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Use of Resources by Adult Karner Blue Butterflies (*Lycaeides melissa samuelis*) in Michigan: Comparisons of Nectar Selections on Native and Non-native Species

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Use of Resources by Adult Karner Blue Butterflies (*Lycaeides melissa samuelis*) in Michigan: Comparisons of Nectar Selections on Native and Non-native Species

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Abstract

The endangered Karner blue butterfly (*Lycaeides melissa samuelis*) is at risk due to the decline of oak barrens and dry sand prairies, the butterfly's sole habitat. Survival depends on effective conservation of remaining habitat and creation of new viable habitats. Successful management must consider resource needs for both larval and adult stages. Information on adult nectar requirements is limited (Grundel *et al.* 2000, Pickens & Root 2008). Sufficient nectar increases lifespan and fecundity of butterflies (Murphy *et al.* 1983, Hill & Pierce 1989, Fischer & Fielder 2001), and deters emigration. Encroachment of non-native nectar species into butterfly habitat warrants further investigation into how non-natives are being utilized by Karner blues before they are eradicated. The Huron-Manistee National Forest in Michigan currently hosts some of the largest populations of Karner blue butterflies left in the wild (USFWS 2003). In order to provide optimal resources for adults, we had three main objectives: determine if occupied sites provide sufficient nectar resources for both generations, investigate possible nectar preferences and establish if adults are relying on non-native nectar species. During the summers of 2019 and 2020 we investigated nectar selection preferences of Karner blue butterflies and used logistic regression analyses to compare selected nectar species with other species available within 2 m. Additionally, we examined how non-native species were being used by adult Karner blues. Logistic regression analyses were used to compare selected nectar plants with unselected nectar plants available within a 2 m radius. Results found that during first generation flight periods, nectar sources were limited in abundance and diversity and butterflies had an affinity for Northern dewberry (*Rubus flagellaris*). Resources available for second

generation butterflies were more abundant and diverse. However, population numbers are generally three to four times larger in this brood and additional resources may be needed (Lawrence & Cook 1989, Schweitzer 1989, Bleser 1992, Leach 1993, USFWS 2003, Pickens & Root 2008). Second generation butterflies preferred butterfly weed (*Asclepias tuberosa*), horsemint (*Monarda punctata*) and a non-native species, spotted knapweed (*Centaurea stoebe*). Recommendations include: bolster nectar plant abundance and diversity available during the first generation, increase abundance of butterfly weed and horsemint for second generation butterflies and restore the pollinator community with sufficient native nectar resources before eradicating spotted knapweed.

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Chapter 1: Introduction

The Karner blue butterfly (*Lycaeides melissa samuelis)* is a federally endangered species that inhabits the upper Midwest and Northeast regions of the United States. Originally extant in 12 states and the province of Ontario, Canada, Karner blue populations are now less than 1% of their original size (USFWS 2003) and have been extirpated from Canada and at least seven states (D. Marsh, personal communication, February 27, 2023). Today, small remnant populations can be found in Ohio, Michigan, New Hampshire, New York and Wisconsin (D. Marsh, personal communication, February 27, 2023). Wisconsin and Michigan currently support the largest number of local populations, with the greatest number of butterflies (Kleintjes *et al*. 2003, USFWS 2019).

Historically the Karner blue butterfly flourished in oak barrens and dry sand prairies (Dirig 1994, USFWS 2003). Maintained by frequent disturbance and characterized by low tree density and canopy cover, the understory of oak barrens are dominated by grasses and herbaceous species (Curtis 1959, Nuzzo 1986). Urbanization and agricultural practices have led to fragmentation, development and degraded succession that have destroyed 99% of these lands (Dirig 1994, Grundel *et al.* 1998, USFWS 2003). Many species, like the Karner blue, have come to rely upon the unique composition of plant species and shade heterogeneity that these ecotones provide. Open sunny areas foster behaviors such as foraging and mating, and provide a variety of nectar plants for adult butterflies (Dirig 1994, Grundel *et al.* 1998), while the shade gradient along the borders of openings provides shade-related trade-offs between the Karner blue's sole larval host plant, wild lupine (*Lupinus perennis*) and ovipositing females (Grundel *et al*. 1998).

Survival of the Karner blue butterfly depends on effective conservation of remaining habitat and creation of new viable habitat. Successful management plans must include necessary requirements for larval and adult stages (Grundel *et al*. 1998, Schultz *et al*. 2003). Though research on the Karner blue's larval host plant is extensive, information on resources for adults is limited (Grundel *et al.* 2000, Pickens & Root 2008). Nectar provides butterflies with essential water, sugar and amino acids that aid in maintenance and reproduction (Boggs 1987, Schultz & Dlugosch 1999). To reproduce successfully, females must obtain sufficient nectar to meet energetic and nutritional requirements necessary for egg production and survival (Boggs & Ross 1993, Fischer & Fiedler 2001, Boggs & Freeman 2005, Janz 2005, Chau *et al*. 2020), while males must procure adequate nectar to survive and find mates (Scharf *et al*. 2013, Chau *et al*. 2020).

Nectar resources are critical in maintaining butterfly populations (Hill 1992, Schultz & Dlugosch 1999), and adult butterfly population densities have long been shown to have a direct relationship with nectar abundance and diversity (Gilbert 1971, Ehrlich & Gilbert 1973, Murphy 1983, Grossmueller & Lederhouse 1987, Schultz & Dlugosch 1999). For example, Britten & Riley (1994) found that sites with increased nectar abundance were more likely to support populations of the uncompahgre fritillary (*Boloria acrocnema*), and Williams (1998) determined that population sizes in Gillett's checkerspot (*Euphydryas gillettii*) increased with greater nectar diversity. Similarly, insufficient nectar sources can limit Karner blue butterfly populations (Lawrence & Cook 1989, Dunn 2008). Sites that are more abundant in nectaring plants tend to support larger populations of Karner blues (Lawrence 1994, Bidwell 1995), and distribution of the butterfly is restricted in comparison to the range of its host plant, suggesting that other requirements are needed for inhabitation (Schweitzer 1989, Bleser 1992, Leach 1993, Dirig

1994, Grundel *et al*. 1998, Grundel *et al.* 2000, USFWS 2003); as was observed when the Karner blue was officially extirpated from Ontario, Canada after a series of droughts drastically decreased nectar resources available for adults (Packer 1994, USFWS 2003).

Previous studies have found that adult butterfly longevity and fecundity increase with sufficient nectar (Murphy *et al*. 1983, Hill & Pierce 1989, Fischer & Fielder 2001). For example, when sugar content was increased in the diets of Edith's checkerspot (*Euphydryas editha*), it prolonged life of the butterflies, increased total egg production, produced larger egg masses and extended the period of egg production (Murphy *et al*. 1983). Furthermore, unlaid oocytes in female Mormon fritillary (*Speyeria mormonia*) declined in direct proportion to increased sugar content provided in diets, indicating that resources can be allocated away from reproduction when butterflies are under resource stress (Boggs & Ross 1993). Lastly, when sufficient sugar was provided in diets of the common imperial blue butterfly (*Jalmenus evagoras*), females laid three times more eggs (Hill & Pierce 1989). Butterflies also compensated for lower sugar in diets by feeding for longer periods of time (Hill & Pierce 1989). Allocating more time to feeding can leave butterflies less time for behaviors such as mating and ovipositing (Schultz & Dlugosch 1999) and increases susceptibility to predators (Hill & Pierce 1989).

In Michigan, the largest Karner blue butterfly populations are located in remnant oak barrens and sand prairies within the Huron-Manistee National Forest (HMNF) Baldwin/White Cloud Ranger District (USFWS 2012). Occupied Karner blue habitat is actively managed with frequent burns and cuttings directed by the United States Department of Agriculture Forest Service (USDAFS). Understanding foraging behaviors of Karner blue adults and investigating possible preferred nectar plants would give valuable information for management, as these areas are re-seeded with new nectar species after being disturbed. Providing optimal nectar sources for

adults could increase population sizes and provide more stability, as populations with low numbers are at a greater risk of extirpation from unusual weather events, disease and predation (Dunn 2008).

Past research on nectar selections by Karner blue butterflies suggests that they are opportunistic foragers, but some studies indicate that specific nectar species are used selectively rather than in proportion to availability (Schweitzer 1989, Lane 1994, Lawrence 1994, Herms 1996, Grundel *et al*. 2000). Optimal foraging theory predicts that animals will select resources that provide the greatest reward (Pyke *et al*. 1977, Rhiannon & Schultz 2016). Selection of specific nectar species may indicate that these particular resources are of more value to the Karner blue (Manly *et al*. 2002, Ezzedine & Matter 2008, Rhiannon & Schultz 2016).

It's also essential to determine if Karner blue adults are exploiting non-native species in managed occupied habitat. Non-native and invasive species can compete for nutrients, water, light and space with native species (Brown *et al*. 2002), and native plant species have been shown to decline in habitats where aggressive exotics have taken over (Brown *et al.* 2002, Schultz *et al*. 2003). Lack of native nectar sources can limit population sizes of butterflies, as was found in investigations of nectar use by the Fender's blue (*Icaricia icarioides fenderi*), a species that is also in the Family *Lycaenidae* and has similar life history traits to the Karner blue (Schultz and Dlugosch 1999). Estimates of Fender's blue butterfly resources found that population sizes did not correlate with overall abundance of flowers or total available flower nectar, but were correlated with abundance of native nectar sources and total native nectar (Schultz and Dlugosch 1999).

There is evidence that removal of non-native nectar species can increase abundance of native nectar plants (Brown *et al*. 2002, Basket *et al*. 2011). However, there is also the potential for pollinator populations to crash following the widespread removal of non-native species that are common (Gibson *et al*. 2006, Carvhaleiro *et al*. 2008, Basket *et al*. 2011). Previous research has shown that native butterflies utilize nectar from non-native plants (Bergerot *et al*. 2010, Thomas & Schultz 2016, Staab *et al.* 2020, Rivest *et al*. 2023). Empirical evidence comparing phenologies of natives and non-natives, suggests that non-natives can supplement nectar when natives are no longer in bloom, as non-natives can flower for longer and flower at different times (Gerlach & Rice 2003, Pearson *et al*. 2012, Rivest *et al*. 2023). However, the quality of nectar found in non-natives is questionable, as non-natives often trade lower quality nectar for increased growth rate and showy flowers (Basket *et al*. 2001). It's crucial to understand the impact that non-natives have on an ecosystem before drastic eradication, especially when considering that a fragile species may be relying on them.

To provide optimal resources for Karner blue adults in the HMNF we investigated the foraging habits of Karner blue adults during the summers of 2019 and 2020. Our main objectives were to determine if these areas provide sufficient nectar resources for both first-and second-generation butterflies, examine possible nectar selection preferences and establish if butterflies are relying on non-native or invasive nectar species. This information can then be used by the USDAFS to help aid in future management of adult resources in occupied sites within the HMNF Baldwin/White Cloud Ranger District.

