Aphis nerii*) were present on the host plant [34]. In the current study, higher abundances of non-predatory arthropods, only a few of which sequester cardenolides, may have favored monarch survival because predators, such as spiders and ants, may have preferentially fed on these other arthropods. Optimal foraging theory demonstrates unequivocally that even slight differences in profitability can cause a prey species to be eliminated from the diet of a predator [81,83]. Indirect effects might explain why monarch survival in our study was more closely associated with the taxon richness and abundance of non-predatory arthropods than it was to the taxon richness or abundance of predatory arthropods. If so, then monarch conservation activities might benefit from activities that promote high biodiversity and functional arthropod communities.

The role of arthropod biodiversity on monarch egg and larval survival seems to vary extensively among the few studies that have examined it. As found in our study, several studies have found that high biodiversity or indirect top-down effects favor monarch recruitment and survival [29,35,36,45,84]. However, a number of other studies have found that either biodiversity has no influence on survival or it has a negative influence on survival [33,37,40,61]. For example, in Minnesota there was a direct relationship between the presence of spiders and low monarch survival. In that study, the presence of aphids was also associated with lower survival among monarch eggs and larvae [40]. Similarly, in Wisconsin, there was reduced survival when host plants held both ants and aphids [37]. In Michigan, it was found that monarch egg survival was lower in plots with higher plant diversity than it was in plots with low plant diversity [33]. Lastly, in Nebraska it was found that there was little difference in monarch recruitment and survival in urban

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

gardens, where arthropod diversity is expected to be low, and tallgrass prairies, where arthropod diversity would be high [61].

We think that some of these differences are due to geography and site characteristics. In our study we focused on the arthropods associated with host plants and we uncovered significant variation among plants. On a larger scale, Lynch and Martin [31] documented considerable variation in monarch survival among study sites in north Texas and northwestern Louisiana. It may be important that the study sites used by Lynch and Martin [31] were all pastures and pastures vary considerably in plant diversity according to how intensively they are managed. Our study area is specifically managed to promote high plant diversity. High plant diversity, in turn, is correlated with high arthropod diversity [85,86]. In addition, it may be important that all of the studies that failed to find a positive influence of arthropod diversity on monarch survival were studies that occurred at higher latitudes. In North America arthropod diversity is higher at low latitudes and lower at high latitudes. Arthropod diversity is particularly high in Texas and Oklahoma [46], where most first generation monarchs originate. For this reason, the influence of arthropod biodiversity on monarch survival might be more important in southern latitudes than in northern latitudes. We suggest that in order for effective monarch conservation to occur, consideration must be given to the influence that functional arthropod communities have on monarch survival in addition to simply adding more milkweed to the landscape. Clearly more research needs to be done on this topic for appropriate and successful conservation strategies to be developed.

The monarch butterfly was once an abundant species with a continent-wide distribution. It seems likely that the decline of monarch butterflies in North America is tied to the global and serious issue of declining terrestrial arthropods in general [87–90]. If so, this further emphasizes the need to frame the conservation of monarch butterflies within a broader framework of restoring terrestrial arthropod diversity and the ecological function of the associated arthropod communities.

Author Contributions: Conceptualization, J.G.K; methodology, M.N., K.L.H., A.S., K.C., and J.G.K.; formal analysis, M.N. and J.G.K.; investigation, M.N., K.L.H., A.S., K.C., and J.G.K.; resources, J.G.K; data curation, J.G.K; writing—original draft preparation, M.N.; writing—review and editing, M.N., K.L.H., A.S., K.C., and J.G.K.; visualization, M.N. and J.G.K.; supervision, J.G.K.; project administration, J.G.K.; funding acquisition, J.G.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Texas Comptroller of Public Accounts, Economic Growth and Endangered Species Management Division, grant numbers 5975LV and 6192CS. In-kind matching funds were provided by the College of Science and Engineering, Texas A&M University – Commerce. The APC was funded by Texas A&M University – Commerce.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank the numerous field assistants who aided in the collection of field data: Emily Casper, Nathan Connon, Hannah Dill, Nikki Dawson, and Beth Fortner. Thanks, are also extended to Howard Crenshaw, TPWD Wildlife Division, for assistance working on the Cooper Wildlife Management Area and to Kody Waters for his assistance with working on the Cooper Lake State Park property. Mike Quinn, Curatorial Associate at The University of Texas at Austin, is thanked for assistance in arthropod identification.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

PAGE LEFT INTENTIONALLY BLANK FOR EIDTORIAL PURPOSES

doi:10.20944/preprints202105.0653.v1

Appendix A

Appendix A. Arthropod taxa associated with 210 monarch egg and larva host plants. Rows highlighted in yellow represent predatory taxa. Rows highlighted in green represent herbivorous taxa known to feed on milkweed or observed to do so in this study. All other taxa were considered to be either nectaring, harboring, or transient. Percent frequency is the percentage of eggs that a taxon was associated with.

