Endangered *Oarisma poweshiek* butterfly larval foraging and adult habitat interactions in Manitoba, Canada.

by

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Abstract

The Poweshiek skipperling (*Oarisma poweshiek*) is endemic to the tall grass prairie in North America, and is now critically endangered globally. Existing populations are scattered amongst tall grass prairie remnants. However, the host food plants eaten by Poweshiek skipperling larvae, the vegetative and microclimatic descriptions of immature and adult microhabitats as well as *O. poweshiek* behaviour in Manitoba are unknown. I observed the foraging behaviour of larval *O. poweshiek* in natural habitat to determine the plant species that they consume and document their development. I also followed adults in prairie patches to identify locations in which various activities, such as egg laying or nectar feeding, were facilitated by the habitat. I measured vegetative, structural and microclimatic attributes of microhabitats to determine potential characteristics which facilitate various adult activities and larval development. Larvae appeared to navigate microhabitats to locate host food plant species, alternating between shoots of various species throughout their development. Adults flew almost exclusively in the prairie plant community, rarely flew in shrub or ephemeral wetland communities and were never observed flying in wetland or forest communities. Adult activities appeared to be distributed along a soil moisture gradient, with egg laying associated with the mesic section of the moisture gradient, resting and/or basking associated with the drier section and nectar feeding generally associated with sections throughout the moisture gradient. My research contributes knowledge about larval *O. poweshiek* foraging and adult habitat interactions in prairies in Manitoba, Canada. Discoveries from my research may guide habitat stewardship to ensure that high quality habitat is available for every life stage and inform reintroduction activities to ensure potential release locations contain required habitat features. Novel descriptions of locations which facilitate larval development and various adult activities may inform provincial and federal recovery strategies to increase the chances of *O. poweshiek*'s survival. My findings may also initiate further research about the Poweshiek skippering and possibly guide the strategies to recover other Lepidopterans-at-risk. Now with a greater understanding of larval foraging and adult interactions, we may hopefully generate potential causes which explain *O. poweshiek*'s decline and identify possible solutions to facilitate its successful recovery!

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Contributions. This research (Chapters 2 and 3) has been prepared as manuscripts to be published with Dr. Richard Westwood. R. W. acquired funding, R. W. and I co-designed the research, I led field data collection, analysis, as well as drafting of manuscripts and R. W. and I revised the final manuscripts. Dr. Andrew Park, Bill Watkins and Dr. Gard Otis provided invaluable reviews of the thesis version of drafts for which we are grateful.

Chapters are manuscript drafts, in anticipation of the following publications:

Chapter 2: Henault, J. and Westwood, R. In preparation. Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada.

Chapter 3: Henault, J. and Westwood, R. In preparation. Adult habitat interactions of the endangered *Oarisma poweshiek* butterfly are associated along a soil moisture gradient in tall grass prairie in Manitoba, Canada.

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Chapter 1: Introduction

The Poweshiek skipperling (*Oarisma poweshiek*, (Parker, 1870), Lepidoptera: Hesperiidae) is a species endemic to the tall grass prairie in North America (Catling and Lafontaine 1986; Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2014). Once occurring in suitable locations in southern Manitoba, Canada, several north-central states and eastern Michigan in the United States of America, this species is now endangered in each jurisdiction and critically endangered globally (Canada - COSEWIC 2014; Canada Gazette 2019; United States of America - United States Fish and Wildlife Service 2015; internationally - Red List of Threatened Species of the International Union for the Conservation of Nature by Royer 2020). Approximately 99% of the tall grass prairie ecosystem has been destroyed (Samson and Knopf 1994), resulting in remnant Poweshiek skipperling populations in southern Manitoba, Canada and Michigan, United States of America (COSEWIC 2014; United States Fish and Wildlife Service 2015). Remnant colonies have also declined range-wide since approximately the late 1980s (Committee on the Stats of Endangered Wildlife in Canada 2014; Smith et al. 2016).

The Poweshiek skipperling in Manitoba flies in most years between the last week of June to the second week of July, although in some years skipperlings fly during late July and early August (Semmler 2010; COSEWIC 2014). Adults lay eggs throughout their flight period, eggs hatch and larvae feed during the summer-fall until they enter diapause when we assume developmental conditions (perhaps temperatures) deteriorate at the start of winter (potentially September-December). Then, larvae likely resume feeding in the spring before pupating during mid-late June (Layberry et al. 1998; COSEWIC 2014). Researchers have reported several egg laying and larval host food plant species in Canada (Dupont-Morozoff 2013) and the United States (Holzman 1972; McCabe and Post 1977; Borkin 1995; Dana 1999 unpublished; Pointon 2015; Smith et al. 2016; Belitz et al. 2019), but the host species used in Manitoba are still unclear. Past authors derived larval host food requirements from observing individual feeding bouts in natural habitats, but *O. poweshiek* larvae have not yet been tracked throughout their life cycle in the field.

Several Lepidopterists have researched *O. poweshiek* in Manitoba, including Paul Klassen who contributed numerous occurrence localities in the 1980s (R. Westwood pers. comm.

2021). Catling and Lafontaine (1986) first reported *O. poweshiek* in western academic literature in 1985. They also described grasses, forbs, trees and swamp graminoids such as *Andropogon gerardi* Vitman (big bluestem), *Liatris ligulistylis* (A. Nelson) K. Schum. (meadow blazingstar), *Populus tremuloides* Michx. (trembling aspen) and *Eleocharis elliptica* Kunth respectively, that were associated with *O. poweshiek* observations. Catling and Lafontaine (1986) also noted that higher elevation prairie, forested areas and swamps were adjacent to each other in areas supporting this skipper. Semmler (2010) reported that *Rudbeckia hirta* L. (black-eyed susan) and *Solidago ptarmicoides* (Torr. & A. Gray) B. Boivin (upland white goldenrod) were most commonly used as nectar sources. Also, that locations where more time had passed since being burned contained greater abundances of these forbs than locations more recently burned (Semmler 2010). The records which describe the way in which fires were started (prescribed burn, wildfire, accidentally started by human activities) at the locations in Semmler (2010) are not detailed. This general area where Poweshiek skipperlings were supported has been occasionally managed with fire (Grantham et al. 2021), therefore both wildfires and prescribed burns were possible. Locations where fires occurred three to five years before observations supported the greatest abundance of skippers (Dupont-Morozoff 2013). Dupont-Morozoff (2013) also attempted to determine the adult population size using mark-release-recapture techniques but a small sample size prevented a robust estimate. Dupont-Morozoff (2013) observed one female sequentially lay eggs on *A. gerardi* (grass), *Melilotus* (L.) Mill. spp. (sweet clover; forb), *Solidago* L. spp. (goldenrod; forb) and *Quercus macrocarpa* Michx. (bur oak; tree). Dearborn and Westwood (2014) developed an adult emergence prediction model using degree days accumulated at a nearby weather station to estimate development in natural habitats. A habitat suitability model was created using vegetative and physical attributes of locations supporting *O. poweshiek* by Hooshmandi (2016) to locate possible unknown habitat in Manitoba that could support this skipper. Henault (2017) discovered that sites with higher abundances of adult *O. poweshiek* were relatively drier (as indicated by the presence of *H. hirsuta*) and were also correlated with potential host plant species *Sporobolous heterolepis* (A. Gray) A. Gray (prairie dropseed), *A. gerardi* and *Schizachyrium scoparium* (Michaux) Nash (little bluestem) compared to sites with lower adult abundances (as indicated by Juncaceae Juss. spp. (rush family)). Henault (2017) also found that *R. hirta* occurred at higher densities than the sites with lower *O.*

poweshiek abundances. The habitat suitability model developed by Hooshmandi (2016) was refined and applied to the local ecoregion in Manitoba in 2020 (Westwood et al. 2020).

Butterflies lay eggs in microhabitats containing host food plants and the microclimatic conditions required for immature stages to develop (Ashton et al. 2009; Krämer et al. 2012; Ewing et al. 2020). If laid on the ground or an otherwise unpalatable substrate, larvae search for host food plants once hatched (Kopper et al. 2000; Hellmann 2002). In general, females butterflies use cues (host plants, plant chemical composition) to identify suitable microhabitats (Wiklund 1984; Lund et al. 2019).

The developmental rates throughout the growth stages of *O. poweshiek* is not well understood. The number of degree days accumulated during the Poweshiek skipperling life cycle in occupied microhabitats in natural areas in Manitoba has not been reported. In Manitoba degree day accumulations using air temperatures recorded at a nearby weather station are annually calculated (based on larval thermal development) to time adult population surveys with adult emergence (Dearborn and Westwood 2014; Henault and Westwood unpublished).

Tall grass prairie habitat supporting *O. poweshiek* in Manitoba is contained within the Manitoba Tall Grass Prairie Preserve (MTGPP; interpretive centre approximately 49.153° N, 96.729° W). This tall grass prairie area is relatively mesic and contains grassland openings, forest stands and ephemeral or permanent wetlands (Catling and Lafontaine 1986). Typical mesic prairies include the grasses *A. gerardi* and *S. heterolepis*, forbs *Solidago rigida* L. (rigid goldenrod) and *L. ligulistylis*, trees *P. tremuloides* and *Q. macrocarpa* with wetter areas supporting species including *E. elliptica* and Juncaceae spp. (Catling and Lafontaine 1986; COSEWIC 2014).

Poweshiek skipperlings have been consistently observed within the prairie type of plant communities during annual adult surveys in the MTGPP (Grantham et al. 2020), but vegetative and physical characteristics supporting egg laying, nectar feeding and other adult interactions are unknown. Tall grass prairies where I conducted research contain physical slopes (Catling and Lafontaine 1986), possibly providing a soil nutrient and moisture gradient (Liu et al. 2020) which creates a change of plant species (Zalatnai and Körmöczi 2004). Ravenscroft (1994) and Grundel et al. (1998) have shown butterflies lay eggs and conduct other activities in specific areas containing certain vegetation species and physical architectures. Therefore, I aspired to determine how adults use prairie environments in Manitoba by evaluating whether locations used for specific survival behaviours were different (*i.e.* are eggs laid in different locations than nectar is consumed?).

