Temporal and spatial changes in monarch butterfly population synchrony in Michigan and density-dependent influences on the incidence of the protozoan parasite, *Ophryocystis elektroscirrha.*

Joshua E. Armagost and Andrew C. Johnson

Mentored by Stephen Malcolm, D. Phil.

Department of Biological Sciences

Western Michigan University

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Abstract

Much is known about the migration, mating, physiology, and host plant use of the monarch butterfly, *Danaus plexippus*, however, little is known about the dominant phase of their life history when their annual populations increase during the summer. Our research aimed to shed light on this topic by examining temporal and spatial changes in the numbers of monarchs arriving to southwest Michigan as well as the incidence and prevalence of the neogregarine monarch parasite *Ophryocystis elektroscirrha*. Throughout the summer we sampled adult monarchs as well as their subsequent eggs and larvae to better understand patterns of synchrony among successive generations. We found trends to suggest that the population is initially highly synchronized and behaves predictably during times of reproductive effort. Our data show that the weights of the adult monarchs vary significantly with the age of the butterfly and also that weight and age vary significantly through time. We also looked at how various degrees of infection of *O. elektroscirrha* and the proportion of butterflies infected varies significantly through time. Our data suggest that monarch arrivals to Michigan are synchronized and predictable as indicated by the weekly fluctuations in egg and larvae densities per milkweed hostplant ramet, the sudden incidence of young and healthy adult monarchs at specific points in time, and the changing proportions of adult monarchs infected to varying levels with *O. elektroscirrha* during key life-cycle events.

Introduction

The monarch butterfly, *Danaus plexippus*, is an iconic example of insect migration by virtue of its predictable, long-distance annual migration in North America between over-wintering locations in Mexico and breeding habitat distributed across the USA and southern Canada east of the Rocky Mountains (Urquhart, 1960; Johnson, 1969; Calvert and Brower, 1986; Malcolm, 1987; Brower and Malcolm, 1991; Brower, 1995, 1996).

From early wing-tagging studies by Nora and Fred Urquhart in Canada (Urquhart, 1960; Urquhart and Urquhart, 1976, 1978) and later studies coordinated by Orley Taylor of Monarch Watch at the University of Kansas (http://www.monarchwatch.org/index.html) (Hobson *et al.*, 1999) we know that adult monarchs fly each autumn to twelve locations in oyamel fir forests above 3,000 m altitude in the Sierra Transvolcanica mountains of central Mexico, west of Mexico City (Calvert and Brower, 1986; Brower, 1985, 1995, 1996). There the butterflies remain for up to five months overwintering in tightly aggregated clusters until their return migration north in spring at the end of March. We also know that the same butterflies that left the USA and southern Canada in the autumn and spent the winter in Mexico, return to the southern USA where they mate, lay eggs and die (Cockrell *et al.*, 1993; Malcolm *et al.*, 1993). The offspring of these migrants from Mexico then feed on southern hostplant species and upon emergence as adults continue the migration north to recolonize the full spatial extent of their milkweed hostplants across North America, east of the Rocky Mountains (http://www.learner.org/jnorth/monarch/index.html) (Malcolm *et al.*, 1993; Howard and Davis, 2009).

In addition to their spectacular annual migration in North America, monarch butterflies are well known for their specialized larval feeding on milkweeds in the genus *Asclepias* and their ability to sequester toxic steroids known as cardenolides for use in defense against natural enemies such as birds (Brower and Brower, 1964; Alonso-Mejia and Brower, 1994; Malcolm, 1995). Interestingly, each *Asclepias* hostplant species generates a different pattern of sequestered cardenolides in adult monarchs and Malcolm *et al.* (1993) used these chemical "fingerprints" as indicators of migratory strategy in spring migrants. Malcolm *et al.*, found that monarchs migrate using a "successive brood" strategy (Figure 1). In "successive brood" migration the migrating spring butterflies would reach the southern USA from Mexico, mate, lay their eggs on southern *Asclepias* species (Figure 1) and die, leaving their offspring to continue the migration north. Evidence from both cardenolide fingerprints (Malcolm *et al.,* 1993) and isotopic signatures (Wassenaar and Hobson, 1998; Hobson *et al.,* 1999) show that spring migration does occur via successive broods.

While much is known about migration, mating behavior and physiology, hostplant use, and the operation of defense against natural enemies in monarch butterflies, we still know little about the dominant phase of their life history when their annual populations increase during the summer. Michigan is in the center of the extensive distribution of the common milkweed, *Asclepias syriaca*, which is the most important hostplant for monarchs (Malcolm *et al.,* 1989) out of the 108 species of *Asclepias* milkweeds described from North America by Woodson (1954). It is in their northern range that monarchs generate up to three successive generations (Borkin, 1982; Malcolm *et al.,* 1987) to establish the large numbers of butterflies that fly to Mexico each autumn. This annual cycling of butterflies brings up many questions about population dynamics and the predictability of changes in behavior as well as physiology. It is known that the entire

population of monarch butterflies migrate, mate, produce offspring, and over winter during roughly the same time periods each year that appear to be closely synchronized with the spring and autumn equinoxes, however, what is not known is how "synchronous" the spread of successive generations in space and time might be. Based on the knowledge we have about the monarch migration and life history, we would expect the synchrony of broods to be higher at the start of the breeding season as monarchs begin to arrive from the south and then become less and less defined as the season progresses due to the over-lapping of successive broods. We would also expect synchrony to be re-established suddenly at the autumn equinox in September when monarchs enter reproductive diapause (Herman, 1985) and begin migrating south for the overwintering sites in Mexico.