Taxon	Common Name	Total Abundance	Frequency	Percent Frequency
Hemiptera, Aphidoidea	Aphid	10792	80	38.10
Hymenoptera, Formicidae, others	Other Ants	907	37	17.62
Hymenoptera, Formicidae, *Monomorium minimum*	Little Black Ant	855	74	35.24
Hymenoptera, Formicidae, *Solenopsis invicta*	Red Imported Fire Ant	633	69	32.86
Coleoptera, Curculionidae, Baridinae	Flower Weevil	272	40	19.05
Arachnida, Acari, Mites	Mite	268	62	29.52
Araneae, Salticidae	Jumping Spider	246	116	55.24
Coleoptera, Curculionidae, Molytinae	Stem Weevil	167	42	20.00
Coleoptera, Dermestidae	Dermestid Beetle	139	28	13.33
Hemiptera, Cicadomorpha	Leafhopper	137	77	36.67
Coleoptera, Chrysomelidae, Alticini	Flea Beetle	128	48	22.86
Diptera, unknown	Other Flies	116	72	34.29

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

Hemiptera, Lygaeidae, *Oncopeltus fasciatus*	Large Milkweed Bug	108	38	18.10
Aranea, Unknown	Other Spider	59	41	19.52
Orthoptera, Caelifera	Grasshopper	50	34	16.19
Coleoptera, Chrysomelidae	Other Leaf Beetle	43	29	13.81
Coleoptera, Unknown	Other Beetles	33	22	10.48
Coleoptera, Curculionidae, Entiminae	Broad-nosed Weevil	31	20	9.52
Thysanoptera	Thrip	29	18	8.57
Hemiptera, Heteroptera	Other True Bugs	28	24	11.43
Hymenoptera, Apocrita, unknown wasps	Wasp	25	20	9.52
Arachnida, Opiliones	Harvestman	24	24	11.43
Araneae, Araneidae	Orb-weaver Spider	22	19	9.05
Diptera, Chironomidae	Midge Fly	22	18	8.57
Hemiptera, Lygaeidae, *Lygaeus kalmii*	Small Milkweed Bug	19	16	7.62
Araneae, Oxyopidae	Lynx Spider	18	17	8.10
Araneae, Thomisidae	Other Crab Spider	18	15	7.14
Hemiptera, Lygaeidae, unknown	Other Seed Bug	17	9	4.29

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

Araneae, Lycosidae	Wolf Spider	14	12	5.71
Othoptera, Tettigoniidae	Katydid	13	12	5.71
Myriapoda, Diplopoda	Millipede	13	8	3.81
Collembola	Springtail	12	8	3.81
Diptera, Muscidae	House Fly	11	11	5.24
Araneae, Thomisidae, *Misumena vatia*	Goldenrod Crab Spider	11	8	3.81
Phasmatodea	Stick Insect	10	10	4.76
Coleoptera, Coccinellidae, *Coccinella septempunctata*	Seven-spotted Ladybeetle	10	10	4.76
Coleoptera, Cerambycidae	Longhorn Beetle	10	7	3.33
Insecta, Unknown egg	Insect Egg	10	1	0.48
Araneae, Tetragnathidae	Long-jawed Orb Weaver	8	7	3.33
Coleoptera, Coccinellidae, *Harmonia axyridis*	Asian Ladybeetle	8	7	3.33
Diptera, Calyptratae	Other Calyptrate Fly	8	5	2.38
Hymenoptera, Apidae, *Xylocopa* sp.	Carpenter Bee	6	6	2.86
Hemiptera, Reduviidae	Assassin Bug	6	4	1.90
Arachnida, Acari	Tick	5	3	1.43

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

Hemiptera, Coreidae	Leaf-footed Bug	5	3	1.43
Othoptera, Grylidae	Field Cricket	4	4	1.90
Coleoptera, Carabidae	Ground Beetle	4	4	1.90
Hymenoptera, Anthophila, Unknown	Other Bee	4	4	1.90
Blattodea, Isoptera	Termite	4	4	1.90
Isopoda	Isopod	4	3	1.43
Neuroptera, adult	Lacewing	4	3	1.43
Neuroptera, larvae	Lacewing Larva	4	2	0.95
Araneae, Agelenidae	Grass Spider	3	3	1.43
Hemiptera, Miridae	Plant Bug	3	3	1.43
Coleoptera, Cantharidae	Soldier Beetle	3	3	1.43
Hymenoptera, Apidae, *Bombus* sp.	Bumblebee	3	3	1.43
Hymenoptera, Apidae, *Apis* sp.	Honey Bee	3	2	0.95
Coleoptera, Elateridae	Click Beetle	2	2	0.95
Coleoptera, Tenebrionidae	Darkling Beetle	2	2	0.95
Coleoptera, Staphylinidae	Rove Beetle	2	2	0.95