In my B.Sc. Honours thesis (Henault 2017) I predicted that various host species would make up different proportions of larval diets, which larvae access potentially by moving between shoots of species during development. I also hypothesized that adults conduct specific activities in locations with certain vegetation and structural attributes to facilitate these activities in prairies. Researchers have not tracked the foraging activities of *O. poweshiek* larvae over entire seasons (host plants consumed by larvae and larval behaviour) or described the vegetative and microclimatic attributes where larvae occur in tall grass prairies in Manitoba. These aspects of Poweshiek skipperling ecology became my focus. I separated my research into two chapters, both prepared as academic manuscripts to be published. Hypotheses and objectives are described in their respective research chapters. During the research in Chapter 2, I followed Poweshiek skipperling adults in their natural habitat to locate microhabitats where eggs were laid and to observe larval foraging behaviour and development. This research was designed to identify the larval host food plants and document feeding behaviour in natural tall grass prairie habitat Manitoba, examine vegetative and physical microhabitat characteristics, and document *O. poweshiek* development rates. I discuss how the knowledge generated may contribute to a better understanding of Poweshiek skippering, in terms of oviposition biology and the attributes within suitable microhabitats of immatures. The results may contribute to their recovery by guiding the development of appropriate diets for reared larvae and guide the identification of potential habitats to reintroduce *O. poweshiek*. Identifying the larval host plants utilized in natural habitats for Poweshiek skipperling is critical to prioritizing future research directions to recover this species.

In Chapter 3, I report research where I compared the vegetative, structural and edaphic characteristics of locations within prairie patches where adults laid eggs, rested or interacted in some other way. I aspired to determine how adults use tall grass prairies in Manitoba and simultaneously how microhabitats within this habitat may facilitate these adult interactions. I sought to identify the plant communities where Poweshiek skipperling conducts various activities (*e.g.*, how do *O. poweshiek* use specific locations for egg laying, nectar feeding, and other activities?). Although prairie patches likely contain similar plant species and configurations plant communities, I assume each prairie patch is unique. By describing vegetative and physical attributes that characterise adult interaction types, I hoped to ensure that my research could be applied to stewardship of, and reintroduction to, any prairie in Manitoba and potentially North America.

Discoveries from my research may guide disturbance strategies to provide suitable vegetation to facilitate all activity types, inform rearing of Poweshiek skipperlings in captivity, support the assessment of potential reintroduction sites and contribute to continuous revisions of recovery approaches. All in an effort to improve the likelihood of long-term *O. poweshiek* survival!

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Chapter 2: Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada.

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The Poweshiek skipperling (*Oarisma poweshiek*) is endemic to the tall grass prairie in North America, and is now critically endangered globally. Existing populations are scattered amongst tall grass prairie remnants. However, the host food plants eaten by Poweshiek skipperling larvae, the vegetative and microclimatic descriptions of immature and adult microhabitats as well as *O. poweshiek* behaviour in Manitoba are unknown. I followed Poweshiek skipperling adults in their natural habitat to locate microhabitats where eggs were laid and to observe larval foraging behaviour and development. I measured vegetative, structural and microclimatic characteristics of microhabitats used by immatures and documented document the host species larvae consumed, their general behaviour (including movement distance and orientation measurements) and their stage of development in microhabitats. Larvae ate *A. gerardi* (big bluestem), *M. richardsonis* (mat muhly), *S. heterolepis* (prairie dropseed) and *S. scoparium* (little bluestem) in natural tall grass prairie. Larvae appeared to navigate microhabitats to locate host food plant species, alternating between shoots of various species throughout their development. Microhabitats appeared to be more open and have drier microclimates than areas where eggs were not laid. Equipped with an improved understanding of specific larval feeding patterns, adult behaviours and microhabitat attributes we can hopefully narrow the list of possible causes of decline and facilitate the identification of potential solutions to recover the Poweshiek skipperling.

Introduction

The Poweshiek skipperling (*Oarisma poweshiek*, (Parker, 1870) (Lepidoptera: Hesperiidae)) is an obligate tall grass prairie butterfly once commonly found in the north central United States of America and southern Manitoba, Canada (McCabe and Post 1977; Catling and Lafontaine 1986; Klassen et al. 1989; Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2014; Belitz et al. 2018). The destruction of 99.9 % of tall grass prairie (Samson and Knopf 1994) was likely one of the main causes that reduced the number of Poweshiek skipperling colonies (COSEWIC 2014; Smith et al. 2016). The abundance of adults in remnant colonies has declined since approximately the 1980s to potentially a few hundred individuals across its range (abundance estimates in late 1980s by P. Klassen and R. Westwood unpublished; COSEWIC 2014; Smith et al. 2016; Grantham et al. 2020). However, reasons for these population declines in remnant colonies are unknown (Committee on the States of Endangered Wildlife in Canada 2014; Smith et al. 2016), but possibly due to unsuitable habitat management (Swengel and Swengel 1999). Remaining *O. poweshiek* populations occur at the margins of the historical range in Michigan, United States of America (Belitz et al. 2018) and in Manitoba, Canada (COSEWIC 2014). Poweshiek skipperling is listed as endangered in both Canada and the United States of America (COSEWIC 2014; United States Fish and Wildlife Service 2015; Canada Gazette 2019) and critically endangered on the planet (Red List of Threatened Species of the International Union for the Conservation of Nature by Royer 2020).

In Manitoba, the Poweshiek skipperling occurs in wet to mesic tall grass prairie patches surrounded by stands of *Quercus macrocarpa* Michaux (Fagaceae) (bur oak) and *Populus tremuloides* Michaux (Salicaceae) (trembling aspen) intermixed with both permanent and ephemeral wetlands (Catling and Lafontaine 1986; COSEWIC 2014), within a specific range of patch sizes (Westwood et al. 2020). In Manitoba during the 2000s, dozens of adults were consistently observed within individual research sites (approximately 1 quarter section parcels) totally up to several hundred individuals (COSEWIC 2014). The abundance of Poweshiek skipperling across its range has since declined to approximately a few hundred individuals (COSEWIC 2014; Smith et al. 2016; Grantham et al. 2020). Henault (2017) reported that sites with higher abundances of adult *O. poweshiek* were relatively drier than those with lower adult abundances, with high abundance locations indicated by the presence of *Hypoxis hirsuta*

(Linnaeus) Coville (Hypoxidaceae) (star-grass) (in 2017 thesis erroneously reported as *Bouteloua curtipendula* (Michx.) Torr. (Poaceae), J. Henault correction 2021), medium abundance by the presence of *Deschampsia cespitosa* (L.) P. Beauv. (Poaceae) and low abundance by the presence of Juncaceae Juss. spp.. *Rudbeckia hirta* L. (Asteraceae) occurred at relatively high densities in sites supporting higher abundances of *O. poweshiek* adults compared to sites supporting lower abundances of skipperlings (Henault 2017).

The Poweshiek skipperling in Manitoba flies in most years between the last week of June to the second week of July, although in some years skipperlings fly in late July to early August (Semmler 2010; COSEWIC 2014). Adults lay eggs throughout their flight period, eggs hatch and larvae feed during the summer-fall until they enter diapause when we assume developmental conditions (perhaps temperatures) deteriorate at the start of winter (potentially beginning during September to December). Larvae then resume feeding in the spring and likely pupate during mid-late June (Layberry et al. 1998; COSEWIC 2014). Dupont-Morozoff (2013) observed ovipositions on *Andropogon gerardi* Vitman (Poaceae) (big bluestem; grass), *Melilotus* (L.) Mill. spp. (sweet clover; forb), *Solidago* L. spp. (goldenrod; forb) and *Quercus macrocarpa* Michx. (bur oak; tree).

A female and male were observed mating in the canopy of graminoids at a visually mesic location, after which the female immediately flew approximately three metres to the eventual oviposition location and laid an egg on a *Sporobolus heterolepis* (A. Gray) A. Gray (Poaceae) (prairie dropseed) leaf (J. Henault unpublished observation 2015; Supplementary Figure 2.1, please see below for observations of activities prior to mating in 2017 – Supplementary Video 2.2). Observations of egg laying in the United States (Wisconsin, Minnesota and North Dakota: tall grass prairies; Michigan: tall grass prairie fens) report adults using several substrates: the Poaceae Barnhart spp. (grasses) *S. heterolepis*, *Schizachyrium scoparium* (Michaux) Nash (little bluestem), *A. gerardi*, *Hesperostipa spartea* (Trin.) Barkworth, *Muhlenbergia richardsonis* (Trin.) Rydb. (mat muhly) and *Muhlenbergia glomerata* (Willd.) Trin.; the Cyperaceae Juss. spp. (sedges) *Eleocharis elliptica* Kunth, *Eleocharis* R. Brown spp. (spike rush), *Carex sterilis* Willd. (little prickly sedge) and unidentified Cyperaceae Juss. spp.; the forb *Silphium terebinthinaceum* Jacquin (Asteraceae) (prairie dock) and the shrub *Potentilla fruticosa* L. (Rosaceae) (shrubby cinquefoil) (Wisconsin: Borkin 1995; Minnesota: Dana 1999 unpublished; Michigan: Holzman 1972; Pointon 2015; Belitz et al. 2019; North Dakota: McCabe and Post 1977).

The host plants consumed by larvae in natural habitats have not been reported in Canada, but several observations have occurred in the United States of America. Borkin (1995) in Wisconsin observed larvae during individual feeding bouts in natural prairie habitat. Larvae were observed eating *S. heterolepis* and *S. scoparium* (Borkin 1995). Larvae collected as eggs from local females were observed in prairie plug pots dug into a natural tall grass prairie habitat in Minnesota (Dana 2020 pers. com.), where they ate the previous species as well as *A. gerardi* (Dana 1999 unpublished). Feeding marks, but no direct observations, were observed on *Carex inops* subspecies *heliophila* (Mack.) Crins and an unidentified graminoid (Dana 1999 unpublished). Potential larval feeding marks were observed on *M. richardsonis* leaves where *O. poweshiek* females had laid eggs in Michigan (Pointon 2015), however larval feeding was not observed. *Oarisma poweshiek* in a zoo rearing-setting (Minnesota Zoo, United States of America) have been observed eating *Carex pensylvanica* Lam. for at least part of their life cycle (Smith et al. 2016). Based on egg host plants and feeding marks near hatched eggs in natural habitat in the United States of America, researchers have also suggested *B. curtipendula*, *E. elliptica* and *Carex* spp. may be larval food species (McCabe and Post 1977; Selby 2005; COSEWIC 2014; Smith et al. 2016). Observations tracking the life history of individual Poweshiek skipperling larvae in natural habitats in North America have not been reported. Larvae are thought to overwinter at the base of the host plant or nearby just above the soil surface (McAlpine 1972; Borkin 1995; COSEWIC 2014).

Butterflies lay eggs in microhabitats containing host food plants and the microclimatic conditions (such as temperature or humidity) required for immature stages to develop (Ashton et al. 2009; Krämer et al. 2012; Ewing et al. 2020). If laid on the ground or an otherwise unpalatable substrate, larvae have been observed searching for host food plants once hatched (Kopper et al. 2000, Hellmann 2002). In general, female butterflies use cues (host plants, plant chemical composition) to identify suitable microhabitats (Wiklund 1984, Lund et al. 2019).

