Population dynamics and nectar preference of the Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae].

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This is to certify that I have examined this copy of a master's thesis by

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DEDICATION

To all (people and critters) who love wild places.

To people: may the Karner blue butterfly remind us of the miracles in nature and joy of being connected to the natural world.

To the Karner blues: keep flying

ABSTRACT

We researched population dynamics and suitable habitat of the endangered Karner blue butterfly, *Lycaeides melissa samuelis*. We analyzed six years of population and weather data from Ft. McCoy, Wisconsin and using autocorrelation functions we found two population dynamic patterns: a long term trend and an alternating generational cycle. To determine adult floral preference, we calculated a visitation rate for each visited flower species that incorporated the number of feeding visits and flower species abundance. Summer flight Karner blue butterflies had high visitation rates to the following five species: *Asclepias tuberosa*, *Amorpha canescens*, *Asclepias verticillata*, *Helianthus occidentalis,* and *Monarda punctata,* indicating preference. Spring flight males displayed a within-site floral species preference and three species may be preferred. We also found evidence that *Lupinus perennis*, the larval host plant, may not be an important nectar plant.

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CHAPTER 1

Introduction

Recovery plans for endangered insects require an understanding of population dynamics and suitable habitats. Population dynamics knowledge can help determine the population patterns (Turchin and Taylor 1992), population structure (Levins 1970), and limiting factors (Ehrlich et al. 1975; Ehrlich and Murphy 1987) of a species. Population patterns can illuminate trends and cycles (Turchin and Taylor 1992) that allow a timely response to a population decline in an endangered species. Knowledge of a population cycle provides a basis for predicting population changes, thus allowing managers to plan recovery activities appropriately. Population structure also has recovery implications, and many insect species are thought to live in a metapopulation structure (Levins 1970; Harrison et al. 1988) where several relatively discrete local populations are connected by dispersal. Within this metapopulation structure multiple theoretical spatial structures are possible and the specific spatial structure of a species will affect management and reserve design decisions (Harrison et al. 1988). Finally, if limiting factors are identified they may help direct and improve conservation efforts for an imperiled species (Ehrlich et al. 1975; Ehrlich and Murphy 1987).

Understanding the suitable habitat can greatly improve recovery efforts for an endangered species because it describes the minimal habitat requirements that are necessary for long-term survival (Busenberg and Velasco-Hernandez 1994). Suitable butterfly habitats need both adequate larval and adult resources, and while considerable information about larval resources is available and generally well-integrated into conservation plans for many imperiled butterfly species (Thomas 1984; New et al. 1995), a lack of information about the needs of adult butterflies limits the ability to include adult resource information in conservation planning (Baz 2002; Dennis et al. 2003; Tudor et al. 2004).

We studied the population dynamics and suitable habitat of the endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae]. *L. m. samuelis* is a bivoltine butterfly that lives in oak savanna and pine barren ecosystems in the northern Midwest and northeastern part of the United States (U.S. Fish and Wildlife Service 2003). The larvae feed exclusively on *Lupinus perennis* L. and adults feed from a variety of flowering plants (Haack 1993).

In chapter 2, we present an analysis of the population dynamics of the Karner blue butterfly. We analyzed long-term population and weather data at Ft. McCoy, Wisconsin. Utilizing autocorrelation and partial autocorrelation functions we found two population patterns: a long term trend and an alternating generational cycle. The long term trend observed at 7 of the 11 sites showed population decrease followed by an increase over six years. We also found density-dependent population growth and a positive relationship between early summer precipitation and survival over the concurrent spring-to-summer generation change. The ability of Karner blue butterfly populations to rebound after a decrease could be due to beneficial habitat characteristics. Therefore, Ft. McCoy may be a good location for further studies of Karner blue butterfly suitable habitat. The density-dependent summer generation growth and relationship between precipitation and summer growth may help future researchers discover factors limiting Karner blue butterfly population growth.

In chapter 3, we present an analysis of suitable adult habitat by studying adult floral preference. Butterfly floral preference is likely related to recognition of flower species (Swihart 1970; Bernard 1979; Scherer and Kolb 1987) and butterflies can learn to prefer floral species if their behavior is appropriately rewarded (Lewis and Lipani 1990). Many butterfly species display a naive preference for specific colors but can switch and prefer a color associated with the greatest likelihood of a nectar reward (Swihart 1970, 1971; Weiss 1995, 1997). We studied nectar preference of both male and female Karner blue butterflies in multiple locations during the summer flight. Both sexes preferred three species and two additional species were preferred by males only. In addition, the abundance of one preferred floral species greatly affected the mean visitation rate over time. Finally, we found that males had higher visitation rates to flowers than females overall. In the western part of the *L. m. samuelis* range, including Minnesota and Wisconsin, we recommend that managers concentrate effort on increasing preferred nectar species.

We also studied nectar preference in chapter 4 and we examined male nectar feeding behavior to determine if they display floral preferences during the spring flight. We investigated nectar preference by independently quantifying individual foraging behavior and floral species abundance within their natural habitat. Within sites, males displayed a floral species preference and three species may be preferred. Due to a lack of repetition of frequently visited floral species at multiple sites we were unable to determine if they preferred the same floral species at several sites. We also found evidence that flower morphology may inhibit the use of certain species. Further research is needed to identify specific preferred floral species for both sexes. Until preferred species are discovered we recommend habitat management to focus on increasing nectar plants listed in the Karner blue butterfly recovery plan with easily accessible nectaries.

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CHAPTER 2

Population dynamics of the Endangered Karner Blue Butterfly at Ft. McCoy, Wisconsin

Abstract

The endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae], has become a symbol of both endangered insects and ecosystems. To improve our understanding of the population dynamics of this species, we analyzed six years of population and weather data from eleven sites at Ft. McCoy, Wisconsin. Adult *L. m. samuelis* butterflies were surveyed approximately weekly at each site using a straight line transect method. Using autocorrelation and partial autocorrelation functions we found two population dynamic patterns: a long term trend and an alternating generational cycle. Several monitored *L. m. samuelis* sites showed a decline from the summer flight in 1997 to spring flight in 1999 and then a gradual increase from the summer flight in 1999 to the spring in 2003. We also found density-dependent growth and a positive relationship between early summer precipitation and survival over the concurrent spring-to-summer generation change. The ability of Karner blue butterfly numbers to rebound after a decrease could be due to beneficial habitat characteristics. Therefore, Ft. McCoy may be a good location for further studies of Karner blue butterfly suitable habitat. The density-dependent summer generation growth and relationship between precipitation and summer growth may help future researchers discover factors limiting Karner blue butterfly population growth. The methods used in this study could be applied to other imperiled butterfly species to learn more about their population dynamics.

Introduction

Insect conservation has become a distinct field recognizing that conservation efforts need to address the specific requirements of this important taxon (New et al. 1995). Historically, conservation efforts focused on large vertebrate or plant species; however these conservation efforts do not ensure the survival of insect species. On the contrary, in several situations insect species have gone extinct while the population sizes of surrounding plant and animal species remain stable (New et al. 1995). The number of endangered invertebrate species continues to increase (Murphy et al. 1990), and to reverse this trend insects need additional conservation attention. Butterflies are an important flagship group that can draw attention and resources to insect and ecosystem conservation (New et al. 1995).

The Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae], has become a symbol of both endangered insect species and the disappearing ecosystem it inhabits. This attractive, small, endangered butterfly (U.S. Fish and Wildlife Service 2003) is one of 25 species chosen by the National Wildlife Federation to celebrate the 25th anniversary of the Endangered Species Act of 1973 (www.nwf.org). The Karner blue butterfly is also a symbol of the disappearing oak savanna and pine barren ecosystems in the northeastern and northern Midwest parts of the United States. Savannas are unique ecosystems between the prairie and woodland, and are one of the most threatened United States ecosystems, with only 0.02% of their pre-settlement area remaining (Nuzzo 1986). A better understanding of Karner blue butterfly population dynamics can improve conservation efforts for this flagship species, and ecosystem-based conservation efforts will help preserve their unique ecosystem.

Long term research projects increase understanding of insect population dynamics by identifying population trends and discovering factors that limit population growth (Murphy and Weiss 1988). Measuring population size through time allows researchers to examine how the population changes as the environment changes; this is especially important due to the dramatic human effects on the environment (Ehrlich and Murphy

1987). Decades of research on the population dynamics of several checkerspot butterfly species, *Euphydryas* spp., in the western United States (Ehrlich et al. 1975; Ehrlich and Murphy 1987) have identified several factors that limit these butterfly populations. For example, topographic diversity is important because it provides a variety of microclimates that buffer the effects of environmental stochasticity on larval survival. *Euphydryas* spp. larvae living on north-facing slopes survival well in most years, but during wet years larvae on south-facing slopes survive better. Therefore, suitable habitat for the checkerspot butterfly includes areas in which host plants grow on both north and south facing slopes in close proximity (Ehrlich and Murphy 1987).

Information on population dynamics can be used to improve conservation efforts. For example, understanding the importance of larval habitat diversity has improved monitoring efforts (Murphy and Weiss 1988) and reserve designs for the checkerspot butterfly (Ehrlich and Murphy 1987). Multiple years of larval abundance monitoring data linked to microclimate data allows researchers to better understand how this relationship affects long-term persistence (Murphy and Weiss 1988). Understanding which habitat factors are important can also help identify optimal locations for reserves and can help determine if current unoccupied habitats are likely to support a butterfly population in the future (Murphy et al. 1990).

To increase our knowledge of *L. m. samuelis* population dynamics and ultimately improve conservation efforts of this species, we analyzed long term population data covering six years from eleven sites at Ft. McCoy, Wisconsin. Our objectives were to identify population patterns, quantify population changes, and identify potentially limiting factors for this species.

Methods

<u>Study organism and site</u>

The phenology of *L. m. samuelis* is linked to its exclusive larval food species, wild lupine, *Lupinus perennis* (Fabaceae), a perennial legume. *Lupinus perennis* starts growing in mid-April and peak flowering is typically in early June, followed by seed dispersal and senescence in mid-July (Dirig 1994). Phenology varies with weather and local microclimates, especially shade; *L. perennis* will grow in open and closed tree canopies, but does not flower under closed canopies (Boyonoski 1992). In late April/early May, *L. m. samuelis* larvae hatch and consume *L. perennis* leaves for approximately three weeks. Larvae pupate on vegetation near the soil and enclose after approximately 8 to 11 days (Andow et al. 1994). Larvae, especially older instars, and pupae are tended by ants (Savignano 1994). In late May/early June, spring flight adults emerge and feed on nectar from numerous flower species (Haack 1993). The mean adult life span is estimated by mark-release-recapture data to be four days, but researchers think they can live two to three weeks (U.S. Fish and Wildlife Service 2003). Females lay eggs individually on or near *L. perennis* stems and leaves. These eggs hatch in approximately four days and immature development of the summer generation follows the same pattern as the spring generation. The summer flight adults emerge in late July/early August and females lay eggs on *L. perennis* and on nearby vegetation, and these eggs over-winter and hatch the following spring (U.S. Fish and Wildlife Service 2003).

<u>Study area</u>

The 24,282 ha Department of Army military training base, Ft McCoy (44° 01'N, 90°41'W), is located in southwest Wisconsin. *L. m. samuelis* populations have been documented on 95% of approximately 15 km^2 of the mapped *L. perennis* at Ft. McCoy (Maxwell 1998). In a report discussing Karner blue butterfly status in Wisconsin, Bleser (1994) described Ft. McCoy as one of the most important *L. m. samuelis* habitats in the state. In 1996, Army biologists choose eleven *L. m. samuelis* sites for long term monitoring because they had relatively high adult butterfly densities in each region of the base (Figure 1). They defined the site boundaries by locating areas where both *L. perennis* and potential nectar plants were found in close proximity to areas where *L. m. samuelis* were observed. The *L. m. samuelis* sites are identified by a letter and a number based on the military training areas where they are located.

L. m. samuelis surveys

Adult *L. m. samuelis* butterflies were surveyed using a straight line transect method (Brown and Boyce 1996). After identifying site boundaries, Army biologists established several permanent transects at each site. They randomly selected the location for the first transect and placed additional permanent transects 20-40 m apart depending on the site size; at large sites transects were placed further apart, while those in small sites were placed closer together. Monitoring began in 1996 and spanned two generations per year, except 1996 and 1997, when only the larger summer generation was surveyed. In 1996, only two sites were surveyed but this increased to eleven sites by 1999. About 95% of the survey data through 2003 were collected by a single person following a standard protocol (Wilder 1999). Each site was surveyed approximately every seven days during the spring and summer adult flights.

Correlation functions and population patterns

Our first step was to summarize the multiple survey data points for each generation into a single population index for each site. Following the technique described in Manly (1976), we used trapezoidal integration to compute the area under the curve of population size versus time. This population index is influenced by the number of adults emerging in the site, the amount of time each adult lives and stays in the site, and adult immigration from other sites. When necessary, we extrapolated the survey data to zero population size at each end of the phenology curve using a seven day sampling interval. Because surveys occasionally started late or ended early compared with the phenology curve, we extrapolated the zero time to fourteen days, when the first or last adult count was above 60 individuals. We transformed the population index to the natural log to account for exponential population growth.

The population index we used is not the same as population size. Biologically it would be inaccurate to equate this index to the total number of butterflies emerging in the site because it is also affected by adult longevity, emigration and immigration. However, the population index may be related to the number of eggs laid in the site for the next generation. If sites have proportionally similar numbers of males and females, the index could be considered to be proportional to egg abundance in the next generation.

We then used autocorrelation (ACF) and partial autocorrelation functions (PACF) to analyze the population dynamics occurring at each site. Analysis of an ACF graph reveals population patterns that are more difficult or impossible to observe in the original time series (Turchin and Taylor 1992). The shape of the ACF can illustrate if the population is regulated and this helps determine an appropriate population model. The ACF shape of an unregulated population is consistent with a random walk, and the autocorrelation values (ACs) will slowly dampen to zero. The ACs of a regulated and stationary population will dampen quickly to zero, while the ACs of a population that is regulated with a trend will dampen slowly. Once the data with a trend are detrended, the ACs will dampen faster (Royama 1992).

The PACF graphs remove the correlation that occurs due to the lower order correlations in the ACF. A significant partial autocorrelation (PAC) value occurs when the line is outside the 95% confidence interval (Barlett bands) and reveals the minimum number of independent factors that need to be included in a population model (Turchin and Taylor 1992).

We constructed the ACF and PACF graphs in Excel following methods in Royama (1992). At several sites we observed that the ACF of the original data did not drop to zero quickly. Because this is evidence of a long term population trend, we detrended by differencing (Royama 1992) and calculated an ACF and PACF on the detrended data.

Between-generation population change

To explain Karner blue butterfly population patterns, we examined several aspects of the between-generation population change. We determined between-generation population change by calculating the difference between the log transformed population indexes of the summer flight and the spring flight for each site in each year (n = 55 site-year combinations). To create an overall summary statistic, we calculated the mean of these differences for all sites in the same year (n=5) and determined an overall mean and confidence intervals using these five values. We back-transformed the mean and confidence intervals to the arithmetic scale to compare the population growth to other studies.

We also used between-generation population change to test for density-dependent growth. To estimate initial spring population density at each site, we subtracted the logged total transect length from the logged spring population index. We then regressed between-generational population change against this initial population density measure. We also calculated between-generation population change from summer-to-spring (over-winter) and regressed this value against summer population densities using same methodology. For each data set, we used a general linear model in SAS to test for density-dependent growth (SAS Institute 1997).

In addition, we examined several weather variables as potential explanatory factors of the yearly variation of between-generation population change. We utilized weather data from the closest accessible NOAA weather station (43°56' N, 90°49' W), located in Sparta, WI, 20 km west of the Ft. McCoy headquarters (Sparta station, http://www.ncdc.noaa.gov). We used a general linear model in SAS (SAS Institute 1997) to regress between-generation population change and weather variables. For the spring-to-summer generation change, we included the following variables in the model: cumulative rainfall (1 June to 31 July), mean air temperature in June, and mean air temperature in July. For the summer-to-spring (over-wintering) generation change we examined: fall heat (number of days when daily maximum air temperature exceeded 29° C between 1 August and 30 November); winter snow (total snow fall amount between 1 November and 31 March, number of days with continuous snow cover at 2.54 cm or above); winter cold (number of days daily minimum air temperature was below -23° C, number of days daily minimum 24 hour temperature was below -12° C, number of days minimum 24 hour temperature was below -12° C without snow cover); and spring weather (mean temperature 1 May to 31 May, cumulative rainfall 15 April to 31 May).

The winter temperatures, -12° C and -23° C, were chosen to test the effect of moderate and extreme air temperatures.

Finally, to assess whether population patterns were affected by site size, we regressed the mean population index against approximate site area for each site. To calculate approximate site area, we multiplied the total transect length by the distance between each transect.

Results

Correlation functions and population patterns

The original data shown on three graph series in Figure 2 (population series, ACF, and PACF) illustrated two patterns: a long term trend and an alternating generational cycle. The original data in the population series and ACF graphs showed a U-shaped pattern indicating a long term trend for seven of the eleven sites: A5, B7, B16, B13-3, B8, E13-1, and D6-1 (Figure 2). For these sites, the ACF generally decreased for lags one to five, then increased between lag six through ten. Site E13-1 illustrated this pattern particularly well while other sites, similar to B8, still showed this U-shaped pattern but the curve was not as smooth. For the population series that showed the trend, the U-shape was also more difficult to discern but still noticeable. Generally the first generation graphed has the highest population index, and then the index dropped and reaches its lowest value in the middle of the times series. This is followed by an increase that reaches a higher value near the end of the time series. Because this U-shape was consistently evident at multiple sites, it suggests the presence of a long term trend with a half period length of approximately five generations. After the data were detrended, the U-shape pattern was removed and the detrended ACF graphs oscillated around and damped to zero (Figure 2).

The data also revealed an alternate generational pattern. At all 11 sites, in the detrended ACF graphs, lag one was a negative correlation, while lag two was positive, and this alternate pattern continued with most odd lags showing a negative correlation while even lags were positive. Past researchers have observed that generally the summer adult flight is larger than the spring flight (U.S. Fish and Wildlife Service 2003) and these observations correspond with the pattern in the detrended ACF graphs. Odd lags, which compared summer-to-spring and spring-to-summer flights, were negatively correlated, and even lags, which compared summer-to-summer and spring-to-spring flights, were positively correlated.

The PACF graphs also illustrate the dramatic difference between the original and detrended data. At lag one, the original series PACFs showed a mix of positive and negative PACs, while for the detrended data, all the sites illustrated a strong negative PAC value (Figure 2). Furthermore, site E13-2 showed a significant positive PAC at lag one but after detrending this changed to a negative correlation. In addition, within the original data no sites had significant negative PACs, but the detrended data showed four sites (C11, B8, D6-1, D9) with significant negative PACs at lag one. This means at least one independent factor affected population dynamics of lag one at these sites (Turchin and Taylor 1992).

The alternate generational pattern was partially masked in the original population series data by the long term trend. This long term trend also affected the strength of the ACs and PACs. When the original data were detrended, the alternate generational pattern was more noticeable in both the population series and the ACF and PACF graphs.

We examined approximate site size as a potential explanation for the variation between sites, but we did not notice any patterns. We did not find a correlation between approximate site size and the mean population index for each site ($R^2 = 0.0027$, $P > 0.05$, Figure 3). Also the four sites (A1-1, C11, D9, E13-2) that did not show the long term trend did not share similar characteristics of either site size or mean population index.

Between-generation population change

To quantify the alternate generational pattern illustrated by the ACF and PACF, we estimated the seasonal change from the spring to the summer generation. The mean generation change on the back-transformed arithmetic scale was 2.126 (95% C.I.: $0.0335 \leq x \leq 8.456$). This means that during an average year the population doubles from the spring to the summer flight, but there is high variation as the confidence interval spans from zero to an eight fold change. Furthermore, one of the five years, 2003, was outside of the 95% confidence interval.

Density-dependent growth

We found evidence of density-dependent growth during both the summer and over-winter generation. For the summer generation change we found overall evidence of consistent density-dependent growth (Table 1, Figure 4a). When examining year to year differences, we consistently found negative slopes and all slopes were significantly different than zero. Because the slope values varied each year, these results indicate that the strength of density dependence varies from one year to the next. In comparison, for the over-winter change we did not find consistent overall density-dependent growth but did find significant changes between years (Table 1, Figure 4b). Only one year, 1998-1999, showed evidence of significant negative density-dependent growth and all other years had no evidence of density-dependent growth because slope values were not significantly different than zero.

Weather factors

During the summer generation change (spring to summer), we found a significant positive relationship between survival and early summer rainfall (Table 2, Figure 5); as the amount of precipitation increased the population change from spring to summer also increased. During the over-winter generation (summer to spring), we found a surprising significant positive relationship between the population change and the number of days with extremely cold temperatures. Specifically we found a significant relationship between population change and both the number of days the minimum 24 hour temperature was below -12° C and the numbers of days the minimum 24 hour temperature was below -12° C without snow cover. These results were surprising because it implied that increased exposure to cold with or without snow cover increased egg survival. This relationship was strongly influenced by a single unusual year with a high number of cold days that also had positive population change. Therefore this result may be spurious and unlikely to predict future relationships. In addition, this result was inconsistent with other regression tests using cold and snow data that resulted in no relationship (Table 2).