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 May 2021 doi:10.20944/preprints202105.0653.v1

Hymenoptera, Vespidae	Vespid Wasp	2	2	0.95
Diptera, Sarcophagidae	Flesh Fly	2	2	0.95
Diptera, Tachinidae	Tachinid Fly	2	2	0.95
Diptera, Syrphidae, adult	Flower Fly, adult	2	2	0.95
Coleoptera, Coccinellidae, Larva	Ladybeetle Larva	2	2	0.95
Araneae, Philodromidae	Running Crab Spider	2	2	0.95
Diptera, Tipulidae	Cranefly	2	2	0.95
Hemiptera, Pseudococcidae	Mealybug	2	2	0.95
Hemiptera, Pentatomidae, Asopinae	Predatory Stink Bug	2	1	0.48
Coleoptera, Scarabaeidae	Scarab Beetle	1	1	0.48
Hemiptera, Pentatomoidea	Stink bug, non-predatory	1	1	0.48
Araneae, Salticidae, *Myrmarachne* sp.	Ant-mimic Jumping Spider	1	1	0.48
Lepidoptera, larva	Caterpillar	1	1	0.48
Mecoptera	Scorpion Fly	1	1	0.48
Trichoptera	Caddisfly	1	1	0.48
Lepidoptera, Heterocera	Moth	1	1	0.48