Purpose

The purpose of this study is to gain a better understanding of adult resource use by the endangered Karner blue butterfly, as information on adult needs is limited (Grundel *et al.* 2000, Pickens & Root 2008), and is absent from occupied Karner blue habitat within the HMNF Baldwin/White Cloud Ranger District. Furthermore, determining if non-native nectar species are being used as a resource for this butterfly is critical before removing non-native species from occupied habitat. Information gained from observing foraging behaviors will be useful in future management of Karner blue habitat, specifically in decisions about nectar species used to re-seed areas that have been newly disturbed and in the management of non-natives.

Scope

In order to investigate nectar selection preferences and possible use of non-native species by adult Karner blue butterflies, we investigated the foraging habits of Karner blue butterflies in the HMNF in Otto Township, Oceana County, Michigan during 2019 and 2020. We observed 273 selections made by 172 first generation butterflies, and 520 selections made by 311 second generation butterflies. Logistic regression analyses were used to compare observations and determine the probability of a Karner blue selecting a nectar species when other species were available within 2 m. Though previous studies have investigated nectar selections made by Karner blue butterflies, few have compared how frequency of nectar plants impact selection and even fewer have quantified this with statistical analyses. Additionally, concerns of how to manage non-native species requires further investigation into use of non-natives by Karner blue adults, especially considering that resources have been reported to be low in abundance during this time (Schweitzer 1989, Bleser 1992, Haack 1992, Leach 1993).

This study focused on populations of Karner blue butterflies in occupied sites located within the HMNF, and limits this study to subpopulations inhabiting this specific location. Though these results can be used to gain a better understanding of resource use and needs for Karner blue adults, results do not represent use of nectar plants by the entire pollinator community. We also cannot definitively conclude what these observations interpret about selection preferences of butterflies without more in-depth research into the quality and quantity of nectar provided by nectar species observed in this study.

Assumptions

Butterflies initially test nectar species in order to detect sufficient sugar and nutrients before feeding (Bakowski *et al*. 2010), and in this study we assumed that when a butterfly inserted their proboscis for less than five seconds, the flower was being tested and was not selected by the butterfly to feed upon. Based on optimal foraging theory (Pyke *et al*. 1977), we also assumed that butterfly satiation is equal among butterflies we observed, and when a nectar species was estimated to be more likely selected, the preferred species was more frequently selected because it offered a greater reward in either quality or quantity of nectar.

Objectives

In order to provide optimal resources for Karner blue adults in the HMNF we aimed to address three main objectives: 1) determine if occupied habitat provided sufficient nectar resources in bloom for both first-and second-generation butterflies, 2) investigate possible nectar selection preferences for adults and 3) establish if butterflies utilize any non-native or invasive nectar species as a resource.

Significance

The Karner blue butterfly is considered an indicator species for oak barren habitats. Sites that are occupied by Karner blue populations indicate that the habitat is meeting the environmental conditions needed to support early successional dependent species. The oak barrens of Michigan support various imperiled groups which include: two species of mammals, three birds, six reptiles and amphibians, 14 invertebrates and 50 species of plants (USFWS 2003). Some noteworthy species include: Eastern box turtle (*Terrapene c. carolina*), prairie warbler (*Dendroica discolor*), frosted elfin (*Callophrys irus*), phlox moth (*Schinia indiana*) and prairie smoke (*Geum triflorum*). Increased survival and fitness of the Karner blue is likely to increase the success of numerous plants, pollinators and animals that also inhabit these ecosystems.

Chapter 2: Review of Literature

The Karner blue butterfly (*Lycaeides melissa samuelis)* is a federally endangered species that inhabits the upper Midwest and Northeast regions of the United States. Originally extant in 12 states and the province of Ontario, Canada, Karner blue populations are now less than 1% of their original size (USFWS 2003), and have been extirpated from Canada and at least seven states (D. Marsh, personal communication, February 27, 2023). Today, small remnant populations can be found in Ohio, Michigan, New Hampshire, New York and Wisconsin (D. Marsh, personal communication, February 27, 2023). Wisconsin and Michigan currently support the largest number of local populations, with the greatest number of butterflies (Kleintjes *et al*. 2003, USFWS 2019). However, these populations are unstable and are at risk of extirpation.

Karner blue butterflies are a bivoltine species. First generation adults typically emerge in late May and end in late June, while second generation butterflies generally begin to emerge in early July and last through August (Grundel *et al.* 1998, Pickens & Root 2008, USFWS 2003, Dunn 2008). Second generation butterflies lay eggs that overwinter for nine months and become the first brood the following year (Grundel *et al*. 1998, Pickens & Root 1998, USFWS 2003). Emergence can vary from year to year and depends heavily on geographic location and local weather conditions (Haack 1992, USFWS 2003). Broods typically start to peak two weeks after emergence begins (USFWS 2003). The ratio of male to female butterflies changes throughout this duration and indicates the stage of the peak (USFWS 2003). During peak flight periods, the sex ratio of butterflies usually exceeds 50% males. As the flight period progresses, the ratio of males declines (USFWS 2003, Dunn 2008).

Historically the Karner blue butterfly flourished in oak barrens. Maintained by frequent disturbance and characterized by low tree density and canopy cover, the understory of oak barrens is dominated by grasses and herbaceous species (Curtis 1959, Nuzzo 1986). Urbanization and agricultural practices have led to fragmentation, development and degraded succession that have destroyed 99% of these lands (Dirig 1994, Grundel *et al.* 1998, USFWS 2003). Many species, like the Karner blue, have come to rely upon the unique composition of plant species and shade heterogeneity that these ecotones provide. Open sunny areas foster behaviors such as foraging and mating, and provide a variety of nectar plants for adult butterflies (Dirig 1994, Grundel *et al.* 1998), while the shade gradient along the borders of openings provides shade-related trade-offs between the Karner blue's sole larval host plant, wild lupine (*Lupinus perennis*) and ovipositing females (Grundel *et al*. 1998). Wild lupine is essential for viable populations of Karner blues (USFWS 2003). Host plants grown in partial shade are higher in nitrogen content, which improves larval growth and survival (Grundel *et al*. 1998). Heterogenous shade also gives females an option to lay eggs on host plants that are in various phenological stages and increases survival of larvae (Grundel *et al*. 1998).

Survival of the Karner blue butterfly depends on effective conservation of remaining habitat and creation of new viable habitats. Karner blue sites are maintained in an early successional state by prescribed fire, mechanical thinnings and chemical treatments. Newly disturbed habitat is then re-established with wild lupine and native nectar plants. Providing sufficient quality and quantity of host plants and adult resources is critical. Successful management plans must consider requirements necessary for larval and adult stages (Haack 1992, Schultz & Dlugosch 1999, Grundel *et al*. 2000, Pickens & Root 2008). Though research on the Karner blue's larval host plant is extensive, information on resources for adults is limited

(Grundel *et al.* 2000, Pickens & Root 2008). Adult Karner blues spend 7-23% of their time feeding (Grundel *et al*. 1998). Nectar provides butterflies with water, sugar and amino acids that are essential for maintenance as well as for egg production (Boggs 1987, Schultz & Dlugosch 1999). Insufficient nectar in diets can limit energy and time used to forage, mate and oviposit (Kingsolver 1983, Shreeve 1992, Doak *et al*. 2006, Chau *et al*. 2020). To reproduce successfully, females must obtain sufficient nectar to meet energetic and nutritional requirements necessary for egg production and survival (Boggs & Ross 1983, Fischer & Fiedler 2001, Boggs & Freeman 2005, Janz 2005, Chau *et al*. 2020), while males must procure adequate nectar in order to survive and find mates (Scharf *et al*. 2013, Chau *et al*. 2020).

Nectar resources are critical in maintaining butterfly populations (Hill 1992, Schultz & Dlugosch 1999), and adult butterfly population densities have long been shown to have a direct relationship with nectar source abundance and diversity (Gilbert 1971, Ehrlich & Gilbert 1973, Murphy 1983, Grossmueller & Lederhouse 1987, Schultz & Dlugosch 1999). Previous research on how abundance and diversity of nectar influence population dynamics of butterflies includes a study by Britten & Riley (1994), that used pairwise comparisons to investigate occupied and unoccupied habitat of the uncompahgre fritillary (*Boloria acrocnema*). Occupied sites were paired with unoccupied sites based on similar elevations, slopes, aspects, geographical locations and percent of host plant abundance. Results of this study found that occupied sites had increased diversity of nectar sources. This study modeled a similar study by Williams (1998), where ten habitat variables, including host plant and nectar plant abundance, were compared with population sizes of Gillett's checkerspot (*Euphydryas gillettii*). Results of this study found that population sizes were correlated with availability of nectar sources and not with overall abundance of the butterfly's host plant. Similarly, insufficient nectar sources can limit Karner

blue butterfly populations (Lawrence & Cook 1989, Dunn 2008). Quantity and quality of nectar plants available for Karner blue butterflies are a concern throughout its range, especially during the Karner blue's second flight period which is generally three to four times larger than the first (Lawrence & Cook 1989, Schweitzer 1989, Bleser 1992, Leach 1993, USFWS 2003, Pickens & Root 2008,). Sites that are more abundant in nectaring plants tend to support larger populations of Karner blues (Lawrence 1994, Bidwell 1995), and distribution of the butterfly is restricted in comparison to the range of its host plant, suggesting that other requirements are needed for inhabitation (Schweitzer 1989, Bleser 1992, Leach 1993, Dirig 1994, Grundel *et al*. 1998, Grundel *et al.* 2000, USFWS 2003); as was demonstrated in a review of the status of the Karner blue butterfly in Michigan by Lawrence (1994), where he found that butterfly abundance was lower in sites with sufficient wild lupine but lacking in nectaring plants. Butterflies have also been shown to emigrate from sites with abundant host plants but insufficient nectar sources (Grossmueller & Lederhouse 1987, Schultz & Dlugosch 1999, Fred & Brommer 2009). Emigration from sites with unstable population numbers can be detrimental, as was shown when the Karner blue butterfly was officially extirpated from Ontario, Canada, after a series of droughts drastically decreased nectar sources available for adults (Packer 1994, USFWS 2003).

Previous studies have found that adult longevity and fecundity increase when sufficient nectar is available for butterflies (Murphy *et al*. 1983, Hill & Pierce 1989, Fischer & Fielder 2001). For example, when Murphy *et al*. (1983) manipulated sugar and amino acids in diets of Edith's checkerspot (*Euphydryas editha*), they found that sugar had the greatest impact on reproductive success. Increased sugar in diets prolonged life of the butterflies, increased total egg production in later egg masses and extended the period of egg production. Additionally, Boggs and Ross (1993), found that when they varied sugar in diets of Mormon fritillary (*Speyeria*

mormonia), they did not find a correlation between diet and lifespan of male butterflies, but did find that diet had a strong association with lifespan and fecundity in females. They also discovered that oocytes remaining in the ovaries of females declined in direct proportion to increased sugar content provided in diets, demonstrating tangible evidence that resources can be allocated away from reproduction when butterflies are under resource stress. Lastly, Hill & Pierce (1989) found that fecundity increased as much as threefold when sufficient sugar was provided in diets of female common imperial blue (*Jalmenus evagoras*). This study also found that as sugar content in diets increased, so did the stimulation to feed, and females compensated for lower sugar in diets by feeding for longer periods of time. Allocating more time to feeding can leave butterflies less time for behaviors such as mating and ovipositing (Schultz & Dlugosch 1999) and increases susceptibility to predators (Hill & Pierce 1989).