In addition to characteristic patterns of host plant use facilitated by migration, monarch butterflies are also subject to attack by a neogregarine protist parasite, *Ophryocystis elektroscrirrha,* and both incidence and prevalence of the parasite vary through the annual cycle of monarchs (Altizer *et al.,* 2000) and also with host plant species (De Roode *et al.,* 2008). This danaid-specific pathogen is well adapted to take advantage of specific events during the life cycle of the monarch butterfly. *O. elektroscrirrha* parasites persist in the gut of developing monarch larvae and reproduce until the adult monarch ecloses. Heavily infected adults emerge from their chrysalis covered with *O. elektroscrirrha* spores (McLaughlin and Myers, 1970) and sometimes this parasite load can disrupt the development of the emerging adult, resulting in deformation or death (McLaughlin and Myers, 1970; Leong *et al.,* 1997). Surviving monarchs are carriers of the parasite and can transmit the infection horizontally to other adult monarchs during mating and vertically to eggs laid by infected females (Altizer *et al.,* 2000; De Roode *et al.,* 2008). Because *O. elektroscrirrha* spores are unable to reproduce on fully formed adult

butterflies, transmission from female to egg is the most common vehicle for infection (Altizer *et al.,* 2000). Spores deposited on or near eggs are consumed by the emerging larva during feeding and allow the parasite to enter the gut; thus completing the cycle. Although much is known about *Ophryocystis elektroscrirrha,* little is known about possible changes in the incidence or prevalence of this parasite in relation to increasing density of monarchs across the three generations that characterize their life history in the Great Lakes region (Malcolm *et al.,* 1987).

It has been proposed that the long-distance migration of monarch butterflies, as well as other migratory species, can affect the prevalence within a host population of a pathogen or parasite such as *O. elektroscirrha* (Bartel *et al.,* 2011). Two migratory mechanisms; "migratory escape" (Loehle, 1995) and "migratory culling" (Bradley and Altizer 2005) are proposed as explanations of this phenomenon. The "migratory escape" mechanism predicts that parasite densities and rate of transmission will increase with time spent in a habitat and migration provides a mechanism for spatial escape from areas with large parasite densities. "Migratory culling" predicts that highly infected migrants will die during the strenuous act of long-distance migration resulting from the negative effects of the parasite such as reductions in body size (De Roode *et al.,* 2007) and dispersal ability (Bradley and Altizer, 2005) thus allowing migrants to escape the parasites spatially.

We investigated the changing nature of spatial and temporal synchrony of monarch generations through the summer at similar latitudes in southwest Michigan as well as the relationship between generation synchrony and both monarch density and the incidence of a parasitic disease by testing the following hypotheses: Null (1), adult monarch arrivals to Michigan and their occurrence throughout the summer are random. Alternatively (1a), adult monarch arrivals to Michigan are highly synchronized and predictable in May and June, losing

synchrony during the summer until finally restoring synchrony with the approaching autumnal equinox in Michigan. Null (2), the incidence of spores of *O. elektroscirrha* on monarch adults in the field is random and density independent and does not change with time. Alternatively (2a), *O. elektroscirrha* spores on monarch adult abdomens are positively density dependent and increase with time through the summer. Or (2b), that *O. elektroscirrha* spore densities vary predictably through the summer in relation to the synchrony in the monarch life cycles.

Methods

Field

Adults and eggs of the monarch butterfly, *Danaus plexippus*, were collected from spring arrival in late May until their departure in mid-September 2011, with an emphasis on eight locations across southwest Michigan (Figure 2). The locations varied in habitat and included the common milkweed species *Asclepias syriaca*, *A. incarnata*, and *A. tuberosa*. Milkweed genets were identified at each location and mapped with a Thales Navigation MobileMapper™ GPS/GIS receiver. Genet mapping was performed by walking the perimeter of each genet to determine the boundaries and area of each using the "area" function of the MobileMapper™. Ramets within the mapped genets were then surveyed for monarch eggs and larvae on a weekly basis while searching for adult monarchs. For a few select genets, each individual ramet was uniquely tagged and measured bi-weekly for height and the number of leaves, flower inflorescences, and seedpods present. Locations of eggs and larvae were marked with a waypoint using a Garmin eTrex Legend HCx GPS unit along with the date, time, location and host plant species. Adult monarchs were also collected during this time with records of time, date, location, behavior prior to capture, and GPS waypoint information. All eggs and adults captured were placed in glassine

envelopes and frozen at -20 ^{o}C in sealed plastic bags to prevent desiccation until laboratory processing at Western Michigan University.

Laboratory

In the laboratory, adult monarchs were removed from the freezer and immediately weighed on a Mettler balance (accuracy of 0.01 mg) to determine the wet weight of each individual butterfly. The wings were carefully removed from the thorax and digital photographs were taken of the wings and abdomen in both the dorsal and ventral orientation to further analyze color and wing wear as indicators of butterfly age. A small ruler was included in each photograph for scale. Once photographed, the right forewing length was measured from base to tip in a straight line and a wing bore 5 mm in diameter was cut from the discal cell of the right hind wing using a cork borer. The wing bore was mounted on a labeled microscope slide for a digital measure of scale loss and a second measure of wing wear. Wing wear was then measured qualitatively using the photographs on a scale from 0 to 5; 0 showing no evidence of scale loss and 5 showing very high scale loss. Each monarch was sampled for the neogregarine parasite, *Ophryocystis elektroscirrha*, by wrapping the ventral surface of the abdomen with a disk (1cm^2) of clear Scotch^{TM} tape to remove a thin layer of scales with any attached spores. The tape disk was then mounted on the microscope slide next to the wing bore from the same butterfly. A second microscope slide was placed on top of the samples and securely taped to prevent the sample from moving. The monarchs were returned to the glassine envelopes in the freezer ready for subsequent dissection, freeze drying and cardenolide analysis. Each slide was examined for *Ophryocystis elektroscirrha* using a Nikon™ Microphot microscope set at 100x magnification. The 1cm^2 disk of tape was scanned systematically so as to avoid counting spores twice and the

total spore count was recorded for each sample. Highly infected samples of 1,000 spores or more were recorded as such and further sub-sampling is needed to obtain a more accurate estimate of the total parasite load.

Statistical Analysis

Statistical analysis was performed with IBM™ SPSS software. Correlation, independent t-tests and ANOVA were utilized in the analysis. ESRI's Arc Map 10.0 was used to visualize spatial and temporal trends in the data. The data obtained using the Thales Navigation MobileMapper™ GPS/GIS receiver and the Garmin eTrex Legend HCx GPS unit were imported into ESRI's ArcGIS 10.0 for analysis in the Western Michigan University GIS lab.