Coleoptera, Curculionoidea, Unknown	Other Weevil	1	1	0.48

References

1. Oberhauser, K.S.; Nail, K.R.; Altizer, S. *Monarchs in a Changing World: Biology and Conservation of an Iconic Butterfly*; Cornell University Press, 2015; ISBN 978-0-8014-5559-9.
2. Gustafsson, K.M.; Agrawal, A.A.; Lewenstein, B.V.; Wolf, S.A. The monarch butterfly through Time and Space: The Social Construction of an Icon. *BioScience* **2015**, *65*, 612–622, doi:10.1093/biosci/biv045.
3. Flockhart, D.T.T.; Wassenaar, L.I.; Martin, T.G.; Hobson, K.A.; Wunder, M.B.; Norris, D.R. Tracking Multi-Generational Colonization of the Breeding Grounds by monarch butterflies in Eastern North America. *Proc. R. Soc. B Biol. Sci.* **2013**, *280*, 20131087, doi:10.1098/rspb.2013.1087.
4. Nail, K. Monarch (Danaus Plexippus) Species Status Assessment Report, Version 2.1 September 2020. 126.
5. Lyons, J.I.; Pierce, A.A.; Barribeau, S.M.; Sternberg, E.D.; Mongue, A.J.; Roode, J.C.D. Lack of Genetic Differentiation between monarch butterflies with Divergent Migration Destinations. *Mol. Ecol.* **2012**, *21*, 3433–3444, doi:https://doi.org/10.1111/j.1365-294X.2012.05613.x.
6. Scott, J.A. *The Butterflies of North America: A Natural History and Field Guide*; Stanford University Press, 1992; ISBN 978-0-8047-2013-7.
7. Thogmartin, W.E.; Wiederholt, R.; Oberhauser, K.; Drum, R.G.; Diffendorfer, J.E.; Altizer, S.; Taylor, O.R.; Pleasants, J.; Semmens, D.; Semmens, B.; et al. monarch butterfly Population Decline in North America: Identifying the Threatening Processes. *R. Soc. Open Sci.* **2017**, *4*, 170760, doi:10.1098/rsos.170760.
8. Brower, L.P.; Williams, E.H.; Dunford, K.S.; Dunford, J.C.; Knight, A.L.; Daniels, J.; Cohen, J.A.; Van Hook, T.; Saarinen, E.; Standridge, M.J.; et al. A Long-Term Survey of Spring monarch butterflies in North-Central Florida. *J. Nat. Hist.* **2018**, *52*, 2025–2046, doi:10.1080/00222933.2018.1510057.
9. Petition for Protection of the monarch butterfly Under the Endangered Species Act | Xerces Society Available online: https://xerces.org/publications/policy-statements/petition-for-esa-protection-monarchs (accessed on 17 February 2021).
10. Voorhies, K.J.; Szymanski, J.; Nail, K.R.; Fidino, M. A Method to Project Future Impacts From Threats and Conservation on the Probability of Extinction for North American Migratory Monarch (Danaus Plexippus) Populations. *Front. Ecol. Evol.* **2019**, *7*, doi:10.3389/fevo.2019.00384.
11. Flockhart, D.T.T.; Pichancourt, J.-B.; Norris, D.R.; Martin, T.G. Unravelling the Annual Cycle in a Migratory Animal: Breeding-Season Habitat Loss Drives Population Declines of monarch butterflies. *J. Anim. Ecol.* **2015**, *84*, 155–165, doi:https://doi.org/10.1111/1365-2656.12253.
12. Semmens, B.X.; Semmens, D.J.; Thogmartin, W.E.; Wiederholt, R.; López-Hoffman, L.; Diffendorfer, J.E.; Pleasants, J.M.; Oberhauser, K.S.; Taylor, O.R. Quasi-Extinction Risk and Population Targets for the Eastern, Migratory Population of monarch butterflies (Danaus Plexippus). *Sci. Rep.* **2016**, *6*, 23265, doi:10.1038/srep23265.
13. Oberhauser, K.; Wiederholt, R.; Diffendorfer, J.E.; Semmens, D.; Ries, L.; Thogmartin, W.E.; Lopez-Hoffman, L.; Semmens, B. A Trans-National monarch butterfly Population Model and Implications for Regional Conservation Priorities: Regional Monarch Conservation Priorities. *Ecol. Entomol.* **2017**, *42*, 51–60, doi:10.1111/een.12351.
14. Grant, T.J.; Bradbury, S.P. The Role of Modeling in monarch butterfly Research and Conservation. *Front. Ecol. Evol.* **2019**, *7*, doi:10.3389/fevo.2019.00197.
15. Flores-Martínez, J.J.; Rendón-Salinas, E.; Martínez-Pacheco, A.; Salinas-Galicia, R.; Munguía-Carrara, M.; Rickards, J.; Sarkar, S.; Sánchez-Cordero, V. Policy Implementation Halts Deforestation in Winter Habitat of monarch butterflies in Mexico. *BioScience* **2020**, *70*, 449–451, doi:10.1093/biosci/biaa038.
16. Flores-Martínez, J.J.; Martínez-Pacheco, A.; Rendón-Salinas, E.; Rickards, J.; Sarkar, S.; Sánchez-Cordero, V. Recent Forest Cover Loss in the Core Zones of the monarch butterfly Biosphere Reserve in Mexico. *Front. Environ. Sci.* **2019**, *7*, doi:10.3389/fenvs.2019.00167.