Discussion

Correlation functions and population patterns

Populations of *L. m. samuelis* showed a long term trend at Ft. McCoy, declining from the summer flight in 1997 to spring flight in 1999 and then gradually increasing from the summer flight in 1999 to the spring in 2003. It is possible that this trend is part of a long term population cycle with a half period length of approximately five generations or two and a half years. We could be observing a part of a long term cycle or the trend may be a unique feature of this time period. It is unlikely this trend is due to specific management activities at Ft. McCoy because management has been site specific and there has not been a coordinated management effort similar for all sites during this time period (Appendix A, Wilder 1999). It would be beneficial to discover if this trend is a cycle and if it occurs on a larger spatial scale. If *L. m. samuelis* populations have a long term cycle it would provide a basis to predict population changes, thus allowing managers to schedule habitat management plans in relation to the population size. For example, a prescribed burn that is likely to increase the population in the long term but decrease the population in the short term could be planned when the *L. m. samuelis* population is high and avoided when populations are predictably low.

Determining population patterns and trends is important for endangered species because their survival can rely on a timely response to a population decline. The "masking" effect of the long term trend is especially concerning because it can make it difficult to identify a population decrease. For example, a population could be in decline due to habitat changes but the decrease may be difficult to observe if the population is on the upward swing of its population cycle. However, when the long term cycle begins to decrease and the population continues to decline the result will appear to be a sudden dramatic drop because the initial decline was masked by the population cycle. In addition, the annual population cycles of bivoltine species with dramatically different population sizes each generation can mask overall trends. Our analysis allowed a close inspection of population dynamics, and such techniques could be used in other systems to identify a declining population trend. If a declining trend is detected early, it might be possible to direct management efforts to appropriate habitats and reverse the trend.

This analysis also provides information regarding possible metapopulation dynamics of the Karner blue butterfly. An important precondition for a true metapopulation to improve population persistence is that the subpopulations fluctuate asynchronously (Levins 1970). The eleven subpopulations at Ft. McCoy appear to fluctuate in synchrony. This suggests that the Karner blue butterfly does not function as a true metapopulation, contrary to the suggestion of several researchers (Givnish et al. 1988), and may function as a patchy population or core-satellite populations at Ft. McCoy (Harrison et al. 1988).

Between generation population change

The alternate generational pattern corresponds to the influence of seasons. The summer generation change, June to July, is shorter and has less extreme weather than the over-winter generation change, August to May. These factors may partially explain why the summer generation generally has an increased population index relative to the spring generation. Previous researchers have suggested that population densities during the summer flight are often three to four times higher than the preceding spring flight, however in some years the summer flight is smaller than the spring flight (U.S. Fish and Wildlife Service 2003). Due to the high variability between years, the mean generation change can be misleading; therefore we advise emphasizing the high variability amongst years rather a single number describing the average change between the generations.

Density-dependent growth

Summer density-dependent growth may be caused by competition for high quality oviposition sites, nectar, or protection from natural enemies. Several researchers have found that *L. perennis* quality affects larval survival and have suggested that higher quality *L. perennis* is often found in partial or closed sub-habitats (Grundel et al. 1998; Maxwell 1998; Lane and Andow 2003). In high density years there may be an inadequate number of oviposition sites in partial and closed sub-habitats, resulting in more spring adults laying eggs on lower quality *L. perennis* and consequently lower survival rates. The summer density-dependent growth could also be a result of adults competing for a limited amount of nectar (Savanick, Chapter 4 this volume). If there is an inadequate amount of nectar, butterfly longevity may decrease, resulting in a lower population index for the summer generation. Finally, density-dependent growth may be due to a limited supply of tending ants. Researchers have found that larval survival rates increase with ant tending (Savignano 1994). If at relatively high larvae densities there are an inadequate number of tending ants, larval survival will decrease. Further research could focus on what specific factors are causing this summer density-dependent growth, allowing managers to reduce these limiting factors.

Weather factors

The significant relationship between rainfall in June and July and increased summer generation survival could be mitigated through the host plant, *L. perennis*. Plant quality and plant influenced microclimate are two probable mechanisms. Both Lane (1999) and Grundel et al. (1998) found that *L. m. samuelis* larval development time is slower on wilted *L. perennis* leaves than on leaves from well-watered plants. Because host plant quality likely increases with precipitation, this would result in higher larval survival as precipitation increases. The plant-influenced microclimate could also affect larval survival. For most plants, water vapor escapes through stomata as a plant transpires during the day (Kramer and Boyer 1995), creating a humid microclimate at the leaf surface compared to the surrounding air (Willmer 1989). To avoid dehydration, plants close stomata in response to both internal factors, for instance low leaf water status, and external factors, including light intensity, temperature and humidity (Kramer and Boyer 1995). Closed stomata during dry conditions decreases humidity at the leaf surface (Willmer 1989), and may decrease larval survival because lepidopteran larval survival is affected by relative humidity (Singh and Ashby 1985). Overall, the plant quality mechanism is likely to affect larvae every year, while the microclimate mechanism may only decrease larval survival in years with extremely dry summers. The microclimate mechanism may explain the steep decline that occurred during the 2003 summer generation change.

During the over-winter generation change, we did not find a significant relationship between *L. m. samuelis* population change and continuous snow cover or total snow amounts. Because the southern range boundary of *L. m. samuelis* populations corresponds with the line of 80 to 120 days of continuous snow cover, Dirig (1994) hypothesized that the range of *L. m. samuelis* was associated with this snowline. He thought the insulating properties of snow cover might be important for egg survival during extremely cold winter temperatures. However, we did not find a relationship between snow cover and subsequent population change. In addition, all five years of this study had fewer than 80 days of continuous cover with a range between 17 and 71 days. Furthermore, the year with the highest over-winter survival, 2002-2003, had one of the shortest periods of continuous snow cover, only 19 days. These data show that duration or depth of snow cover does not help predict the over-winter generation change, however the *L. m. samuelis* geographic range may still be related to the 80 days of continuous snow cover through a different mechanism.

Phenological overlap of *L. perennis* and *L. m. samuelis* in the spring is likely affected by spring weather. Weiss et al. (1993) showed that coordination of resources with the insect stage requiring the resource is an ongoing struggle for insects living in varied habitats. A spring weather variable may indicate how well Karner blue butterfly egg hatch and *L. perennis* growth is synchronized each year. We found that May mean temperature had a negative correlation with population change and was close to significant at the 0.05 level (Table 2). Mean temperature in May could have a non-liner relationship where both colder and warmer mean temperatures decrease survival and mid-temperatures have a positive impact on survival. It would also be informative to measure temperature at a more representative location, such as lupine leaf surface temperature, rather than air temperature.

Over-winter weather factors likely have a significant effect on population change. However, discovering which weather factors are most important may be difficult due to the long time period over which this generation is exposed to the elements. Over a nine month time period, multiple weather variables, all the possible interactions between variables, and influence of a specific location may all influence subsequent survival. Collecting microclimate weather data at several Karner blue butterfly sites may help reduce the extraneous variability and help researchers identify key influential weather factors.

Habitat Heterogeneity

Numerous researchers have argued that habitat heterogeneity is important for long term insect population persistence through variable weather (Ehrlich and Murphy 1987; Weiss et al. 1988; Kindvall 1995; Hanski 2003). In addition, several researchers have suggested that *L. m. samuelis* may live in a savanna ecosystem in order to survive unpredictable weather, because weather factors may be filtered differently by different sub-habitats (Grundel et al. 1998; Maxwell 1998; Lane and Andow 2003). The relationship between summer precipitation and larval survival suggests that greater precipitation increases larval survival. This weather effect could be mitigated through different sub-habitats. For example, shady closed canopy may be the best sub-habitat for larval survival if the summer is hot and dry. Maxwell (1998) found that a greater proportion of larvae were in shady habitats during an extremely dry year than more moderate years. However, the open canopy may be the best if the summer is cool because larvae may develop more quickly in a warmer sub-habitat. Because precipitation and other weather factors may affect sub-habitats differently, suitable habitat for long term persistence of the Karner blue butterfly may depend on diverse sub-habitats in close proximity.

In this study, we found overall stable *L. m. samuelis* populations at eleven sites at Ft. McCoy. This is a positive result for a species that has been declining over the last several decades. The ability of Karner blue butterfly numbers to rebound after a decrease could be due to beneficial Ft. McCoy habitat characteristics. Therefore, Ft. McCoy may be a good location for further studies of Karner blue butterfly suitable habitat. The density-dependent summer generation growth, and relationship between precipitation and summer growth may help future researchers discover factors limiting Karner blue butterfly population growth. Further research could confirm our proposed explanations and integrate our increased knowledge of Karner blue butterfly population dynamics into management plans to help increase the overall population density. Finally this study could be used as model to learn more about the population dynamic of other imperiled butterfly species using monitoring and weather data.

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Appendix A. Site-specific management and disturbances at Ft. McCoy Karner blue butterfly sites, 1997-2004

SITE A1-1

Oct. 2000: Young jack pine trees that were encroaching into the site opening were cut with chainsaws.

April 2001: Young jack pine trees cut in Oct. 2000 were burned.

SITE A5

September 2001: A large brush cutting machine was used to clear small trees and woody plants from approximately 50% of the site.

SITE B7-1

No management conducted.

SITE B8

March 1997: A large brush cutting machine was used to clear small trees and woody plants from a part the site.

Sept./Oct. 2001: A large brush cutting machine was used to clear small trees and woody plants from the same part of the site cleared in March.

SITE B13-3

November 2000: Trees and woody plants cut with chainsaws.

Jan-March 2001: Brush piles cut in Nov. 2000 were burned. Due to dry conditions the burn was larger than the brush piles and approximately 50% of the site burned.

Summer 2002: A spot application of herbicide was applied to spotted knapweed. 5% or less of site was sprayed and little to no lupine was sprayed.

Summer 2003: A spot application of herbicide was applied to spotted knapweed.

April 2004: A spot application of herbicide was applied to small trees and brush in 0.1 hectare area.

Summer 2004: Knapweed hand pulled within site.

September 2004: A spot application of herbicide was applied to small trees and brush in 0.7 hectare area.

SITE B16

April 2000: A prescribed burn occurred over approximately 80% of site.

SITE C11

February 2001: Woody plants cut and piled a small amount of brush in less than 5% of the site.

March 2003: Wildfire burned approximately 80% of site.

Summer 2004: Spotted knapweed hand-pulled from site edge.

SITE D6-1

November 2003: A large brush cutting machine was used to clear small trees and woody plants from approximately 0.9 hectare area.

SITE D9

November 2000: A large brush cutting machine was used to clear small trees and woody plants from approximately 50% of site.

SITE E13-1

October-November 2001: Small trees and woody plants cut and piled.

SITE E13-2

November 2003: A large brush cutting machine was used to clear small trees and woody plants in a 1.2 hectare area.

Table 1. Analysis of Variance of year and initial population density for each generation change

Generation change	Source	df	Type I SS	*F*	*P*
Summer					
	Year	4	34.96	22.44	**<0.0001**
	Density	1	32.19	82.64	**<0.0001**
	Density*Year	4	7.02	4.50	**0.0038**
	Error	45	17.53		
Year	**Slope estimate**		**Standard Error**	***T***	***P***
1999	-1.04		0.20	-5.16	**<0.0001**
2000	-0.91		0.12	-7.68	**<0.0001**
2001	-0.37		0.16	-2.31	**0.0254**
2002	-0.36		0.17	-2.17	**0.0355**
2003	-0.35		0.16	-2.25	**0.0294**

Generation change	Source	df	Type I SS	*F*	*P*
Over-winter					
	Year	4	68.98	19.06	**<0.0001**
	Density	1	1.03	1.14	0.2916
	Density*Year	4	14.35	3.97	**0.0077**
	Error	45	40.71		
Year	**Slope estimate**		**Standard Error**	***T***	***P***
1998-1999	-1.21		0.33	-3.73	**0.0005**
1999-2000	0.44		0.36	1.21	0.2339
2000-2001	0.69		0.57	1.21	0.2329
2001-2002	-0.13		0.33	-0.41	0.6869
2002-2003	0.06		0.32	0.18	0.8602

Table 2: Regression analysis of the population index change as a function of weather variables for the summer and over-winter generation change.

Summer weather variables	*F*	*P*
Rainfall, Ln, 6/1 - 7/31	19.17	**<0.001**
Air temperature, monthly mean, 6/1 - 6/30	2.21	0.1445
Air temperature, monthly mean, 7/1 -7/31	0.19	0.6632
Over-winter weather variables	*F*	*P*
Snow fall, Ln, 9/1 - 3/31	1.91	0.1744
Days of continuous snow cover, Ln, 9/1 - 3/31	1.35	0.2515
Days minimum 24 hour temperature < -23 C, Ln, 9/1 - 3/31	0.65	0.4245
Days minimum 24 hour temperature < -12 C, without snow cover, Ln, 9/1 - 3/31	9.37	**0.0038**
Days minimum 24 hour temperature < -12 C, Ln, 9/1 - 3/31	8.59	**0.0054**
Days maximum 24 hour temperature > 29 C, Ln, 8/1 - 9/30	2.24	0.1418
Air temperature, monthly mean, 5/1 - 5/31	4.03	0.0509
Rain, Ln, 4/15-5/31	0.57	0.4536

FIGURES

Figure 1	Map of *L. m. samuelis* sites in Ft. McCoy, Wisconsin,
Figure 2	Autocorrelation function and partial autocorrelation function and population index graphs of *L. m. samuelis*
Figure 3	Regression line of mean population indexes as a function of the approximate mean area (m) of each site. All values are log transformed. $R^2 = 0.0027$, $P > 0.05$
Figure 4	Regression lines of the population index generation change as a function of initial population density for each year and generation change. All values are log transformed. A) summer flight subtracted from previous spring flight and spring population density for each year. B) spring flight subtracted from previous summer flight and previous summer population density for each year.
Figure 5	Site specific regression lines of the population index generation change (summer flight subtracted from previous spring flight) as a function of rain (cm) in June and July for each site. All values are log transformed.

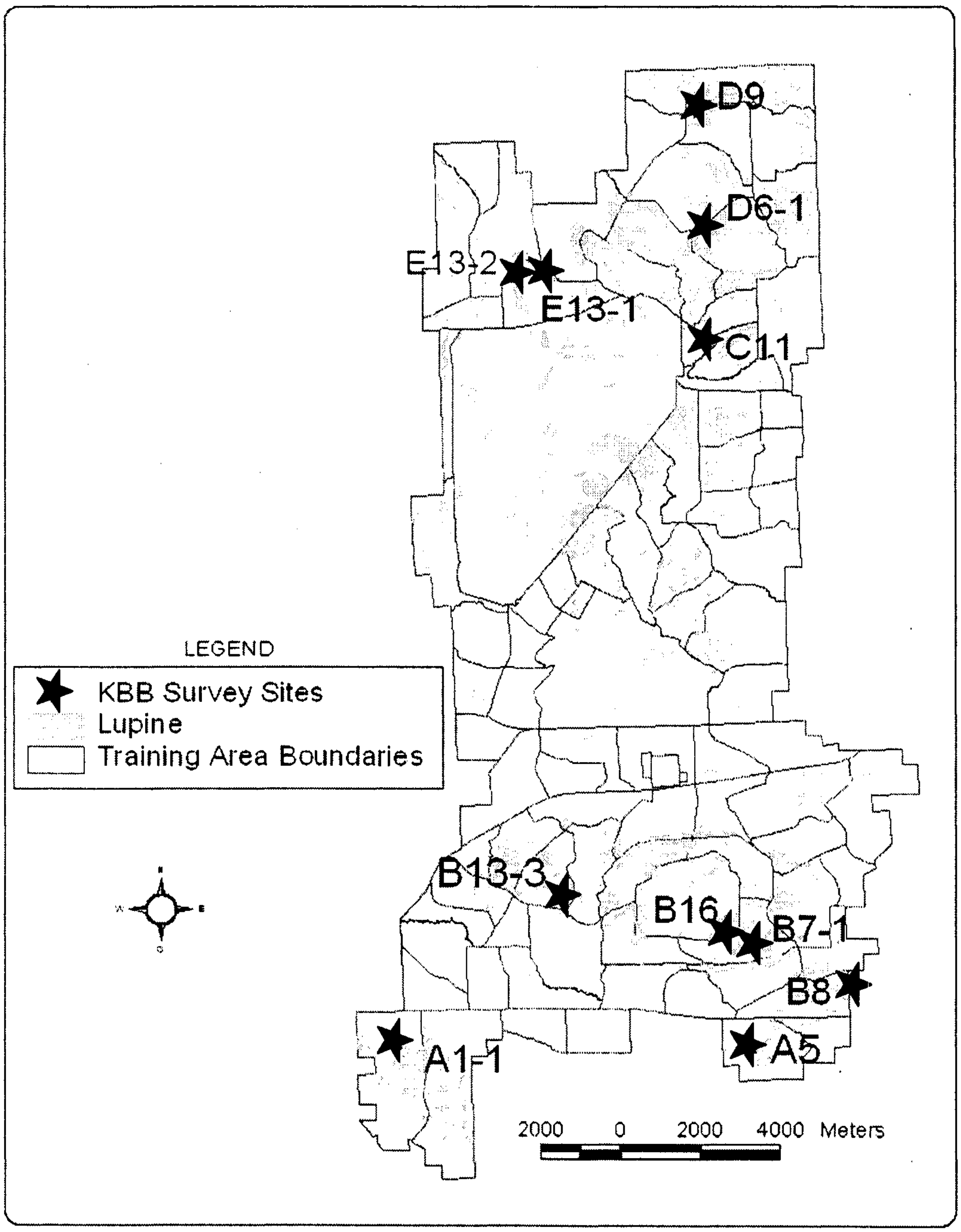

D9
D6-1
E13-2
E13-1
C11
LEGEND
KBB Survey Sites
Lupine
Training Area Boundaries
B13-3
B16
B7-1
B8
A1-1
A5
2000 0 2000 4000 Meters