The developmental rates throughout the growth stages of *O. poweshiek* are not well understood. The number of degree days accumulated during the Poweshiek skipperling life cycle in occupied microhabitats in natural areas in Manitoba has not been reported. In Manitoba, degree day accumulations using air temperatures recorded at a nearby weather station are annually calculated (based on estimated larval thermal development) to synchronise the timing of adult population surveys with adult emergence (Dearborn and Westwood 2014; Henault and Westwood unpublished). In addition to increasing biological knowledge, a better understanding of immature development may conceivably help conservation partners time annual adult population surveys (Westwood et al. 2012; Grantham et al. 2020) with increased accuracy.

The host food plants eaten by Poweshiek skipperling larvae in Manitoba tall grass prairies are unknown. The vegetation, physical structure, soil characteristics and microclimate attributes in suitable microhabitats of immature skipperlings have also not been described. Identifying the larval host plants that Poweshiek skipperling use in natural habitats is critical to prioritizing future research directions and contributing to the recovery of this species. Our research documents larval feeding biology and microhabitat requirements in natural habitats to increase knowledge of larval behaviour and development, and in part to guide captive-rearing programs and the assessment of potential reintroduction locations. The objectives of this research were to: 1) identify Poweshiek skipperling larval host plants in natural tall grass prairie sites in Manitoba, 2) examine the vegetative and physical characteristics, including the size, of microhabitats where eggs were laid, 3) document larval feeding behaviour of wild Poweshiek skipperling during their life cycle in natural habitats and 4) report degree day accumulations for life cycle stages in natural habitat. We hypothesised that A) females lay eggs in microhabitats with a diversity of host food plants and range of microclimates (with subsequent temperatures and humidities), and B) larvae locate host food species at specific locations within microhabitats. We predict that females would choose microhabitats with generally suitable attributes (potentially the abundance and diversity host food plants as well as the general microclimate within the microhabitat), thus facilitating larval survival by enabling larvae to reposition themselves while feeding, resting or moulting if necessary.

Methods

Field research was carried out within the MTGPP in southeastern Manitoba, Canada (interpretive centre approximately 49.153° N, 96.729° W). In the warmest month, temperatures average 18.8 °C (July) and in the coldest month average -16.6 °C January (average of 1981-2010 at Zhoda, MB; Government of Canada 2021). Precipitation averages 101.1 mm in the wettest month (June) and averages 17.9 mm in the driest month (February) (Government of Canada 2021a). This area is located within the Steinbach ecodistrict, the Interlake Plain ecoregion and the Boreal Plains ecozone (Agriculture and Agri-Food Canada 2013). The bedrock is limestone, glacial residue occurs consistently and soils are Dark Gray Chernozems as well as Peaty Gleysol and Mesisol soils (Ecological Stratification Working Group 1995).

The MTGPP has been surveyed for the Poweshiek skipperling adults annually between 2006 and 2020 (Westwood et al. 2012; Grantham et al. 2020). Sites were surveyed using a meandering transect walk technique (Royer et al. 1998) where observations of Poweshiek skipperling in each site are recorded and summed during each survey year (number of adults observed/number of survey hours). Sites were surveyed along pre-established paths that include habitats with various soil moistures that are dominated by graminoids. At least one or both authors surveyed in the field as part of these collective efforts since 2006. During 2006 and 2007, surveyors only recorded the number of adults but not time spent surveying (R. Westwood unpublished). While surveying, we noted adult behaviours such as egg laying and their approximate locations in each site. For this study, we selected a site with a consistent annual presence of Poweshiek skipperling between 2007 and 2017 as well as where we had previously observed ovipositions. The research site was a tall grass prairie complex containing prairie patches adjacent to wetlands and forests in one quarter section parcel of land (0.64 km^2). Within this complex we studied Poweshiek skipperling at three prairies patches isolated from each other by wetlands/forests (three prairie sections approximately 0.20 km^2 area and 2.6 km perimeter in total). A wildfire last disturbed the site in autumn of 2011. The Nature Conservancy of Canada removes woody encroaching perennial species using prescribed fires, by cutting vegetation or applying herbicides when required (Grantham et al. 2021).

In 2018, we followed adult female Poweshiek skipperlings every day during their flight period between 25 June to 4 July and 6 July and from approximately 10 am and 5 pm in the study site to observe egg laying. We saw females from 27 June to 3 July 2018 and males and/or females from 26 June to 4 July 2018 (dates inclusive for both date ranges). The bases of shoots where females were observed laying eggs were marked with a metal stake and recorded with a GPS unit (Garmin Oregon 700; approximate accuracy 3 m). After the female flight period, the shoot supporting the egg was identified to species and the stake repositioned if necessary. A typical reposition involved placing the stake next to the egg, instead of at the base of the host plant which would allow us to locate eggs as leaves moved in the wind.

Egg enclosures – July 2018 to July 2019

Our main goal was to make sure areas within egg enclosures were as similar as possible to natural larval conditions in a prairie. We centred large black plastic refuse pails (which we previously cut in half) around egg laying locations, breaking the ground surface to a depth of 3 cm to prevent larval escape (Fig. 2.1). Enclosures were 49 cm wide at the base and extended 28 cm above the ground surface (area at the ground approximately 0.19 m^2). The circumference of comparison plot rings matched the outside edge of egg enclosures. However, the opening of the black refuse pails flared slightly so the egg enclosure area at the ground surface was slightly smaller than the comparison plot rings. We also spray-painted the enclosures white (in an effort to reduce heat radiation) and secured with metal stakes pushed into the ground.

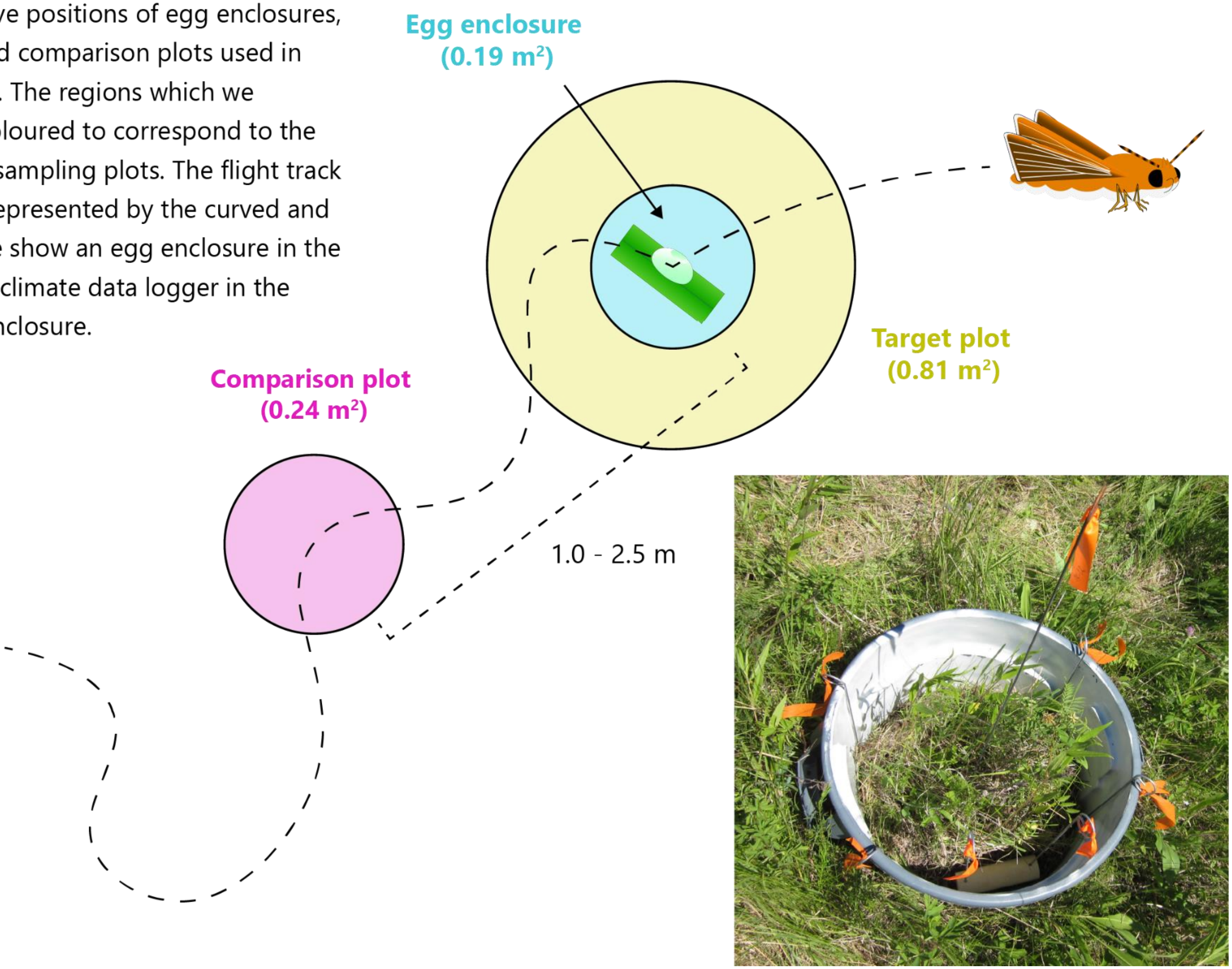

Fig. 2.1. Relative positions of egg enclosures, target plots and comparison plots used in 2018 and 2019. The regions which we sampled are coloured to correspond to the three types of sampling plots. The flight track of an adult is represented by the curved and dotted line. We show an egg enclosure in the field; the microclimate data logger in the shade of the enclosure.

Enclosures were left without mesh to mimic ambient microclimates; mesh may shade and therefore alter larval behaviour. Our design risked larvae crawling out or predators accessing larvae, but we think that our design also maintained the natural conditions that a typical larva experiences. We cut vegetation 3-5 cm around the inside edge of enclosures (just above the layer of dead vegetation) to reduce the likelihood of larval escape. Any vegetation around the outside of enclosures that could potentially allow larvae to escape was also cut. Vegetation was clipped approximately once every week. However, we stopped cutting vegetation after larvae were determined not to be present in an enclosure (after 2 weeks with no larval observations). Cuts were made following vegetation sampling in August 2018, and also after sampling on 9 May 2019 (sampling dates described in subsequent sections). We left enclosures in place over the winter until the first adults of the next generation were observed (in July). During two weeks of observations starting on 8 May 2019 we did not see larvae, thus we assumed that enclosures were empty. We also stopped clipping vegetation at this time. We hypothesised that the enclosure area would be suitable because larvae are small (approximately 2 – 24 mm in length; McAlpine 1972), and if they moved, they likely would not move outside to the perimeter of this area. Control enclosures at comparison plots to help determine the influences of enclosures to plant growth or ambient microclimates were not placed. We did not want to risk influencing the behaviours of other wildlife using this habitat by obstructing access to potential microhabitats necessary for those species.