17. Stenoien, C.; Nail, K.R.; Oberhauser, K.S. Habitat Productivity and Temporal Patterns of monarch butterfly Egg Densities in the Eastern United States. *Ann. Entomol. Soc. Am.* **2015**, *108*, 670–679, doi:10.1093/aesa/sav054.
18. Malcolm, S.B. Anthropogenic Impacts on Mortality and Population Viability of the monarch butterfly. *Annu. Rev. Entomol.* **2018**, *63*, 277–302, doi:10.1146/annurev-ento-020117-043241.
19. Thogmartin, W.E.; López-Hoffman, L.; Rohweder, J.; Diffendorfer, J.; Drum, R.; Semmens, D.; Black, S.; Caldwell, I.; Cotter, D.; Drobney, P.; et al. Restoring monarch butterfly Habitat in the Midwestern US: 'All Hands on Deck.' *Environ. Res. Lett.* **2017**, *12*, 074005, doi:10.1088/1748-9326/aa7637.
20. Pleasants, J.M.; Zalucki, M.P.; Oberhauser, K.S.; Brower, L.P.; Taylor, O.R.; Thogmartin, W.E. Interpreting Surveys to Estimate the Size of the monarch butterfly Population: Pitfalls and Prospects. *PLOS ONE* **2017**, *12*, e0181245, doi:10.1371/journal.pone.0181245.
21. Saunders, S.P.; Ries, L.; Neupane, N.; Ramírez, M.I.; García-Serrano, E.; Rendón-Salinas, E.; Zipkin, E.F. Multiscale Seasonal Factors Drive the Size of Winter Monarch Colonies. *Proc. Natl. Acad. Sci.* **2019**, *116*, 8609–8614, doi:10.1073/pnas.1805114116.
22. Agrawal, A.A.; Inamine, H. Mechanisms behind the Monarch's Decline. *Science* **2018**, *360*, 1294–1296, doi:10.1126/science.aat5066.
23. Inamine, H.; Ellner, S.P.; Springer, J.P.; Agrawal, A.A. Linking the Continental Migratory Cycle of the monarch butterfly to Understand Its Population Decline. *Oikos* **2016**, *125*, 1081–1091, doi:https://doi.org/10.1111/oik.03196.
24. Ries, L.; Taron, D.J.; Rendón-Salinas, E. The Disconnect Between Summer and Winter Monarch Trends for the Eastern Migratory Population: Possible Links to Differing Drivers. *Ann. Entomol. Soc. Am.* **2015**, *108*, 691–699, doi:10.1093/aesa/sav055.
25. Dockx, C. Differences in Phenotypic Traits and Migratory Strategies between Eastern North American monarch butterflies, Danaus Plexippus (L.): MIGRATION OF EASTERN NORTH AMERICAN MONARCHS. *Biol. J. Linn. Soc.* **2012**, *106*, 717–736, doi:10.1111/j.1095-8312.2012.01916.x.
26. Vidal, O.; Rendón-Salinas, E. Dynamics and Trends of Overwintering Colonies of the monarch butterfly in Mexico. *Biol. Conserv.* **2014**, *180*, 165–175, doi:10.1016/j.biocon.2014.09.041.
27. Castañeda, S.; Botello, F.; Sánchez-Cordero, V.; Sarkar, S. Spatio-Temporal Distribution of monarch butterflies Along Their Migratory Route. *Front. Ecol. Evol.* **2019**, *7*, doi:10.3389/fevo.2019.00400.
28. Malcolm, S.B.; Cockrell, B.J.; Brower, L.P. Spring Recolonization of Eastern North America by the monarch butterfly: Successive Brood or Single Sweep Migration. *Biol. Conserv. monarch butterfly* **1993**, *38*, 253–267.
29. Nail, K.R.; Stenoien, C.; Oberhauser, K.S. Immature Monarch Survival: Effects of Site Characteristics, Density, and Time. *Ann. Entomol. Soc. Am.* **2015**, *108*, 680–690, doi:10.1093/aesa/sav047.
30. Calvert, W.H. Fire Ant Predation on Monarch Larvae (Nymphalidae: Danainae) in a Central Texas Prairie. *J.-LEPIDOPTERISTS Soc.* **1996**, *50*, 149–151.
31. Lynch, S.P.; Martin, R.A. Milkweed Host Plant Utilization and Cardenolide Sequestration by monarch butterflies in Louisiana and Texas. *Biol. Conserv. monarch butterfly* **1993**, *38*, 107.
32. Calvert, W.H. The Effect of Fire Ants on Monarchs Breeding in Texas. *monarch butterfly Biol. Conserv. Cornell Univ. Press Ithaca NY* **2004**, 47–53.
33. Myers, A.; Bahlai, C.A.; Landis, D.A. Habitat Type Influences Danaus Plexippus (Lepidoptera: Nymphalidae) Oviposition and Egg Survival on Asclepias Syriaca (Gentianales: Apocynaceae). *Environ. Entomol.* **2019**, *48*, 675–684, doi:10.1093/ee/nvz046.
34. Koch, R.L.; Venette, R.C.; Hutchison, W.D. Influence of Alternate Prey on Predation of monarch butterfly (Lepidoptera: Nymphalidae) Larvae by the Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae). *Environ. Entomol.* **2005**, *34*, 410–416, doi:10.1603/0046-225X-34.2.410.