When selecting nectar, adult butterflies are generally labeled as generalists, but after more thorough investigation affinity for specific species can often be found. Selections may be reported as opportunistic because butterflies typically visit a wide variety of flowers based largely on abundance and availability (Sharpe *et al.* 1974, Dosa 1999, Bakowski *et al*. 2010). However, choice of flowers is not always random and butterflies often also exhibit flower preferences that differ between species (Jennersten 1984, Murphy *et al*. 1984, Tudor *et al*. 2004, Bakowski *et al.* 2010). Optimal foraging theory predicts that animals select resources that provide the greatest reward (Pyke *et al*. 1977, Rhiannon & Schultz 2016). Selection of these species may indicate that these sources are of more value to the Karner blue (Manly *et al*. 2002, Ezzedine & Matter 2008, Rhiannon & Schultz 2016).

Previous nectar selection observations of Karner blue butterflies suggest that adults have an affinity for specific species. Reviews of previous observations recorded in Michigan include

Papp (1993), that reported that 40% of selections by Karner blue butterflies during observations were on butterfly weed (*Asclepias tuberosa*) and 20% were on horsemint (*Monarda punctata*). Another study by Sferra *et al*. (1993) found that 60% of selections were on horsemint and 40% were on spotted knapweed (*Centaurea stoebe*). Observations in Wisconsin include a study by Leach (1993), where 19% of observed selections of first-generation butterflies were on Northern dewberry (*Rubus flagellaris*) and 16% were on invasive yellow sweetclover (*Melilotus officinalis*), while during the second generation, 38% of selections were on invasive white sweetclover (*Melilotus albus*), 15% on leadplant (*Amorpha canescens*), 12% on invasive leafy spurge (*Euphorbia esula*) and 13% were on horsemint. Also in Wisconsin, Maxwell & Givnish (1994) reported that 50% of first-generation selections were on lyrate rock cress (*Arabis lyrata*) and 14% were on blue toadflax (*Houstonia longifolia*), while during the second generation, 39% of selections were on leadplant, 25% on horsemint and 11% were on whorled milkweed (*Asclepias verticillata*). Lastly, in New York, Schweitzer (1993) suggested an affinity for butterfly weed and spotted knapweed for two years in a row in sites that he observed. However, none of these studies mention how frequency of available nectar species may have influenced selection of species, and abundance cannot be ruled out as a factor.

Very few observations of nectar selections by Karner blue butterflies mention how frequency of nectar species available to butterflies may have influenced selection of species. These include a study by Lawrence (1994) in Michigan, where 88.9% of first generation Karner blue adults fed on Northern dewberry, though this nectar species was observed to be common, However, it was also reported that butterflies did not feed on six other commonly observed nectar species during surveys. These species included: common cinquefoil (*Potentilla simplex*), lance-leaved coreopsis, hairy puccoon *(Lithospermum caroliniense*), yellow hawkweed

(*Hieracium caespitosum*), orange hawkweed (*Hieracium aurantiacum*) and blue toadflax. Additionally, second generation butterflies fed predominantly on butterfly weed (65.7%) and horsemint (20.4%), even though these species were observed to be rare in occurrence. Also in Michigan, Herms (1996) reported that butterfly weed was most frequently used in sites for two years in a row even though it appeared to be consistently rare in all sites. In Minnesota, observations by Lane (1994) also suggested a preference for horsemint, as 93.33% of selections by females and 91.30% by males were on this species. However, this study also noted horsemint was the most common nectar source available in the habitat observed. Lastly, a study by Bidwell (1994) in Wisconsin, found a strong positive correlation between butterfly numbers and abundance of horsemint. This study did not use nectar selections to determine nectar preferences, but instead compared butterfly abundance with abundance of nectar species in occupied habitats. Perhaps the frequency of nectar species recorded in observations are not by chance, as nectar surveys are generally performed in sites where the largest number of butterflies can be found, and Karner blues may be more abundant in these sites because these sites are also more abundant in preferred nectar sources.

Only one previous study has used statistical analyses to investigate how nectar plant abundance may influence nectar selection preferences of the Karner blue butterfly. Grundel *et al*. (2000) compared similarity of nectar plant in diets of Karner blues in the Indiana Dunes Lakeshore using the Brays-Curtis similarity coefficient, and used a floral ranking system to determine if frequency of nectar plants influenced selections. Results from this study found that when more than one species was available within a 2 m radius, the species with the most flowering heads was selected 60.3% of the time. This study also found that in a significant majority of the cases, butterflies chose nectar plant species with white or yellow flowers, though

nectar plants with white and yellow flower colors made up 94.2% of species available in this study. These results may suggest that Karner blues are predominantly opportunistic when selecting. However, comparisons also found that lyre-leaved rock cress (*Arabidopsis lyrata*), lance-leaved coreopsis (*Coreopsis lanceolata*), white sweetclover and Northern dewberry were chosen in a significant percentage of cases when another species was available within 2 m, and in the case of Northern dewberry, preference was not related to the species' local abundance. These results suggest that though Karner blues may increase foraging success by utilizing a large suite of available plants, they also exhibit selection preferences if the reward is more beneficial.

Encroachment of non-native species into butterfly habitat warrants further investigation into the use of non-native nectar plants by butterflies, especially in habitats with few or declining native nectar species. Non-natives can harm endangered species habitat or may provide new resources that species have come to rely on (Zavaleta *et al*. 2001, Rhiannon & Schultz 2016). Non-native and invasive species can compete for nutrients, water, light and space with natives (Brown *et al*. 2002). Competition may reduce the ability of natives to maintain or increase population sizes (Brown *et al*. 2002) and native plants have been shown to decline in habitats where aggressive exotics have taken over (Brown *et al.* 2002, Schultz *et al*. 2003). Nectar of non-native plants often has lower sugar content in comparison to natives, the tradeoff usually being more showy flowers, and rapid growth and spread (Baskett *et al.* 2011). There is evidence that lack of native nectar sources can limit butterfly population sizes, as shown in a study by Schultz & Dlugosch (1999) that investigated resource use by the Fender's blue (*Icaricia icarioides fenderi*), a species that is also in the Family *Lycaenidae* and has similar life history traits to the Karner blue. This study used estimates of resource abundance to analyze how larval and adult resources related to butterfly population sizes. Adult resources were assessed in four

ways: abundance of total nectar flowers, abundance of total native flowers, quantity of nectar from all flowers and quantity of nectar from native nectar flowers. Results found that native nectar flowers were weakly associated with population sizes, while total abundance of native nectar was significantly associated. Interestingly, comparisons of sugar content between natives and non-natives selected in the study found that native nectar plants had significantly higher sugar content, though non-natives still appeared to be heavily used.

There is evidence that removal of invasive nectar species can increase abundance of native nectar plants (Brown *et al*. 2002, Basket *et al*. 2018). However, there is also the potential for pollinator populations to crash following the widespread removal of non-native species that are common (Gibson *et al*. 2006, Carvhaleiro *et al*. 2008, Basket *et al*. 2011). It's crucial to understand the impact non-natives have on an ecosystem before drastically eradicating them, especially when considering that a fragile species may be relying on them.

In Michigan, the largest Karner blue butterfly populations are located in remnant oak barrens and sand prairies within the Huron-Manistee National Forest (HMNF) Baldwin/White Cloud Ranger District (USFWS 2012). Occupied Karner blue habitat is actively managed with frequent burns and cuttings directed by the United States Department of Agriculture Forest Service (USDAFS). Understanding foraging behaviors of Karner blue adults and investigating possible preferred nectar plants would give valuable information for management, as these areas are re-seeded with new nectar species after being disturbed. Providing optimal nectar sources for adults could increase population sizes and provide more stability, as populations with low numbers are at a greater risk of extirpation from unusual weather events, disease and predation (Dunn 2008).

To provide optimal resources for Karner blue adults in the HMNF we must determine if these areas have sufficient nectar resources for both first-and second-generation butterflies, investigate possible nectar selection preferences and establish if butterflies are relying on non-native or invasive nectar species. We investigated the foraging habits of Karner blue adults during the summers of 2019 and 2020 in order to determine possible nectar selection preferences and to ascertain if butterflies are relying on non-native species. This information can then be used by the USFADS and other agencies to help aid in management of adult resources.

Chapter 3: Methodology

Research Subject and Study Sites

Karner blue butterflies are a bivoltine species. First generation adults typically emerge in late May and come to an end in late June, while second generation butterflies generally begin to emerge in early July and last through August (Grundel *et al*. 1998, Pickens & Root 2008, USFWS 2003, Dunn 2008). Second brood butterflies lay eggs that overwinter for nine months and become the first brood the following year (Grundel *et al*. 1998, Pickens & Root 1998, USFWS 2003). Emergence can vary from year to year and depends heavily on geographic location and local weather conditions (Haack 1992).

We studied nectar plant selections of Karner blue butterflies at four sites located within the Huron-Manistee National Forest (HMNF) in Otto Township, Oceana County Michigan (Fig.1). Sites were chosen with help from the United States Department of Agriculture Forest Services (USDAFS) in the Baldwin-White Cloud Ranger District. Sites were selected based on the following criteria: previous surveys by the USDAFS show a recent history of hosting populations of Karner blue butterflies, nectar plants available in the sites were diverse and abundant in comparison to other sites within the HMNF and sufficient wild lupine was found throughout the habitat. All four sites encompass a traditional oak barren vegetation dominated by grasses and forbs. Canopy coverage of these areas was estimated to be less than 15% and consisted mainly of widely spaced mature red oak (*Quercus rubus*), white oak (*Quercus alba*) and black cherry (*Prunus serotina*). Our four observation areas were differentiated with numbers but were treated as a single observation site. Openings were not separated by closed canopy forest, and the distance between them was not great enough to deter travel between them (Dunn 2008), which precluded them from being independent sites. Site 1 (43°28’58.8”N,

86°15’14.5”W), site 2(43°28’59.3”N, 86°14’51.9”W), site 3 (43°28’55.5”N, 86°14’36.9”W) and site 4 (43°28’57.6”N, 86°14’22.4”W) are approximately 1.46 ha, 0.63 ha, 0.85 ha and 2.63 ha, respectively. The total area encompassing observed sites, including the forested vegetation connecting sites was 41.60 ha.

Nectar Selection Surveys

We observed 172 first generation and 311 second generation Karner blue adults during the summers of 2019 and 2020. Observations were carried out during all days that were at least 23.9 °C, there was no precipitation and wind was less than 15 mph (Grundel *et al*. 2000, USFWS 2003). These guidelines were followed in order to limit the chance of flushing butterflies out during conditions that were not ideal for flight, as we wanted to observe butterflies in their most natural state. Surveys were generally conducted between the times of 11:00 a.m. to 4:00 p.m., as previous studies have noted that these are the times that they are most often observed feeding (Lawrence 1994, Dunn 2008, Grundel *et al*. 2000). However, on long hot days observations lasted until approximately 7:00 p.m. due to extended conditions for optimal flight.