Figure 1

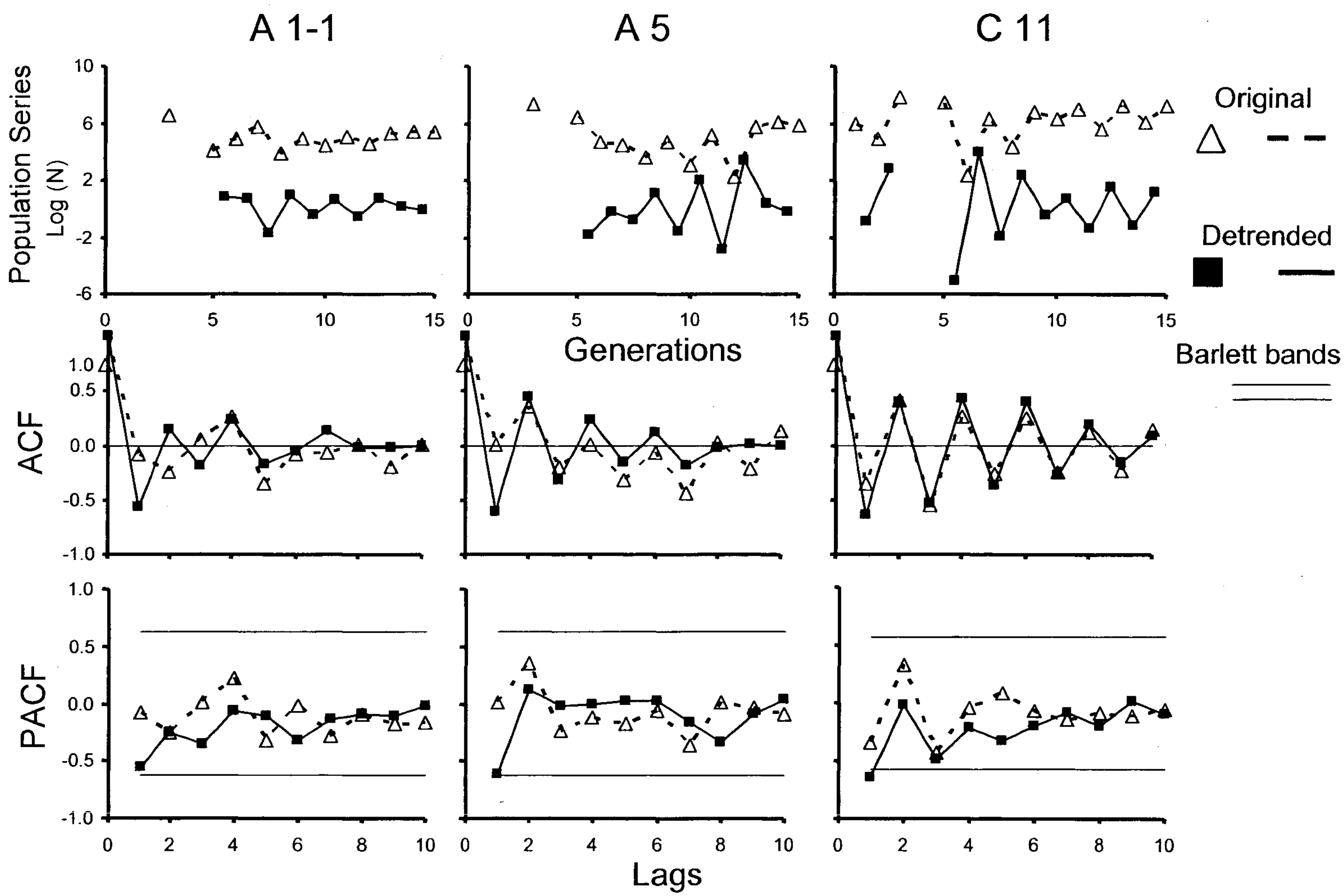
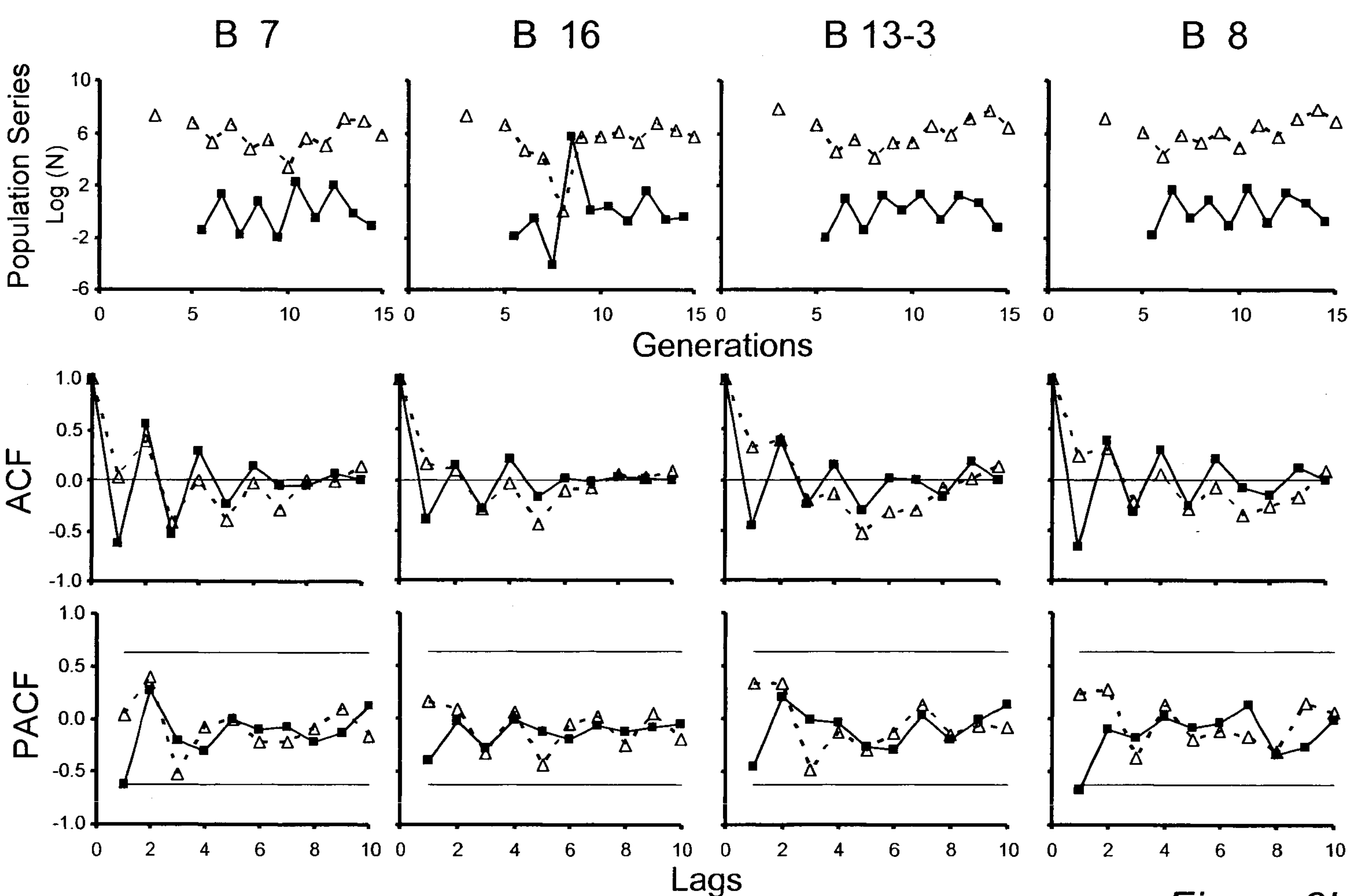

A 1-1
A 5
C 11
Population Series
Log (N)
ACF
PACF
Generations
Lags
Original
Detrended
Barlett bands

Figure 2a


B 7
B 16
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B 8
Population Series
Log (N)
Generations
ACF
PACF
Lags

Figure 2b

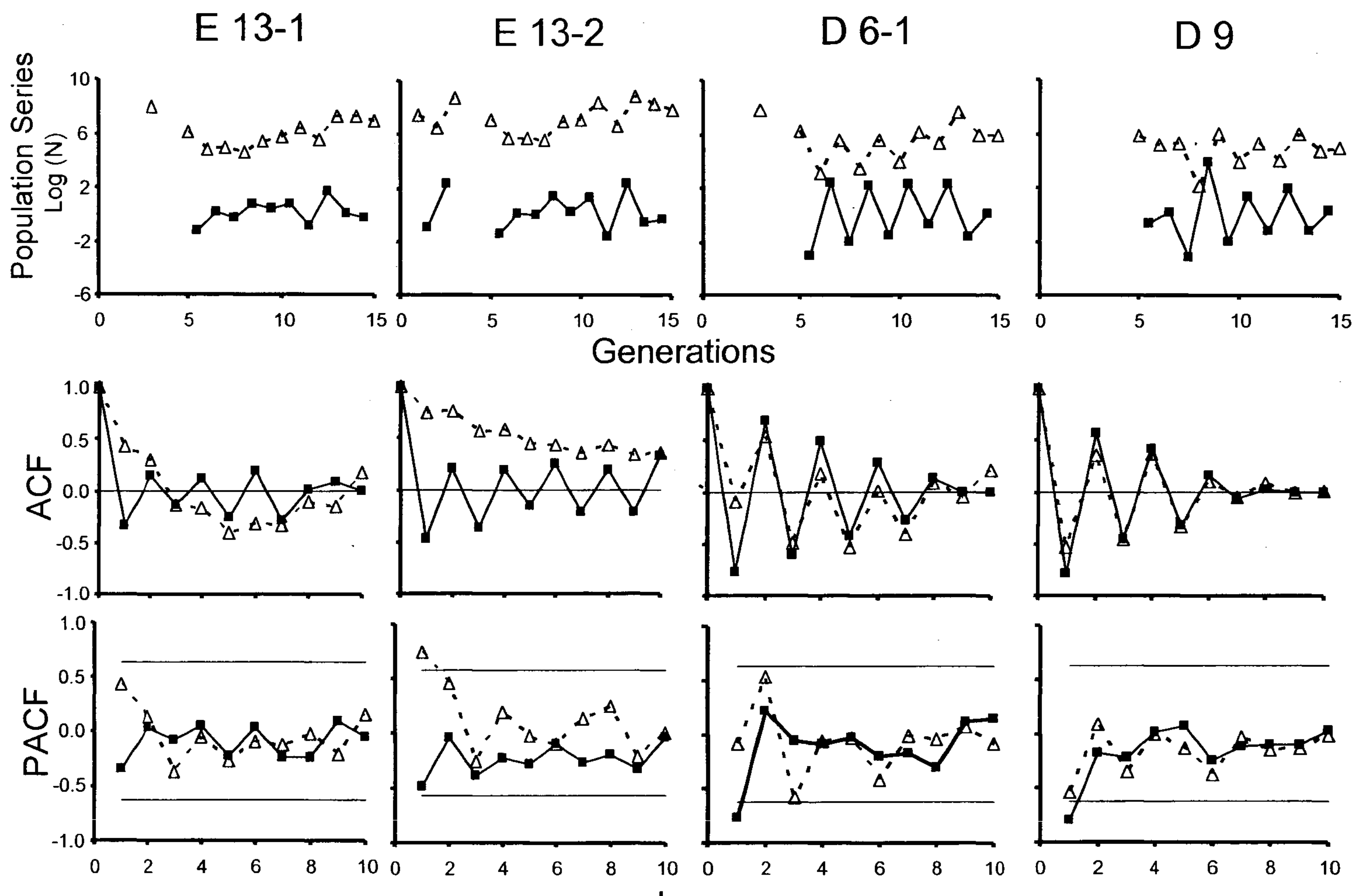

E 13-1
E 13-2
D 6-1
D 9
Population Series
Log (N)
Generations
ACF
PACF
Lags

Figure 2c

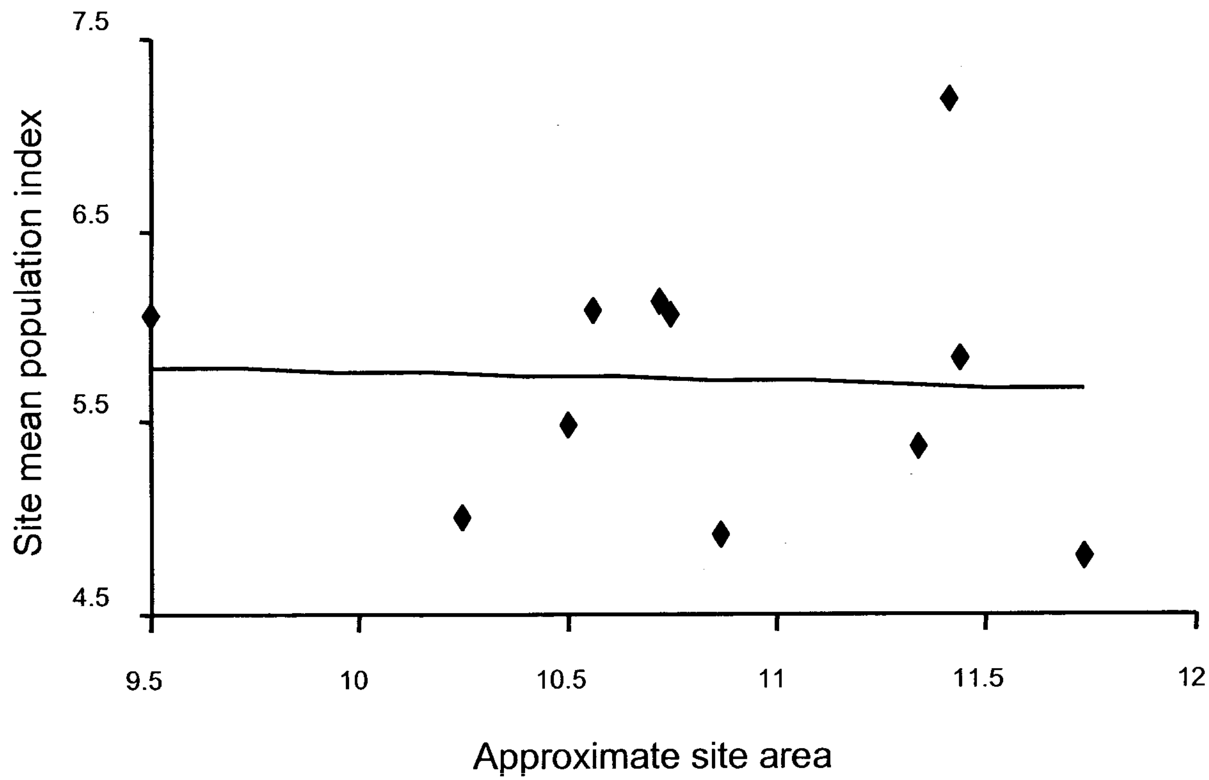
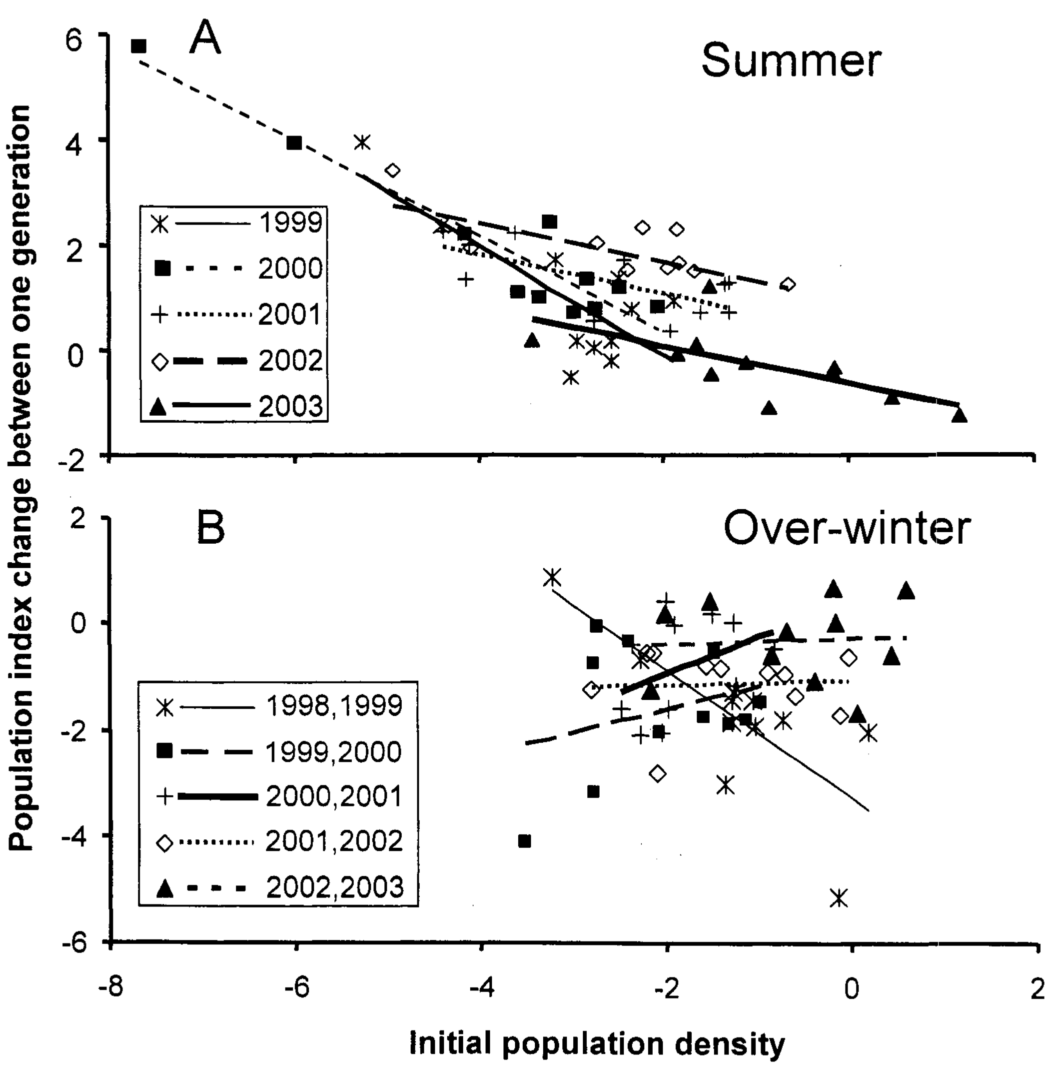

7.5
6.5
5.5
4.5
Site mean population index
9.5
10
10.5
11
11.5
12
Approximate site area

Figure 3


A
Summer
B
Over-winter
Population index change between one generation
Initial population density
1999
2000
2001
2002
2003
1998,1999
1999,2000
2000,2001
2001,2002
2002,2003
6
4
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0
-2
2
0
-2
-4
-6
-8
-6
-4
-2
0
2

Figure 4

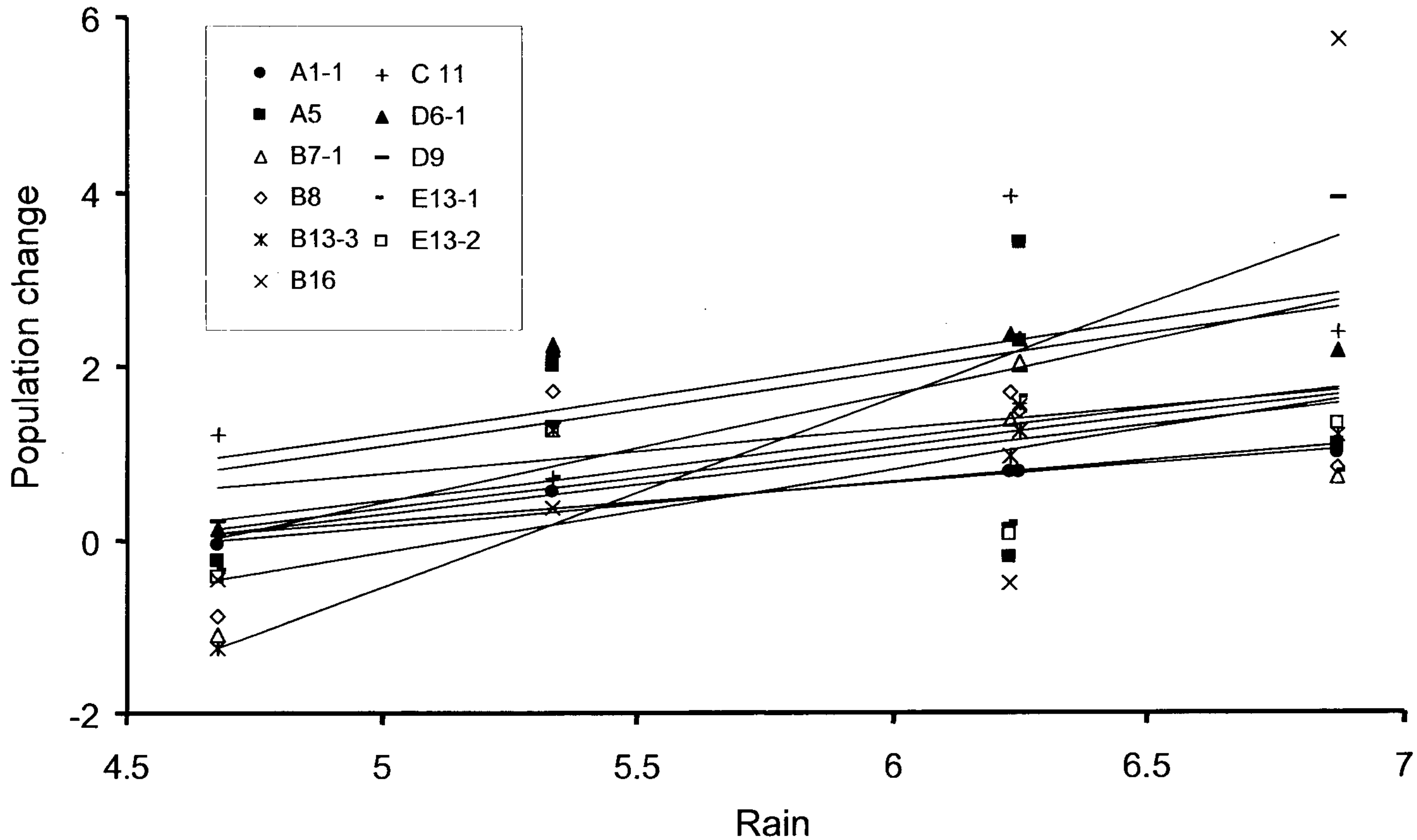
A1-1
A5
B7-1
B8
B13-3
B16
C 11
D6-1
D9
E13-1
E13-2
Population change
6
4
2
0
-2
4.5
5
5.5
6
6.5
7
Rain

Figure 5

CHAPTER 3

Floral preference of an endangered butterfly

Abstract

The endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae], may prefer certain floral species for obtaining nectar, and absence of these species may limit the population size. To determine if summer flight Karner blue butterfly adults have preferences among flower species, we calculated a visitation rate for each flower species that incorporates the number of feeding visits and flower species abundance. We found that Karner blue butterflies had high visitation rates to the following five species: *Asclepias tuberosa* L., *Amorpha canescens* Pursh, *Asclepias verticillata* L., *Helianthus occidentalis* Riddell, and *Monarda punctata* L., indicating preference for these flower species. The visitation rate was sensitive to the abundance of one preferred species, *Am. canescens*, illustrating that the coincidence of adult flight and nectar plant blooming may have important population consequences. Long term persistence of the Karner blue butterfly and other imperiled species may depend on minimum quantities of a suite of preferred species; assuring the presence of these species is a relatively simple management practice that could have significant benefits.