Results

Egg and larval densities per ramet per week are shown in Figure 3 with an initial peak in egg and larval densities during the first week of sampling (June 1) and a decrease during the following week (June 8). Another increase in egg and larval densities occurred in the third week (June 15) with another decrease during the fourth week (June 22) and then a steady rise in egg and larval densities into mid July and our 8th week of observations. We believe that these three peaks in egg and larva density per ramet represent three subsequent generations of monarchs produced by the first arrivals to southwest Michigan in May in addition to eggs laid by later arrivals from the south in June. The disappearace of eggs and larva between the peaks is evidence for generations that are synchronized by monarch migratory behavior and each peak is a reflection of offspring generated by butterflies that initially flew to Michigan and then are the offspring of these migrants. The two-week period between the first two peaks in June may

actually be longer because the first arrivals in May contributed to the first peak and provided the butterflies with sufficient day-degree accumulation to complete a generation.

When fresh weight of adult butterflies was plotted against wing wear as a measure of age (Figure 4) we found that weight (g) decreased significantly as butterflies aged and wing wear increased ($F = 14.34$; $P = 0.001$). We then separated these variables and looked at each individually against time. Weight (g) against time (weeks) shows that weight did not vary significantly among weeks of sampling (Figure 5, ANOVA $F = 0.98$; $P = 0.45$), although at week 5 (June 29 to July 5) there was an increase in the average weight of sampled adult monarchs. In contrast, we did find significant variation in wing wear with time with a decrease in wing wear at week 5, that suggests the emergence of a new generation of adults (Figure 6, ANOVA $F = 2.74$; $P = 0.012$). Together these points indicate that at week 5 there were more adults present with high average weight and low average wing wear suggesting that new and healthy butterflies were being produced at that time.

The prevalence of *O. elektroscirrha* on adult monarchs shows varying degrees of infection against time (Figure 7). The degree to which the adults were infected varied significantly among weeks (ANOVA $F = 2.65$; $P = 0.014$). There was a peak in infection level at week 5 at the same time as butterflies with low wing wear (Figure 6) and a trend towards heavier adults (Figure 5). Thus newly emerged adults at the end of June had the highest infection levels. These infection levels are explored further in Figure 8 where three infection intensities are plotted against time (no spores detected, low spore numbers (1-99), or high spore numbers (100+ spores)). During the first three weeks of sampling there was a sharp decrease in the number of monarchs without any signs of infection (0 spores) and a steady increase in monarchs exhibiting low levels of infection (1-99 spores). This trend continues until at the fourth week of sampling

when all of the monarchs caught had low levels of infection of *O. elektroscirrha* (Figure 8). This is most likely due to an increase in the frequency of direct contact between mating adults during this time period. We then see peaks at weeks 5 and 7 in the proportion of adults that are highly infected (100+ spores). This coincides with the timeframe in which we expect the first brood of monarchs to eclose that were produced from the spring migrants.

Discussion

Our results indicate that adult monarch arrival to Michigan is not random, but highly synchronized, and that the incidence and prevalence of the protozoan parasite, *Ophryocystis elektroscirrha,* also follows this synchrony. Egg and larval densities were highest when we first started our observations in June (Figure 3) and then decreased to June 8, followed by a peak at June 15, another decline and then a steady rise through July to a peak on July 20. This timing allows for the maturation process from egg to adult that takes approximately 23-32 days to complete (Zalucki, 1981, 1982; Cockrell *et al.*, 1993). Using this developmental time, based on day-degree accumulation, any eggs laid by early arriving adults during the week of June 1, 2011 would reach adulthood between June 23 and July 8, 2011. Eggs laid earlier could be responsible for the adults that generated the peak of eggs and larvae on June 15 (Figure 3). Figure 3 also shows that egg and larval densities during the time period where we would expect the adults to eclose are very low to non-existent. This indicates that the initial cohort of eggs laid in early June matured to adulthood synchronously during roughly the same time period.

Further evidence for synchrony is suggested when we analyze the fresh weight of the adult monarchs in relation to their wing wear. For example, there is a strong negative relationship between the weight of the captured adults and the qualitative degree of wing wear

observed (Figure 4). Using wing wear as a measure of age, this shows that as the age of the butterfly increases, the total weight decreases significantly. Our data also indicate that at week 5, from June 29 to July 5, there was an increase in newly emerged, heavier adult butterflies (Figure 5) with almost no wing wear (Figure 6). The simplest interpretation of these data is that these are the first generation of adult monarchs to be produced in Michigan from the first cohort of eggs and larvae that emerge as adults and supports the hypothesis that monarch voltinism is synchronized.

Not surprisingly, the incidence and prevalence of the neogregarine parasite *Ophryocystis elektroscirrha* is also synchronized with their hosts. There were significantly higher average infections in adult monarchs during the fifth week of sampling (Figure 7) which coincides with emergence of new adults. The simplest way to explain this observation is that adult monarchs tend to have the highest density of *O. elektroscirrha* spores immediately following eclosion (McLaughlin and Myers 1970). This relationship demonstrates vertical transmission between infected females to their subsequent eggs because monarch adults with greater than 100 spores are thought to have acquired the parasite during the larval feeding stage (Altizer 2000). We also see an interesting trend when considering horizontal transmission of *O. elektroscirrha* among the adults during this time. This period of time represents the arrival of the adult monarchs to Michigan to begin the summer mating season, thus we would expect direct contact between adults to be more frequent as the population density increases with new arrivals. In figure 8 we see that from early June to early July there is a decreasing trend in the proportion of butterflies that show no infection of *O. elektroscirrha* and an increasing trend in the proportion with low infection (1-99 spores). So much so that by late June all butterflies sampled were infected with

low to high levels of *O. elektroscirrha.* This trend supports the possibility of horizontal transmission among adult monarchs during mating (Altizer 2000).

In summary, our data show that monarch arrivals to Michigan are synchronized and predictable as indicated by the fluctuations in egg and larval densities per larval hostplant ramet per week, the sudden incidence of young and healthy adult monarchs characterized by low average wing wear and high average weight, and the changing proportions of adult monarchs infected to varying degrees with *O. elektroscirrha* during key life cycle events. The prevalence of *Ophryocystis elektroscirrha* in relation to density dependence cannot be determined from our current data, however, the incidence of *O. elektroscirrha* appears to be highly synchronized with the life cycle of developing monarch larvae based on the trends shown in the figures presented. More lab analysis is required to complete the story of synchrony as we have only processed 120 of the close to 400 adult monarchs captured to date.