Karner blue adults were surveyed along fixed transect routes which crossed entire sites and were placed approximately 10 m apart. Observers walked transects until spotting a Karner blue butterfly. Once a butterfly was spotted, a ten-minute nectar selection survey was conducted until the duration of time or until the butterfly left the designated borders of the site. For nectar selection surveys, we recorded the species of selected nectar plants, time spent feeding and sex of the butterfly. Selection of a nectar plant was determined if the butterfly landed on a flower or floret in bloom and inserted its proboscis for more than five seconds (Bakowski *et al*. 2010). Selected plants were flagged with individual numbers so vegetation surveys could be done at the

end of the day. After completing a survey, observers would start on the transect where it was previously left off until spotting a new butterfly to observe. In order to limit the chance of repeat observations, we alternated observing sites 1 and 3 with sites 2 and 4. We also alternated walking transects in the opposite direction to ensure that time of day did not influence feeding patterns.

Vegetation Surveys

Previous studies that have observed Karner blue butterflies note that Karner blues will typically select from an area that is approximately 2 m when selecting a nectar species to feed upon (Leach 1993, Grundel *et al*. 2000). We surveyed and identified all unselected nectar species found within a 2 m radius around each selected nectar plant. Surveys were done at the end of every observation day because we found that the vegetation changed quickly due to the dry hot conditions in the summer. The species of selected and unselected nectar plants, as well as the number of flowers in bloom on selected and unselected nectar plants were recorded. For flowering plants with composite heads consisting of many small florets that were less than 1 mm in size, each composite head was treated as a single flower. Flowers and florets that were not open or were more than 50% wilted were not recorded in surveys. To estimate the abundance of nectar plant species available we used an ACFOR scale to rate the percentage of nectar plants during both generations: Abundant (A) = greater than 20%, Common (C) = 10-19%, Frequent (F) = 5-9%, Occasional (O) = 4-2%, Rare (R) = 1% (Daubenmire 1959) (Table 2, Table 3).

Statistical Analysis

A logistic regression analysis using a 9.4 SAS GENMOD procedure was used to compare the probability of selecting a specific nectar species when other nectar species were available

within a 2 m radius. Significant parameter estimates were used to determine odds ratios. Since species available to the butterflies were the same for both generations each year, data from both generations were combined for 2019 and 2020. A total of 273 and 520 observations were compared for generations 1 and 2 respectively. This same model was used to compare if sex of the butterfly and number of flowers per species were significant predictors of selections.

Chapter 4: Results

The likelihood of Karner blue butterflies selecting specific nectar species were estimated with logistic regression analyses. Estimates with a greater positive number indicate a greater likelihood of a species being selected when other species being compared are available within 2 m, while more negative numbers indicate that the species would be less likely to be selected when other species are nearby. Significant parameter estimates were then used to determine odds ratios of nectar species. Odd ratios greater than 1 indicate greater odds of a species being selected when other species being compared are available within a 2 m radius, while odds ratios less than 1 indicate lower odds of the species being selected when other species are nearby. Each selected species was compared to unselected nectar species unless the sample size was too low. Estimated parameters and odds ratios being reported were found to be significant.

Parameter estimates run on selected nectar plants during the first generation (Fig. 2) found that Northern dewberry was estimated to be more likely selected when yellow hawkweed (*Hieracium caespitosum*) (3.63), common cinquefoil (1.60), and long-leaved bluet (*Houstonia longifolia*) (1.36) were available. Estimates comparing long-leaved bluet and common cinquefoil found that both species were more likely to be selected when yellow hawkweed was nearby (2.03 and 2.27 respectively). The odds ratios of first-generation nectar plants (Fig. 5) determined that the odds ratio of a butterfly selecting Northern dewberry over other cinquefoil was 3.90 times greater, over long-leaved bluet was 4.97 times greater and over yellow hawkweed was 37.65 times greater. The odds ratio of a butterfly selecting long-leaved bluet over yellow hawkweed was 7.58 times greater. The odds ratio of common cinquefoil being selected over yellow hawkweed was 9.65 times greater. Logistic regressions that analyzed the influence of flower

abundance and sex of butterfly on selection preference, found that both these factors were not significant.

Estimated parameters of selections made during the second-generation flight periods (Fig. 3), found that butterfly weed was estimated to be selected over spotted knapweed (0.47), daisy fleabane (*Erigeron strigosus*) (1.98), black-eyed Susan (*Rudbeckia hirta*) (1.62), wild bergamot (*Monarda fistulosa*) (2.12) and yellow hawkweed (2.50). Spotted knapweed was estimated to be more likely to be selected over daisy fleabane (1.51), black-eyed Susan (1.15), wild bergamot (1.65) and yellow hawkweed (2.02). Lastly, horsemint was estimated to be selected before daisy fleabane (1.68), wild bergamot (1.82) and yellow hawkweed (2.20). Odds ratios of second-generation nectar plants (Fig. 5) found that the odds ratio of a butterfly selecting butterfly weed over other spotted knapweed was 1.60 times greater, over black-eyed Susan was 5.05 times greater, over daisy fleabane was 7.21 times greater, over wild bergamot was 8.30 times greater and over yellow hawkweed was 12.05 times greater. The odds ratio of a butterfly choosing spotted knapweed over black-eyed Susan was 3.16 times greater, over daisy fleabane was 4.51 times greater, over wild bergamot was 5.19 times greater and over yellow hawkweed was 7.54 times greater. Lastly, the odds ratio of a Karner blue selecting horsemint over daisy fleabane was 5.37 times greater, over wild bergamot was 6.18 times greater and over yellow hawkweed was 8.98 times greater. Logistic regressions that analyzed the influence of flower abundance and sex of butterfly on selection preferences found that both these factors were not significant.

ACFOR estimates of nectar plants available during the first-generation flight period (Table 2) found that Northern dewberry and yellow hawkweed were abundant nectar species found during this time. However, Northern dewberry was selected 190 times while hawkweed

was only selected twice. Long-leaved bluet and common cinquefoil were both found occasionally throughout sites and were selected 23 and 5 times respectively. Estimates of nectar species abundance during the second flight period (Table 3) found that spotted knapweed and orange hawkweed were abundant during this time. Spotted knapweed was selected 82 times during the survey, while orange hawkweed was not selected at all. Horsemint, daisy fleabane and wild bergamot were common and were selected 59, 21 and 4 times respectively. Black-eyed Susan was selected 27 times and was found frequently. Butterfly weed and yellow hawkweed were rarely observed during surveys. Butterfly weed was selected 97 times while yellow hawkweed was not selected at all. New Jersey tea (*Ceanothus americanus*) was rare, as only one plant was found in 2020 in one site. This was initially overlooked, due to it growing on the fringe of forested habitat and was originally hidden. We observed this plant during the last three days of observations, and it was observed to be selected on all three days.

The total feeding time averages for butterflies differed for first-and second-generation butterflies (Table 2, Table 3). First generation adults fed on nectar plants for an average of 44 seconds, and the average feeding times for specific selected species did not vary greatly. In contrast, average feeding times for second generation butterflies was 82 seconds and the average feeding times of specific selected species varied significantly from each other. New Jersey tea and butterfly weed had the longest feeding time averages of 297 seconds and 132 seconds respectively. These times were two to four times longer than average feeding times of other selected species during this flight period.

Chapter 5: Discussion and Conclusion

Discussion

Sufficient nectar resources available for adult butterflies can increase longevity and fecundity (Murphy *et al*. 1983, Hill & Pierce 1989, Fisher & Fiedler 2001), and can deter emigration (Grossmueller & Lederhouse 1987, Schultz & Dlugosch 1999, Fred & Brommer 2009). In order to provide optimal resources for Karner blue adults in the HMNF we surveyed available resources in occupied sites and observed foraging behaviors during the summers of 2019 and 2020. We aimed to determine if sufficient resources are available during the first- and second-generation flight periods, examine nectar selection preferences and establish if non-native nectar sources are used by adult Karner blues. We found that resources are limited in abundance and diversity during the first generation, especially during the first two weeks of emergence. Resources for second-generation butterflies were more abundant and diverse, but may still need to be increased since these resources support a larger population. Our nectar selection surveys found that first-generation butterflies had an affinity for Northern dewberry, while second-generation Karner blues preferred butterfly weed, horsemint and the invasive spotted knapweed. We also found that abundance of flowers in bloom did not influence selection preferences. Average feeding times varied between the two generations, and second-generation butterflies fed for longer periods of time. Our investigation into non-native resources found that during the first-generation, non-native yellow hawkweed dominated habitat but was not used as a significant resource for butterflies. During the second generation, three non-natives with moderate to high ecological impact were found in occupied habitat, yellow hawkweed, orange

hawkweed and spotted knapweed. Of these species, spotted knapweed was found to be a significant nectar source for adult Karner blues.

Determining if sufficient nectar resources are available for Karner blue butterflies is critical for both first-and second-generation flight periods. Available nectar sources varied between the two generations observed in this study. Nectar species available during the first generation were limited in abundance and diversity (Table 1). Nectar resources were most sparse during the first two weeks of emergence, with sites only providing between two to eight nectar plants. Throughout the first flight period, a total of ten nectar species were available to butterflies, and four of these species were observed to be selected. Only two species were abundant during this time, Northern dewberry and the invasive yellow hawkweed. Northern dewberry was frequently selected, while yellow hawkweed was only selected twice and did not appear to be a significant source for Karner blue adults. All other nectar species were only found occasionally or rarely in the area we observed. Increasing resources for first generation Karner blues, especially during the start of emergence, could help to bolster the number of second generation Karner blue butterfly numbers and number of second-generation eggs. Since eggs laid by second generation butterflies face a high mortality rate due to overwintering (USFWS 2003), increasing the number of eggs laid during the second generation would increase population sizes the following year. Suggestions of early spring species that are listed as resources for the Karner blue butterflies are bastard toadflax (*Comandra umbellata*) (Bleser 1992, Papp 1993, Sferra 1993), and wild strawberry (*Fragaria virginiana*) (Dirig 1976, Schweitzer 1989, Leach 1993, Grundel *et al*. 2000). Both species are listed as early blooming native plant species that are found in oak barren habitat (USFWS 2003). However, further investigation into how these plants influence these habitats is an important step before introducing them.

During the second-generation flight periods, nectar plants available for Karner blues were more abundant and diverse (Table 2). We observed 16 different nectar species in occupied habitat, and eight of these species were selected. Five species were observed to be abundant or common throughout the sites, and four of these species were observed to be used by Karner blues. These observations differed from what we originally expected, as it has been previously reported that resources for second generation butterflies are lacking (Schweitzer 1989, Bleser 1992, Haack 1992, Leach 1993). Though resources available for second generation butterflies were more abundant in comparison to first generation resources, butterfly numbers in the later brood are typically three to four times larger than the first (Lawrence & Cook 1989, Schweitzer 1989, Bleser 1992, Leach 1993, USFWS 2003, Pickens & Root 2009). Additional resources may be needed to support this generation, as forbs in these sites have been steadily declining over the past 20 years (Dunn, J. personal communication, 3/15/2023).