Introduction

Many imperiled butterfly species are in need of urgent conservation action (New et al. 1995). For many species, habitat loss and degradation are the leading causes of population declines (Sibatani 1990; Thomas 1991; Warren 1992). Addressing the conservation needs of threatened species requires knowledge of habitat requirements; only with this knowledge can appropriate habitat reserves and restoration be planned. Butterfly habitats require adequate larval and adult resources, and while considerable information about larval resources is available and generally well-integrated into conservation plans for many imperiled butterfly species (Thomas 1984; New et al. 1995), a lack of information about the needs of adult butterflies limits the ability to include adult resource information in conservation planning (Baz 2002; Dennis et al. 2003; Tudor et al. 2004).

For most butterfly species, floral nectar is a critical adult resource. It is composed of essential nutrients for body functions and reproduction, including carbohydrates, water, and smaller amounts of amino acids and lipids. Nectar feeding can increases adult longevity and reproduction (offspring size and number) in most butterflies. In addition, the size and distribution of butterfly populations can be affected by the amount and spatial arrangement of nectar plants (review in Boggs 1987).

Butterfly species vary in their nectar foraging behavior from generalists to specialists (Tudor et al. 2004), and many species show a preference for one or more flower species (Boggs 1987). Butterfly preference is probably related to recognition of flower species (Swihart 1970; Bernard 1979; Scherer and Kolb 1987) and butterflies can learn to prefer floral species if their behavior is appropriately rewarded (Lewis and Lipani 1990). Many butterfly species display a naive preference for specific colors but can switch and prefer a color associated with the greatest likelihood of a nectar reward (Swihart 1970, 1971; Weiss 1995, 1997).

Because a lack of adult resources reduces fecundity and may limit population size, determining butterfly species' floral preferences could improve the effectiveness of conservation efforts. Although the size of many butterfly populations does not correlate with the total abundance of floral resources, they may be correlated with abundance of preferred flower species. The population size of Fender's blue butterfly [*Icaricia icarioides fenderi* (Macy)], for example, did not correlate with total flower density but was correlated with native nectar supply; this finding is not surprising because *I. i. fenderi* appears to prefer native flower species (Schultz and Dlugosch 1999). Similarly, the population size of the federally endangered Karner blue butterfly [*Lycaeides melissa samuelis* (Nabokov)] does not correlate with total nectar flower abundance (Herms et al. 1996; U.S. Fish and Wildlife Service 2003), but Bidwell (1995) found *L. m. samuelis* numbers correlated with the mean flower counts of *Monarda punctata*. Another researcher suggested that low nectar plant abundance may have limited *L. m. samuelis* population size in the Allegan State Game area in Michigan (Lawrence 1994). If a population of an imperiled butterfly species is nectar limited, increasing preferred nectar species is a relatively simple management practice that could have significant benefits. Our objectives in this study were to determine if summer flight Karner blue butterfly adults displayed preferences among floral species, and to determine which floral species were preferred.

Methods

<u>Karner blue butterfly study system</u>

L. m. samuelis is a bivoltine butterfly that lives in oak savanna and pine barren ecosystems in the northern Midwest and northeastern part of the United States (U.S. Fish and Wildlife Service 2003). Over-wintered *L. m. samuelis* eggs hatch in late April/early May, and the larvae feed exclusively on *Lupinus perennis* L. (Fabaceae). In late May/early June, spring flight adults emerge. The mean adult life span as estimated by mark-release-recapture data is only four days, but researchers think individuals can live for two to three weeks (U.S. Fish and Wildlife Service 2003). After mating, the spring generation females lay eggs that develop into summer adults by late July/early August (Andow et al. 1994).

Even though it has not been explicitly tested, researchers agree that nectar is an important resource for *L. m. samuelis* adults (U.S. Fish and Wildlife Service 2003). Grundel and Pavlovic (1999) found that adults spent 7% to 23% of late morning time feeding. Many researchers have observed adults feeding on flowers, and the Karner blue butterfly recovery plan includes a comprehensive list including 48 floral species available during the spring flight and 72 species available during the summer flight (U.S. Fish and Wildlife Service 2003). In addition, several studies have found that adults visit some species more frequently than others (Packer 1987; Leach 1993; Sferra et al. 1993; Bleser 1994; Lane and Dana 1994; Lawrence 1994; Bidwell 1995; Herms 1996; Maxwell 1998; Grundel and Pavlovic 1999; Lane 1999). A few studies have discussed potentially preferred species but definitive evidence has been absent (Lawrence 1994; Schweitzer 1994; Herms 1996; Grundel and Pavlovic 1999; Lane 1999).

Study areas

We chose study areas with a consistent history of supporting large *L. m. samuelis* populations because availability of preferred nectar resources could be part of the reason the population has maintained large numbers at these locations. To increase the probability of finding important nectar resources, we chose sites within the study areas with high butterfly abundance and high nectar plant diversity. This allowed us to compare choices made by numerous individuals among several nectar plant species.

Ft. McCoy

The Ft. McCoy (24,282 ha, 44° 01'N, 90°41'W) Department of Army military training base is located in southwest Wisconsin 20 km east of Sparta, Monroe county. Several *L. m. samuelis* populations occur in the oak savanna ecosystem on Ft. McCoy, and *L. m. samuelis* have been documented on 95% of approximately 15 square kilometers of the mapped *L. perennis* (Maxwell 1998). In a report discussing Karner blue butterfly status in Wisconsin, Bleser (1994) described Ft. McCoy as one of the most important *L. m. samuelis* habitats in the state. Within Ft. McCoy there are eleven sites monitored annually, each named with a letter and number. We recorded nectar feeding behavior within the following four sites: C11, E13-2, B8, and B13 (see Chapter 2, Figure 1).

Waupaca

This central Wisconsin study area (44° 19'N, 89°13'W), is approximately 185 km west of Ft. McCoy and includes two large Karner blue butterfly sites -- the Emmons Creek Fishery (Fisheries, 2.8 ha) and the Waupaca Field Station (Sawyer, 5.4 ha), located in Portage and Waupaca counties respectively. These locations are approximately 3 km apart. Bleser (1994) and Lane (1999) both noted that these sites have very high densities of Karner blue butterflies.

<u>Observations</u>

Site-visits

We chose sites by walking in high quality *L. m. samuelis* habitat and looking for *L. m. samuelis* butterflies and a diversity of nectar plants. When we found this combination we marked the boundaries of a rectangle to define the site. We conducted these procedures each day and at each location prior to sampling. We re-used a previous rectangle if the butterflies and flowers were both still abundant, but most of the time the sample sites changed in response to flower and butterfly abundance. As a result, each site-visit denotes a specific day and location. In table 1, site-visits are described in three parts including the name of the *L. m. samuelis* habitat (e.g. C-11, Fisheries), a lower case letter describing a unique site, and the date.

Karner blue butterfly nectar feeding

During all site-visits we recorded nectar feeding visits to flowers. We defined a feeding visit by the butterfly's body position in relation to location of the flower's nectar. If it was likely that the butterfly could insert or was inserting its proboscis into the nectar, we counted it as a feeding visit. We carefully observed each butterfly from a distance of at least 1 m with close-focusing binoculars, although the precise position of the proboscis could not always be determined. Sampling occurred in weather conditions suitable for butterfly flight: between 10:00 and 19:00 CST, >18°C, and no precipitation (Thomas 1983, Pollard 1977).

We observed summer generation Karner blue butterflies in 23 site-visits, between July 16 and August 7, 2003, including 10 site-visits at Ft. McCoy and 13 at the Waupaca study area. The density of Karner blue butterflies varied considerably between the two study areas, so we utilized different sampling methods at each location.

At Ft. McCoy, the lower density study area, we used a focal animal method (Martin and Bateson 1993) and followed individual butterflies. We followed 86 males and 72 females, of which 78% and 81%, respectively, visited at least one flower species. We located a butterfly by walking a systematic pattern in the site-visit rectangle. When we spotted the first butterfly we placed a flag at its initial location and started recording its behavior. We followed the butterfly for a maximum of ten minutes, but ended the observation earlier if the butterfly left the site-visit rectangle or was lost from sight. We located subsequent butterflies by returning to the place where the previous butterfly observation began and continued walking the systematic pattern until we located another butterfly.

At Waupaca, the higher density study area, we used a scan method (Martin and Bateson 1993). We walked the entire site-visit rectangle and recorded the sex of each butterfly that we observed feeding and the flower species on which it was feeding. We observed 225 males and 211 females feeding. One census took between 5 to 30 minutes depending on the site-visit. Each census through a site-visit was a replication. We had from one to four replications (mean = 2) for each site-visit with at least 30 minutes between replications.

Nectar plant abundance

During each site-visit we recorded all blooming flower species listed as nectar plants in the Karner blue butterfly recovery plan (U.S. Fish and Wildlife Service 2003). We measured the abundance of each flower species by counting the total number of flower-units, rather than individual flowers. One flower-unit is a flower or the collection of flowers that a butterfly visited by staying stationary or by walking between flowers. Flower-units were distinguished by the need for a butterfly to fly in order to visit another flower-unit. As a result, plants with flowers growing as an inflorescence or spike were usually counted as a single flower-unit.

We measured flower-unit abundance differently in each study area because of differences in flower-unit density. At Waupaca site-visits, we completed a census of all the flower-units. At Ft. McCoy site-visits, we sampled all flower-units within several randomly selected 1 meter wide strip quadrants (Bonham 1989) to sample flower-units, accounting for approximately thirty percent of each site-visit area. Abundances were converted to densities according to the area of each site-visit.

Duration of a visit

We calculated mean duration of a visit to a flower-unit of each flower species in both study areas. At Ft. McCoy, we measured visit duration while following each butterfly. At Waupaca, we sampled butterflies haphazardly and timed part or all of the visit duration. Because some of the duration data were right and/or left censored, we used a parametric survival analysis model to estimate flower visit duration. Because we could not reject the hypothesis that the leaving rate was constant, we fit the data using an exponential model. We calculated the mean duration of a visit to a flower-unit for each flower species separately for each sex (Cox and Oakes 1984). For three flower species, which had fewer than four duration observations, we used the mean duration calculated from all flower species for the appropriate sex. This included *Berteroa incana* (L.) DC.and *Helianthus divaricatus* L. for females and *B. incana* and *Euphorbia corollata* L.for males.

Analysis

Visitation rate

We calculated the visitation rate for each floral species at a site-visit. The visitation rate is the number of feeding visits to a specific flower species for an individual butterfly in an hour. The estimation method was slightly different in each study area. For the focal animal method at Ft. McCoy, the visitation rate for an individual butterfly for each flower species was calculated as follows:

Visitation rate for species A = (number of visits to flower-units of species A/total time the butterfly was followed)/number of flower-units of species A within the study plot

To calculate the visitation rate for each sex at a site-visit, we averaged all individual visitation rates for each flower species.

For the scan method at Waupaca, the visitation rate for each sample was calculated as follows:

Visitation rate for species A = (number of butterflies visiting species A/mean duration of a visit to a flower-unit of species A)/number of flower-units of species A

We completed all the statistical tests on visitation rates using a general linear model on SAS (SAS Institute 1997). We used the following model:

ln visitation rate = flower x site x flower* site .

We also blocked with samples (individuals in Ft. McCoy and replicates in Waupaca).

Butterfly feeding consistency

In order to test if floral preference occurred from similar individual butterfly behavior within each site-visit we completed a rank sum test (Kruskal-Wallis). At Ft. McCoy the sample was an individual butterfly and at Waupaca the sample was a replication. We were not able to run this test at all site-visits in Waupaca because five site-visits only had one replication. The samples were ranked according to visitation rate with the highest visitation rate samples receiving the lowest rank. Then we tested if the mean flower ranks for each species were similar within each site-visit. We completed this test using the NPAR1WAY procedure on SAS (SAS Institute 1997).

Results

Karner blue butterflies visited 15 flowering species during the summer flight in our study. They visited the following species: *Asclepias tuberosa* L., *Asclepias verticillata* L.

(Asclepiadaceae); *Chrysanthemum leucanthmum* L., *Coreopsis palmata* Nutt., *Erigeron annuus* L. Pers., *Helianthus divaricatus* L., *Helianthus occidentalis* Riddell, *Rudbeckia hirta* L. (Asteraceae); *Berteroa incana* DC. (Brassicaceae); *Euphorbia corollata* L., *Euphorbia podperae* Croizat (Euphorbiaceae); *Monarda punctata* L. (Lamiaceae); *Amorpha canescens* Pursch, *Melilotus alba* Medic. (Fabaceae); *Ceanothus americanus* L. (Rhamnaceae). Several species listed in the Karner blue butterfly recovery plan (U.S. Fish and Wildlife Service 2003) as nectar resources were present at these sites but were not visited. The following species, available in at least two site-visits, were not visited: *Achillea millefolium* L.(Asteraceae); *Hypericum perforatum* L. (Hypericaceae); *Lithospermum caroliniense* MacM, *Lithospermum canescens* Michx. (Boraginaceae); *Monarda fistulosa* L. (Lamiaceae); *Linaria vulgaris* Hill (Scrophulariaceae); *Hedyotis longifolia* Hook (Rubiaceae).

Both male and female butterflies showed nectar feeding preferences in both study areas (Table 2); at least one sex showed a preference for the following five species: *As. tuberosa*, *Am. canescens*, *As. verticillata*, *H. occidentalis*, and *M. punctata* (Figures 1 and 2). In all sex and study area combinations in which *As. tuberosa* was available, it was a preferred species and consistently had either the first or second highest mean visitation rate. In three of the four sex and study area combinations Karner blue butterflies showed a preference for *Am. canescens*, and this species consistently had high mean visitation rates. In Waupaca, *As. verticillata* was preferred by both sexes. Because *As. verticillata* was only available at one site-visit in Ft. McCoy, this species could not be included in the analysis for this study area. Likewise, *H. occidentalis* was preferred by males in Waupaca, but this species was not available in Ft. McCoy. *M. punctata* had high mean visitation rates across all sex and study area combinations but a preference for this species was only significant for Waupaca males.

Two additional species had a high mean visitation rate but these rates were high for only one sex. Males preferred *H. occidentalis* while females did not show a preference but had a high visitation rate for both *H. divaricatus* and *H. occidentalis* (Figure 2). Females also had a relatively high visitation rate for *C. palmata* at both study areas; however male visitation rates to this species were relatively low at both study areas. A few additional species were only available at one site-visit; therefore they were not included in the flower by site-visit analysis or shown on figures 1 and 2. The following species had high male visitation at one Ft. McCoy site-visit where only males were observed feeding: *E. podperae,*, *Ch. leucanthmum*, and *Ce. americanus*.

Duration of a visit to a flower-unit, a measurement utilized to calculate visitation rates in the Waupaca study area, probably had a minimal impact on the differences in visitation rates among floral species and the butterfly sexes (Figure 3). We did not find a significant difference in duration between sexes ($F = 0.34$, df =1,15 $P = 0.5808$), or among flower species ($F = 1.28$, df =7,15 $P = 0.3774$). If duration had a strong effect we would expect floral species with short duration to have high visitation rates. *M. alba* had the lowest duration length for both sexes and it did not have unusually high visitation rates (Figure 1 and 2). In addition, similar patterns of preferred species and sex differences occur at both study areas and these similarities would be less likely if duration length had a significant effect.

In general, preference differences among flower species were more readily determined for males than females. Some flower species that were significantly preferred by males were not significantly preferred by females. For example, the difference between males and females for *M. punctata* at Waupaca may in part be due to overall higher visitation rates of males than females. Because male visitation rates are higher, comparison between a visited flower species and species with a visitation rate of zero is more likely to be significantly different. Even though the results for the sex and study area combinations are variable, the visitation rates for the five preferred flower species listed above were relatively high at all sex and study area combinations.

Differences in visitation rate for a particular flower species among site-visits was partially related to differences in the floral arrays and species abundance between site-visits. Three of the four sex and study area combinations had significant flower by site-visit interactions (Table 1, Figures 4). For males at Ft. McCoy, the interaction was driven by the ten fold variation in visitation rate to *Am. canescens* among site-visits (Figure 4a). Female visitation rates for *Am. canescens* also varied dramatically among site-visits, but a particularly high visitation rate to *M. punctata* at one site-visit also contributed to the interaction (Figure 4b). For males at Waupaca the species by site-visit interaction was driven by the interchange of the highest visitation rate between *Am. canescens* and *As. tuberosa* (Figure 4c). The flower by site-visit interaction was not significant for females at Waupaca in part because the absolute difference of visitation rates was small. For example, mean visitation rates to *As. tuberosa* for females varied from 0 to 1.5 (Figure 4d), compared to 0 to 3.0 for males (Figure 4c). Because Karner blue butterflies visit numerous species and preference is likely to change depending on the species available, variation in floral array between site-visits will increase the variation in visitation rates.

The abundance of a preferred species can dramatically affect the visitation rate at different site-visits. *Am. canescens* density greatly affected the mean visitation rate at multiple site-visits. As *Am. canescens* density increased over twelve days at C-11, male and female visitation rates decreased dramatically (Figures 5 a,b,c). At the Fisheries site a similar but opposite pattern occurred over five days; as *Am. canescens* density decreased both male and female visitation rate increased considerably (Figures 5 d,e,f). The decrease of visitation rate as the density increases may be partially explained by a dilution effect. If the number of butterflies feeding stays constant while availability of a flower species increases, the visitation rate to that species will decrease because feeding visits will be spread across more flower-units. Karner blue butterfly nectar feeding behavior was strongly affected by *Am. canescens* density and this influenced the significance of the flower by site-visit interaction. However we did not notice the same visitation rate change with the density of another preferred species, *As. tuberosa*. The observed range of *As. tuberosa* abundance was much smaller than *Am. canescens,* potentially explaining why the dilution effect was not evident in this species.

To determine if overall site-visit preferences are a result of similar individual butterfly preferences, we used a Kruskal-Wallis test to examine the consistency within each site-visit. Because the test showed if mean flower ranks were similar, a significant result showed that the mean ranks of at least two flower species were different from each other, showing consistency of preference. Eleven of 16 site-visits at Ft. McCoy and 13 of 16 site-visits at Waupaca were significant, $P < 0.05$, implying that within a site-visit, individual butterflies have similar flower preferences (Table 1). These results demonstrate strong evidence that within a site-visit, individual or aggregate individual behavior preferences were consistent.

Discussion

Karner blue butterflies do show preferences for particular flower species, and long term persistence at a site may depend on minimum quantities of a suite of preferred species. However, the butterflies visit numerous species and their presence is unlikely to be dependent on one specific floral species (U.S. Fish and Wildlife Service 2003).

To determine which flower species may improve long term survival of the Karner blue butterflies, previous researchers have identified which species are visited frequently (Packer 1987; Leach 1993; Sferra et al. 1993; Bleser 1994; Lane and Dana 1994; Lawrence 1994; Bidwell 1995; Herms 1996; Maxwell 1998; Grundel and Pavlovic 1999; Lane 1999). The authors of the Karner blue recovery plan summarized data from nine studies and listed the following thirteen species as commonly visited by the summer adults: *Am. canescens, As. tuberosa, As. verticillata, B. incana, Centaurea biebersteinii, Euphorbia corollata, Euphorbia podperae, H. occidentalis, Liatris cylindracea, M. alba, M. punctata, R. hirta,* and *Solidago speciosa* (U.S. Fish and Wildlife Service 2003). While these studies show that several floral species are utilized often, they are not conclusive evidence of preference. A frequently visited species may be selected often because it is abundant, not because it is preferred. Preference data, requires the combination of both butterfly visitation and floral abundance data, while frequency data only measures butterfly visitation.

If a butterfly population is nectar-limited, understanding the difference between a frequently visited and preferred species is critical. If a frequently visited species is mistakenly assumed to be a preferred resource, then one may incorrectly expect that increasing the abundance of the species will result in an increased population size. However, this may not be the case, because increased visits may not result in an increased nectar reward. In contrast, variation in the amount of a preferred species is more likely to result in concurrent variation in population size, because butterflies are more likely to receive increased nectar amounts from preferred species. As a result, conclusions from frequency and preference data can be very different and need to be communicated clearly.

Grundel and Pavlovic (1999) studied Karner blue butterfly feeding behavior and floral abundance. While their data showed patterns of visitation frequency, it is unlikely that the results indicate preference because the floral abundance counts were dependent on the presence of an individual nectar feeding Karner blue butterfly. After they observed a Karner blue butterfly feeding visit, they counted the flowers in the two-meter radius surrounding the visited flower. They inferred butterfly preference by examining the total abundance of flower species compared with the individually selected flower species. This methodology assumes each individual butterfly is selecting the flower species to visit among the species in the two meter radius. Because these methods confound individual butterfly choice with flower abundance it is unclear if the results accurately demonstrate preference.