35. Baker, A.M.; Potter, D.A. Invasive Paper Wasp Turns Urban Pollinator Gardens into Ecological Traps for monarch butterfly Larvae. *Sci. Rep.* **2020**, *10*, 9553, doi:10.1038/s41598-020-66621-6.
36. Nestle, R.; Daniels, J.C.; Dale, A.G. Mixed-Species Gardens Increase Monarch Oviposition without Increasing Top-Down Predation. *Insects* **2020**, *11*, 648.
37. Prysby, M.D. Natural Enemies and Survival of Monarch Eggs and Larvae. *monarch butterfly Biol. Conserv. Cornell Univ. Press Ithaca NY* **2004**, 27–37.
38. McCoshum, S.M.; Andreoli, S.L.; Stenoien, C.M.; Oberhauser, K.S.; Baum, K.A. Species Distribution Models for Natural Enemies of monarch butterfly (Danaus Plexippus) Larvae and Pupae: Distribution Patterns and Implications for Conservation. *J. Insect Conserv.* **2016**, *20*, 223–237, doi:10.1007/s10841-016-9856-z.
39. Myers, A.T.; Haan, N.L.; Landis, D.A. Video Surveillance Reveals a Community of Largely Nocturnal Danaus Plexippus (L.) Egg Predators. *J. Insect Conserv.* **2020**, *24*, 731–737, doi:10.1007/s10841-020-00248-w.
40. De Anda, A.; Oberhauser, K.S. Invertebrate Natural Enemies and Stage-Specific Mortality Rates of Monarch Eggs and Larvae. *Monarchs Chang. World Biol. Conserv. Iconic Butterfly* **2015**, 60–70.
41. Hermann, S.L.; Blackledge, C.; Haan, N.L.; Myers, A.T.; Landis, D.A. Predators of monarch butterfly Eggs and Neonate Larvae Are More Diverse than Previously Recognised. *Sci. Rep.* **2019**, *9*, 1–9.
42. Myers, A.T. *The Interacting Influences of Habitat Context and Predators on monarch butterfly (Danaus Plexippus L.) Oviposition and Survival in Agricultural Landscapes*; Michigan State University, 2019;
43. Oberhauser, K.S.; Anderson, M.; Anderson, S.; Caldwell, W.; De Anda, A.; Hunter, M.; Kaiser, M.C.; Solensky, M.J.; De Anda, A.; Hunter, M.D. Lacewings, Wasps, and Flies—Oh My: Insect Enemies Take a Bite out of Monarchs. *Monarchs Chang. World Biol. Conserv. Iconic Insect Cornell Univ. Press Ithaca NY* **2015**, 71–82.
44. Oberhauser, K.S.; Prysby, M.D.; Mattila, H.R.; Stanley-Horn, D.E.; Sears, M.K.; Dively, G.; Olson, E.; Pleasants, J.M.; Lam, W.-K.F.; Hellmich, R.L. Temporal and Spatial Overlap between Monarch Larvae and Corn Pollen. *Proc. Natl. Acad. Sci.* **2001**, *98*, 11913–11918, doi:10.1073/pnas.211234298.
45. Deal, K.A. Impact of Landscape Diversity and Invertebrate Community Dynamics on monarch butterfly (Danaus Plexippus) Oviposition and Survival. Thesis, 2017.
46. Danks, H.V. Regional Diversity of Insects in North America. *Am. Entomol.* **1994**, *40*, 50–55, doi:10.1093/ae/40.1.50.
47. VENTURE, M.J. 2016 Monarch Conservation Implementation Plan.
48. Goddard, M.A.; Dougill, A.J.; Benton, T.G. Scaling up from Gardens: Biodiversity Conservation in Urban Environments. *Trends Ecol. Evol.* **2010**, *25*, 90–98, doi:10.1016/j.tree.2009.07.016.
49. Robertson, B.A.; Rehage, J.S.; Sih, A. Ecological Novelty and the Emergence of Evolutionary Traps. *Trends Ecol. Evol.* **2013**, *28*, 552–560, doi:10.1016/j.tree.2013.04.004.
50. Lepczyk, C.A.; Aronson, M.F.J.; Evans, K.L.; Goddard, M.A.; Lerman, S.B.; MacIvor, J.S. Biodiversity in the City: Fundamental Questions for Understanding the Ecology of Urban Green Spaces for Biodiversity Conservation. *BioScience* **2017**, *67*, 799–807, doi:10.1093/biosci/bix079.
51. Zalucki, M.P.; Kitching, R.L. Temporal and Spatial Variation of Mortality in Field Populations of Danaus Plexippus L. and D. Chrysippus L. Larvae (Lepidoptera: Nymphalidae). *Oecologia* **1982**, *53*, 201–207, doi:10.1007/BF00545664.
52. Zalucki, M.P.; Brower, L.P.; Malcolm, S.B. Oviposition by Danaus Plexippus in Relation to Cardenolide Content of Three Asclepias Species in the Southeastern USA. *Ecol. Entomol.* **1990**, *15*, 231–240.
53. Borkin, S.S. Notes on Shifting Distribution Patterns and Survival of Immature Danaus Plexippus (Lepidoptera: Danaidae) on the Food Plant Asclepias Syriaca. **1982**, *15*, 9.
54. Rawlins, J.E.; Lederhouse, R.C. Developmental Influences of Thermal Behavior on Monarch Caterpillars (Danaus Plexippus): An Adaptation for Migration (Lepidoptera: Nymphalidae: Danainae). *J. Kans. Entomol. Soc.* **1981**, *54*, 387–408.