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Figures and Tables

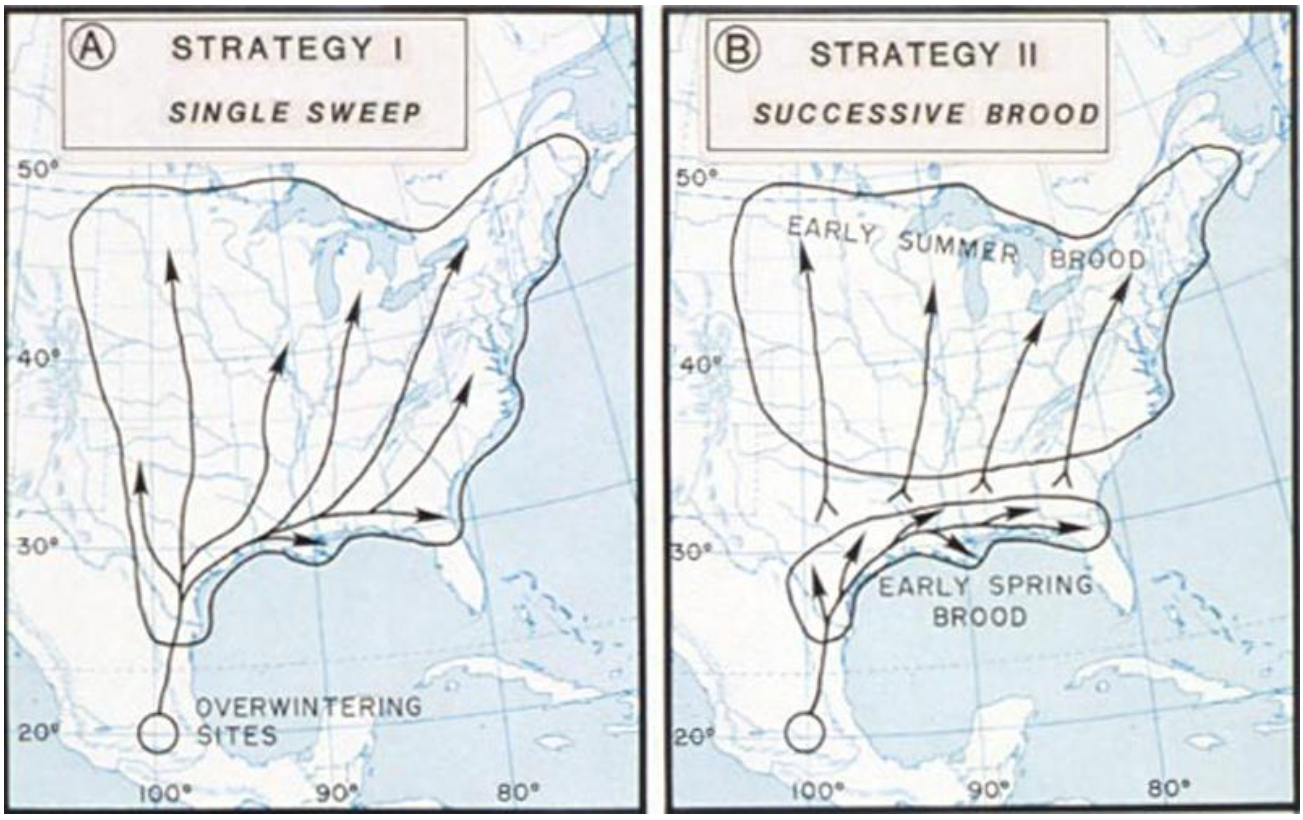

Figure 1. Hypothesized spring migration strategies for monarch butterflies that recolonize North American milkweed breeding habitat east of the Rocky Mountains (Malcolm *et al.,* 1993)