While no other researchers have quantified nectar preference, several have suggested evidence of Karner blue butterfly preference for the following species: *Am. canescens, As. tuberosa, C. americanus, Me. alba,* and *Mo. punctata*. Four studies mentioned that *As. tuberosa* appears to be a preferred species (Lawrence 1994; Schweitzer 1994; Herms 1996; Lane 1999). Lawrence (1994), Lane (1999) and Herms (1996) collected frequency data on butterfly feeding and noted by observation that *As. tuberosa* was rare, suggesting that floral abundance did not explain the high visitation frequency. Lane (1999) also noticed this pattern of high visitation and low floral abundance for another species, *Am. canescens*. Because we found strong evidence of preference for both *As. tuberosa* and, *Am. canescens,* our conclusions are consistent with past observations.

It is unclear if *C. americanus, Me. alba,* and *Mo. punctata* are preferred species. Many researchers have found high visitation frequency to *Mo. punctata* (Leach 1993; Sferra et al. 1993; Lawrence 1994; Herms 1996; Maxwell 1998; Grundel and Pavlovic 1999; Lane 1999), but preference is unclear. Lane (1999) found it was difficult to determine if this species was preferred or visited often because it was abundant. Also, Lawrence (1994) speculated that *Mo. punctata* visitation frequency was higher when *As. tuberosa* was not available. Our results show a moderate preference for this species because mean visitation rate is relatively high but only Waupaca males showed a clear preference. Schweitzer (1994) concluded that both *C. americanus,* and *Me. alba* are preferred species. In this study *C. americanus* had a relatively high mean visitation rate by males at one site-visit. Unfortunately, because this species was only available at one site-visit we were not able to include the species in the analysis. We found butterflies commonly visited *M. alba,* but the visitation rate was low, probably due to the high abundance of this species in our site-visits.

Understanding nectar feeding differences between the sexes may also help determine suitable habitat. The overall visitation rate of males was nearly double the visitation rate of females in both study areas. We did observe more males than females at each study area but the difference in observed numbers is not large enough to explain this result. These observations suggest that males may require more nectar than females. Knutsen et al. (1999) concluded from their data and a review of three other Karner blue butterfly movement studies that males fly more than females.

Summarizing nectar feeding behavior and floral abundance into a visitation rate was an effective method for this system. The number of visits to flower species and flower species abundance both influenced the visitation rate, while duration had a minimal effect. For a different system, duration may have an important influence. The visitation rate was sensitive to floral abundance and this resulted in some interesting patterns with the preferred species, *Am. canescens*. Nectar resources are dynamic and the density change observed for this nectar source illustrates the change in preference over time. Two phenological events, adult flight and important nectar plant blooming, need to correspond. In this study, the butterfly flight was longer than the duration of blooming for this species. The dramatic change of *Am. canescens* floral abundance during a relatively short amount of time illustrates the need to recognize how these two phenological events can vary. When evaluating a species' nectar resource, it is important to consider temporal stochasticity; the coincidence of butterfly flight with the blooming of preferred nectar plants could have important population consequences.

Karner blue butterfly recommendations for the summer flight

In the western part of the *L. m. samuelis* range, including Minnesota and Wisconsin, we recommend that managers concentrate effort on increasing preferred nectar species. Our results and previous research observations show that *As. tuberosa* is consistently a preferred species and we advise efforts to increase this species in Karner blue habitat. We also recommend increasing floral species that both sexes preferred: *Am. canescens*, and *As. verticillata*. Finally, we recommend some effort toward two species that were preferred by one sex: *H. occidentalis* and *M. punctata*.

Due to weather variation from year to year, we suggest that preferred species are encouraged to grow in multiple places along a continuum from open to closed tree canopy. If the same species is planted in a variety of places from sun to shade, the date when individual plants bloom should vary through the season. This extended blooming will help ensure that nectar resources are available during the entire butterfly flight. Some species may not flower in partial or closed canopy, thus extra effort should be directed toward species that do flower in these conditions.

We also recommend inclusion of floral species preference information when assessing Karner blue butterfly habitat. If a species is rare, we advise managers to count the number of flower units for each preferred species blooming during the Karner blue butterfly flight. Quantifying blooming preferred species and comparing these numbers to butterfly abundance may help identify suitable habitat.

Further research is needed to better understand how *L. m. samuelis* preference varies temporally and geographically. In chapter four, we summarize evidence that spring generation adults show floral preferences at the site level, but additional research will help identify if there are particular species preferred across multiple sites. We also recommend further study in different parts of the range to determine preference variations for both generations.

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Table 1. Results of Kruskal-Wallis test for site-visits with two or more samples

Site-visit number	Site-visit description[a]	df[b]	Chi-Square	P	n[c]
	Ft. McCoy Male				
1	C-11 a, 7/16	10	42.67	**<.0001**	9
2	C-11 a, 7/17	10	26.27	**0.0034**	7
3	C-11 b, 7/21	9	88.68	**<.0001**	9
4	C-11 a,c,d 7/24	10	160.49	**<.0001**	18
5	C-11 a,c, 7/28	8	16.94	**0.0307**	2
6	E13-2 b, 7/30	6	24.15	**0.0005**	7
7	B 13 a, 7/18	6	7.06	0.3155	4
8	B 8, 7/23	6	11.49	0.0743	6
9	B 13 b, 7/29	4	3.39	0.4950	2
	Ft. McCoy Female				
1	C-11 a, 7/16	10	11.76	0.3014	5
2	C-11 a, 7/17	10	14.53	0.1504	3
3	C-11 b, 7/21	9	34.85	**<.0001**	11
4	C-11 a,c,d 7/24	9	128.53	**<.0001**	13
5	C-11 a,c, 7/28	8	64.72	**<.0001**	14
6	E13-2 b, 7/30	6	33.74	**<.0001**	3
10	E13-2 a, 7/28	4	13.85	**0.0078**	5
	Waupaca Male				
11	Fisheries a, 7/31	8	43.85	**<.0001**	5
13	Fisheries b, 7/31	11	22.96	**0.0179**	2
14	Fisheries b, 8/1	11	20.86	**0.0349**	2
15	Sawyer, 8/1	8	8.00	0.4335	4
16	Fisheries b,c, 8/5	12	22.30	**0.0343**	2
17	Fisheries e, 8/5	5	16.80	**0.0049**	3
22	Fisheries b,c,d 8/7	13	46.69	**<.0001**	4
23	Fisheries e, 8/7	5	12.77	**0.0256**	3
	Waupaca Female				
11	Fisheries a, 7/31	8	20.49	**0.0086**	5
13	Fisheries b, 7/31	11	22.79	0.0189	2
14	Fisheries b, 8/1	11	17.86	0.0850	2
15	Sawyer 8/1	8	27.44	**0.0006**	4
16	Fisheries b,c 8/5	12	15.49	0.2157	2
17	Fisheries e, 8/5	5	14.75	0.0115	3
22	Fisheries b,c,d 8/7	13	23.79	**0.0331**	4
23	Fisheries e, 8/7	5	15.23	**0.0094**	3

[a] Site-visits with only one transect were not included in this test and are not shown in the table. The site numbers and descriptions for site-visits with only one transect include the following: Waupaca male and female (site-visit 12: Fisheries a, 8/1; site-visit 19: Fisheries a, 8/6; site-visit 20: Fisheries c, 8/6; site-visit 21: Fisheries d, 8/6) and Waupaca female only (site-visit 12: Fisheries a, 8/1). [b] The degree of freedom for these tests follows the number of flower species -1. [c] n denotes number of butterflies for Ft. McCoy site-visits and number of replicates for Waupaca site-visits.

Table 2. ANOVA for each sex and study area combination

Source		*df*	*Type III SS*	*F*	*P*
Male Ft. McCoy					
	flower	12	10.85	7.70	**<.0001**
	site-visit	8	3.42	3.64	**0.0004**
	flower*site-visit	52	8.90	1.46	**0.0239**
	Error	515	60.48		
Female Ft. McCoy	flower	10	0.68	4.19	**<.0001**
	site-visit	6	0.22	2.23	**0.0397**
	flower*site-visit	44	1.75	2.44	**<.0001**
	Error	402	6.55		
Male Waupaca					
	flower	14	40.75	71.85	**<.0001**
	site-visit	11	6.92	15.52	**<.0001**
	flower*site-visit	90	27.48	7.54	**<.0001**
	Error	161	6.52		
Female Waupaca					
	flower	16	14.69	5.96	**<.0001**
	site-visit	12	3.64	1.97	**0.0296**
	flower*site-visit	97	14.59	0.98	0.5452
	Error	167	25.71		

FIGURES

Figure 1

Least squared mean visitation rates with standard error bars across flower species present at two or more site-visits in Ft. McCoy for A) males and B) females. The small letters above the lines separate the means (SNK test). Flower species are ordered from highest to lowest mean visitation rate for Ft. McCoy males, first for flower species available at both study areas (bold type) then for flower species available only at Ft. McCoy. The numbers after the flower species name denotes how many site-visits the flower was available - the first number for males and the second for females.

Figure 2

Least squared mean visitation rates with standard error bars across flower species present at two or more site-visits in Waupaca for A) males and B) females. The small letters above the lines separate the means (SNK test). Flower species are ordered from highest to lowest mean visitation rate for Ft. McCoy males, first for flower species available at both study areas (bold type) then for flower species available only in Waupaca. The numbers after the flower species name denotes how many site-visits the flower was available - the first number for males and the second for females.

Figure 3

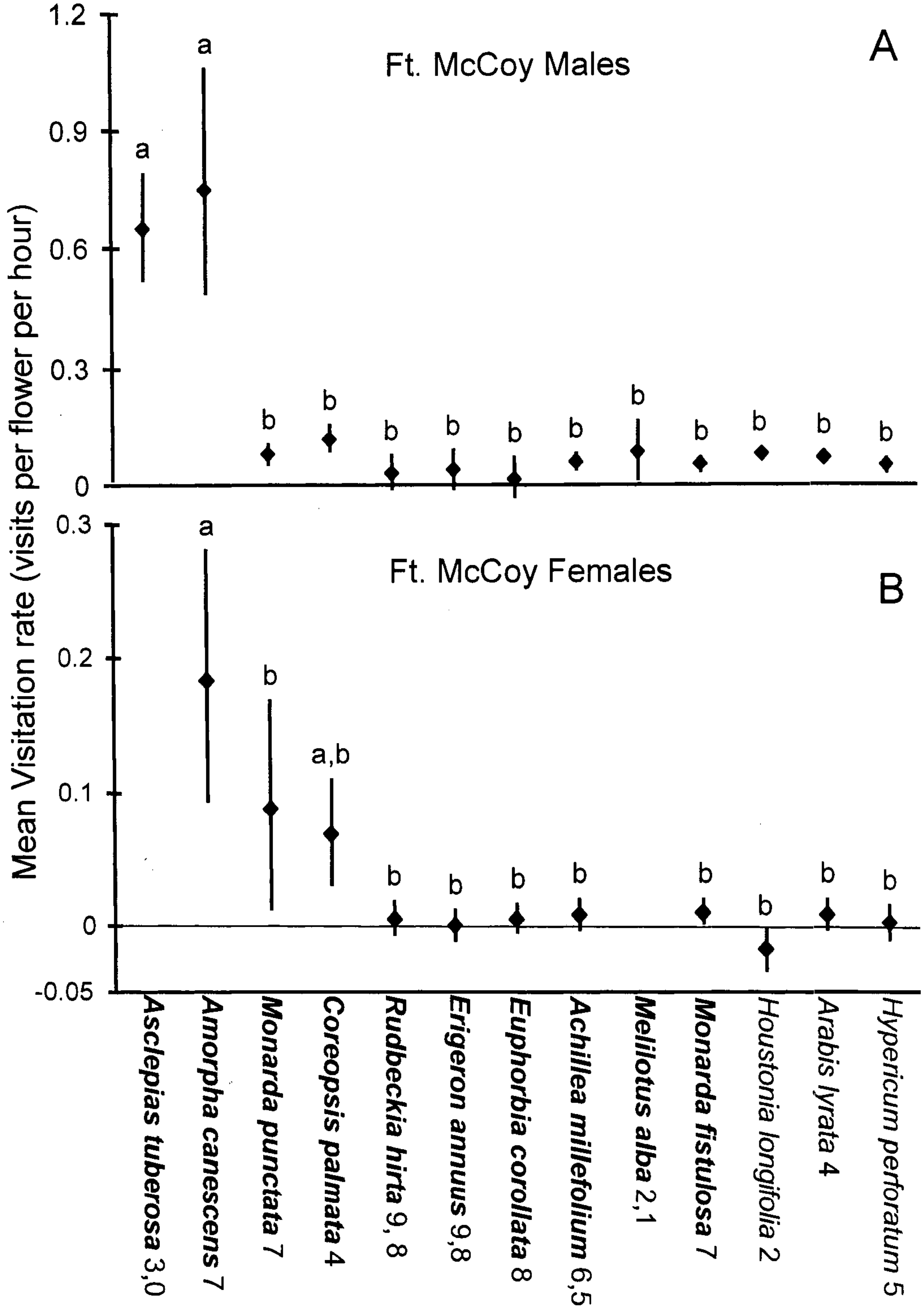
Mean visit duration with standard error bars for flower species with five or more observations for A) males, B) females.

Figure 4

Visitation rate as a function of site-visit and flower species for A) Ft. McCoy males, B) Ft. McCoy females, C) Waupaca males, D) Waupaca females. Values show natural logs of the least squared means for flower species for each site-visit. Site-visit descriptions can be found in Table 2.

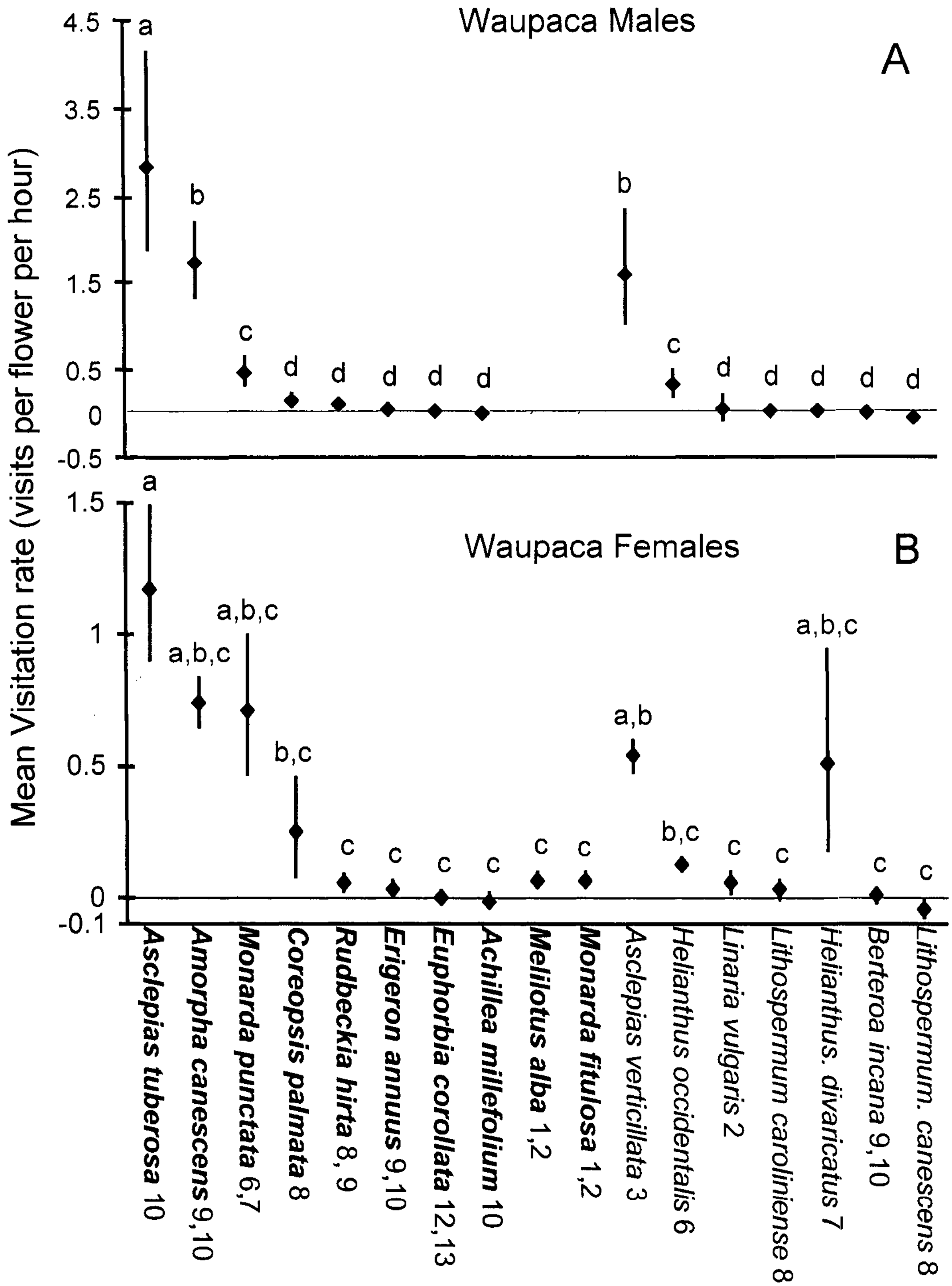
Figure 5

Change of floral abundance and corresponding visitation rates as a function of time for two sites. In Ft. McCoy, site C-11 panels include: A) floral abundance per meter squared as a function of time, B) corresponding male visitation rates, C) corresponding female visitation rates. In Waupaca panels include: D) floral abundance per meter squared as a function of time, E) corresponding male visitation rates, F) corresponding female visitation rates. Natural log least squared mean visitation rate values have been transformed but the scale remains logarithmic.



Ft. McCoy Males
A
Ft. McCoy Females
B
Mean Visitation rate (visits per flower per hour)
1.2
0.9
0.6
0.3
0
0.3
0.2
0.1
0
-0.05
a
a
b
a,b
Asclepias tuberosa 3,0
Amorpha canescens 7
Monarda punctata 7
Coreopsis palmata 4
Rudbeckia hirta 9,8
Erigeron annuus 9,8
Euphorbia corollata 8
Achillea millefolium 6,5
Melilotus alba 2,1
Monarda fistulosa 7
Houstonia longifolia 2
Arabis lyrata 4
Hypericum perforatum 5

Figure 1



Waupaca Males
A
Waupaca Females
B
Mean Visitation rate (visits per flower per hour)
4.5
3.5
2.5
1.5
0.5
0
-0.5
1.5
1
0.5
0
-0.1
a
b
c
d
a,b,c
a,b
b,c
Asclepias tuberosa 10
Amorpha canescens 9,10
Monarda punctata 6,7
Coreopsis palmata 8
Rudbeckia hirta 8, 9
Erigeron annuus 9,10
Euphorbia corollata 12,13
Achillea millefolium 10
Melilotus alba 1,2
Monarda fitulosa 1,2
Asclepias verticillata 3
Helianthus occidentalis 6
Linaria vulgaris 2
Lithospermum caroliniense 8
Helianthus. divaricatus 7
Berteroa incana 9,10
Lithospermum. canescens 8