Larval foraging behaviour

The distance from the egg to the tip of the leaf blade and the height above the ground were recorded. Eggs were monitored daily for hatching. We observed larvae to document the host species they consumed (identified after larval feeding), their general behaviour (including movement distance and orientation measurements) and their stage of development. To monitor larvae, one to three times per week J. H. located a larva and watched it for at least one hour. Field observations began 26 June 2018 and ended in September. We ended our observations using the first date that the temperature dropped below 0° C as a general benchmark. The first 0° C date was 5 September and we ended our observations at 14 September when larvae no longer appeared active. The daily minimum temperature was consistently within a few degrees on either side of 0° C between 28 September until 21 May inclusive. Temperature and photoperiod are likely the most influential factors to induce larval butterfly diapause (Kim et al. 2014), however the diapause-inducing conditions for *O. poweshiek* are unknown. While Dearborn and Westwood (2014) used 6° C as the lower development temperature of *O. poweshiek* based on studies of other Lepidoptera that overwinter as larvae in the region, no study to date has determined the actual minimum threshold of the Poweshiek skipperling. Given this uncertainty, we assumed that larvae would almost certainly be inactive when the temperature was below 0° C, and potentially during the fall season when the photoperiod had shortened compared to the summer. We think the date when temperatures are consistently below 0° C in the fall (28 September in this study) is likely a replicable checkpoint during any year. Therefore, we used this physical checkpoint to enable consistent comparisons in future research. The date at which temperatures become consistenly above 0° C in the spring is potentially also a reliable checkpoint for the termination of diapause, by using the same logic. Thus, we resumed observations on 8 May 2019 after the snow melted earlier in the spring but before we assumed that larvae would have resumed activity.

We measured the horizontal distance between sequential larval feeding or activity locations on shoots. Measurements were recorded whenever larvae moved, sometimes only after multiple days of observations. The locations larvae occupied on plants when feeding, resting and molting was recorded. We measured the distance along a leaf blade from the apex of the larval head to the tip of the blade and vertical distance to the ground. We also recorded orientation of the head (pointing apically or basally on the leaf) and the angle that the dorsal side of the cuticle faced. Larval deviation from a perpendicular orientation to the ground was not measured, however during most observations larvae were generally oriented closer to the vertical plane than the horizontal. We recorded the duration of feeding bouts and inter-feeding resting bouts when the opportunity arose. By chance, J. H. observed all instar transitions immediately after moulting; larvae were observed hardening their exoskeletons on a shoot adjacent to a shriveled exuvia. McAlpine (1972) found exuviae attached to shoots but after the accompanying larvae had vacated the area. These exuviae were used to determine the instar (McAlpine 1972). Our direct observations of moulting may increase precision of the beginning and end of each instar compared to McAlpine (1972). Larvae were measured once each instar 1 to 3 days after moulting (to minimize the risk of damaging them while their cuticle was sclerotizing). Larvae were measured on the plant with a ruler (apex of the head to terminus of the body) without handling the caterpillar, to not displace the it and to potentially reduce stress. Justis Henault observed and classified all adult behaviours (except for two ovipositions and two nectar feeding activities by Richard Westwood), with note-taking assistance from field helpers. Justis Henault solely observed, measured and took notes of egg development as well as larval positions and behaviours.

Microclimate in microhabitats

We placed temperature, relative humidity and dew point data loggers (HOBO® Pro V2 U23-001: Onset Computer Corporation, Bourne, Massachusetts, United States of America) in egg enclosures (n = 4) and comparison plots accompanying egg enclosures (n = 4). We programmed data loggers to record measurements at one-hour intervals between 9 July 2018 and 18 July 2019. Data loggers were placed horizontally at the soil surface, in plastic tubes held in place with metal stakes. Thus, the opening of the tubes allowed air to flow into the data loggers from approximately between the soil surface to a couple of centimetres above the layer of dead vegetation in microhabitats (based on our study, the top of the layer of dead vegetation was approximately 4 cm; Fig. 2.1). We also placed them in areas where they were not exposed to direct sun (egg enclosures: along the south inside edge of plastic pails; comparison plots: to the south of taller vegetation) in an attempt to equalise the degree of shading by data loggers. Although these data loggers on the ground likely recorded different conditions than those experienced by immatures in the plant canopy, we think that they may indicate the general conditions of the microhabitat. We used the data from these data loggers to calculate degree days required to complete developmental stages of immature Poweshiek skipperling and to compare microclimates between egg enclosures and comparison plots. We also acquired temperature data recorded at an Environment and Climate Change Canada weather station located at Emerson, Manitoba (approximately 35 km, 254° from the study site) from an online repository (Government of Canada 2020), to calculate degree days using the temperatures recorded at this station and compare it to field data loggers over the same period. This weather station is currently used to help synchronise adult population surveys in Manitoba and the United States of America (Westwood and Henault unpublished). Calculations used to inform these surveys use data from 1 March through mid-June, likely just before adults eclose. Our objective was to determine if this weather station provided accurate data relative to field microhabitats (the general temperature experienced by larvae in microhabitats, not immediately surrounding larvae on a leaf). Thereby indicating if the weather station could help us precisely synchronise population surveys with adult emergence.

Plant diversity within egg enclosures and target plots – 2018

A ring outside of egg enclosures (extending to a radius of 57 cm from the centre of enclosures) termed “target plots” was also sampled. We sampled these locations on 2 and 3 August 2018. Females flying in search of oviposition locations likely receive continuous stimuli from the environment. If a female is stimulated to lay an egg at an egg-sized location amongst vegetation, does the female evaluate stimuli within an *e.g.*, 10 cm radius, or 50 cm radius of the potential deposition location? By comparing vegetation and measuring physical attributes at different radii from deposited eggs, we hoped to determine the size of prairie assessed by females while laying eggs. The area sampled in target plots (between the egg enclosure and the outside circle) was 0.81 m^2. In egg enclosures and target plots we individually enumerated the number of shoots of the graminoid (Poaceae spp., Cyperaceae spp., Juncaceae spp. and Juncaginaceae Rich. spp.), forb and shrub species. We also estimated to the nearest 1 % the percent cover of the graminoids, forbs, shrubs, duff (dead pieces of vegetation and fine decomposing material) and bare ground. We thought these estimations would be inaccurate so at the time we thought that increasing the degree of precision may increase their accuracy. A metre ruler was used to estimate the height of 95% of the graminoids (estimated by eye; i.e., 2.5% would be taller and 2.5 would be shorter) in each sample plot. Within each sample plot we also measured the depth of duff in three locations. We randomly placed the ruler to record the depth within each plot. We identified plant species using dichotomous keys and visually using Looman and Best (1987) and Leighton and Harms (2014). Then, we updated nomenclature to Tropicos.org (Missouri Botanical Garden 2021), a database that hosts up-to-date, academically-accepted plant nomenclature. We wanted to measure as many attributes of a suitable immature *O. poweshiek* microhabitat as possible. We thought that specific soil moisture may facilitate vegetation and physical attributes that are suitable to immature *O. poweshiek* development. If soil moisture was indicative of suitable locations, then by measuring soil moisture we could predict the approximate location of *O. poweshiek* egg and larval microhabitats in any given prairie (Chapter 3). Soil moisture and electrical conductivity were measured at a depth 10 cm at two locations in egg enclosures with a soil meter (Fieldscout TDR 150; Spectrum Technologies, Incorporated, Aurora, Illinois, United States of America) by pushing a metal probe into the ground randomly and reading the electronic display. We did not want to measure the moisture at the surface of the soil but instead the moisture potentially experienced by the roots of plants. Also, this depth corresponded to the length of the probe that we had. Measurements were conducted after 7 and 8 days without substantial rain showers on 18 and 30 July 2018 respectively (during a couple occasions, enough rain fell to wet the surface of leaves without reaching the ground). The EC (Natural Resources Conservation Service 2012) is a measure of the concentration of molecular ions (or degree of salinity) in the soil.

Plant diversity within egg enclosures and comparison plots - 2019

We also established a circular “comparison plot” 0.24 m^2 in area to accompany each egg observed. To establish comparison plots, we placed a metal rod in the ground at locations where females flew over immediately before laying an egg at a different location while following adults in 2018. We thought the locations of comparison plots may not have stimulated female oviposition (after termed “avoided” locations) but that egg locations had stimulated females above the oviposition threshold to lay an egg (oviposition threshold as described in Singer 1971). We chose comparison plot locations 1.0 to 2.5 m away from egg enclosures and with a similar sun exposure to egg locations when possible. We hoped to compare the attributes of oviposition locations to avoided locations. To mark the edges of the comparison plot while sampling, we used a plastic tube ring.

We sampled egg enclosures and comparison plots at multiple dates to assess host plants available to larvae during the spring. Host plant populations in plots were sampled on 9 May, 30 May, 11 June and 5 July 2019. In egg enclosures and comparison plots, we counted the number of shoots of each host food plant species observed in 2018 and some species of potentially suitable hosts (previously reported in other studies and discussed in our introduction sections; all species which were enumerated during this study are reported in Supplementary Table 2.3). We also estimated the number of shoots belonging to one of two groups as a percentage of total plant shoots in the plot. Specifically, one group contained host food plants observed in 2018 and the second group were all other plants found in the plot (*e.g.*, 50 % shoots of species in the host plant group, 50 % shoots of species in group containing all other plant species). During each of sampling dates the percent cover of graminoids, forbs, shrubs, duff and bare ground were estimated to the nearest 1 %. The height of 95 % of graminoids as a group, as well as sub-portions host grasses and non-host graminoids (graminoid species which we did not observe larvae consuming) were measured with a metre ruler. In 2019, the duff was subdivided into an upper layer of thatch (dead pieces of vegetation) and litter (a lower layer of fine decomposing dead material). The mean duff depth was measured randomly in three random locations within each egg enclosure and comparison plot on 9 May and 30 May, and the depths of thatch and litter in three random locations on 18 July (2018 duff = dead standing and fine decomposing, 2019: thatch = dead standing, litter = fine decomposing). Soil moisture and electrical conductivity were measured at a depth 10 cm at two random locations in both plot types with a soil meter after 6 and 7 days of no precipitation on 9 and 10 May 2019 respectively. The light intensity above the thatch layer and below were measured in immediate sequence using a light metre (measured in footcandles; General Electric Company, United States of America) during zero cloud cover to complement microclimate measurements by data loggers; samples in all plots (one per plot) were recorded on 23 July 2019 within 3.5 hours. The light intensity above thatch was measured by positioning the sensor to break the horizontal plane of the thatch, and the sensor was placed immediately below the thatch material for accompanying sub-thatch measurements.