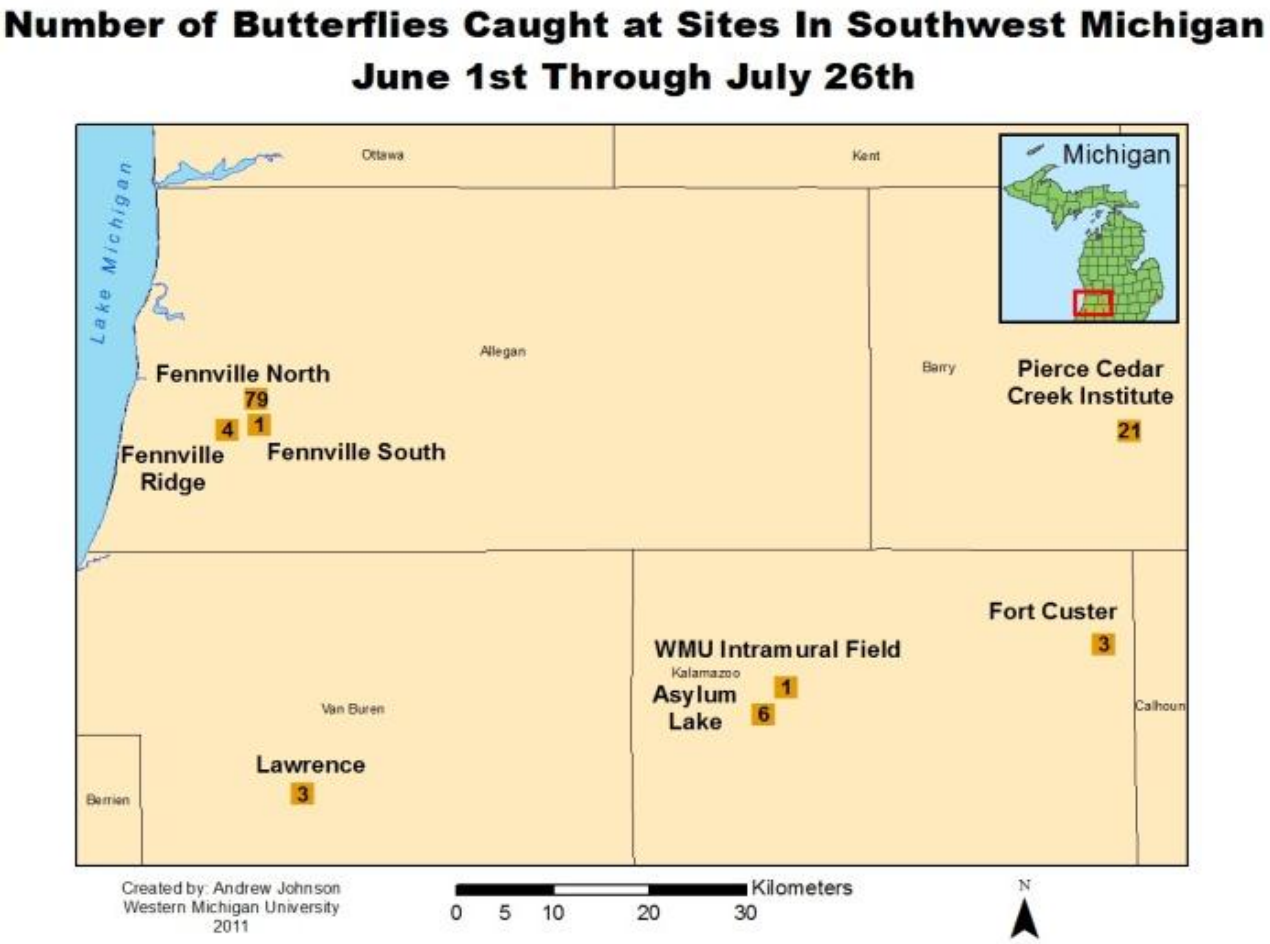

Figure 2. A map of the various sampling sites across southwest Michigan. The yellow boxes indicate the number of adult monarchs sampled at each site.

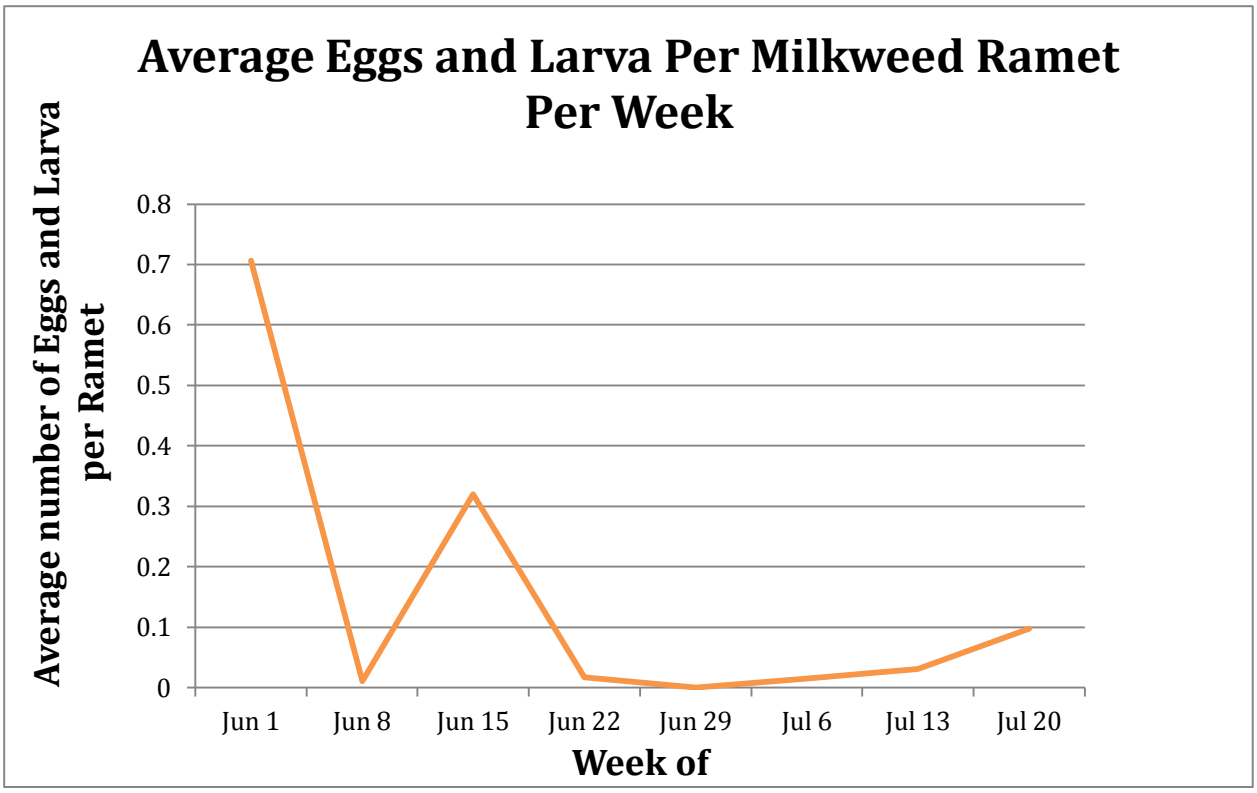

Figure 3. Average number of eggs and larva per milkweed ramet collected from June 1 to July 20, 2011.