Figure 2

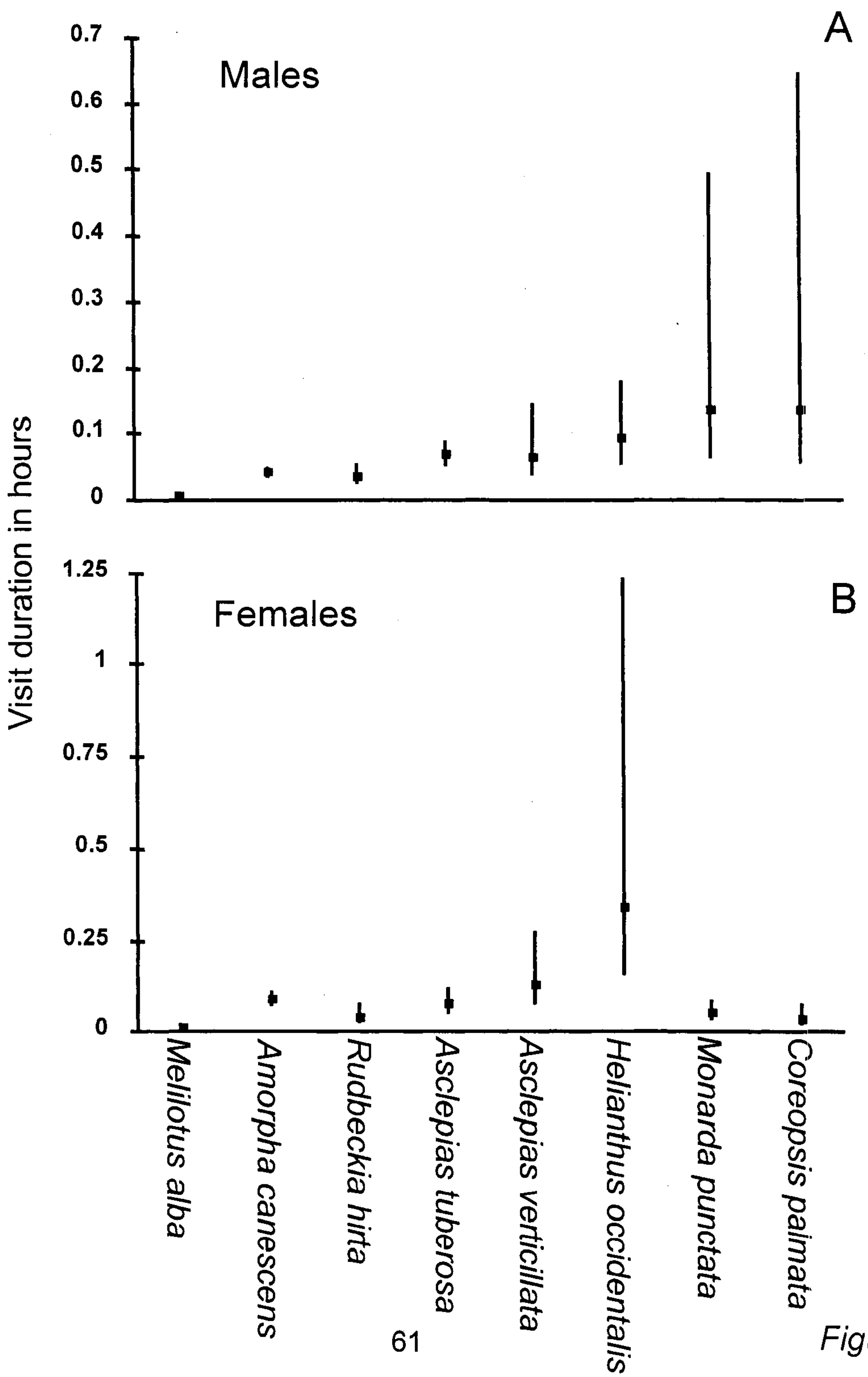
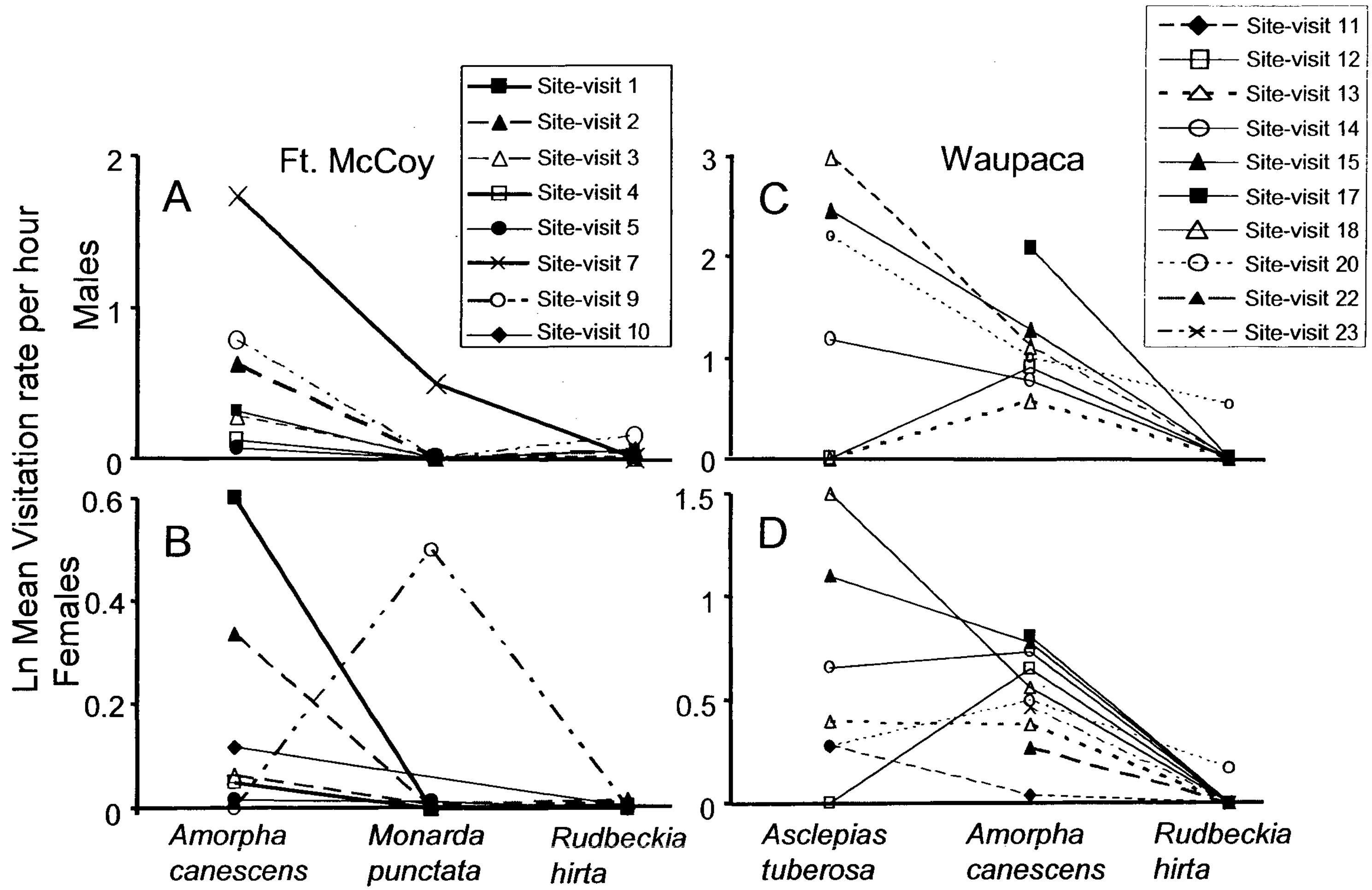

A
Males
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Visit duration in hours
B
Females
1.25
1
0.75
0.5
0.25
0
Melilotus alba
Amorpha canescens
Rudbeckia hirta
Asclepias tuberosa
Asclepias verticillata
Helianthus occidentalis
Monarda punctata
Coreopsis palmata

Figure 3



Ln Mean Visitation rate per hour
Males
Females
Ft. McCoy
Waupaca
A
B
C
D
Site-visit 1
Site-visit 2
Site-visit 3
Site-visit 4
Site-visit 5
Site-visit 7
Site-visit 9
Site-visit 10
Site-visit 11
Site-visit 12
Site-visit 13
Site-visit 14
Site-visit 15
Site-visit 17
Site-visit 18
Site-visit 20
Site-visit 22
Site-visit 23
Amorpha canescens
Monarda punctata
Rudbeckia hirta
Asclepias tuberosa
Amorpha canescens
Rudbeckia hirta

Figure 4

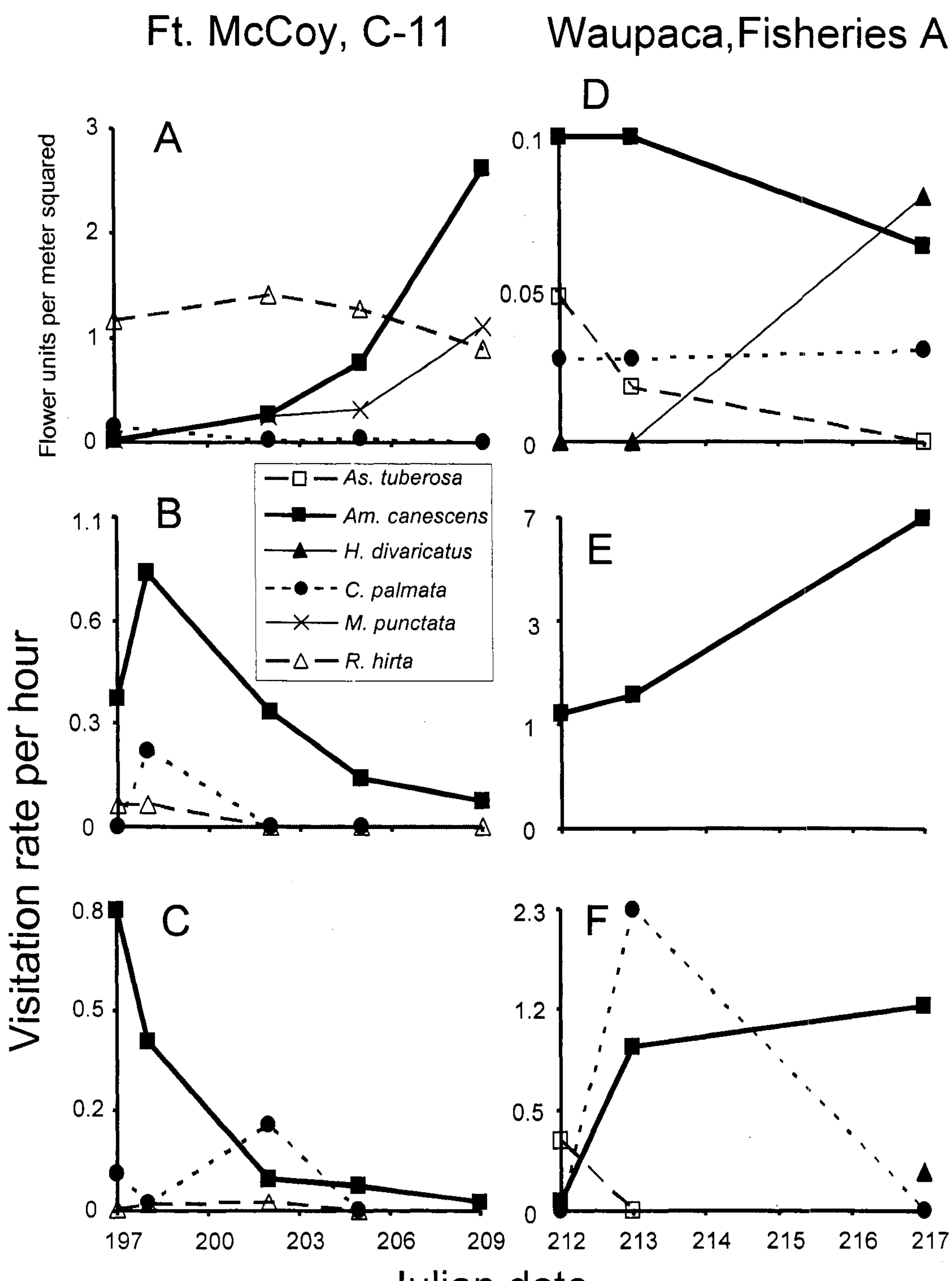

Ft. McCoy, C-11
Waupaca,Fisheries A
A
B
C
D
E
F
Flower units per meter squared
Visitation rate per hour
As. tuberosa
Am. canescens
H. divaricatus
C. palmata
M. punctata
R. hirta
Julian date

Figure 5

CHAPTER 4

Nectar feeding behavior of male Karner blue butterflies during the spring flight

Abstract

The endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae] lives in the disappearing oak savanna and pine barren ecosystems in the United States. To determine if spring flight Karner blue butterfly adults have a flower species preference, we collected data on butterfly nectar feeding visits by following individuals and independently measured flower species abundance in four locations. Using both the behavior and abundance data, we calculated visitation rates to each flower species. Within sites, Karner blue butterfly males displayed a floral species preference and three species (*Arabis lyrata* L., *Hieracium aurantiacum* L., *Potentilla simplex* Michx.) may be preferred. We also found evidence that *Lupinus perennis* L., the larval host plant, may not be an important nectar plant. Until preferred species are determined we recommend focusing management attention on spring nectar plants listed in the Karner blue butterfly recovery plan with easily accessible nectar.

Introduction

The endangered Karner blue butterfly, *Lycaeides melissa samuelis* (Nabokov) [Lepidoptera: Lycaenidae], is a symbol of the disappearing oak savanna and pine barren ecosystem in the northeastern and northern Midwest part of the United States (Andow et al. 1994). Only 0.02% high quality pre-settlement oak savanna is remaining in the Midwest (Nuzzo 1986). Habitat loss and degradation are the leading causes of population declines for this species (U.S. Fish and Wildlife Service 2003). Addressing this threat requires planning habitat reserves and restoring degraded habitat. The success of habitat-related conservation activities will improve as the scientific understanding of suitable habitat increases.

Suitable habitat contains all the necessary resources for species survival and this includes both larval and adult resources for butterflies. For the Karner blue butterfly, considerable information is known about its larval host plant including food quality (Grundel et al. 1998; Lane and Andow 2003) and this information is integrated into the Karner blue butterfly recovery plan (U.S. Fish and Wildlife Service 2003). In contrast, only general information is known about adult nectar feeding behavior. Many researchers have observed adults feeding on flowers, and the Karner blue butterfly recovery plan includes a comprehensive list of nectar species, including 48 floral species available during the spring flight. However there is no conclusive evidence about which, if any, of these species are the most important to the long term survival of this butterfly species (U.S. Fish and Wildlife Service 2003).

If a butterfly population is nectar-limited, understanding the difference between frequently visited species and preferred species is critical. A frequently visited species may be selected often because it is abundant, not because it is preferred. Butterfly preference is likely related to recognition of flower species (Swihart 1970; Bernard 1979; Scherer and Kolb 1987) and butterflies can learn to prefer floral species if their behavior is appropriately rewarded (Lewis and Lipani 1990). Many butterfly species display a naive preference for specific colors but can switch and prefer a color associated with the greatest likelihood of a nectar reward (Swihart 1970, 1971; Weiss 1995, 1997). If a frequently visited species is mistakenly assumed to be a preferred resource, then one may incorrectly expect that increasing the abundance of the species will result in an increased butterfly species population size. However, this may not be the case, because increased visits may not result in an increased nectar reward. In contrast, variation in the amount of a preferred species is more likely to result in concurrent variation in population size, because butterflies are more likely to receive increased nectar amounts from preferred species.

Because a lack of adult resources reduces fecundity and may limit population size (Boggs 1987), determining butterfly species' floral preferences could improve the effectiveness of conservation efforts. Lawrence (1994) suggested that low nectar plant abundance may have limited *L. m. samuelis* population size in the Allegan State Game area in Michigan. If a population of an imperiled butterfly species is nectar-limited, increasing preferred nectar species is a relatively simple management practice that could have significant benefits. Our objective in this study was to determine if spring flight Karner blue butterfly adults displayed preferences among floral species.

Methods

Karner blue butterfly study system

L. m. samuelis is a bivoltine butterfly. Over-wintering *L. m. samuelis* eggs hatch in late April/ early May and consume *L. perennis* leaves for approximately three weeks. In late May/early June, the spring flight adults emerge (Opler and Krizek 1984; Dirig 1994). The mean adult life span estimated by mark release recapture data is only four days, but researchers think individuals can live two to three weeks (U.S. Fish and Wildlife Service 2003). Even though it has not been explicitly tested, researchers agree that nectar is an important resource for *L. m. samuelis* adults and they feed from numerous flower species (Haack 1993).

Study areas

We observed spring generation Karner blue butterflies at four sites within two study areas between June 2 and June 9, 2003. Ft. McCoy (24,282 ha, 44° 01'N, 90°41'W), Department of Army military training base, is located in southwest Wisconsin 20 km east of Sparta, Monroe county. Several *L. m. samuelis* populations occur in the oak savanna ecosystem on Ft. McCoy, and *L. m. samuelis* have been documented on 95% of approximately 15 square kilometers of the mapped *L. perennis* at Ft. McCoy (Maxwell 1998). In a report discussing Karner blue butterfly status in Wisconsin, Bleser (1994) described Ft. McCoy as one of the most important *L. m. samuelis* habitats in the state. Within Ft. McCoy there are eleven sites monitored annually each named with a letter and number. We recorded nectar feeding behavior within the following three sites: C11, E13-1, and E13-2 (see Chapter 2 for map). Our fourth study site, Cuthrell (8.5 ha, 44° 10'N, 89°13'W) is located in Whitewater Wildlife Management Area, Winona county, Minnesota. This is the western most extant population of Karner blue butterfly, and is the only population in Minnesota (Lane and Dana 1994).

Observations

We chose sites to observe butterfly behavior by walking in *L. m. samuelis* habitat and looking for *L. m. samuelis* butterflies and a diversity of nectar plants. When we found this combination we marked the boundaries of a rectangle to define the site. We recorded nectar feeding visits to flowers. We defined a feeding visit by the butterfly's body position in relation to location of the flower's nectar. If it was likely that the butterfly could insert or was inserting its proboscis into the nectar, we counted it as a feeding visit. In general, we did not count non-feeding visits when a butterfly was on a flower in a position where it could not reach the nectaries. However because *L. perennis* is the larval host plant, we recorded both potential feeding and non-feeding visits to this flower species. We carefully observed each butterfly from a distance of one meter or greater. Close focusing binoculars greatly enhanced our ability to observe the butterfly's behavior, although the precise position of the proboscis could not always be determined. Sampling occurred in weather conditions suitable for butterfly flight: between 10:00 and 19:00 CST, >18° C, and no precipitation (Thomas 1983, Pollard 1977).

Karner blue butterfly nectar feeding

We used a focal animal method (Martin and Bateson 1993) and followed individual butterflies. We located a butterfly by walking a systematic pattern in the site. When we spotted the first butterfly we placed a flag at its initial location and started recording the butterfly's behavior. We followed the butterfly for a maximum of ten minutes but ended the observation earlier if the butterfly left the site, or if it was lost from sight. We located subsequent butterflies by returning to the place where the previous butterfly observation began and continued walking the systematic pattern until we located another butterfly.

Nectar plant abundance

At each site we recorded all blooming flower species listed as nectar plants in the Karner blue butterfly recovery plan (U.S. Fish and Wildlife Service 2003) and any additional blooming flower species we observed Karner blue butterflies visiting. We counted flower-units, defined by the behavior of the butterfly, rather than individual flowers. One flower-unit is a flower or the collection of flowers that a butterfly visited by staying stationary or by walking between flowers. If a butterfly would fly to reach the next flower or flowers, they were considered separate flower units. As a result, plants with flowers growing as an inflorescence or spike were usually counted as a single flower-unit. We sampled all flower-units within several randomly selected 1 meter wide strip quadrants (Bonham 1989).

Analysis

We completed a Pearson's Chi square test with the expected frequency of butterfly visits to each flower species based on the proportional flower-unit abundance in each site. When necessary, we grouped floral species with low abundance into an "other" category to meet the minimum required expected frequencies for this test. The species in the "other" category varied at each site: E13-2 included *Fragaria virginiana* Duchesne and *Gaylussacia baccata* K. Koch; C-11 included *Euphorbia corollata* L., *F. virginiana, Hedyotis longifolia* Hook, *Potentilla simplex* Michx., and *Phlox pilosa* L.; Cuthrell included *E. corollata*, *Lithospermum caroliniense* MacM, *Lithospermum canescens* Michx., and *P. pilosa*.

Results

Spring generation male Karner blue butterflies visited six out of the eleven flower species listed as nectar plants in the Karner blue butterfly recovery plan that were found at our sites. Of the 75 males we followed, 53 visited at least one of the following flower species: *Hieracium aurantiacum* L. (Asteraceae); *Euphorbia corollata* L. (Brassicaceae); *Arabis lyrata* L., (Cruciferae); *Gaylussacia baccata* K. Koch (Ericaceae); *Lupinus perennis* L. (Fabaceae); *Potentilla simplex* Michx. (Rosaceae). The following five species were present at our sites but not visited: *Lithospermum caroliniense* MacM, *Lithospermum canescens* Michx. (Boraginaceae); *Phlox pilosa* L. (Polemoniaceae); *Fragaria virginiana* Duchesne (Rosaceae); and *Hedyotis longifolia* Hook (Rubiaceae). All unvisited species were uncommon, comprising 3% or less total flower species abundance at a site, except *P. pilosa* that compromised 17% of the total floral species at E13-2 site (Figure 1a).

A total of four female butterflies were observed during the study at site E13-2 and three individuals visited a total of two flower species. Two individuals visited *H. aurantiacum* and one visited *L. perennis*. The low number of female butterflies observed was possibly due to the timing of the study, which was during the early part of the flight when more males are flying than females (Leach 1993).

Within sites, Karner blue butterfly males displayed a floral species preference. At three of the four sites, we found a significant difference between observed and expected visitation. (Figure 1a-c: E13-2: df = 4, Chi-square = 67.99, $P < 0.001$, C11: df = 2, Chi-square = 50.41, $P < 0.001$; E13-1: df = 1, Chi-square = 38.39, $P < 0.001$). This result shows that Karner blue butterfly males did not visit flower species in relation to floral abundance but preferred one or more species. The Cuthrell site was not significant (df = 2, Chi-square = 4.43, $P = 0.109$) but males still showed high visitation frequency to one species; 49 of 54 visits were to *A. lyrata*. This result was not significant, in part, because this highly visited species also had high flower species abundance: 82% percent of all flower units were *A. lyrata* (Figure 1d).