In order to investigate possible nectar selection preferences of Karner blue adults, we conducted nectar selection surveys during 2019 and 2020 in occupied sites located within the HMNF. Odds ratios for first generation Karner blue butterflies found that Northern dewberry was 3.90 times more likely to be selected over common cinquefoil, 4.97 times more likely to be selected over long-leaved bluet and 37.65 times more likely to be selected over yellow hawkweed. Northern dewberry was one of the most abundant species throughout the sites. However, yellow hawkweed was equally abundant and was only selected twice. Comparisons of yellow hawkweed with other available nectar species found that it was not likely to be selected when other species were available within 2 m. These results reflect observations seen in the field, as it did not appear that butterflies were interested in yellow hawkweed. During our two-year study, observers rarely witnessed Karner blues visiting this species. Previous observations

support our findings that Northern dewberry is a selection preference for Karner blues. For example, Lawrence (1994) reported that in Michigan, Northern dewberry was selected in 88.9% of observations made, though frequency of this species could not be ruled out as the reason why it was most commonly selected, as it was abundant. However, this study also reported that six other nectar plants that were listed as abundant (common cinquefoil, lance-leaved coreopsis, hairy puccoon, yellow hawkweed, orange hawkweed and blue toadflax) were not selected. Another nectar selection study by Grundel *et al*. (2000) in Indiana, analyzed selections and frequency of nectar plants by using a floral ranking system to analyze the relationship between selected species and abundance of flowers. Results of this study found that though butterflies usually chose nectar species with the greatest total number of flowers, Northern dewberry, lance-leaved leaved coreopsis and lyre rockcress were selected in a significant majority of the cases when other nectar species were nearby. In the case of Northern dewberry, preference was not directly related to the species' local flower abundance.

Odds ratios from our nectar selection surveys of Karner blue butterflies during the second flight period found that butterfly weed was 1.60 times more likely to be selected over spotted knapweed, 5.05 times more likely to be selected over black-eyed Susan, 7.21 times more likely to be selected over daisy fleabane, 8.30 times more likely to be selected over wild bergamot and 12.05 times more likely to be selected over yellow hawkweed. Butterfly weed was only found rarely throughout the sites and abundance of this nectar source cannot account for its preference. These results reflect observations made in the field. Multiple butterflies were found feeding on this source at the same time, which was seldom observed for other species. Yellow hawkweed was also found rarely in sites, but was estimated to be less likely to be selected when all other species were nearby. This was expected, as this species was rarely selected when it was abundant

during the first generation. Spotted knapweed and orange hawkweed were both abundant throughout the sites. Spotted knapweed was found to be 3.16 times more likely to be selected over black-eyed Susan, 4.51 times more likely to be selected over daisy fleabane, 5.19 times more likely to be selected over wild bergamot and 7.54 times more likely to be selected over yellow hawkweed. Butterflies never fed on orange hawkweed in any observations, and Karner blues appeared to avoid patches of this species in the field. Orange hawkweed is considered a noxious weed in five states, due to the fact it tends to dominate areas it grows in. In habitat we observed in the HMNF we found this was the case, as patches of orange hawkweed were generally monospecific. As a result, this species wasn't present in vegetation surveys and is not included in our analyses. Considering that orange hawkweed was abundant and was never selected, suggests that it is not a nectar source for Karner blue butterflies. Horsemint, daisy fleabane, and wild bergamot were all common in the sites. Horsemint was found to be 5.37 times more likely to be selected over daisy fleabane, 6.18 times more likely to be selected over wild bergamot and 8.98 times more likely to be selected over yellow hawkweed. Observers in this study noted that selections observed on horsemint and daisy fleabane were frequent, while wild bergamot was seldomly selected, even when it was the most common species available within 2 m. New Jersey tea is not mentioned in our analyses, as the sample size was too low for statistical analysis. One plant was discovered near the edge of site 4, and was observed during the latter part of 2020 for three days. However, it's important to note that five to twelve butterflies were observed feeding on this plant at the same time on all three days it was observed. More butterflies were found feeding on this species at one time than any other species observed. New Jersey tea is listed as a resource for Karner blue butterflies in several states which include Indiana (Martin 1992, Grundel *et al*. 2000), Minnesota (Martin 1994), New Hampshire (Bidwell

1991, Hemoldt *et al*. 1994), New York (Dirig 1976, Fried 1987, Schweitzer 1989) and Wisconsin (Bleser 1992, Swengel and Swengel 1993) and also Ontario, Canada (Packer 1987, Packer 1990), and is listed as a preferred nectar source for Karner blues in Indiana, New Hampshire, New York and Ontario, Canada (Schweitzer 1989, Blesser 1992, Haack 1992). This is a potential resource that should be considered when reseeding newly disturbed Karner blue habitat.

Reviews of previous nectar selections by second generation Karner blue butterflies in Michigan support the preferred species found in this study: butterfly weed, horsemint and spotted knapweed. For example, Lawrence (1994) found that 65.7% of second-generation butterflies selected butterfly weed, and another 20.4% fed on horsemint. He also reported that though both species were present at each site, they were very low in frequency, and when the two species were near each other, butterfly weed appeared to be preferred over horsemint. Additionally, Herms (1996) reported that she observed butterfly weed being most frequently used two years in a row in habitat he observed even though it was consistently rare on all sites. Other studies have similar results, but do not specify if abundance of nectar plants were accounted for. These studies include observations made by Papp (1993), that documented that 40% of selections by Karner blue butterflies he observed were on butterfly weed and 20% were on horsemint; and Sferra (1993) that found that 60% of nectar selections she observed were on horsemint, and 40% of selections were on spotted knapweed.

Reviews of nectar selections by second generation Karner blue butterflies in other states demonstrate some similar selection preferences, as well as some differences. Similar findings include a two-year study in New York, by Schweitzer (1989), where he proposed that Karner blues had an affinity for butterfly weed and spotted knapweed in sites he observed, while in

Minnesota, Lane (1994) found that horsemint was the most frequently selected nectar plant, as it was selected 92% of the time. However, she also noted that this was the most abundant nectar species available to butterflies and frequency may have influenced selections. Bidwell (1994) also suggested an affinity for horsemint in Wisconsin, as he found a strong correlation between abundance of horsemint in sites and number of Karner blue butterflies. Further studies in Wisconsin produced varying results. Observations by Maxwell & Givnish (1994), reported that 39% of selections by Karner blues were on leadplant and 25% were on horsemint, while a study by Leach (1993) suggested a preference for white sweetclover, as it was selected 33% of the time. In Indiana, observations by Grundel *et al*. (2000), also found a preference for white sweetclover, as it was the most commonly selected species during the second generation even when other species were nearby. Inconsistent selection preferences found in these studies may be due to differing locations of the surveys. Different environments present variations in the suite of plants available for butterflies, and species grown in different locations will also have differing phenologies (USFWS 2003, Bakowski *et al*. 2010).

In order to determine if flower abundance influenced nectar selections by Karner blue butterflies, we compared the total number of flowers of selected species with total flower counts of unselected species available within 2 m. Results from this study found that abundance of flowers did not significantly influence selections made by first or second generation Karner blues. In contrast, Grundel *et. al* (2000) found that when more than one species was available within a 2 m radius, the species with the most flowering heads was selected 60.3% of time. However, Grundel *et al.* (2000) used a floral ranking system to estimate flower abundance instead of a true count of flowers available. Conflicting results may be due to differing methodologies, study locations or climates.

Comparisons of average feeding times in this study found that they varied greatly between generations. During the first generation, the total average feeding time was 44 seconds, and average feeding times between selected species did not vary greatly; while during the second generation, the total average feeding time was almost twice as long as the first generation (82 seconds), and differences between feeding time averages for specific selected species varied greatly from each other. The longest feeding time averages were on New Jersey tea (297 seconds) and butterfly weed (132 seconds). These times are two to four times longer than average feeding times recorded for other species. We are not able to definitively conclude what these differences mean without further knowledge about the quality and quantity of the nectar found in selected species. Based on optimal foraging theory (Pyke *et al*. 1977), we predict that in this study butterflies fed for longer periods of time when the reward was greater, meaning there was less need to search for additional sources. However, there is also the possibility that butterflies fed for longer periods of time because they were not able to gain sufficient requirements as quickly from the resource. Pickens & Root (2008) observed similar differences in feeding time averages between generations of Karner blues they observed, and found that foraging rates were significantly higher in second-generation butterflies. First-generation butterflies in this study never foraged for longer than seven minutes, while second generation butterflies often fed for the full 15-minute observations they conducted. Pickens and Root (2008) suggest that longer feeding times during the second generation may be due to decreased nitrogen content found in wild lupine leaves available to second-generation larvae. This theory is based on previous research that shows that as wild lupine plants start to senescence, leaves of the plant decrease in nitrogen content (Grundel *et al*. 1998) and also evidence that adult butterflies rely

more on nectar sources when host plant nutrition is low at the larval stage (Mevi-Schutz et al. 2003).

Our investigation into non-native nectar use by Karner blue adults in the HMNF found that during the first generation, three non-native species were observed in the habitat, yellow hawkweed, yellow goat's beard (*Tragopogon dubius*) and hairy vetch (*Vicia villosa*). Neither yellow goat's beard nor hairy vetch were observed to be selected in this study, and both species are considered to have a low ecological impact. Yellow goat's beard has the potential to over dominate in a few select environments (Cohen *et al*. 2014), but this was not the case in the habitat we observed, as it was found rarely. Hairy vetch is considered to be thoroughly naturalized in these habitats. Yellow hawkweed is considered an aggressive non-native species, as it can dominate areas it's grown in and can establish monocultures (Cohen *et al*. 2014). In our observations in the HMNF we found that this was one of the most abundant species. Though this species may limit diversity, other species still appeared to grow throughout its range in the field. This species was only selected twice during both years it was observed, and does not appear to be a resource that is used by Karner blues.

During the second generation, four non-native species were reported: spotted knapweed, orange hawkweed, yellow hawkweed and hairy vetch. Three of these non-natives are considered to have a moderate to high ecological impact on ecosystems. Orange hawkweed is considered a noxious weed in five states because of its capability to over dominate areas (Cohen *et al*. 2014), as was found in the habitat we observed. Patches of orange hawkweed were large and precluded other species from growing within and around it. Karner blue adults never fed on this species during the two years we surveyed nectar use, and it appeared that Karner blues avoided patches of the plant. Spotted knapweed is considered a prohibited noxious weed in Michigan. It tends to

dominate areas and is an allelopathic species (Cohen *et al*. 2014). Areas we observed found that this species dominated large areas, but still allowed for other species to grow within these patches. Spotted knapweed was a preferred nectar source for Karner blue adults and was estimated to be selected over daisy fleabane, wild bergamot, black-eyed Susan, and yellow hawkweed. Therefore, it will be critical to establish sufficient native nectar sources before drastic removal of spotted knapweed is considered.

Conclusions

Nectar selection results from this study, coupled with previous reports suggest that Northern dewberry is a preference for first generation Karner blue adults, while during the second-generation flight period, butterflies have a preference for butterfly weed, spotted knapweed, and horsemint. New Jersey tea was not included in analyses, but observers noted that it appeared to support multiple butterflies and is an additional resource to consider. Orange hawkweed was also not included in analyses due to its monospecific state, but considering that it was abundant and was never selected, we assume that this is not a nectar source for Karner blue butterflies.