Lepidopteran nomenclature initially follows Pohl et al. (2018), and secondarily GBIF.org (Global Biodiversity Information Facility 2021) if not discussed in the first article. Pohl et al. (2018) is the most recent classification of Lepidoptera in Canada and Alaska (United States of America) and GBIF.org is an online database containing classifications developed by using academic literature.

Analysis

The distance larvae ate or rested from the plant tip is reported as mean ± range and the distance from the ground as mean ± 95 % confidence intervals (C. I.). While measuring the distance of larvae from the tip, we were able to place the ruler alongside the leaf blade. We think that this ruler placement combined with the relatively small distances which we measured, enabled us to record measurements in centimetres to two decimals precisely. When measuring the distance from the ground, we were not confident that our measurements would be exactly perpendicular to the surface of the soil and thought that the relatively greater distance increased the likelihood that we would make errors at a similar degree of precision. Given these uncertainties, we measured the distance from the ground using centimetres to only one decimal place. We did not want to disturb larvae by recording measurements. If the conditions were windy thus increasing the likelihood that we might displace the larvae during measurements or if larvae flinched while we were measuring, we ceased recording measurements of that specific larval behaviour event. We returned later in the day to resume observations of new larval behaviours. Our decision to avoid disturbance to larvae resulted in recording only the distance of larvae to the tip at some events, only the distance of larvae to the ground at others and only an observation of larval feeding/resting without any measurements recorded at some events. We report the total number of observations of each larval activity in the relevant sections. The duration which larvae were observed feeding and resting is reported as mean ± 95 % C. I.. For these calculations, we only used feeding and resting observations where the larvae alternated between feeding and resting at least one time. Therefore, some observations of resting were unaccompanied by a feeding observation thus were not included in these calculations. The unused resting observations were included in the calculation of the total amount of time which we observed immatures. The degree of larval activity was assessed by calculating the proportion of observations in which a larva was observed on a different shoot than the previous observation (frequency at which larvae moved since last observation). Since plant leaves that larvae occupied were vertically positioned, a larva's degree of exposure to the sun depended on the larvae's orientation (which direction the larvae faced its dorsal side) instead of whether larvae were situated on the dorsal or ventral side of blades. The mean angle, directionality (r) and significance of direction (Rayleigh test) that larvae oriented on host plants while feeding and resting were calculated as in Batschelet (1981). At least five samples are required to conduct the Rayleigh test (Batschelet 1981).

Temperature data from the Emerson weather station between 30 June through July 8 2018 were used to supplement egg-period microclimate data from data loggers. Degree day accumulations were calculated using biofix dates, as well as lower and upper development thresholds to develop standard and double sine models as in Dearborn and Westwood (2014). The model calculations start from a calibration date for accumulations (biofix date), then the technique subtracts the hypothesized lower development temperature (6 °C) from the day average temperature (standard model) or accounts for degree day accumulations between the lower and an upper development threshold (32 °C) (double sine model) to calculate accumulated degree days. The first dates of each development period (see below) are the biofix dates during our study. Microclimate data recorded by data loggers (temperature as °C, humidity as % and dew point as °C) were standardized by adding or subtracting values relative to the average recording by all data loggers, to calibrate for individual measurement differences among data loggers. To calibrate, we operated the data loggers in a building with a consistent program to maintain the temperature and humidity for three days. We calculated the mean of the measurements for each hour across units, and subtracted/added the mean from the measurement recorded by each unit to determine their bias. Then, for every field recording we added/subtracted the respective value for each datalogger (degrees, %) before making calculations or conducting analyses. Degree days calculated using recordings from data loggers in egg enclosures and comparison plots were compared using 95 % C. I.s.

We assessed the microclimate near the soil surface of microhabitats during what we hypothesised are important developmental periods of the Poweshiek skipperling's life cycle during any given year. The 2018-19 life cycle was divided into the following components: 30 June to 27 September – first egg laid and active larvae, 28 Sept to 21 May – diapause, 22 May to 10 July – break of diapause to first adult eclosion and 30 June 2018 to 10 July 2019 – egg laid to adult eclosion. We did not observe larvae completing development for their entire life from egg to adult. Therefore, the date ranges of each theoretical period are estimations. We used the dates when temperatures became consistently below 0° C in the fall (28 September) until temperatures resumed consistently above 0° C in the spring (22 May) to define the diapause period when we assumed conditions would not be suitable for larval development. We think that the temperature of 0° C is a biologically relevant date for future researchers studying diapause in similar taxa. Although the start of larval activity in the spring is not known, the "break of diapause to adult emergence" developmental period encompasses all degree days that could be accumulated following winter by larvae. To compare our calculations to those by the only previous study which used dates from 1 March to the first adults observed (Dearborn and Westwood 2014), we also calculated degree day accumulations from 1 March through 10 July (first adult eclosion). We used these five periods when comparing degree day accumulations.

Since we did not start to record the temperature, humidity and dew point in the field immediately after the eggs were laid and could not acquire humidity and dew point data from another source, we instead analysed these three microclimate variables from the date when the first eggs hatched. The periods for which we compared microclimate variables recorded by data loggers and comparison plots were: 11 July to 27 September – active larvae, 28 Sept to 21 May – diapause, 22 May to 10 July – break of diapause to adult eclosion and 11 July 2018 to 10 July 2019 – egg hatch to adult eclosion. We calculated the mean and 95% confidence intervals of the mean, minimum and maximum temperature, relative humidity and dewpoint in each of the egg enclosures and comparison plots during these four development periods.

We standardised shoot counts in all plots to the same area (0.19 m^2 of egg enclosures) for analysis. We converted 0.24 m^2 (comparison plots) and 0.81 m^2 (target plots) to the size of egg enclosures (0.19 m^2). We chose 0.19 m^2 instead of to 1 m^2 because scaling up would add shoots that we did not actually count in the field. We thought that scaling down to 0.19 m^2 made the most sense. Using RStudio (RStudio Team 2021), we calculated the mean of the duff depth recorded in each plot. Data were transformed (shoot counts: $\log_{10}$; physical variables: arcsine squareroot or $\log_{10}$ plus 1) so the accompanying residuals met normality before we compared recordings between plot types using Welch two sample t-tests. If the data residuals did not meet normality and continued to fail these assumptions after transformations, the 95 % C. I. of data for each plot type were assessed instead. The species composition and abundance of shoots of each species were compared using permutation multivariate analysis of variance (PerMANOVA) (observed values compared to the random expected values, then assessed by a permutation test) (Anderson 2001; McCune and Mefford 2011), using the Sorensen (Bray-Curtis) distance measure (calculates similarity based on weighing species equally while integrating the abundance of each species, and is suitable for continuous and heterogeneous data sets) (Bray and Curtis 1957; Magurran 1988; McCune and Mefford 2011). We calculated the mean of the soil moisture and electrical conductivity measurements across sample days and compared the results within years (2018 and 2019) but not between, because seasonal soil moisture likely fluctuated. Footcandles of light were converted to metric lux; light intensity was derived by calculating the percentage of light passing through the thatch layer (light intensity (%) = ($\text{lux}_{\text{below thatch}}$ / $\text{lux}_{\text{above thatch}}$) X 100), then transformed (arcsine squareroot) before a Welch two sample t-test comparison between egg enclosures and comparison plots.

RStudio (RStudio Team 2021) using R version 4.1.2 as the software base (R Development Core Team 2021) was used for Welch two sample t-tests. We used the R packages “ggplot2” (Wickham et al. 2020) to create the relative host plant figure and used “svglite” (Wickham et al. 2021) to export it. We created the relative host plant figure in RStudio to obtain accurate bar proportions, then imported plots into Adobe Illustrator to improve the resolution, colours and add plant illustrations. PerMANOVA was conducted using PC-ORD (McCune and Mefford 2011). Plant and butterfly stage illustrations were created (J. Henault) using Adobe Illustrator (Adobe Inc. 2021a). We used Adobe Premiere Pro (Adobe Inc. 2021b) to stabilise the videos before production.

Results

Oviposition

Poweshiek skippering females were observed laying six eggs during five separate oviposition episodes (up to 5 different females, but please recall that we did not mark individuals). All egg laying occurred on separate plants: grasses *A. gerardi* - 4 eggs; *S. heterolepis* - 1 egg; and the forb *H. hirsuta* - 1 egg. Eggs were laid between 30 June and 3 July 2018. An egg enclosure was established at each location an egg was laid (n = 6).

Before laying an egg, females were observed flying close to vegetation, hovering above a small area, occasionally landing (but not laying eggs) and finally landing and laying an egg before elevating and departing. Prior to oviposition females were often observed landing on leaf blades and touching the leaf blade with the tip of their abdomen but not laying an egg. On one occasion, a female inspected three separate possible oviposition locations, laid an egg at a fourth location, then inspected three more locations before she laid a second egg at a separate location (eight locations searched and two eggs laid). One female was observed nectar feeding immediately before searching for locations to deposit eggs.

In 2017 before formal observations, J. H. observed a female and male attempting to mate at a visually mesic location (Supplementary Video 2.2). The female landed on the leaf of a graminoid near the top of the vegetative canopy and the male landed on the same shoot but below the female. The male crawled alongside the female and attempted to swing the tip of its abdomen under its body to meet the tip of the female's abdomen. The female then crawled away further towards the tip of the leaf tip. Then, the female held its wings horizontally to its body, in a similar position which we observed while adults basked and flew. The male crawled up the leaf blade to below the female and swung its abdomen in a similar way to that observed previously. The female's wings appeared to partially impede 1) the male from crawling closely alongside and 2) the tips of the abdomens from touching. After the male swung its abdomen a few times without mating, the female flew to land a few metres away and the male followed. This set of behaviours occurred two or three times before the skipperlings mated. While mating, the pair perched on the graminoid for approximately 30 – 60 sec before separating. Following this, the female immediately flew approximately three metres away and laid an egg on a Cyperaceae spp. (sedge). These behaviours were similarly observed during the mating observation in 2015 which was mentioned earlier.

Observations of immatures

Eggs were pale green when first laid and turned pale yellow prior to hatch, as also noted by McAlpine (1972). Larval colour pattern and body shape were as described in McAlpine (1972). However, in the first instar descriptions, McAlpine (1972) did not report the bumps that we observed – each with a small black spot – regularly occurring across the cuticle of first instar larvae. The markings on the dorsal surface of the head capsule noted as faint in third instar larvae by McAlpine (1972) were bold in larvae we observed. Larvae were always on host plants during our observations, whether they were feeding, resting or moulting.