Due to the varying floral arrays in each site, our data can only identify relative nectar preference. Three species (*A. lyrata, H. aurantiacum, P. simplex*) are relatively preferred over the other species at a site. *Arabis lyrata* was visited in all sites, and at two of the four sites the visitation frequency was very high. However, it is unclear if *A. lyrata* is preferred because the high visit frequency could be a result of high floral abundance (Figure 1). *H. aurantiacum* was only available at one site and the visitation frequency was very high while floral species abundance was moderate at this site (Figure 1a). Similarly *P. simplex* was only available at one site and the visitation frequency was very high (19 visits) while floral species abundance was low (0.06%). *P. simplex* was included in the category "other" in Figure 1b.

Male Karner blue butterflies land on *L. perennis* flowers but it is unclear if they can feed from these flowers. Males often sat on *L. perennis* flowers in a position that was impossible for nectar feeding, with their heads directed up toward the sky rather than toward the flower's nectar. We found more male non-feeding visits to *L. perennis* than visits that had the potential to be for feeding. At two of the three sites where males visited *L. perennis* flowers, non-feeding visits were double the frequency of possible-feeding-visits (Figure 2). In addition, it was impossible to confirm during the possible-feeding-visits that the butterfly proboscis's could reach the floral nectary because the flower petals obscured access to the floral nectary. Bees easily accessed *L. perennis* nectaries by landing on the upper petals causing the lower petal to open, allowing the bee to access the inner parts of the flower. A Karner blue butterfly that landed on *L. perennis*, on the other hand, did not create a clear opening between flower petals to the nectaries. To gain access to the nectar a butterfly would have to squeeze between petals on the side of the flower, which was never observed.

Even if Karner blue butterflies were able to feed from *L. perennis*, it was not a frequently visited or a preferred nectar species for male Karner blue butterflies. *L. perennis* is rarely visited compared to other species (Figure 1). In fact, when all sites were combined, possible *L. perennis* feeding visits only accounted for .045 percent of all feeding visits. This low frequency of *L. perennis* visits is not explained by the lack of *L. perennis* plants. At three of the four sites, *L. perennis* was at least the second most abundant species available (Figure 1). Because possible *L. perennis* feeding visits are low even with high availability, *L. perennis* is not a preferred nectar species.

Discussion

Karner blue butterfly males show flora feeding preferences within sites. However the Karner blue butterfly will visit numerous species, and its presence does not appear to be dependent on one specific floral species (U.S. Fish and Wildlife Service 2003).

To determine which flower species may improve long term survival of Karner blue butterflies, previous researchers have identified which species are visited frequently (Leach 1993; Lawrence 1994; Maxwell and Givnish 1994; Grundel and Pavlovic 1999). The authors of the Karner blue butterfly recovery plan summarized data from five studies and listed the following eight species as commonly visited by the spring flight: *A. lyrata, H. longifolia, H. aurantiacum, L. perennis, Melilotis offincionalis* L. (Pallas), *P. simplex*, *Rubus flagellaris* Willd., and *Rubus sp.* IN (U.S. Fish and Wildlife Service 2003). While these studies show that several floral species are utilized often, they are not conclusive evidence of preference because a frequently visited species may be selected often because it is abundant. Frequency data can show potentially preferred species, but conclusive preference data requires the combination of both butterfly visitation and floral abundance data.

Our results suggest that *A. lyrata* is an important nectar resource for the spring generation. Similar to our results, other researchers have found high visitation frequency to *A. lyrata* (Maxwell and Givnish 1994; Grundel and Pavlovic 1999). *Arabis lyrata* may also be an important resource because it appears to be a common and widespread species in Karner blue butterfly habitats; previous studies conducted in four states and one Canadian province, have observed feeding on this species (Packer 1987; Leach 1993;

Bleser 1994; Martin 1994; Maxwell 1998; Grundel and Pavlovic 1999). It was abundant in all four sites in this study. *Arabis lyrata* could be very important because the presence of this one species may provide nectar for many Karner blue butterfly populations.

Previous researchers also found that *P. simplex* and *H. aurantiacum* were frequently visited (U.S. Fish and Wildlife Service 2003).

While many researchers have recorded *L. perennis* as a nectar plant (Packer 1987; Leach 1993; Sferra et al. 1993; Bleser 1994; Helmbolt and Amaral 1994; Maxwell and Givnish 1994), there is evidence that *L. perennis* nectar may not be accessible to Karner blue butterflies. The morphology of *L. perennis* flowers may make it difficult or impossible for this small butterfly to access the nectar. While there is limited research on this specific floral species, there is research on similar species. According to Proctor and Yeo (1972), lupines have flowers similar to most flowers in the pea family which force insects to be in a specific position to access the pollen or nectar. As a result, the number of insect species that can access the pollen or nectar is reduced. Specifically, Knuth (1908) describes lupines as having a pumping arrangement where pollen is extruded out from the tip of the keel due to the pressure on the flower from the insect visit. Dunn (1956), who researched several lupine species in the group Micranthi, which ranges from British Columbia to Mexico, stated that the morphology of the lupine flower prevents insects other than bees from efficiently accessing the pollen. He noticed that other insects land on lupine, but only bees were able to trigger the lupine flower to provide easy access to pollen. Nectar production in lupine species may be limited or non-existent. Knuth (1908) stated that lupines are nectar-less, bee flowers. Dunn (1956) described a channel-like flower structure that bees probe even though lupines do not have nectar glands. In 1999, Grundel and Pavlovic found in a red dye staining test that *L. perennis* did not have floral nectaries. Schultz and Dlugosch (1999) found that the related species *Lupinus sulphuresu* ssp. *kincaidii*, the larval host plant for the Fender's blue butterfly [(*Icaricia icarioides fenderi* (Macy)] produced only a small amount of nectar. Kaye (1999) found that this lupine species may require bee pollination visits to produce seeds. However, *Lupinus sulphuresu* ssp. *kincaidii* is listed as a nectar plant species for *I. i. fenderi*

(Schultz et al. 2003) because the butterfly has been observed landing on it but it is unclear if researchers have conclusive evidence of the butterfly extracting nectar.

Male Karner blue butterflies may land on a *L. perennis* flowers for reasons other than nectar feeding. These include searching for a mate and regulating body temperature. Because females oviposit on or near *L. perennis,* males may perch on *L. perennis* flowers to find a potential mate. Researchers have documented, for other butterfly species, that males attempt to find mates at oviposition sites (Courtney and Parker 1985; Lederhouse et al. 1992). In addition, Karner blue butterfly males may rest on *L. perennis* flowers to thermoregulate. *L. perennis* flowers are often the highest growing plant in open areas and the large flower surface could be a good platform for thermoregulation.

Three additional species that are listed as spring nectar plants in the Karner blue butterfly recovery plan have flowers with difficult to access nectaries, therefore these species may not have accessible nectar. Boggs (1987) shows that proboscis length affects nectary access. Only a butterfly with a 3 inch long proboscis, for example, will be able to reach a nectary at the end of a 3 inch long corolla tube. Also, Corbet (2000) showed that butterfly species with short probosicis's did not visit deep flowers. Because Karner blue butterflies are small and are likely to have a corresponding short proboscis, they may have difficulty accessing nectar in flower species with long corolla tubes, such as *L. caroliniense*, *L. canescens*, and *P. pilosa*. The long corolla tube in these species may explain why Karner blue butterfly males did not visit these species during this study, even though they were present at our sites.

L. m. samuelis recommendations for the spring flight

Further research is needed to identify preferred species for both sexes during the spring flight. Until preferred species are discovered, we recommend that managers increase species that are listed in the Karner blue recovery plan with easily accessible nectaries in habitats with low spring nectar plant abundance.

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FIGURES

Figure 1

The total number of butterfly visits and flower species composition in each site. Ft. McCoy study area: A) E13-2, B) C-11, C) E13-1, Whitewater study area: D) Cuthrell.

Figure 2

Visits to *L. perennis* categorized as either possible feeding visits or non-feeding visits.

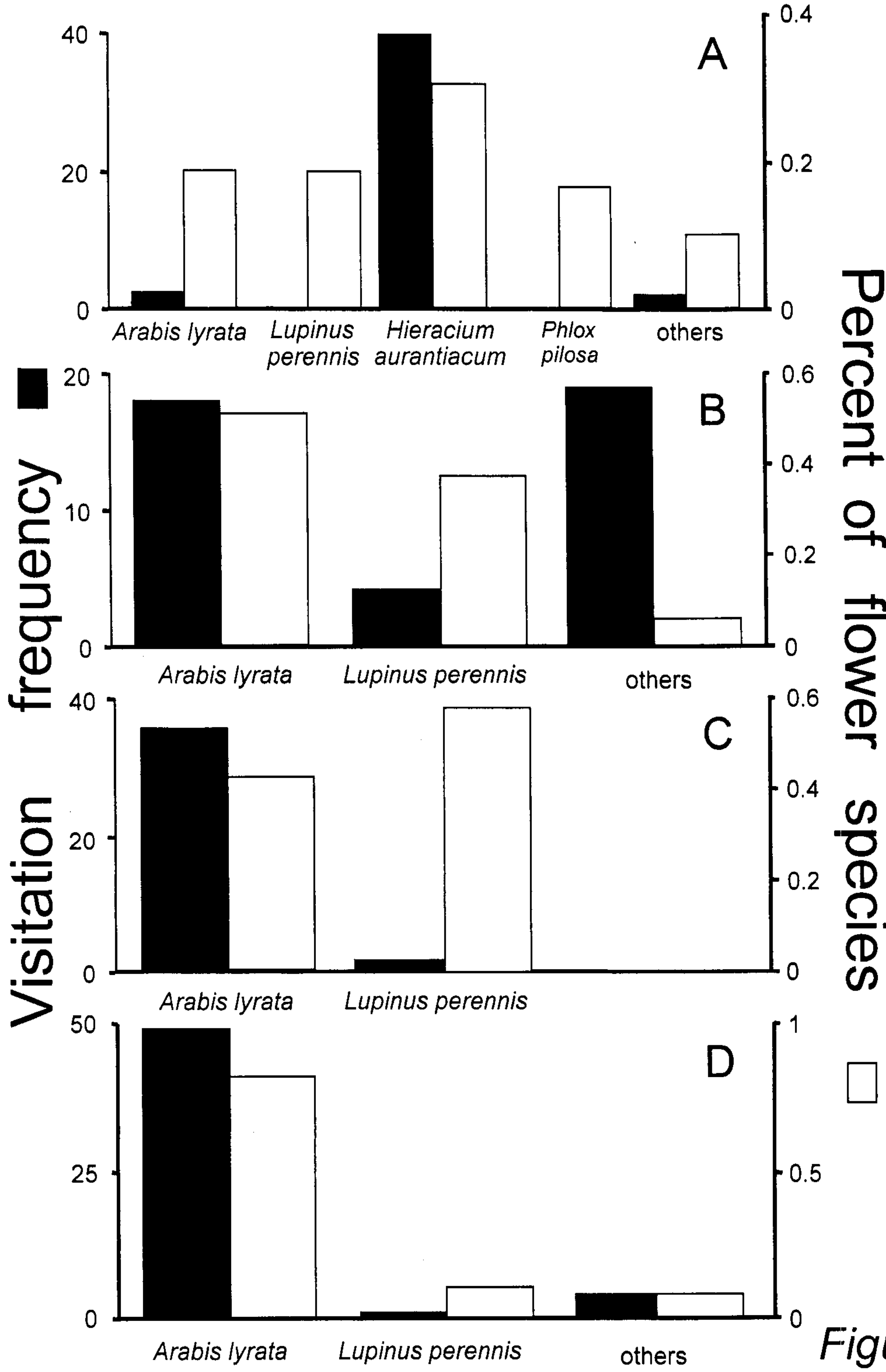
A
B
C
D
Visitation frequency
Percent of flower species
Arabis lyrata
Lupinus perennis
Hieracium aurantiacum
Phlox pilosa
others
0
20
40
10
25
50
0.2
0.4
0.6
0.5
1

Figure 1

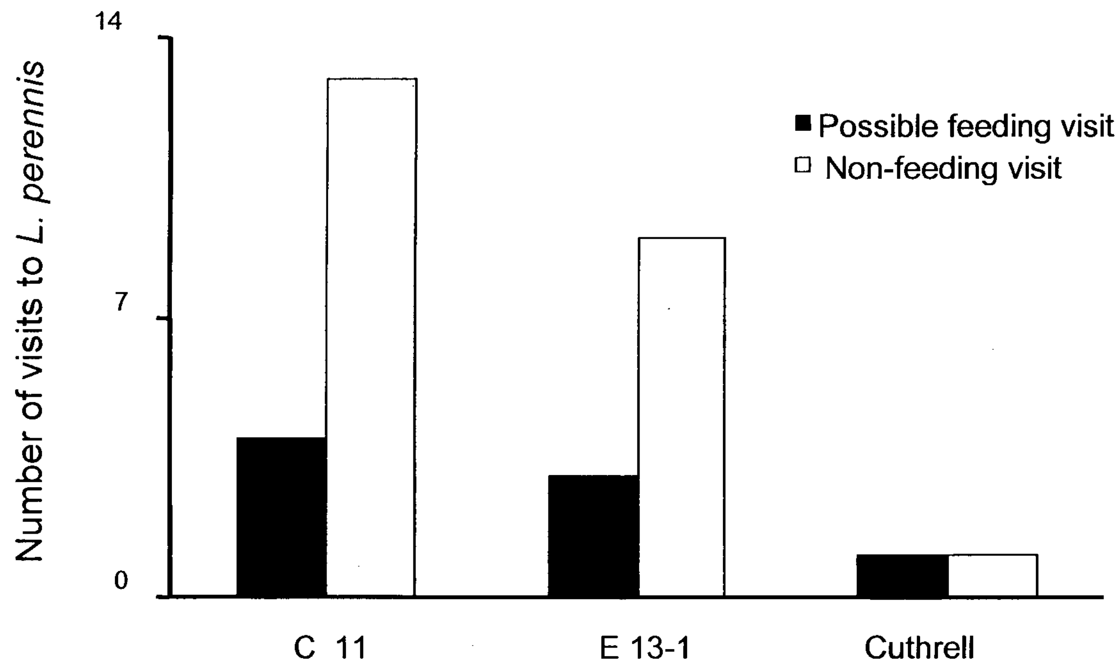
Number of visits to L. perennis
14
7
0
Possible feeding visit
Non-feeding visit
C 11
E 13-1
Cuthrell

Figure 2

APPENDIX A

Powerpoint Presentation Script for "The Story of the Karner Blue Butterfly"

The following script corresponds to "The Story of the Karner Blue Butterfly" Powerpoint presentation that consists of 55 slides to provide a visual aid for teaching about Karner blue biology, ecology, and conservation. The numbers and bold headings below describe each slide in the presentation. The Powerpoint slides are available on a CD that can be purchased by contacting:

Monarchs in the Classroom
University of Minnesota
Department of Fisheries, Wildlife and Conservation Biology
1980 Folwell Ave., 200 Hodson Hall
St. Paul, MN 55108
612-624-8706
www.monarchlab.umn.edu

Goals of the presentation

- Increase appreciation and understanding of natural world interconnections through explanation of the biology and ecology of Karner blue butterflies.
- Increase awareness of the savanna/ barrens ecosystems, understanding of ecological management and the Karner blue butterfly role as a flagship species.
- Increase understanding of the role of governmental and non-governmental organizations in conservation.

Abbreviations

WWMA: Whitewater Management Area
DNR: Department of Natural Resources

Introduction

1. Three male Karner blue butterflies on hand
Introduction of the presentation and the presenter
(The picture was taken by holding the camera in the hand that didn't have Karner blues perched. The males pictured are likely drinking perspiration off the person's hand).

2. Female Karner blue
People are concerned about the survival of the Karner blue butterfly species due to range wide population declines from habitat loss and other factors. To increase the likelihood of survival it was listed as a federally endangered species in 1992.

3. Karner blue historic range
The blue on map shows the historic range of the Karner blue (Figure 4 modified: Lane and Weller 1994). Currently the only place in Minnesota that Karner blues can be found

is in the Whitewater Wildlife Management Area, WWMA, (north of Whitewater State Park). A population in Cedar Creek natural history area in Anoka county disappeared prior to 1994 (Andow et al. 1994).

4. Several Karner blues on orange flower (butterfly weed)
The three main sections of this slide presentation are Karner blue biology, ecology, and conservation. The biology section focuses on the Karner blue species. The ecology section focuses on interactions of the Karner blue and its habitat. The conservation section focuses on how people interact with Karner blues and their habitat.

Biology

5. Male Karner blue
This section includes three topics: identification, taxonomy and life cycle.

Identification

6. Monarch, great spangled fritillary, and Karner blues on butterfly weed (Inset shows Karner blues with wing open. Circle shows Karner blue with wings closed)
The Karner blue butterfly is a small butterfly, approximately a 1" wing span, compared with approximately a 3" wing span of the monarch and great spangled fritillary.

7. Male and female Karner blue wings open, without labels
Guess which one is male and which one is female.

8. Male and female Karner blue wings open, with labels
Did you guess correctly? The upper surface of the wing varies between males and females. Both are blue with a narrow black band and white fringe. The male is violet blue while the female blue coloration varies from violet blue near the body to dark grey brown near the upper wing tips. The female also has several orange crescents on its upper hind wing.

9. Three pictures (wings closed): Karner blue, eastern tailed blue, spring azure
Karner blues have relatives with similar appearances that are often found in the same habitat. Compare the Karner blue to the eastern tailed blue and spring azure. A person must observe carefully to identify the correct species. The amount of orange on the under surface of the wing helps identification. The spring azure doesn't have any orange, and the eastern tailed blue has a smaller area with orange than the Karner blue.

10. Eastern tailed blue, wings open
The eastern tailed blue can also be distinguished by a small white extension on the hind wing referred to as a "tail". Eastern tailed blues are commonly seen in Minnesota.

Taxonomy

11. Karner blue on a person's thumb

The **common name** of the Karner blue originated from a the name of a railroad stop, Karner in New York State, close to where the butterfly was first identified. Even though common names are often interesting and easier to remember, the use of common names can be confusing. Because common names aren't standardized, sometimes a species has numerous common names. It's similar to a person having several nicknames. Therefore each species is also given one scientific name, which is used in all languages.

Many species consist of two or more **subspecies** that may be able to interbreed, but are different enough that they are given a different name. The subspecies is listed after the genus and species in the scientific name. The scientist who named the species, last name is found after the scientific name in parenthesis with the year the information was published. The Karner blue was named in 1944 by Vladimir Nabokov. Vladimir Nabokov was also a famous novelist from Russia who wrote several books, including Lolita.

Science is dynamic. As new information is discovered scientific names can change. Many people think that the Karner blue should be a species rather than a subspecies but the research has not been completed to determine if and how the scientific name should change.

12. Subspecies map

There are six *Lycaeides melissa* subspecies. This map shows the geographic distribution of each subspecies (Lane and Weller 1994). The Karner blue range does not overlap with any of the other subspecies; therefore, it does not have the opportunity to interbreed in nature. In Minnesota, its range is closest to *Lycaeides melissa melissa,* or the Melissa blue. The Karner blue is the only subspecies in which the larvae exclusively eat wild lupine (*Lupinus perennis*), but other subspecies' larvae eat wild lupine and/or other lupine species.

13. Two pictures, wings closed. Melissa blue, Karner blue – both males.

The Melissa blue has more orange on the underside of the wing than the Karner blue.

14. Two pictures, one male and one female Melissa blue

The female Melissa blue has more orange banding on the upper surface of the fore wing than the Karner blue. The upper, blue surface of the male looks very similar in both species.

Life cycle

15. Four pictures of the four life cycle stages

Like all butterflies, Karner blues go through four stages: egg, larvae, pupa, and adult. (Magnification varies in each picture.)

16. Egg

This egg is on a lupine stem (the picture is magnified). Eggs are approximately 0.7 mm (.03 inches) in diameter. Females lay eggs singly on the lupine or plants near the lupine (U.S. Fish and Wildlife 2003).

17. Lupine leaf, hatched egg and newly hatched larvae

Just above the white egg case with the tiny hole in the middle is a newly hatched larva. Larvae are approximately 1.5 mm (.06 inches) long when they hatch and they immediately start eating lupine (Herms et al. 1996). Unlike many other butterflies, Karner blue larvae don't eat their egg case.

18. Close up of larvae

The larval stage is focused on eating and growing. You can also see the black pile of frass, or feces, in this photo. Karner blue larvae go through four instars while growing from 1.5 to 8.5 mm (.06 inches to .33 inches). This development typically occurs in about 17 days. Development time varies with temperature and food quality (Herms et al.1996). The follow slides show more details of the larvae stage. (This photo is taken through a dissecting microscope.)

19. Feeding damage on lupine

This feeding damage is a referred to as "window pane-ing." A larva eats most of the leaf but leaves the upper epidermis or "skin" of the plant. The feeding damage is transparent like a window pane.

20. Monarch larva on butterfly weed

Monarch larvae look extremely different from Karner blue due to their brightly colored stripes and large size. These differences are due to different strategies to avoid natural enemies (predators and parastoids). The monarch eats milkweed species and becomes poisonous to predators by sequestering a toxin found in milkweed. The bright coloration warns predators that it tastes bad. The next few slides show strategies the Karner blue larvae uses to avoid natural enemies.