55. Fisher, K.E.; Hellmich, R.L.; Bradbury, S.P. Estimates of Common Milkweed (Asclepias Syriaca) Utilization by Monarch Larvae (Danaus Plexippus) and the Significance of Larval Movement. *J. Insect Conserv.* **2020**, *24*, 297–307, doi:10.1007/s10841-019-00213-2.
56. Murtaugh, P.A. In Defense of P Values. *Ecology* **2014**, *95*, 611–617.
57. Wagenmakers, E.-J.; Farrell, S. AIC Model Selection Using Akaike Weights. *Psychon. Bull. Rev.* **2004**, *11*, 192–196, doi:10.3758/BF03206482.
58. Grodsky, S.M.; Saul-Gershenz, L.S.; Moore-O'Leary, K.A.; Whitney, J.P.; Hernandez, R.R. Hare Don't Care! Consumption of a Rare, Desert Milkweed Containing Phytochemicals by the Black-Tailed Jackrabbit. *J. Arid Environ.* **2020**, *174*, 103991, doi:10.1016/j.jaridenv.2019.103991.
59. Li, Y.; Liu, J.S. Robust Variable and Interaction Selection for Logistic Regression and General Index Models. *J. Am. Stat. Assoc.* **2019**, *114*, 271–286, doi:10.1080/01621459.2017.1401541.
60. Greenland, S.; Mansournia, M.A.; Altman, D.G. Sparse Data Bias: A Problem Hiding in Plain Sight. *bmj* **2016**, *352*.
61. Geest, E.A.; Wolfenbarger, L.L.; McCarty, J.P. Recruitment, Survival, and Parasitism of monarch butterflies (Danaus Plexippus) in Milkweed Gardens and Conservation Areas. *J. Insect Conserv.* **2019**, *23*, 211–224, doi:10.1007/s10841-018-0102-8.
62. Grant, T.J.; Flockhart, D.T.T.; Blader, T.R.; Hellmich, R.L.; Pitman, G.M.; Tyner, S.; Norris, D.R.; Bradbury, S.P. Estimating Arthropod Survival Probability from Field Counts: A Case Study with monarch butterflies. *Ecosphere* **2020**, *11*, e03082, doi:https://doi.org/10.1002/ecs2.3082.
63. Cohen, J.A.; Brower, L.P. Oviposition and Larval Success of Wild monarch butterflies (Lepidoptera: Danaidae) in Relation to Host Plant Size and Cardenolide Concentration. *J. Kans. Entomol. Soc.* **1982**, *55*, 343–348.
64. Zalucki, M.P.; Brower, L.P. Survival of First Instar Larvae OfDanaus Plexippus (Lepidoptera: Danainae) in Relation to Cardiac Glycoside and Latex Content OfAsclepias Humistrata (Asclepiadaceae). *Chemoecology* **1992**, *3*, 81–93, doi:10.1007/BF01245886.
65. Haan, N.L.; Landis, D.A. Grassland Disturbance Increases monarch butterfly Oviposition and Decreases Arthropod Predator Abundance. *Biol. Conserv.* **2019**, *233*, 185–192, doi:10.1016/j.biocon.2019.03.007.
66. Manly, B.F. Stage-structured populations. In *Stage-Structured Populations*; Springer, 1990; pp. 1–6.
67. Johnson, E.A.; Miyanishi, K.; Kleb, H. The Hazards of Interpretation of Static Age Structures as Shown by Stand Reconstructions in a Pinus Contorta–Picea Engelmannii Forest. *J. Ecol.* **1994**, 923–931.
68. Prysby, M.D.; Oberhauser, K.S. Temporal and Geographic Variation in Monarch Densities: Citizen Scientists Document Monarch Population Patterns. 12.
69. Betz, R.F.; Rommel, W.R.; Dichtl, J.J. Insect Herbivores of 12 Milkweed (Asclepias) Species. In Proceedings of the Proceedings of the Fifteenth North American Prairie Conference; The Natural Areas Association Bend, OR, 1997; pp. 7–19.
70. Fishbein, M.; Venable, D.L. Diversity and Temporal Change in the Effective Pollinators of Asclepias Tuberosa. *Ecology* **1996**, *77*, 1061–1073, doi:https://doi.org/10.2307/2265576.
71. Liaw, S.-W. The Structure of the Gynostegium, Breeding System, and Pollination Ecology of Spider Milkweed, Asclepias Viridis Walter (Apocynaceae). *Okla. Native Plant Rec.* **2016**, *16*, 10–44, doi:10.22488/okstate.17.100121.
72. Vasconcellos-Neto, J.; Messas, Y.F.; da Silva Souza, H.; Villanueva-Bonila, G.A.; Romero, G.Q. Spider–plant interactions: an ecological approach. In *Behaviour and ecology of spiders*; Springer, 2017; pp. 165–214.
73. Dyer, L.A.; Floyd, T. Determinants of Predation on Phytophagous Insects: The Importance of Diet Breadth. *Oecologia* **1993**, *96*, 575–582, doi:10.1007/BF00320516.
74. Smithers, C.N. A Note on Natural Enemies of "Danaus Plexippus" (L.) (Lepidoptera: Nymphalide) in Australia. *Aust. Entomol.* **1973**.