Assessment of resources available to Karner blues in occupied habitat within the HMNF found that resources are lacking in abundance and diversity during the first flight, especially during the first two weeks of emergence. Increasing nectar species that bloom in early spring could help bolster population numbers. Species available to second generation butterflies are more abundant and diverse. However, since the second generation is typically three to four times larger (Lawrence & Cook 1989, Schweitzer 1989, Bleser 1992, Leach 1993, USFWS 2003, Pickens & Root 2009), enhancing available species during this time may still be necessary.

Based on the results from our nectar selection surveys, it would benefit butterflies to increase butterfly weed and horsemint in particular.

Concerning the management of non-natives and aggressive species in these habitats, three species appear to pose a threat: yellow hawkweed, orange hawkweed and spotted knapweed. These species tended to dominate large portions of occupied habitat, and decreased diversity. The only non-native nectar source that appeared to be used by Karner blue adults was spotted knapweed. Providing sufficient native nectar sources to replace spotted knapweed is crucial before drastic eradication. It's important to note that this study only observed use of nectar plants by adult Karner blues. During this study orange hawkweed was observed to be selected by other species of butterflies, specifically various swallowtails. We also observed numerous species of bees on species that were not listed as selection preferences for Karner blues, specifically wild bergamot. However, decreasing patches of monocultures and increasing native nectar resources would increase diversity and stability for all pollinators in the community.

Future research needs include analyses to determine quality and quantity of nectar in selected species would be beneficial. Understanding how the content of nectar found in native and non-native influence feeding behaviors of adult Karner blue butterflies could give valuable insight into future management of non-native species in these habitats. Also, a secondary study to determine additional early blooming nectar sources for first generation Karner blues would be valuable, as this should be investigated before new species are introduced.

Tables

Table 1. Nectar plant species available to first generation Karner blue butterflies

Plant species	Plant abundance (ACFOR scale)	Total times selected	Average feeding time (s)	Standard dev. (s)	Native species (yes/no)	Habitat implications for non-native species
Coreopsis lanceolata, sand coreopsis	X	0	0	0	Yes	
Hieracium caespitosum, yellow hawkeed	A	2	26	38	No	Moderate to high ecological impact
Houstonia longifolia, long-leaved bluet	O	23	43	43	Yes	
Nuttallanthus canadensis, blue toadflax	R	0	0	0	Yes	
Oenothera biennas, common evening primerose	X	0	0	0	Yes	
Potentilla simplex, common cinquefoil	O	5	18	13	Yes	
Rubus flagellaris, Northern dewberry	A	190	45	40	Yes	
Tragopogon dubius, yellow goat's beard	X	0	0	0	No	Low impact
Vicia villosa, hairy vetch	X	0	0	0	No	Considered thoroughly naturalized
Viola pedata, bird's foot violet	R	0	0	0	Yes	

Abundance of nectar species were estimated using an ACFOR scale: Abundant (A) = greater than 20%, Common (C) = 10-19%, Frequent (F) = 5 -9%, Occasional (O) = 4-2%, Rare (R) = 1%. Species than had less than six individuals present in the observed areas were not included in the ACFOR rating and are denoted with (X).

Table 2. Nectar plant species available to second generation Karner blue butterflies

Plant species	Plant abundance (ACFOR scale)	Total times selected	Average feeding time (s)	Standard dev. (s)	Native species (yes/no)	Habitat implications for non-native species
Achillea millefolium, common yarrow	X	0	0	0	Yes	
Asclepias syriaca, butterfly weed	R	97	132	133	Yes	
Ascelpias tuberosa, common milkweed	O	0	0	0	Yes	
Ceanothus americanus, New Jersey tea	X	3	297	325	Yes	
Centaurea stoebe, spotted knapweed	A	82	59	70	No	Prohibited noxious weed in Michigan
Coreopsis lanceolata, praire coreopsis	X	0	0	0	Yes	
Erigeron strigosus, daisy fleabane	C	21	76	74	Yes	
Euphorbia corollata, flowering spurge	O	6	47	50	Yes	
Helianthus mollis, downy sunflower	X	0	0	0	Yes	
Hieracium aurantiacum, orange hawkweed	A	0	0	0	No	Moderate to high ecological impact
Hieracium caespitosum, yellow hawkweed	R	0	0	0	No	Moderate to high ecological impact
Lespedeza capitata, hairy bushclover	R	0	0	0	Yes	
Monarda fistulosa, wild bergamot	C	4	49	50	Yes	
Monarda punctata, horsemint	C	59	53	63	Yes	
Rudbeckia hirta, black-eyed susan	F	27	52	79	Yes	
Vicia villosa, hairy vetch	X	0	0	0	No	Considered thoroughly naturalized

Abundance of nectar species were estimated using an ACFOR scale: Abundant (A) = greater than 20%, Common (C) = 10-19%, Frequent (F) = 5 -9%, Occasional (O) = 4-2%, Rare (R) = 1%. Species than had less than six individuals present in the observed areas were not included in the ACFOR rating and are denoted with (X).

Figures

Figure 1. Sites used in observation surveys during 2019 and 2020 in the Huron-Manistee National Forest, Otto Township, Oceana County, Michigan. Site 1 (43°28'58.8"N, 86°15'14.5"W), site 2(43°28'59.3"N, 86°14'51.9"W), site 3 (43°28'55.5"N, 86°14'36.9"W) and site 4 (43°28'57.6"N, 86°14'22.4"W) are approximately 1.46 ha, 0.63 ha, 0.85 ha and 2.63 ha, respectively. The total area encompassing observed sites, including the forested vegetation connecting sites is 41.60 ha.

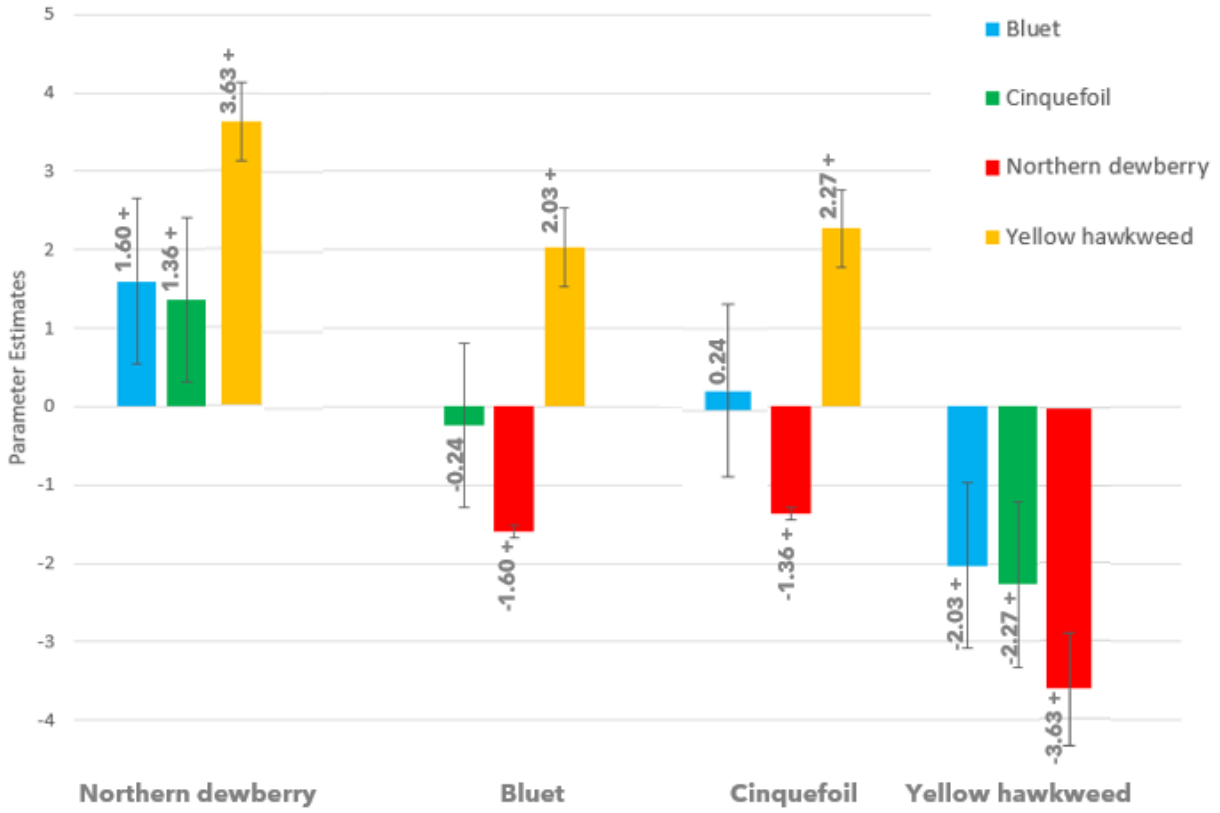

Figure 2. Logistic regression parameter estimates of nectar selections by first generation Karner blue butterflies. Estimates with greater positive numbers indicate a greater likelihood that the species being compared will be selected when other species in bloom are available within a 2m radius. More negative estimate parameters indicate that the species being compared will be less likely to be selected to the compared species within 2m. Estimates denoted with (+) were significant.

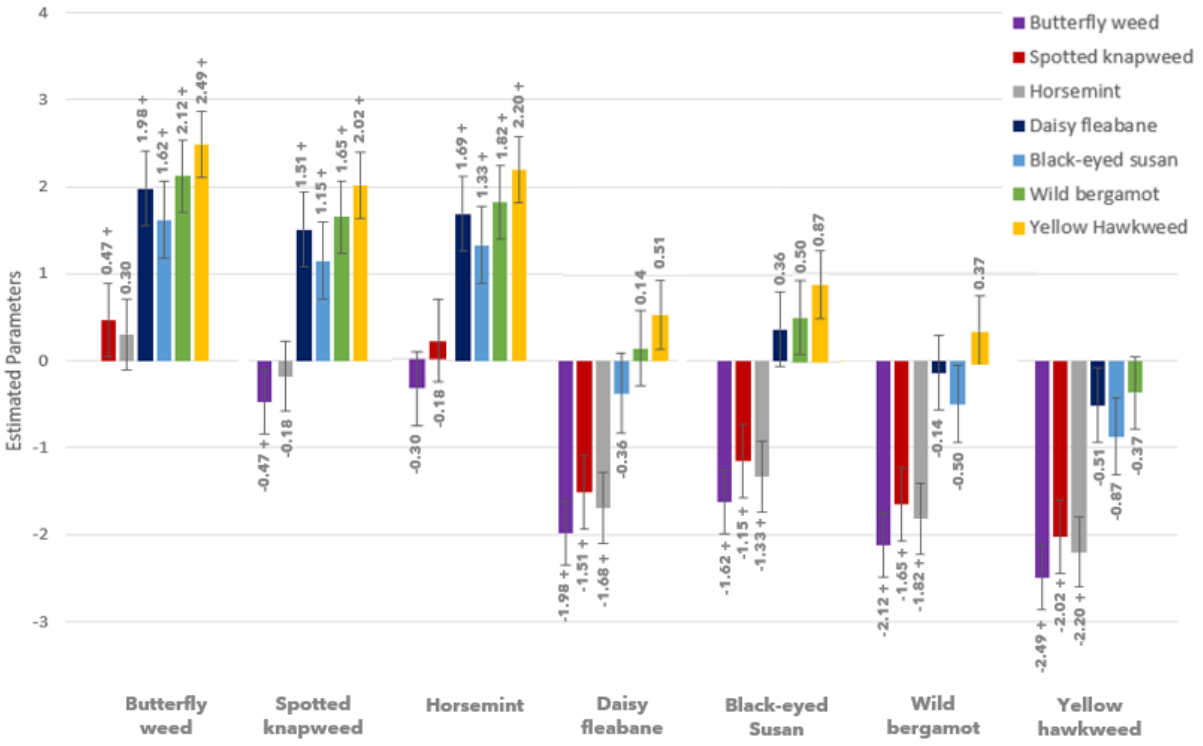

Figure 3. Logistic regression parameter estimates of nectar selections by second generation Karner blue butterflies. Estimates with greater positive numbers indicate a greater likelihood that the species being compared will be selected when other species in bloom are available within a 2m radius. More negative estimate parameters indicate that the species being compared will be less likely to be selected to the compared species within 2m. Estimates denoted with (+) were significant.