21. Larva under lupine leaf

See if you can find the larva in this picture.

22. Larva under lupine leaf, yellow circle

The larva is inside the yellow circle. The Karner blue uses camouflage as a strategy to avoid natural enemies.

23. Larva with ants in the center of a lupine leaf

The Karner blue also attracts ants to avoid natural enemies. Larvae attract ants by secreting a liquid from glands on the top of their body. The ants feed on the liquid from these glands. Ant presence increases the survival of Karner blue larvae, but the exact method that increases survival is unknown. The ants' presence may simply deter natural enemies from attempting to attack the larvae or the ant may actively defend the larva. There are several ant species that "tend" Karner blue larvae (U.S. Fish and Wildlife Service 2003). Ant tending is common in other butterfly species related to the Karner blue.

24. Pupa

After approximately three weeks the larva changes into a pupa. The larva uses its silk gland to attach to a surface, commonly oak leaves.

25. Emerging adult crawls out of the pupa case

After approximately seven days the adult crawls out from the pupa case.

26. Recently-emerged adult on oak leaf straightening its wings

The adult must quickly pump its wings full of blood and let them dry in order to fly. The adult stage is adapted for flight and drinking liquids. The colorful wings of a butterfly are made from tiny scales. The colors and patterns of the wings help a butterfly recognize the opposite sex of its own species.

27. Karner blue perched on person's thumb

Butterfly mouth parts make a dramatic change, from a larva jaw adapted to eating leaves to an adult straw. The arrow is pointing to a specialized mouth part, the proboscis, which works like a straw and allows a butterfly to drink liquids. Butterflies often use their proboscis to ingest nectar from flowers. The butterfly pictured here is probably drinking perspiration from the person's thumb. Salt is an important nutrient for male butterflies. It is common for groups of male butterflies to drink liquids from moist soil or mud puddles.

28. Mating

After mating, females lay eggs on or nearby lupine. Adults live a short time, only about one week.

29. Phenology Diagram

Karner blues have two generations each year. Eggs laid during the summer flight stay in the egg stage all winter and will not hatch until the following spring. In early May the eggs hatch, and spring flight adults emerge in late May/early June. These adults mate, lay eggs, and then die. Their eggs start development immediately and they emerge as adults in late June/early July (summer flight). The eggs laid by the second flight adults over-winter and the cycle continues. Karner blue butterflies don't migrate.

Ecology

30. Male Karner blue

The ecology section focuses on interactions of the Karner blue and it's habitat.

Habitat

31. Field with trees in background

Karner blue butterflies live in oak savanna and pine barrens habitats. These habitats are located where the prairie begins to transition into woodland, and typically contain numerous prairie grasses and flowers with patches of oak or pine trees. Butterfly habitat must contain food for the caterpillar and adult.

32. Wild lupine

Wild lupine (*Lupinus perennis*), pictured here, is the only food the Karner blue caterpillar can eat. Because the Karner blue is specialized to eat only one plant species, without this specific species the butterfly cannot survive.

33. Three pictures: horsemint, butterfly weed, leadplant

The adults, however, will drink nectar from numerous plant species. Three of the 80 plants Karner blues have been seen feeding from are picture here. Butterfly weed (*Asclepias tuberosa*) is the orange flower, horsemint (*Monarda punctata*), is the pink flower, and leadplant (*Amorpha canescens*) is the dark purple flower.

34. Trees and short ground cover

A mixture of tree cover is important for Karner blue survival (Lane and Andow 2003). Larvae generally survive better in shaded areas partially because they grow faster when eating from lupine that is growing in shade (Lane 1999). However, adults are commonly seen in open, sunny areas where blooming flowers are common. Oak savanna and pine barrens are good Karner blue habitats because they naturally provide a mixture of open areas with a variety of shade from tree cover.

Ecosystem Processes

35. Hillside with lupine in the foreground, shrubs and trees in the background

Ecosystems change throughout time. Succession and disturbance are two main forces that change ecosystems and a combination of these forces is critical for the continuation of Karner blue survival. **Succession** is a natural process by which plant communities change. For example, an open area will often change from grass-to-shrub-to-tree-dominated with time.

36. Three pictures: Elk, fire, steep hillside

Disturbances caused by wild fires, animal activity, disease and topography reverse effects of succession. These disturbances can subtly or dramatically reduce the tree and shrub cover.

Fire was a very important disturbance that naturally occurred in savanna/ barrens. A low intensity fire may only kill young trees, but a high intensity fire can kill large trees and remove a significant percent of the canopy. Fires naturally occurred in this landscape and the savanna/ barrens ecosystem evolved with this disturbance. Bur and black oak trees, for instance, have thick bark to protect themselves from fire.

The elk pictured here along with bison and deer lived in the Minnesota savanna/barrens prior to European settlement. **Animal** activity, including grazing and trampling of young trees and shrubs by these mammals, could have reduced the likelihood of savanna becoming woodland (U.S. Fish and Wildlife Service 2003).

Oak wilt is a **disease** that can kill mature oak trees. Because this disease can be spread by the roots, small openings in woodland are created when oak wilt kills a group of trees.

Topography can also cause disturbances that reverse or prevent tree and shrub growth. In this photo there are no trees growing on the steep slope in the foreground. Due to soil erosion it is more difficult for trees to become established on steep slopes especially with sandy soil. In addition, a south-facing hillside tends to be drier, thus reducing plant growth (U.S. Fish and Wildlife Service 2003). In WWMA several remaining Karner blue habitat patches are found on dry, steep, sandy hillsides. (The steep hillside picture is from WWMA)

37. Trees with fall colors
Ideally several different successional states will occur in savanna/ barrens. This allows wildlife, including Karner blue butterflies, to respond to the constantly changing habitat through dispersal. (The opening in the trees in the middle of the slide was created to improve the Karner blue habitat in WWMA.)

Conservation

38. Karner blue male
The conservation section explains ways in which people interact with the Karner blue and its habitat. This section includes the following topics: threats, management, flagship species, research, organization, and how you can help.

39. Historic sites map
In other to help Karner blues survive we need to know the **threats**. Habitat loss and degradation are the two main threats to Karner blue survival. Both pine barrens and oak savanna are threatened ecosystems due to dramatic habitat loss since European settlement. For example, only 0.02% of pre-settlement oak savanna acreage remains in Minnesota (Nuzzo 1986). The red squares on this map show places where Karner blues lived in the past that no longer support populations. **Habitat loss** occurs when humans dramatically change the native habitat. This occurs, for instance, when a city is built or native plants are plowed to create an agriculture field. **Habitat degradation** occurs in savannas and barrens when these areas become woodlands due to succession. (Note: historic sites marked on the map only include sites outside of the recovery and potential recovery areas. This map is found on this compact disk in the Karner Blue Recovery Plan, Appendix B).

Management

40. Four pictures: prescribed fire, tree stump, carrying seedlings, machine
Management is critical for the maintenance of savanna/ barrens habitat. **Prescribed fire** is an effective management method used to maintain savanna and barrens habitat. However none of the life stages of a Karner blue can survive a fire, therefore managing habitat where a small population exists is challenging. In WWMA managers only burn outside of a Karner blue occupied area. In areas where a large population of Karner blues live the population is able to re-colonize naturally after a fire.

The picture of the **tree stump** from WWMA shows another management option that results in reducing tree and shrub cover. Woody vegetation can be cut or mowed. Specialized equipment can be used to clear larger areas; a **machine** aptly called a "shedder" is pictured here and has been used to manage Karner blue habitat at Fort McCoy, WI. Some shrubs and trees will quickly re-sprout after cutting and spot herbicide is often used to reduce their growth.

In this photo butterfly weed **seedlings** are being carried into Karner blue habitat. The seedlings were planted into a WWMA valley to increase the nectar plant diversity and quantity for Karner blues. Increasing numbers of desirable plants by scattering seeds is another common management practice.

Flagship species

41. Female Karner Blue on leadplant
When a charismatic species attracts considerable attention it is referred to as a flagship species. The Karner blue is an unusual flagship species because most others are large vertebrates like whales, bears, and tigers. Flagship species can help attract resources (financial and others) to assist recovery of the species and the species' habitat. As a result, efforts to help one species can result in survival of numerous species that share the same ecosystem. The following slides show some of the species that share the savanna/ barrens ecosystem with the Karner blue, and would benefit from ecosystem management (U.S. Fish and Wildlife Service 2003).

42. Three pictures, two flowers and one insect
Goat's rue *(Tephrosia virginian)* is classified as MN special concern.
(bottom left -white and pink flower)
Rough-seeded fame flower (*Talinum rugospermum*) is classified as MN endangered. It is hard to find when it is not blooming and it only blooms during the afternoon and evening.
(top left - pink flower, yellow stamens)
Phlox flower moth (*Schinia indiana*) is classified in WI as state endangered and federal special concern. (right - pink flower with a moth at the center)
Nine additional rare Minnesota plants are associated with Karner blue habitats. (The names of these nine species can be found on this compact disk in the Karner Blue Recovery Plan, Appendix D.)

43. Two pictures, turtle and snake
The eastern hognose (*Heterodon platyrhinos*) is a rare species found in Karner Blue habitats in Minnesota.
Blandings turtle (*Emydoidea blandingii*) is classified in MN as state threatened and federal special concern.

44. Three pictures, one flower and two butterfly
Black eyed susan (*Rudbeckia hirta*) - yellow flower brown center
Eastern tiger swallow tail (*Papilio glaucus*) - black and yellow butterfly
Monarch butterfly (*Danaus plexippus*) - orange and black butterfly

45. Three pictures, one flower, one bird, one fawn
Butterfly weed (*Asclepias tuberosa*) - orange flower
Indigo Bunting (*Passerina cyanae*) - blue bird
White tailed deer fawn

Research

46. Person in the lab with Karner blue larvae and lupine leaves
Research can help guide management decisions. While a graduate student at the University of Minnesota, Cynthia Lane studied the different habitat needs of larvae and adult Karner blues. She found that larval survival was higher in areas where lupine grew in shade, but that sunny areas provide adults with more nectar plants. Her research implies a variety of tree cover in close proximity is an important habitat characteristic that managers can work toward (Lane 1999). Margaret Savanick studied adult nectar feeding preferences. While Karner blue adults drink nectar from over eighty different plant species, they may prefer particular species. Additional plants of the preferred species could help increase the Karner blue population. The woman in this picture is helping to raise Karner blues for Margaret's study.

47. Three pictures: cage, dish with eggs, person in lab
Captive rearing is used in both research and management projects. Captive rearing dramatically increases the number of individuals that reach the adult stage. This provides insects for experimentation and/or population supplementation. The following slides show some steps involved in captive rearing. Females caught in the field are transported to the lab and placed in a cage with lupine and nectar sources, like the cage on the top left. After laying eggs for several days the females are released where they were captured. Eggs are carefully removed from the plant and placed in a container. The small dots in the petri dish on the lower left are eggs. The woman on the right is feeding the larvae fresh lupine and cleaning out their dishes, which needs to be done approximately every other day. (The cage was purchased from Bioquip, it is called a "bugdorm".)

48. Three pictures: larvae dish, rearing chamber, person in field
The upper left picture is a typical larva-rearing dish. Floral tubes keep the lupine fresh, and oak leaves provide a pupa attachment surface. Insect rearing chambers, like the one on the right, provides a predator-free artificial habitat with suitable temperature, light,

and humidity. Captively reared Karner blues are released back into their habitat and studied. The woman on the lower left is observing a recently-released adult.

49. U.S. Fish and Wildlife Service recovery plan map
This map shows the recovery areas for Karner blue. The recovery units, colored yellow, are first priority and potential recovery units, colored blue, are second priority. These areas were chosen by a team of experts who wrote the recovery plan for the U.S. Fish and Wildlife Service. (For more information see the Karner Blue Recovery Plan, Appendix B)

Organizations

50. Yellow diamond sign
Numerous governmental and non-governmental organizations have been involved in the conservation efforts for the Karner blue. Many efforts involve partnerships between several organizations.

The **U.S. Fish and Wildlife Service** is responsible for the implementation of the Endangered Species Act.

Minnesota's and Wisconsin's **Department of Natural Resources** play a very active role in Karner blue conservation. The Minnesota DNR has been actively monitoring and managing the site where the only remaining Minnesota population is found. The Wisconsin DNR has led an innovative, grassroots approach to organize 26 traditional and non-traditional conservation partners in a statewide plan.

Fort McCoy, an army training base, in Wisconsin has been actively managing, monitoring, and studying Karner blue populations and habitats found on the army land. (This yellow diamond sign, from Ft. McCoy, is a standard sign used in Army bases to mark areas where endangered species live.)

51. Karner blue on yellow flower
Non-governmental organizations play an important role in Karner blue conservation.

The **National Wildlife Federation** is very involved in education programs about endangered species.

The Nature Conservancy is involved in Karner blue conservation in several states. In Indiana, Karner blue populations have been supplemented through captive rearing programs supported by The Nature Conservancy.

The **Toledo Zoo** and **The Nature Conservancy** have joined resources to reintroduce the Karner blue to Ohio. Over 1,300 Karner blues, captively reared at the Toledo Zoo, have been released on local Nature Conservancy land between 1998 and 2003.

Waupaca Field Station efforts illustrate the impressive conservation impact of a private landowner. Bob Welch and Deb Martin own and manage 7,500 ares of restored prairie and oak savanna land adjacent to their home in central Wisconsin. In addition, the Waupaca field station is surrounded by 119,500 acres of public land with similar management objectives. They started the private, nonprofit organization Waupaca Field Station to facilitate opportunities for education, conservation and research on their land. Bob Welch is a middle school teacher in Waupaca, and his students volunteer with numerous research projects. Their land is willed to The Nature Conservancy.

52. Karner blue on thumb

How you can help the Karner blue and other endangered species.

a. Educate others – Introduce friends and family to the Karner blue
b. Personal action – Minimize the resources you use in your day to day life. For example: reduce, reuse, recycle; conserve water; support mass transit; when possible bike or walk rather than traveling by car.
c. Support conservation organizations - volunteer, financial support
d. Political support - Encourage your elected representatives to support conservation efforts. For example, encourage government officials to increase funding for the Endangered Species Act. The Act requires actions but enacting a law does not directly provide funding for those actions. There is considerable debate on the effectiveness of the Endangered Species Act. According to one analysis "current funding is less than 20 percent of the amount we estimate it will take to get the job done. This illustrates that the passage of legislation such as the Endangered Species Act does not guarantee the funding that is essential to fulfill the law's mandates. If the public values biodiversity and endangered species, more congressional funds will need to be appropriated for the endangered species program." (Miller et al. 2002)

53. Male Karner blue

A quote from novelist Vladimir Nabokov: In a 1948 edition of The New Yorker he said, "I confess I do not believe in time. I like to fold my magic carpet, after use, in such a way to superimpose one part of the pattern upon another. Let visitors trip. And the highest enjoyment of timelessness – in a landscape selected at random – is when I stand among rare butterflies and their food plants. This is ecstasy. . ." (Andow et al. 1994)

54. Credits

55. Acknowledgements

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Additional Information on the Karner Blue Butterfly

Karner Blue Butterfly Recovery Plan
An electronic copy of the recovery plan is included on this compact disk.

Endangered Species Act
Federal endangered species status adds considerable legal assistance to conservation of this species. The species and its habitat are legally protected under the Act. It is illegal to kill, harm or harass an endangered species. Special permits are required to research the Karner blue and manage their habitat. In addition, the U.S. Fish and Wildlife Service is required to develop a recovery plan for every listed species that details the actions needed to recover the population.

National Wildlife Federation
www.nwf.org
The National Wildlife Federation is very involved in endangered species conservation. For the 25 year anniversary of the Endangered Species Act the National Wildlife Federation started the "Keep the Wild Alive" campaign and they chose 25 endangered species to profile, including the Karner blue. "Keep the Wild Alive employs education, advocacy, policy and conservation initiatives to implement local and national projects that support endangered species conservation and to help people understand how their actions can help protect wildlife and wild places" (NWF website). The campaign emphasizes education and local conservation initiatives. The species recovery fund is another important aspect of the campaign. Each year this fund provides financial support for local conservation efforts to support conservation of imperiled species.

Non-traditional organizations
A diversity of organizations play a role in Karner blue butterfly conservation because the contemporary habitat of the Karner blue includes altered habitat where lupine and nectar plants grow. Examples of altered habitats include: roadsides, airports, logged forests, restored agricultural fields, military bombing ranges, mowed right-of ways for electric or gas lines.

Wisconsin Statewide Habitat Conservation Plan
www.dnr.state.wi.us/org/land/er/publications/karner/karner.htm
Through the Wisconsin Statewide Habitat Conservation Plan the Wisconsin DNR has led a grassroots approach to organizing traditional partners (eg. The Nature Conservancy) and non-traditional partners (eg. Department of Transportation, paper companies, electric and power companies) in the development of a large scale conservation plan. As a result a total of 26 partners plan to apply the conservation plan on over 250,000 acres (U.S. Fish and Wildlife Service 2003).

Save the Pine Bush

www.savethepinebush.org

This organization in Albany, NY helped identify the importance of the Karner blue butterfly. Also, they have protected local habitat where the Karner blue was first identified.

Toledo Zoo

In Ohio, the local Karner blue population went extinct in the late 1980's or early 1990's (Andow et al. 1994). Starting in 1998 the Toledo Zoo and The Nature Conservancy have joined resources to reintroduce the Karner blue to Ohio. For the last five years reintroduction of captively reared individuals has occurred on local Nature Conservancy land. According to Dr. Peter Tolson, the Director of Conservation and Research at the Toledo Zoo "We've released 1364 Karner Blue Butterflies to the Nature Conservancy's Kitty Todd Preserve from 1998-2003. This is the only site so far, although two additional sites, one in the Oak Openings Metropark (Campbell Prairie) and other in a separate area of KTP (Moseley Barrens) are being prepared for additional releases. Our measure of success is numbers of Karner Blue Butterflies (evaluated by transect counts and Lincoln Peterson mark-recapture estimates) at KTP equivalent to or exceeding numbers at sites in the Allegan State Game Area, Allegan, MI where the founder stock (wild female butterflies) was obtained." (personal communication, 2003)

Butterfly Conservation Initiative

A partnership between the US Fish and Wildlife Service and a non-profit zoo group, American Zoo and Aquarium Association, created the Butterfly Conservation Initiative. The goal of this initiative is to focus on conservation of the federally endangered butterflies (22 butterflies were on the list in 2002). The Butterfly Conservation Initiative organized "The Karner blue Butterfly Recovery Implementation Workshop" at the Toledo Zoo in June of 2002. Recovery efforts of the Karner blue is planned to be used as a model for conservation of other species.

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APPENDIX B

A Karner blue butterfly male lifespan record

A male Karner blue butterfly was still alive after 29 days. This captively-reared male emerged on July 10th then lived in a cage for five days due to inclement weather. On July 15th it was released in to Cuthrell valley in the Whitewater Wildlife Management Area in Minnesota. (For more information on the release area, see Chapter 2) I re-sighted this butterfly 24 days later, on August 8, 2003, recognizable by a single dot from a sharpie pen mark on its wing. I marked all butterflies emerging on the same day with a unique group marking. Its wings were extremely worn and almost transparent. I observed it feeding and flying. This individual lifespan data is significant because the mean adult lifespan estimated by mark release recapture data is four days, but researchers think they can live for two to three weeks (U.S. Fish and Wildlife Service 2003). This is the longest recorded lifespan of a Karner blue butterfly living in a natural ecosystem. Previous data from mark-release-recapture studies have recorded maximum time between recapture as 18 days for a female, 14 days for a male (Bidwell 1995), and a 14.95 days for both sexes (Knutson et al. 1999).

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APPENDIX C

Female Karner blue butterfly with an unusual wing color pattern

In Cuthrell valley of Whitewater Wildlife Management Area in Minnesota on June 17, 2002 a female Karner blue butterfly was found with an unusual color pattern on her hind wings. Females typically have several orange crescents near the margins of the upper (dorsal) side of the hind wing (Klots 1979). This individual did not have any orange crescents on the dorsal side of her wings (Figure 1). She did have orange crescents on the underside of her wing (Figure 2). She was collected and held in a cage to collect eggs for a captive rearing project. She laid 112 eggs, more than any other female collected, over three days. Unfortunately she died in captivity from an unknown cause. We did not keep exact records on her progeny but the offspring, reared from 8 mothers, did have a mixture of color patterns, some had orange crescents and some did not have orange crescents on the dorsal hind wing. The captive-reared adults, including her progeny, were released into Cuthrell and Lupine valley on both July 22 and 26, 2002.

Figure 1

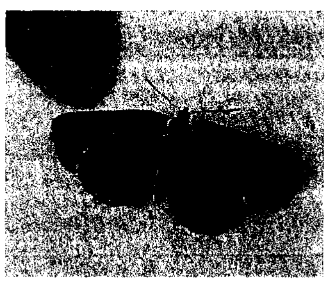

Figure 2

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