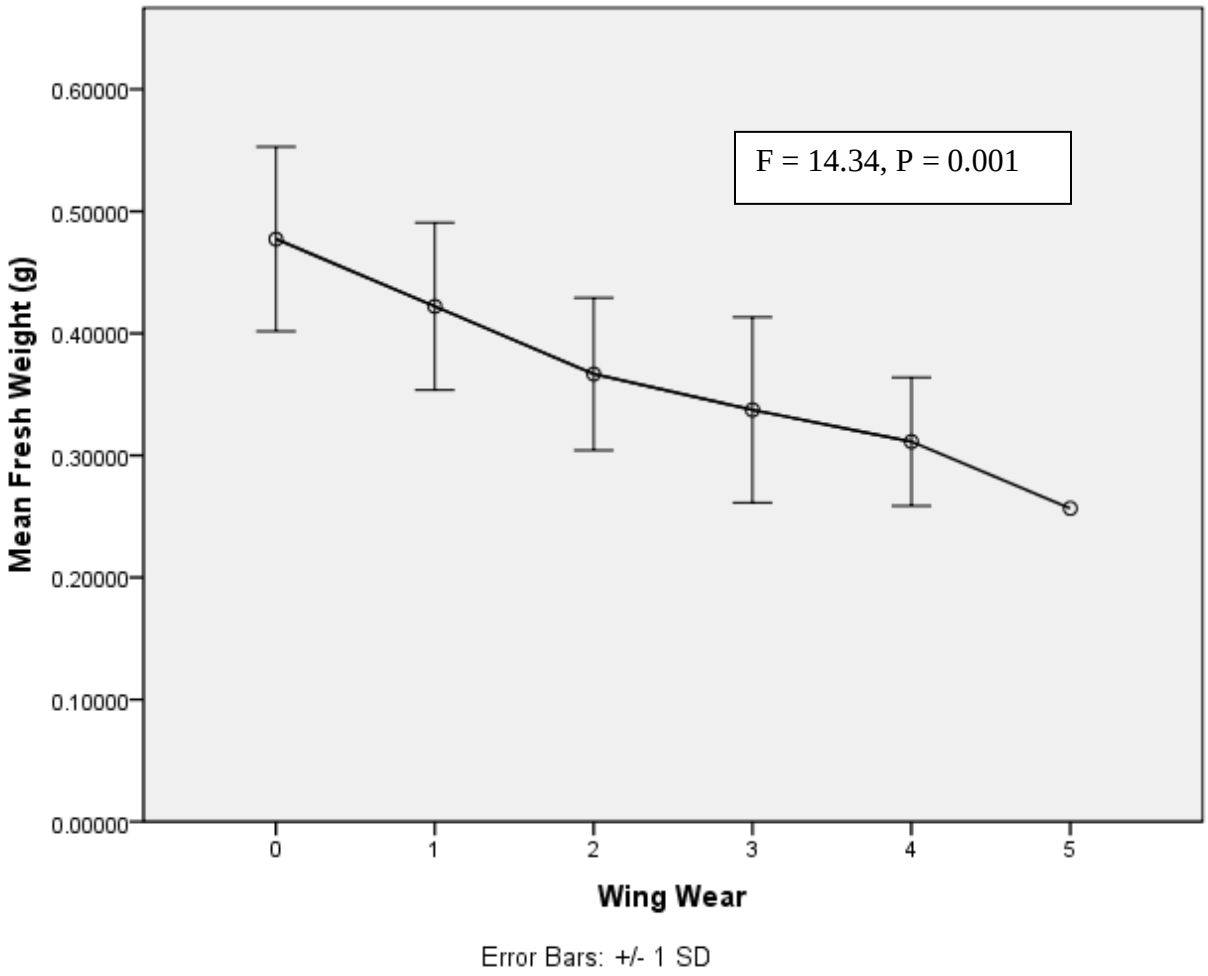

Figure 4. Mean adult monarch fresh weight (g $\pm$1 SD) against wing wear. Wing wear was measured on a qualitative scale of 0-5 (0 = no wing wear, to 5 = very high wing wear).

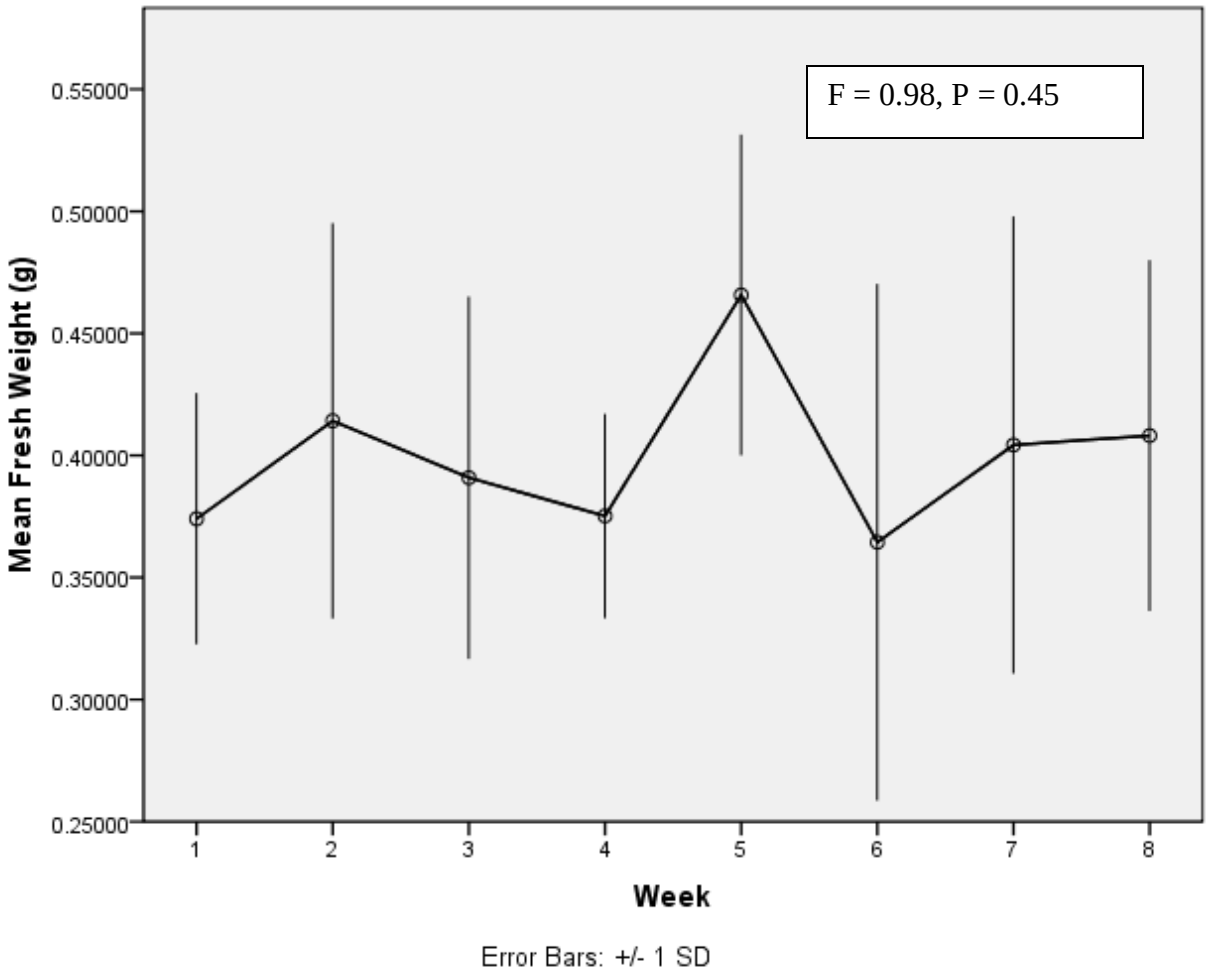

Figure 5. Mean adult monarch fresh weight (g ±1 SD) plotted against time (week).