Immature 1. The egg was laid on the dorsal surface of a blade of *H. hirsuta*. After consuming the top of its eggshell to hatch, the larva also ate the sides but not the base of the eggshell attached to the leaf. Immature 1 was next observed 1 day later on a shoot of *A. gerardi* 12.4 cm to the east (90°). It ate the tip of the youngest developing leaf of this *A. gerardi* (we display a picture of Immature 3 feeding in Fig. 2.2). Immature 1 ate at the leaf tip (n = 3, mean = 10 min, 95 % C. I. = 9 – 12 min), then crawled down the leaf blade and rested (n = 2, mean = 54 min, 95 % C. I. = 45 – 62 min), before repeating, crawling to the leaf tip to feed. It ate the tip of the leaf during all feeding events which we observed (n = 3, 0.00 cm from the leaf tip during all observations). Immature 1 rested a mean of 10.1 mm (n = 2, observed 0.93 and 1.09 cm) from the tip of the leaf and a mean of 9.9 cm (n = 2, 95 % C. I. = 3.8 – 16.0 cm) from the ground. Immature 1 was observed resting on the dorsal surface of this leaf (n = 3), with its head pointing towards the ground. We observed four resting events during Immature 1's development.

Fig. 2.2. Immature 3 (instar 2) feeding at the tip of a *S. heterolepis* leaf.

Immature 1 was observed for 12 days during the egg and first instar developmental stages. It was 0.36 cm during instar 1. Immature 1 was not found during later searches.

Immature 2. The egg was laid on a *S. heterolepis* leaf. Immature 2 was laid on the dorsal surface 7.0 cm from the tip and 15.0 cm above the ground. It ate the top and sides of its egg shell, but not the base, behaving similarly in this way to Immature 1. Immature 2 moved from the species it was deposited as an egg to *M. richardsonis*.

Immature 2 ate exclusively *M. richardsonis* during the first instar, *S. heterolepis* and *M. richardsonis* during the second instar, *S. heterolepis* and *S. scoparium* during the third instar and only *S. heterolepis* during the fourth instar (Table 2.1). Resting locations were on shoots used for feeding, except on one occasion when J. H. accidentally disturbed Immature 2 during larval observations. It crawled 1.3 cm to a leaf at a similar height, and was found resting in the original undisturbed location the next day.

Table 2.1. Proportion of feeding observations on host plant species amongst all instars (percent (number of observations)).

Immature	***A. gerardi***	***M. richardsonis***	***S. heterolepis***	***S. scoparium***
1	100 (3)			
2		29 (8)	67 (18)	4 (1)
3			100 (14)	

Immature 2 moved between shoots, using living and dead vegetation to travel (Fig. 2.3). Each time it moved, Immature 2 travelled a range of 5.6 to 9.0 cm. It appeared to move more frequently during later instars (Table 2.2). Larval movement in all instars combined was directional at mean 286° ± angular deviation 46° ($n = 7$; $r = 0.677$; Rayleigh test; $p = 0.032$). Immature 2 moved 60.6 cm in 60 days (from egg hatch through instar 4).

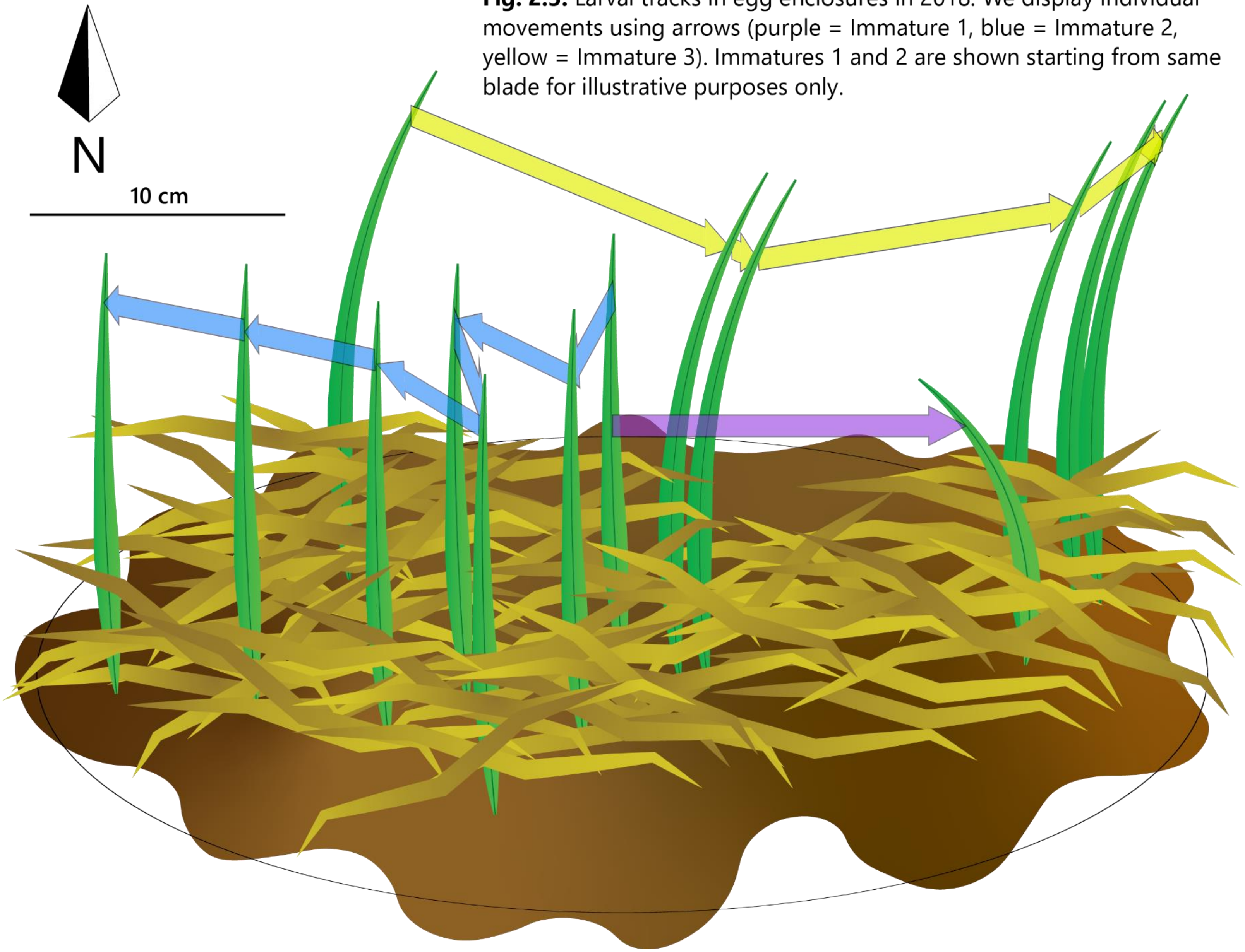

Fig. 2.3. Larval tracks in egg enclosures in 2018. We display individual movements using arrows (purple = Immature 1, blue = Immature 2, yellow = Immature 3). Immatures 1 and 2 are shown starting from same blade for illustrative purposes only.

Table 2.2. Movements by Immatures as larvae (observed number of movements). Larval movement distance between shoots (mean ± range) and frequency (%) that larvae travelled to different host shoots between activity bouts.

Stage	Distance (cm)	Frequency (%)
Immature 1		
Instar 1 (1)	12.4	100
Immature 2		
Instar 1 (1)	5.6	25
2 (1)	6.4	33
3 (3)	7.2 (6.1 to 9.0)	60
4 (3)	6.5 (5.2 to 8.9)	100
Immature 3		
Instar 1 (3)	9.7 (1.3 to 15.8)	60
2 (2)	3.7 (1.2 to 6.2)	100

The tips of host plants were eaten during instar 1, and the sides of blades, in addition to the tip, were consumed during instars 2 to 4. Feeding by Immature 2 produced staggered notches on both edges of a leaf (mean length of two adjacent notches = 0.7 cm (range 0.2 – 1.1 cm) or longer stretches of one side of a leaf (mean 0.9 cm (range 0.5 – 1.3 cm)). Both feeding patterns were similar to observations in McAlpine (1972). To eat the long sections, Immature 2 chewed from the edge of a blade to the midvein. Then it returned to the edge of blade before once again eating to the vein. Each of these feeding passes were as wide as Immature 2's head capsule.

Immature 2 fed at or near the tip (n = 4, mean = 21 min, 95 % C. I. = 15 – 27 min) crawled closer to the ground and rested on a leaf blade (n = 11, mean = 95 min, 95 % C. I. = 54 – 136 min), then repeated by crawling to the tip to feed. Immature 2 ate near the tip amongst all instars (Fig. 2.4). The 95 % C. I. of its feeding locations from the ground amongst all instars ranged between 19.8 and 7.1 cm (n = 21). It appeared to rest closer to the ground during later instars, although we could not robustly test this difference. We report the numerical values of feeding and resting locations on shoots relative to the leaf tips and the ground in Supplementary Table 2.4. Immature 2 rested with its head pointing towards the ground during 95 % of observations (n = 21). Immature 2 consumed and rested on shoots that were vertically oriented, except for one occasion. Its dorsal surface while resting was not oriented in a specific direction (n = 12; r = 0.404; Rayleigh test; p = 0.147). Its orientation while feeding appeared directional (0° ± angular deviation 32°) but a small sample size prevented significance testing (n = 3; r = 0.846). We observed 19 resting events during Immature 2's development.