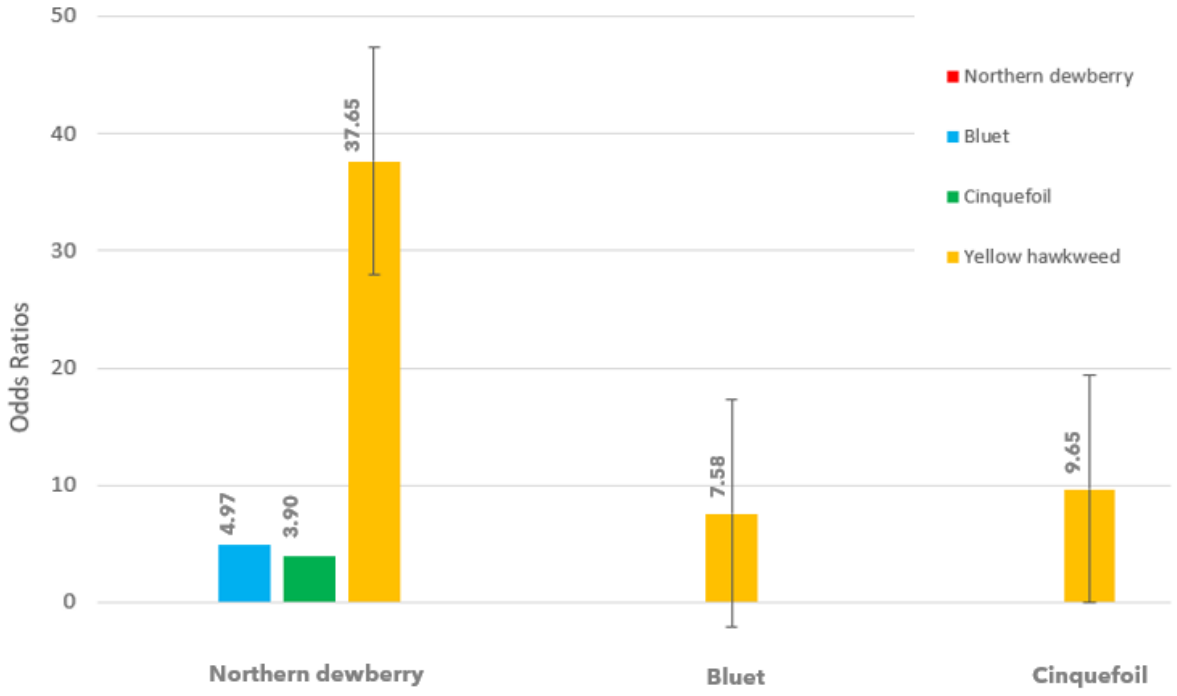

Figure 4. Odds ratios of nectar selections by first generation Karner blue butterflies. Odds ratios greater than 1 indicate greater odds of a species being selected when other species being compared are within a 2m radius. Odds ratios were determined on selected and unselected species unless the sample size was too low. All odds ratios were found to be significant.

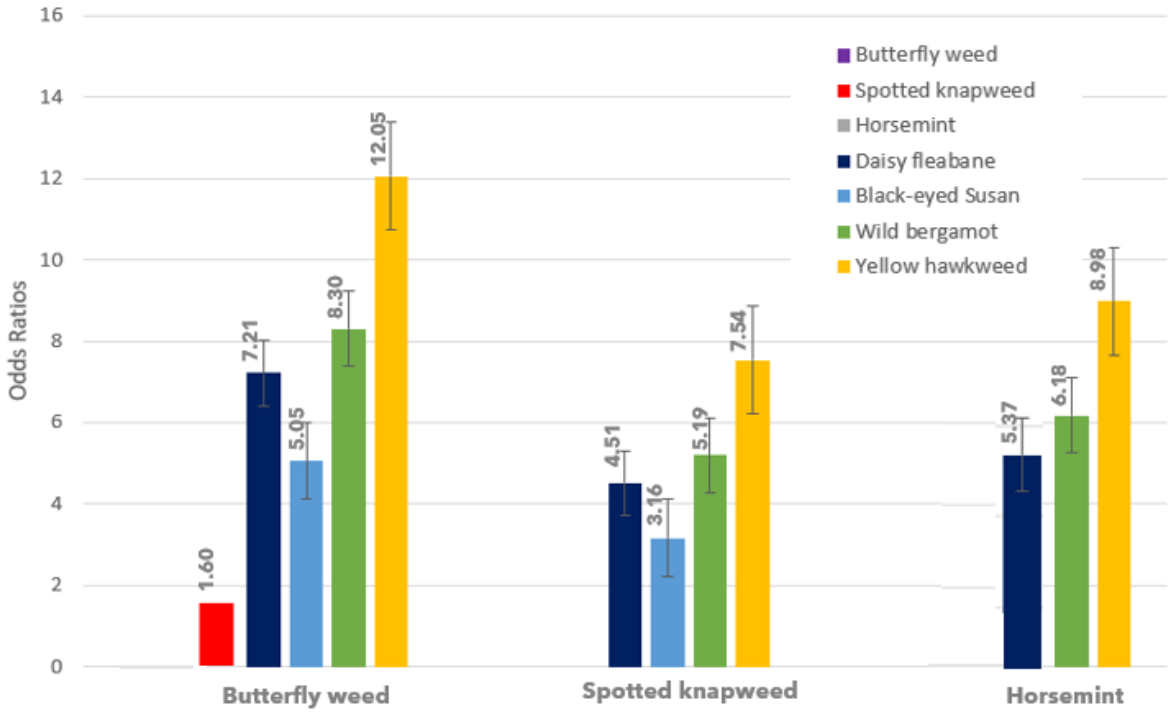

Figure 5. Odds ratios of nectar selections by second generation Karner blue butterflies. Odds ratios greater than 1 indicate greater odds of a species being selected when other species being compared are within a 2m radius. Odds ratios were determined on selected and unselected species unless the sample size was too low. All odds ratios were found to be significant.

Bibliography

Bakowski, M., A. Filipiak & Z. Fric (2010). Foraging behavior and nectar use in adult large copper butterflies, *Lycaena dispar* (Lepidoptera: *Lycaenidae*). Entomologicia Fennica 21: 49-57.

Baskett, C.A., S.M. Emery & J.A. Rudgers (2011). Pollinator visits to threatened species are restored following invasive plant removal. International Journal of Plant Science 172(3): 411-422.

Bidwell, A.D. (1991). A habitat-based preserve plan for the Karner blue butterfly (*Lycaeides Melissa samuelis*) in Concord, New Hampshire. Unpublished report. The Nature Conservancy, New Hampshire Field Office, Bedford, NH.

Bidwell, A.D. (1994). Mark-release-recapture of Karner blue butterflies (*Lycaeides melissa samuelis*) at Fort McCoy Military Reservation. Unpublished report. U.S. Fish and Wildlife Service, St. Paul, MN.

Bidwell, A.D. (1995). Karner blue butterfly (*Lycaeides melissa samuelis*) dispersal and habitat disturbance at Fort McCoy Military Reservation, Wisconsin. M.S. Thesis. University of Wisconsin, Stevens Point, WI.

Bleser, C.A. (1992). Status survey, management and monitoring activities for the Karner blue butterfly (*Lycaeides melissa samuelis*) in Wisconsin, 1990-1992. Unpublished report. Wisconsin Department of Natural Resources, Bureau of Endangered Resources, Madison, WI.

Boggs, C.L. (1987). Ecology of nectar and pollen feeding in Lepidoptera. In: nutritional ecology of insects, mites, spiders and related invertebrates. Insect Science Applications 10(2): 369-391.

Boggs, C.L. & C.L. Ross (1993). The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: *Nymphalidae*). Ecology 74: 433-441.

Bogs, C.L., & K.D. Freeman (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia 144: 353-361.

Britten, H.G. & L. Riley (1994). Nectar source diversity as an indicator of habitat suitability for the endangered uncompahgre fritillary, *Bolaria acrocnema* (*Nymphalidae*). Journal of Lepidoptera Society 48: 173-179.

Brown, B.J., M.J., Randall & S.A. Graham (2002). Competition for pollination between an invasive species (Purple loosestrife) and a native congener. Ecology 83(8): 2238-2336.

Carvalheiro, L.G., E.R.M., Barbosa & J. Memmott (2008). Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study. Journal of Applied Ecology 45: 1419-1427.

Chau, S.N., L.V. Bristow, R. Grundel & J.J. Hellmann (2020). Resource segregation at fine spatial scales explains Karner blue butterfly (*Lycaeides melissa samuelis*) distribution. Journal of Insect Conservation 24: 739-749.

Cohen, J.G., M.A. Kost, S. Bradford, S. Slaughter & A.A. Dennis (2014). A Field Guide to the Natural Community of Michigan. Michigan State University Press. Lansing, MI.

Curtis, J.T. (1959). The Vegetation of Wisconsin: an ordination of plant communities. The University of Wisconsin Press, Madison, WI pp. 657.

Daubenmire, R.F. (1959). Canopy coverage methods of vegetation analysis. Northwest Science 33: 43-64.

Dirig, R. (1994). Historical notes on wild lupine and the Karner blue butterfly at the Albany Pine Bush, New York. In: Karner butterfly: a symbol of a vanishing landscape. Minnesota Agricultural Experiment Station. University of Minnesota, St. Paul, MN pp. 23-96.

Dirig, R. (1976). Karner's famous blue butterfly. In: Pine Bush-Albany's last frontier. Pine Bush Historic Preservation Project. Albany, New York pp. 197-210.

Dirig, R. & J.F. Cryan (1976). The Karner blue project: January 1973 to December 1976. Atala 4: 22-26.

Doak, P., P. Kareiva & J. Kingsolver (2002). Fitness consequence of choosy oviposition for a time-limited butterfly. Ecology 87: 395-408.

Dosa, G. (1999). Flower visitation patterns of butterflies and burnet moths in Aggtelek-Karst (Hungary). Nota Lepidoptera 22: 58-66.

Dunn, J.P. (2008). Investigations of adult dispersal, habitat quality analysis, and the development of monitoring techniques for the Karner blue butterfly within the Muskegon Recovery Unit. Final report for the USFWS. St. Paul, MN.