 doi:10.20944/preprints202105.0653.v1

75. Isman, M.B.; Duffey, S.S.; Scudder, G.G.E. Cardenolide Content of Some Leaf- and Stem-Feeding Insects on Temperate North American Milkweeds (*Asclepias* Spp.). *Can. J. Zool.* **1977**, *55*, 1024–1028, doi:10.1139/z77-130.
76. Rayor, L.S. Effects of Monarch Larval Host Plant Chemistry and Body Size on Polistes Wasp Predation. *monarch butterfly Biol. Conserv. Cornell Univ. Press Ithaca NY* **2004**, 39–46.
77. Wootton, J.T. The Nature and Consequences of Indirect Effects in Ecological Communities. *Annu. Rev. Ecol. Syst.* **1994**, 443–466.
78. Mittelbach, G.G.; McGill, B.J. *Community Ecology*; Oxford University Press, 2019; ISBN 978-0-19-257286-8.
79. Vidal, M.C.; Murphy, S.M. Bottom-up vs. Top-down Effects on Terrestrial Insect Herbivores: A Meta-analysis. *Ecol. Lett.* **2018**, *21*, 138–150, doi:10.1111/ele.12874.
80. Frago, E.; Godfray, H.C.J. Avoidance of Intraguild Predation Leads to a Long-Term Positive Trait-Mediated Indirect Effect in an Insect Community. *Oecologia* **2014**, *174*, 943–952.
81. Prado, S.G.; Frank, S. Optimal Foraging by an Aphid Parasitoid Affects the Outcome of Apparent Competition. *Ecol. Entomol.* **2014**, *39*, 236–244.
82. Krey, K.L.; Cooper, W.R.; Renkema, J.M. Revealing the Diet of Generalist Insect Predators in Strawberry Fields: Not Only Pests, But Other Predators Beware. *Environ. Entomol.* **2020**, *49*, 1300–1306, doi:10.1093/ee/nvaa125.
83. Giraldeau, L.-A.; Danchin, E. Solitary Foraging Strategies. *Behav. Ecol.* **2008**, 233–256.
84. Ali, J.G.; Agrawal, A.A. Asymmetry of Plant-Mediated Interactions between Specialist Aphids and Caterpillars on Two Milkweeds. *Funct. Ecol.* **2014**, *28*, 1404–1412, doi:10.1111/1365-2435.12271.
85. Hertzog, L.R.; Meyer, S.T.; Weisser, W.W.; Ebeling, A. Experimental Manipulation of Grassland Plant Diversity Induces Complex Shifts in Aboveground Arthropod Diversity. *PLoS One* **2016**, *11*, e0148768.
86. Welti, E.; Helzer, C.; Joern, A. Impacts of Plant Diversity on Arthropod Communities and Plant–Herbivore Network Architecture. *Ecosphere* **2017**, *8*, e01983, doi:https://doi.org/10.1002/ecs2.1983.
87. Forister, M.L.; Pelton, E.M.; Black, S.H. Declines in Insect Abundance and Diversity: We Know Enough to Act Now. *Conserv. Sci. Pract.* **2019**, *1*, e80, doi:https://doi.org/10.1111/csp2.80.
88. Wagner, D.L. Insect Declines in the Anthropocene. *Annu. Rev. Entomol.* **2020**, *65*, 457–480, doi:10.1146/annurev-ento-011019-025151.
89. van Klink, R.; Bowler, D.E.; Gongalsky, K.B.; Swengel, A.B.; Gentile, A.; Chase, J.M. Meta-Analysis Reveals Declines in Terrestrial but Increases in Freshwater Insect Abundances. *Science* **2020**, *368*, 417–420, doi:10.1126/science.aax9931.
90. Hallmann, C.A.; Sorg, M.; Jongejans, E.; Siepel, H.; Hofland, N.; Schwan, H.; Stenmans, W.; Müller, A.; Sumser, H.; Hörren, T.; et al. More than 75 Percent Decline over 27 Years in Total Flying Insect Biomass in Protected Areas. *PLOS ONE* **2017**, *12*, e0185809, doi:10.1371/journal.pone.0185809.