Note:

Week	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Dates	Jun 1-7	Jun 8-14	Jun 15-21	Jun 22-28	Jun 29-Jul5	Jul 6-12	Jul 13-19	Jul 20-26

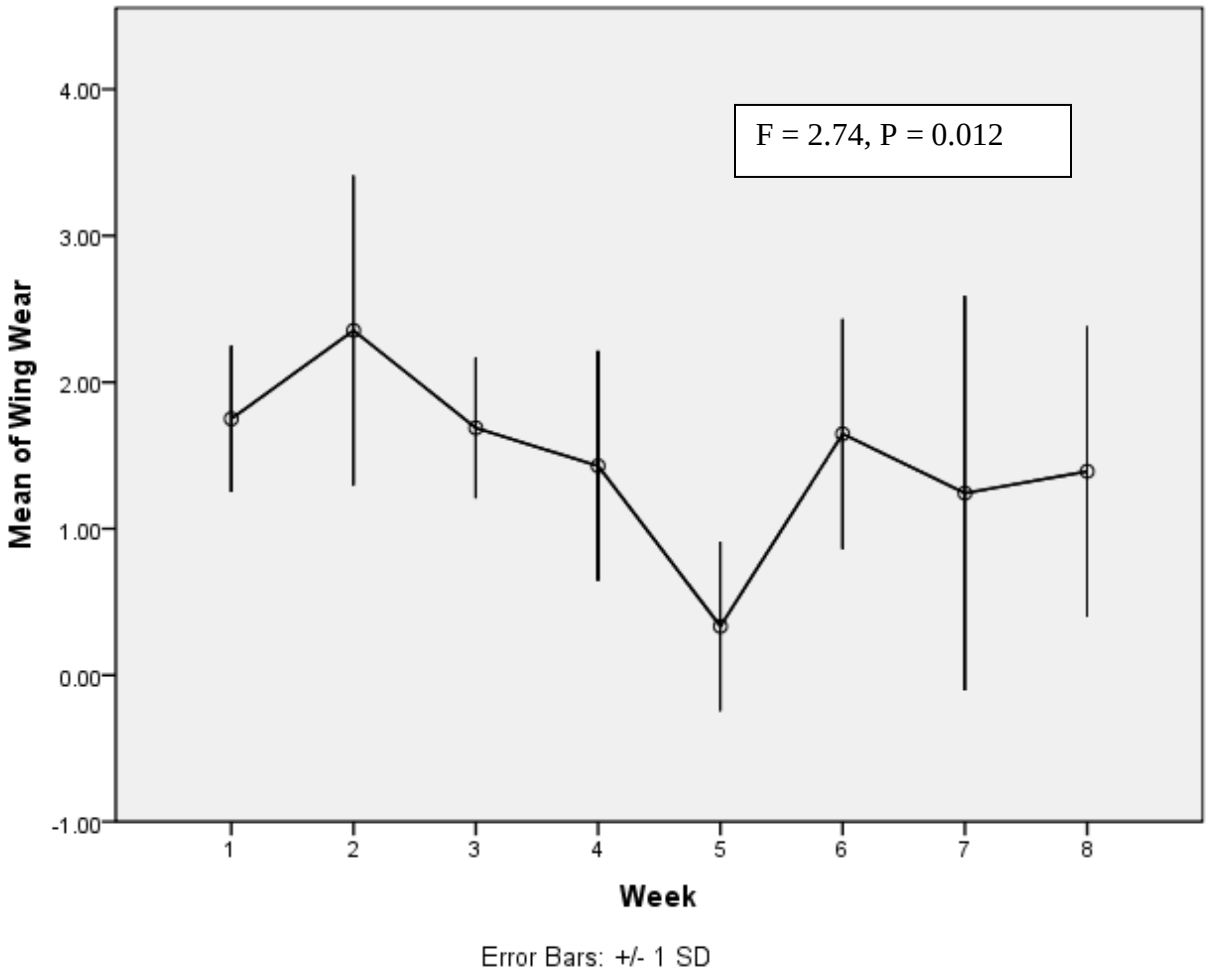

Figure 6. Mean adult monarch wing wear (±1 SD) plotted against time. Wing wear was measured on a qualitative scale of 0-5 (0 = no wing wear, to 5 = very high wing wear).

Note:

Week	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Dates	Jun 1-7	Jun 8-14	Jun 15-21	Jun 22-28	Jun 29-Jul5	Jul 6-12	Jul 13-19	Jul 20-26