Ehrlich, P.R. & L.E. Gilbert, (1973). Population structure and dynamics of a tropical butterfly *Heliconius ethilla*. Biotropica 5(2): 69-82.

Ezzeddine M. & S.F. Matter, (2008). Nectar flower use and electivity by butterflies in sub-alpine meadows. Journal of Lepidopterists' Society 62: 138-142.

Fischer, K. & K. Fiedler, (2001). Effects of adult feeding and temperature regime on fecundity and longevity in the butterfly *Lycaena Hippothoe* (*Lycaenidae*). Journal of the Lepidopterists' Society 54: 91-95.

Fred, M.S. & J.E. Brommer (2009). Resources influence dispersal and population structure in an endangered butterfly. Insect Conservation and Diversity 2: 176-182.

Fried, C. S. (1987). Dispersal of the Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov) in the Albany Pine Bush. Unpublished report. Endangered Species Unit of the New York State Department of Environmental Conservation, Albany, NY.

Gibson, R.H., I.L. Nelson, G.W. Hopkins, B.J. Hamlett & J. Memmott (2006). Pollinator webs, plant communities and the conservation of rare plants: arable weeds as a case study. Journal of Applied Ecology 43: 246-257.

Gilbert, L.E. (1971). The effect of resource distribution on population structure in the butterfly *Euphydryas editha*: Jasper Ridge vs. Del Puerto Canyon colonies. Ph.D. Thesis. Stanford University, Stanford, CA.

Grossmeuller, D.W. & R.C. Lederhouse (1987). The role of nectar source distribution in habitat use and oviposition by the tiger swallowtail butterfly. Journal of Lepidoptera Society 41: 159-165.

Grundel, R., N.B. Pavlovic & C.L. Sulzman (1998). Habitat use by the endangered Karner blue butterfly in oak woodlands: the influence of canopy cover. Biological Conservation 85: 47-53.

Grundel, R., N.B. Pavlovic & C.L. Sulzman (2000). Nectar plant selection by the Karner blue butterfly (*Lycaeides melissa samuelis*) at the Indiana Dunes National Lakeshore. American Midland Naturalist 144: 1-10.

Haack, R.A. (1992). Endangered and threatened insects in the United States and Michigan. Newsletter. Michigan Entomology Society 37: 1-8.

Helmbolt, K. M. & M. Amaral (1994). Status of the Karner blue butterfly in New Hampshire. In: Karner blue butterfly: a symbol of a vanishing landscape. St. Paul Minnesota Agricultural Experiment Station. St. Paul, MN pp. 123-128.

Herms, C.P. (1996). The endangered Karner blue butterfly (Lepidoptera: *Lycaenidae*). In: Michigan: habitat suitability, potential impacts of gypsy moth (Lepidoptera: *Lycaenidae*) suppression, and laboratory rearing. M.S. Thesis. Michigan State University, East Lansing, MI.

Hill, C.P. (1992). Temporal changes in abundance of two lycaenid butterflies (*Lycaenidae*) in relation to adult food resources. Journal of Lepidopterists' Society 46: 174-181.

Hill, C.J. & N.E. Pierce (1989). The effect of adult diet on the biology of butterflies in the common Imperial blue, *Jalmenus evagoras*. Oecologia 81: 249-257.

Janz. N. (2005). The relationship between habitat selection and preferences for adult and larval food resources in the polyphagous butterfly *Vanessa cardui* (Lepidoptera: *Nymphalidae*). Journal of Insect Behavior 18: 767-780.

Kingsolver, J.G. (1983). Ecological significance of flight activity in colias butterflies: Implications for reproductive strategy and population structure. Ecology 64: 546-551.

Kleintjes, P.K., J.M. Sporrong, C.A. Raebel & S.F. Thon (2003). Habitat type and restoration for the Karner blue butterfly: a case study from Wisconsin. Ecological Restoration 21(2): 107-115.

Lane, C.P. (1994). Habitat preferences of the Karner blue butterfly in Minnesota. In: Karner blue butterfly: a symbol of a vanishing landscape. Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, MN pp. 63-72.

Lawrence, W.S. (1994). Karner blue butterfly populations in the Allegan State Game Area, Michigan. In: Karner blue butterfly: a symbol of a vanishing landscape. Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, MN pp. 53-62.

Lawrence, W.S. & A.C. Cook (1989). The status and management of Karner blue (*Lycaeides melissa samuelis*) populations in the Allegany State Game Area, Michigan. Unpublished report. Nature Conservancy, Michigan Field Office, East Lansing, MI 65 p.

Leach, M. (1993). Status and distribution of the Karner blue butterfly at Fort McCoy, Wisconsin:

final report in a two-year study. Unpublished report. Natural Resources Management Division, The Nature Conservancy, Stevens Point, WI 50 p.

Manly, B.F., L.L. McDonald, D.L. Thomas, T.L. McDonald & W.P. Erickson (2002). Resource selection by animals: statistical design and analysis for field studies. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Martin, M.L. (1992). Karner blue butterfly (*Lycaeides melissa samuelis*) in Indiana: 1990 status report. Unpublished report. Indiana Department of Natural Resources, Indianapolis, IN 13 p.

Maxwell, J. & T. Givnish (1994). Research on the Karner blue butterfly at Fort McCoy, Wisconsin: progress report for the 1994 field season. US Fish and Wildlife Service and U.S. Department of Defense, Fort McCoy, Wisconsin.

Mevi-Schutz, J. M. Goverde &A, Erhardt (2003). Effects of fertilization and elevated CO2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. Behavioral Ecology and Sociobiology 54: 36-43.

Murphy, D.D. (1983). Nectar sources as constraints on distribution of egg masses by the checkerspot butterfly, *Euphydryas chalcedona* (Lepidoptera: *Nymphalidae*). Environmental Entomology 12: 463-466.

Murphy, D.D., Launer, A.E. and Ehrlich. P.R. (1983). The role of adult feeding and egg production and population dynamics of the checkerspot butterfly, *Euphydryas editha*. Oecologia. 56: 257-263.

Murphy, D.D., M.S. Menninger & P.R. Ehrlich (1984). Nectar source distribution as a determinant of oviposition host species in *Euphydryas chalcedona*. Oecologia 62: 269-217.

Nuzzo, V.A. (1986). Extent and status of Midwest oak savanna: presettlement and 1985. Natural Areas Journal. 6: 6-36.

Packer, L. (1987). Status report on the Karner blue butterfly, *Lycaeides melissa samuelis* Nabokov, in Canada. Unpublished report. Wildlife Fund and the Ontario Ministry of Natural Resources, Wildlife Branch, Ontario, Canada 66p.

Packer, L. (1990). The status of two butterflies, Karner blue (*Lycaeides melissa samuelis*) and frosted elfin (*Incisalia trus*), restricted to oak savanna in Ontario. In: conserving

Carolinian Canada. Waterloo, Ontario p. 253-271.

Packer, L. (1994). The extirpation of the Karner blue butterfly in Ontario. In: Karner blue butterfly: a symbol of a vanishing landscape. Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, MN pp. 143-151.

Papp, C. (1993). Habitat study of the endangered Karner blue butterfly (*Lycaeides Melissa samuelis* Nabokov) in Michigan oak savanna. Preliminary report. Department of Natural Resources, Wildlife Division, Lansing, MI.

Pickens, B.A. & K.V. Root (2008). Factors affecting host-plant quality and nectar use for the Karner blue butterfly: implications for oak savanna restoration. Natural Areas Journal 28: 210-217.

Pyke, G.H., H.R. Pulliam & E.L. Charnov (1977). Optimal foraging: a selective review of theory and tests. Quarterly Review of Biology 52: 137-154.

Rhiannon, T.C. & C.B. Shultz (2016). Resource selection in an endangered butterfly: females select native nectar species. Journal of Wildlife Management 80(1): 171-180.

Scharf, I. F. Peter & O.Y. Martin (2013). Reproductive trade-offs and direct costs for males in arthropods. Evolutionary Biology 40: 169-184.

Schultz, C.B. & K. Dlugosch (1998). Nectar and host plant scarcity limit populations of an endangered Oregon butterfly. Oecologia. 119: 231-238.

Schultz, C.B., P.C. Hammond & M.V. Wilson (2003). Biology of the Fender's blue butterfly (*Icaricia icarioides fenderi* Macy), an endangered species of Western Oregon native prairies. Natural Areas Association 23(1): 61-71.

Schweitzer, D.F. (1989). Fact sheet for the Karner blue butterfly with special reference to New York. Unpublished report. The Nature Conservancy, Albany, NY.

Schweitzer D.F. (1993). Prioritizing Karner blue (*Lycaeides melissa samuelis* Nabokov) habitats for protection activities. In: Karner blue butterfly: a symbol of a vanishing landscape. Minnesota Agricultural Experiment Station. University of Minnesota, St. Paul, MN pp. 22-24.

Sferra, N.J., D.N. Ewert, C.A. Clampitt, H.E. Ballard, J.M. Aguiar & T. Darnell (1993). Management of oak savanna and oak barrens habitat in Newaygo and Muskegon

Counties, Michigan, for Karner blue butterflies (*Lycaeides melissa samuelis*) and other oak savanna invertebrates. Unpublished report. The Nature Conservancy, Muskegon, MI.

Sharp, M.A., D.R. Parks, & P.R. Ehrlich (1974). Plant resources and habitat selection. Ecology 55: 870-875.

Shreeve, T.G. (1992). Adult behavior. In: the ecology of butterflies in Britain. Oxford University Press, Oxford pp 22-45

Swengel, A. & S. Swengel (1993). Observations of Karner blues and the barrens butterfly community in Wisconsin. Manuscript. Baraboo, WI. pp. 36.

Swengel, A. B. & S. R. Swengel (1996). Factors affecting abundance of adult Karner blues (*Lycaeides melissa samuelis*) (Lepidoptera: *Lycaenidae*) in Wisconsin Surveys 1987-95. The Great Lakes Entomologist 29: 93-105.

Tudor, O., R. Dennis, J. Greatorex-Davies & T. Sparks (2004). Flower preferences of woodland butterflies in the UK: nectaring specialists are species of conservation concern. Biological Conservation 119: 397-403.

U.S. Fish and Wildlife Service (2003). Karner Blue Butterfly Recovery Plan (*Lycaeides melissa samuelis*). Department of the Interior, U.S. Fish and Wildlife Service, Great Lakes-Big Rivers Region (Region 3), Fort Snelling, MN.

U.S. Fish and Wildlife Service. (2012). Karner Blue Butterfly (*Lycaeides melissa samuelis*) 5 Year Review: Summary and Evaluation. Department of Interior, U.S. Fish and Wildlife Service, Great Lakes-Big Rivers Region (Region 3), Fort Snelling, MN.

Voss, E.G. & A.A. Reznicek (2012). Field Manual of Michigan Flora. The University of Michigan Press. Ann Arbor, Michigan.

Williams, E.H. (1988). Habitat and range of *Euphydryas gillett*ii (*Nymphalidea*). Journal of Lepidoptera Society 42: 37-45.

Zavaleta, E.S., R.J. Hobbs & H.A. Mooney (2001). Viewing invasive species removal in a whole-ecosystem context. Trends in Ecology and Evolution 16:454-459.