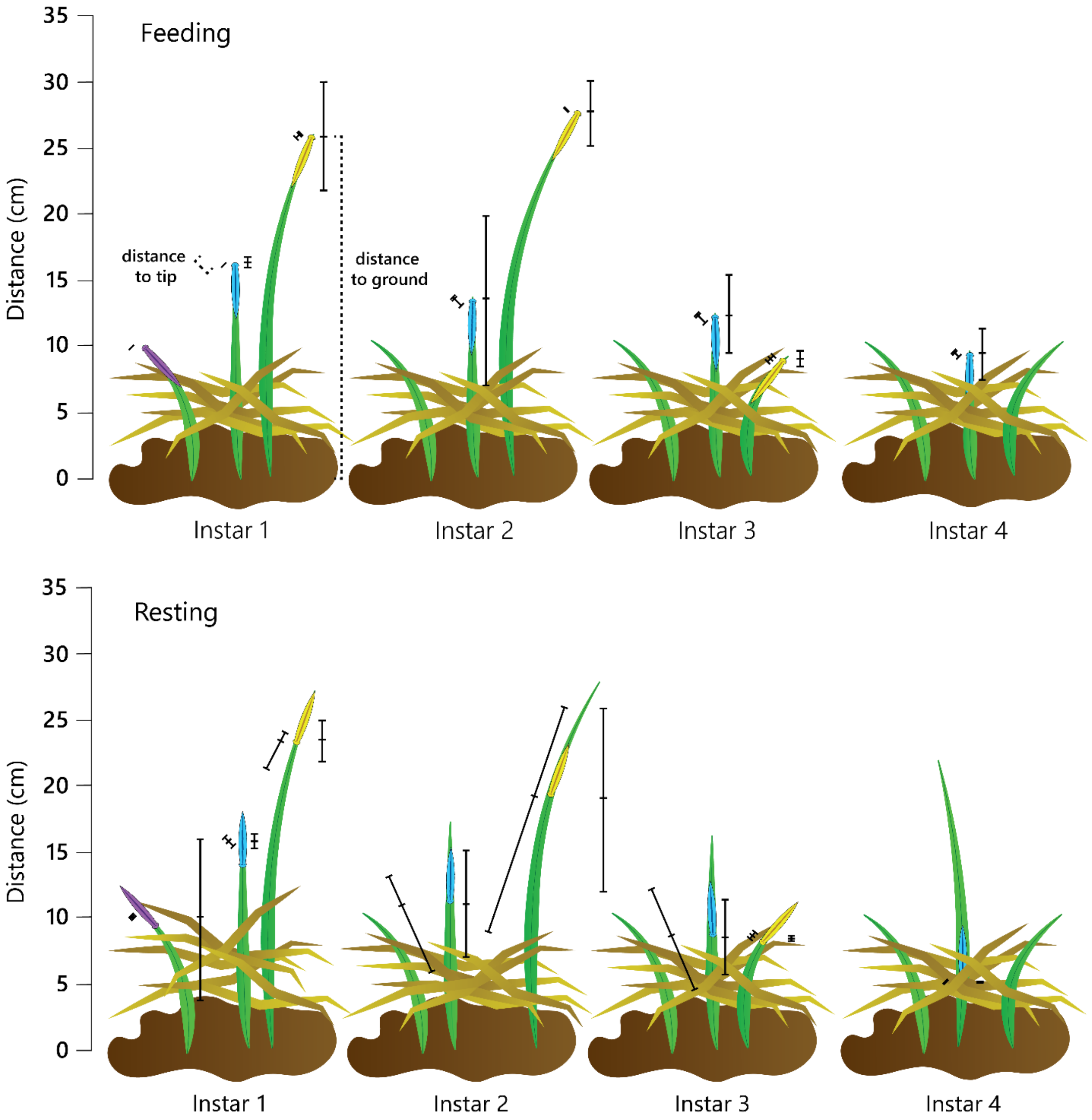

Fig. 2.4. Larval host plant feeding and resting locations on shoots. We display larvae during each instar in which they were observed (Immature 1 = purple, Immature 2 = blue, Immature 3 = yellow). Larvae are approximately 7x size to scale. The distance (cm) along graminoid shoots from the apex of the larval head to the tip of the blade and the vertical distance to the ground where larvae ate or rested is shown along the y-axis. Bars on the left side of larvae are the distance to the tip (mean and range) and on the right are the distance to the ground (mean and 95 % C. I.). Since we did not record measurements which Immature 1 fed from the ground during instar 1, we display this larva at the approximate distance which it rested from the ground during this instar. Duff is represented to scale at the base of plants.

This individual was observed for 70 days (from egg laid through instar 4). Immature 2 exuviae were observed attached to a blade as Immature 2 rested nearby on the same leaf. The length of Immature 2 measured in the third instar was 0.85 cm. Immature 2 was last observed resting at the base of a host plant approximately 5.2 cm above the ground on 9 September, 2018. The location was searched in May 2019 but Immature 2 was not located.

Immature 3. Immature 3 was laid on *A. gerardi*. Oviposition occurred on the dorsal surface of the leaf blade, 2.5 cm from the leaf tip and 36.8 cm above the ground. Immature 3 consumed the top and sides of the eggshell but did not eat the base of the egg. It moved from its natal plant to a *S. heterolepis* to feed.

Immature 3 exclusively ate *S. heterolepis* during instars 1, 2 and 3. Resting locations during all instars were on shoots consumed before or following resting. It moved to multiple host plant species and amongst several shoots to feed and rest (Fig. 2.3). Immature 3 movements ranged between 1.2 to 15.8 cm amongst instars and activity was greater during the older instar (Table 2.2). Movements by Immature 3 were not oriented in a specific direction (n = 5; r = 0.471; Rayleigh test; p = 0.338). Immature 3 moved 36.5 cm in 26 days (from egg hatch through the third instar).

Immature 3 consumed the tip from the end of the leaf blade, or consumed leaf portions the width of its head, and passed from one edge to the opposite edge, slicing the leaf tip (Supplementary Video 2.5). Feeding marks also included notches on alternate or the same edge (s) of a blade. On one occasion, a longer section of leaf (approximately ½ the length of this larva) was consumed to the midvein on both sides at the same location. Immature 3 ate with its head pointing toward the tip of the leaf it was consuming during 100% of observations.

Immature 3 fed near the leaf tip (n = 7, mean = 25 min, 95 % C. I. = 11 – 39 min), crawled further from the tip to rest (n = 7, mean = 87 min, 95 % C. I. = 50 – 124 min)), and crawled to feed at the shoot again. The 95 % C. I. of its feeding locations from the amongst all instars ranged between 30.3 and 8.5 cm above the ground (n = 12) and its resting locations between 25.8 and 8.2 cm (n = 13; Fig. 2.4). Immature 3 rested with its head facing down 100 % of observations (n = 12). It fed on blades that were vertical, eating with the dorsal portion of its body significantly directed at a mean 307° ± angular deviation 35° (n = 5; r = 0.815; Rayleigh test; p = 0.026) and resting at 357° ± 34° (n = 7; r = 0.827; Rayleigh test; p = 0.004). We observed 14 resting events during Immature 3's development.

This individual was observed during the egg and instars 1 through 3 stages, but not observed after day 36. When molting was observed, Immature 3 was resting on a leaf blade nearby an old exuviae attached to the leaf. Immature 3 was measured at 0.61 cm and six days later at 0.62 cm during the second instar.

Immature 4. Immature 4 was laid on *A. gerardi*. The location was searched on 17 dates over the study and no larva was found. **Immature 5.** This egg was laid on *A. gerardi* 4.9 cm from the leaf tip and 29.6 cm from the ground. No larva was found during 16 subsequent visits during the study. **Immature 6.** Immature 6 was also laid on *A. gerardi*, but no larva was found during searches at this location on 17 dates throughout the study period. The egg laid in 2017, laid 7.9 cm from the tip of the shoot and 26.1 cm above the ground (we did not compare these measurements to those in 2018 because vegetation in the site may have grown differently and thus created a relatively different selection of possible microhabitat). This location contained all host food species that we observed larvae eating in 2018 and contained relatively high abundances of *M. richardsonis* shoots. The location where we observed this oviposition did not have soil nutrients and textures clearly similar to that observed at different positions along a soil moisture gradient (as described in chapter 3 of this thesis).

We observed larvae (not including visits to monitor eggs) for 48.1 hours during 2018. We observed 42 feeding bouts, 30 resting bouts and 14 movements between shoots amongst all larvae. Other Lepidopterans (*e.g.*, Family Geometridae) were also observed feeding, and their feeding marks were similar to those of Poweshiek skipperling larvae. Thus, we did not record marks which we were not certain that *O. poweshiek* made. Ants and jumping spiders were observed in the enclosures, however no direct predation was observed.

Larval development

Using data recorded by data loggers in egg enclosures we calculated that larvae required a mean of 1700.0 degree days (standard model; n = 4, 95 % C. I. = 1644.5 – 1755.5 degree days) and 1723.9 degree days (double sine model; n = 4, 95 % C. I. = 1654.3 – 1793.5 degree days) to develop from the first egg laid to the first adult that emerged (30 June 2018 to 10 July 2019).

During this same period, we calculated that data loggers in comparison plots accumulated a mean of 1693.4 degree days (standard model; n = 4, 95 % C. I. = 1590.7 – 1796.0 degree days) and 1722.0 degree days (double sine model; n = 4, 95 % C. I. = 1655.9 – 1788.0 degree days). The degree days calculated for egg enclosure and comparison plot locations using each model were not significantly different based on a lack of overlapping confidence intervals. Calculation of degree days using the nearest Environment Canada weather station located at Emerson, Manitoba during the same period resulted in 1715.1 degree days (standard model) and 1737.0 degree days (double sine model).

We display the degree days required by larvae to complete each instar in 2018 in Table 2.3. In Supplementary 2.6 we report our calculated degree day accumulations in egg enclosures and comparison plots during the active larval, diapause and break of diapause to adult eclosion periods.

Table 2.3. Degree day accumulations in egg enclosures calculated using two models (mean ± SD), and the number of calendar days required to complete each stage (egg and instars 1 to 3). Data from data loggers in all egg enclosures were used (n = 4), calculating degree days calibrated to larva that completed stages. Immature 2 and 3 accumulated the same number of degree days to complete each stage.

Stage	Standard	Double Sine	Days
Immature 1			
Egg	180.0 ± 0.9	163.6 ± 2.9	10
Immature 2			
Egg	182.1 ± 1.0	163.9 ± 2.9	10
Instar 1	125.9 ± 1.7	108.5 ± 6.7	9
2	196.7 ± 4.4	184.8 ± 8.7	16
3	417.3 ± 11.8	384.7 ± 16.3	34
Immature 3			
Egg	182.1 ± 1.0	163.9 ± 2.9	10
Instar 1	125.9 ± 1.7	108.5 ± 6.7	9
2	196.7 ± 4.4	184.8 ± 8.7	16

During the period used in general to synchronise adult surveys (1 March to 10 July in our study), we calculated that microhabitats in egg enclosures accumulated 635.7 degree days (standard model; n = 4, 95 % C. I. = 609.8 – 661.5 degree days) and 706.6 degree days (double sine model; n = 4, 95 % C. I. = 699.9 – 713.3 degree days). A total of 667.2 degree days (standard model) and 695.3 degree days (double sine model) were calculated using temperature data from the Emerson weather station during this period.

Microclimate in microhabitats

During the active larval period, the relative humidity (mean and minimum) was lower most of the data loggers amongst egg enclosures than in comparison plots (Table 2.4). The maximum relative humidity in egg enclosures was lower using some data loggers and higher in others than in comparison plots during the diapause and the break of diapause to adult emergence periods. The temperature (mean, mean minimum and mean maximum) and dew point recorded in egg enclosures was typically not different from that recorded in comparison plots during any development period. Occasionally, the data recorded by one data logger (of the four amongst egg enclosures) was different than data loggers in comparison plots. These main trends also occur during their entire recorded life cycle from the date when first egg hatched to when the first adult eclosed (Supplementary Table 2.7).