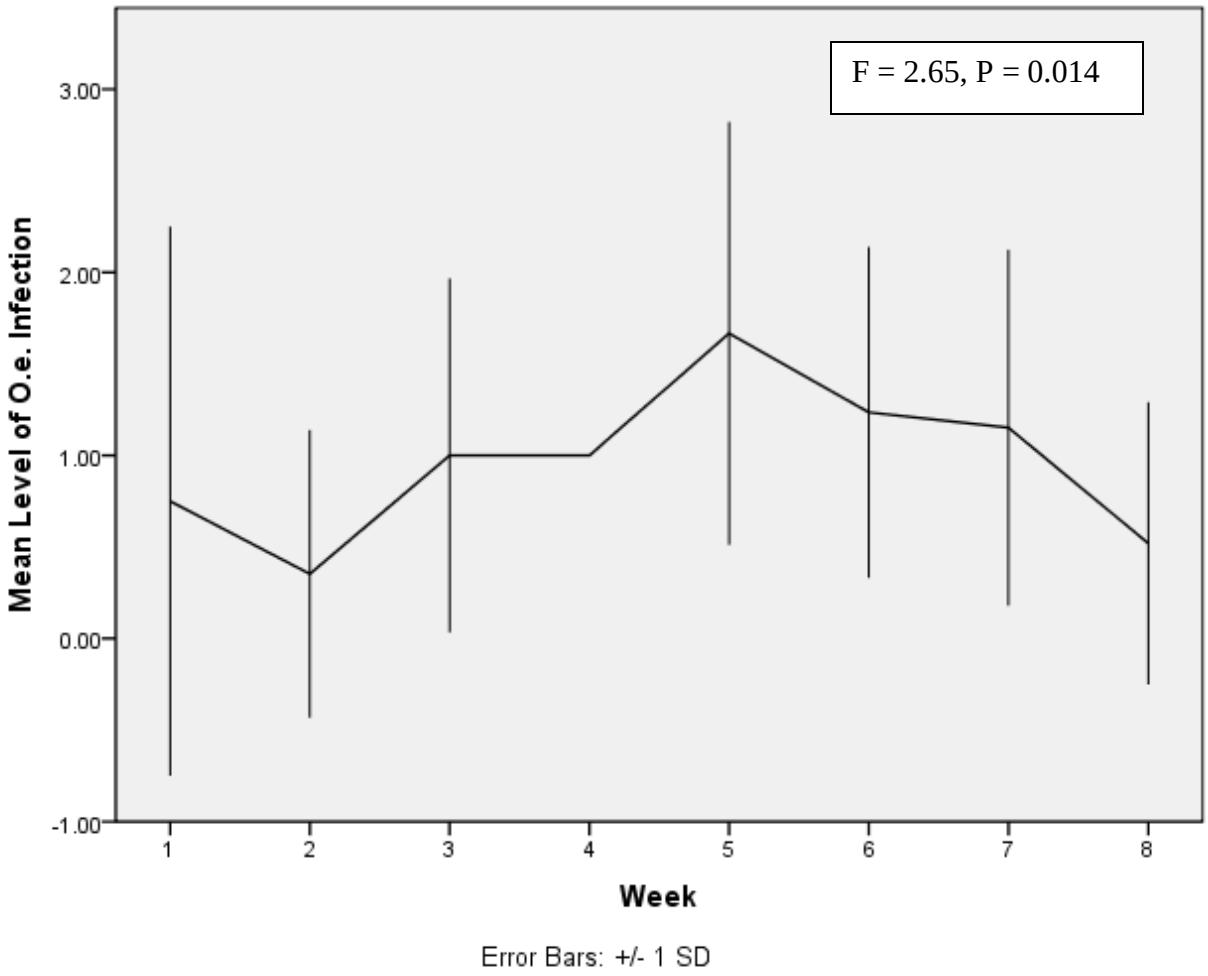

Figure 7. Mean level of infection (±1 SD) by the parasite *Ophryocystis elektroscirrha* plotted against time. Degrees of infection are described as 0 = no spores, 1 = 1-99 spores, 2 = 100-499 spores, and 3 = 500+ spores.

Note:

Week	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Dates	Jun 1-7	Jun 8-14	Jun 15-21	Jun 22-28	Jun 29-Jul5	Jul 6-12	Jul 13-19	Jul 20-26

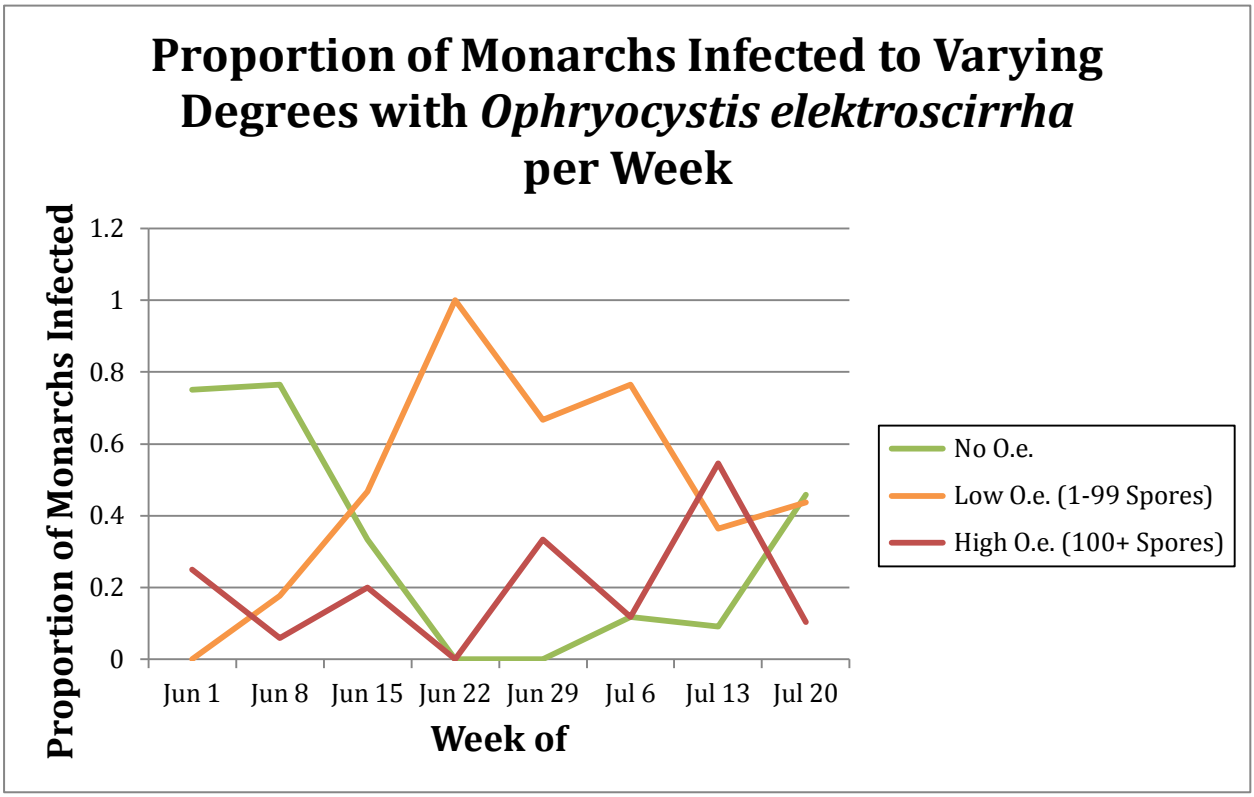

Figure 8. The proportion of adult monarchs infected to varying degrees with *O. elektroscirrha* against time (week).