Table 2.4. The **A,** temperature (° C); **B**, relative humidity (%) and **C**, dewpoint (° C) during development periods (mean (95% C. I.)) recorded in egg enclosures and comparison plots. The development periods spanned the inclusive date ranges: active larvae – 11 July to 27 September, diapause – 28 Sept to 21 May, break of diapause to adult eclosion – 22 May to 10 July. We bolded the font of means (95% C. I.) recorded by data loggers containing confidence intervals that did not overlap with at least 3 data loggers from the other type (α = 0.05).

A

Plot	**Temperature$_{mean}$**	**Temperature$_{min}$**	**Temperature$_{max}$**
		Active larval period	
Egg			
1	16.6 (15.6 to 17.7)	8.3 (7.3 to 9.3)	25.5 (24.2 to 26.9)
2	16.4 (15.4 to 17.4)	8.1 (7.1 to 9.0)	25.4 (24.0 to 26.8)
3	16.8 (15.7 to 17.9)	7.0 (6.0 to 8.0)	**27.6 (26.0 to 29.1)**
4	16.7 (15.7 to 17.7)	8.0 (7.1 to 9.0)	26.4 (25.0 to 27.8)
Comparison			
5	16.7 (15.7 to 17.7)	8.7 (7.7 to 9.7)	24.6 (23.3 to 25.9)
6	16.6 (15.6 to 17.6)	8.4 (7.4 to 9.4)	25.7 (24.1 to 27.2)
7	15.5 (14.2 to 16.9)	8.5 (7.3 to 9.7)	23.5 (21.6 to 25.4)
8	16.3 (15.4 to 17.2)	8.8 (7.9 to 9.7)	24.2 (22.9 to 25.5)
		Diapause	
Egg			
1	-1.0 (-1.7 to -0.3)	-4.9 (-5.4 to -4.4)	3.0 (1.9 to 4.1)
2	-1.0 (-1.7 to -0.3)	-4.6 (-5.1 to -4.2)	2.7 (1.6 to 3.8)
3	-0.7 (-1.4 to -0.1)	-4.4 (-4.9 to -3.9)	3.1 (2.0 to 4.2)
4	0.0 (-0.6 to 0.6)	-3.0 (-3.4 to -2.5)	3.3 (2.3 to 4.3)
Comparison			
5	-0.5 (-1.2 to 0.2)	-4.1 (-4.6 to -3.7)	3.3 (2.2 to 4.5)
6	-0.8 (-1.6 to -0.1)	-4.8 (-5.3 to -4.3)	3.1 (2.0 to 4.3)
7	-0.1 (-0.7 to 0.4)	**-2.9 (-3.4 to -2.5)**	2.8 (1.8 to 3.7)
8	-0.6 (-1.2 to 0.1)	-3.6 (-4.2 to -3.1)	2.5 (1.5 to 3.5)
		Break of diapause to adult eclosion	
Egg			
1	16.7 (15.6 to 17.7)	8.0 (6.8 to 9.2)	25.7 (24.1 to 27.3)
2	16.8 (15.7 to 17.8)	7.3 (6.0 to 8.6)	26.3 (24.7 to 27.9)
3	16.7 (15.6 to 17.8)	7.8 (6.6 to 9.0)	25.6 (24.0 to 27.1)
4	16.5 (15.6 to 17.5)	8.8 (7.7 to 9.8)	25.3 (23.9 to 26.8)
Comparison			
5	17.2 (16.2 to 18.3)	7.8 (6.5 to 9.1)	26.7 (25.0 to 28.4)
6	17.3 (16.2 to 18.4)	7.6 (6.3 to 8.9)	27.6 (25.7 to 29.4)
7	16.6 (15.6 to 17.5)	8.7 (7.5 to 9.8)	25.3 (23.8 to 26.8)
8	16.7 (15.7 to 17.7)	8.5 (7.4 to 9.6)	26.1 (24.6 to 27.7)

Table 2.4 continued.

B

Plot	**Relative Humidity$_{mean}$**	**Relative Humidity$_{min}$**	**Relative Humidity$_{max}$**
		Active larval period	
Egg			
1	**77.2 (75.3 to 79.1)**	**50.8 (47.9 to 53.8)**	98.3 (97.6 to 99.1)
2	81.1 (79.2 to 82.9)	55.3 (52.2 to 58.5)	**100.3 (99.7 to 100.8)**
3	**75.7 (74.0 to 77.4)**	**48.4 (45.5 to 51.3)**	95.4 (94.9 to 95.9)
4	**76.6 (74.9 to 78.2)**	**52.7 (49.9 to 55.5)**	94.7 (94.0 to 95.4)
Comparison			
5	**82.9 (81.1 to 84.7)**	**64.0 (60.9 to 67.1)**	97.7 (97.2 to 98.2)
6	**82.9 (81.2 to 84.6)**	**61.1 (57.7 to 64.4)**	98.1 (97.7 to 98.4)
7	76.9 (71.5 to 82.3)	60.2 (54.9 to 65.5)	89.7 (83.9 to 95.4)
8	**80.8 (79.4 to 82.3)**	**64.9 (62.4 to 67.3)**	**93.0 (92.3 to 93.7)**
		Diapause	
Egg			
1	83.0 (81.3 to 84.7)	69.6 (66.7 to 72.4)	94.5 (93.6 to 95.3)
2	87.4 (85.9 to 89.0)	75.6 (72.6 to 78.5)	**97.0 (96.4 to 97.7)**
3	82.1 (80.7 to 83.6)	70.4 (67.7 to 73.1)	**91.3 (90.7 to 91.9)**
4	82.2 (80.6 to 83.8)	72.7 (70.0 to 75.4)	**90.0 (89.2 to 90.8)**
Comparison			
5	84.6 (83.0 to 86.1)	72.5 (69.7 to 75.3)	94.1 (93.4 to 94.8)
6	85.6 (84.0 to 87.1)	73.4 (70.6 to 76.2)	95.0 (94.3 to 95.6)
7	**89.0 (87.7 to 90.2)**	79.0 (76.7 to 81.3)	95.9 (95.3 to 96.5)
8	80.1 (78.8 to 81.4)	70.7 (68.4 to 72.9)	**86.9 (86.1 to 87.6)**
		Break of diapause to adult emergence	
Egg			
1	71.6 (68.3 to 75.0)	**42.8 (38.5 to 47.1)**	97.5 (96.0 to 99.0)
2	75.8 (72.6 to 79.1)	47.5 (42.8 to 52.2)	**100.0 (99.1 to 100.9)**
3	73.0 (70.2 to 75.9)	49.2 (45.1 to 53.2)	**94.3 (93.2 to 95.5)**
4	74.6 (71.6 to 77.6)	51.2 (46.8 to 55.6)	**93.4 (92.4 to 94.5)**
Comparison			
5	76.2 (72.5 to 80.0)	53.2 (47.6 to 58.9)	96.9 (95.9 to 97.9)
6	75.0 (71.4 to 78.6)	48.5 (43.0 to 54.0)	97.4 (96.5 to 98.3)
7	79.6 (76.5 to 82.7)	56.0 (51.1 to 60.9)	98.2 (97.5 to 98.8)
8	74.9 (72.1 to 77.7)	53.7 (49.2 to 58.1)	91.5 (90.7 to 92.3)

Table 2.4 continued.

C

Plot	**Dewpoint$_{mean}$**	**Dewpoint$_{min}$**	**Dewpoint$_{max}$**
	Active larval period		
Egg			
1	12.1 (11.2 to 13.1)	6.3 (5.3 to 7.4)	16.4 (15.4 to 17.4)
2	12.7 (11.7 to 13.7)	6.6 (5.5 to 7.6)	17.4 (16.3 to 18.5)
3	11.9 (11.0 to 12.9)	5.2 (4.2 to 6.2)	16.8 (15.7 to 17.9)
4	12.2 (11.3 to 13.2)	5.8 (4.7 to 6.8)	17.1 (16.1 to 18.2)
Comparison			
5	13.2 (12.2 to 14.1)	6.4 (5.3 to 7.5)	18.2 (17.2 to 19.2)
6	13.0 (12.1 to 14.0)	6.3 (5.2 to 7.4)	18.6 (17.5 to 19.6)
7	12.3 (11.2 to 13.5)	5.8 (4.7 to 6.9)	18.0 (16.5 to 19.4)
8	12.6 (11.7 to 13.5)	5.8 (4.7 to 6.8)	18.3 (17.2 to 19.3)
	Diapause		
Egg			
1	-3.9 (-4.5 to -3.3)	-7.4 (-8.1 to -6.7)	-1.2 (-1.8 to -0.5)
2	-3.3 (-3.8 to -2.7)	-6.3 (-6.9 to -5.7)	-0.8 (-1.5 to -0.2)
3	-3.6 (-4.1 to -3.0)	-6.8 (-7.5 to -6.1)	-1.1 (-1.7 to -0.5)
4	-2.7 (-3.3 to -2.2)	**-5.5 (-6.2 to -4.9)**	-0.6 (-1.2 to 0.0)
Comparison			
5	-3.6 (-4.2 to -3.0)	-7.3 (-8.0 to -6.7)	-0.5 (-1.3 to 0.2)
6	-3.8 (-4.4 to -3.2)	**-7.7 (-8.4 to -7.0)**	-0.7 (-1.4 to 0.1)
7	-2.4 (-3.0 to -1.9)	-5.8 (-6.4 to -5.2)	0.3 (-0.4 to 1.0)
8	-4.0 (-4.6 to -3.3)	-7.6 (-8.4 to -6.9)	-1.2 (-1.9 to -0.4)
	Break of diapause to adult emergence		
Egg			
1	10.6 (9.4 to 11.8)	5.9 (4.5 to 7.2)	**14.4 (13.2 to 15.7)**
2	11.6 (10.4 to 12.9)	5.9 (4.5 to 7.2)	15.8 (14.5 to 17.2)
3	11.3 (10.1 to 12.5)	5.6 (4.3 to 6.9)	15.6 (14.3 to 16.9)
4	11.5 (10.3 to 12.7)	6.2 (5.0 to 7.5)	15.8 (14.4 to 17.1)
Comparison			
5	12.0 (10.8 to 13.2)	5.1 (3.6 to 6.5)	17.5 (16.2 to 18.7)
6	11.8 (10.6 to 12.9)	5.1 (3.7 to 6.5)	17.1 (15.8 to 18.5)
7	12.3 (11.1 to 13.4)	5.6 (4.2 to 7.0)	17.7 (16.5 to 18.9)
8	11.6 (10.4 to 12.7)	4.9 (3.6 to 6.2)	17.6 (16.2 to 18.9)

Plant diversity within egg enclosures and target plots – 2018

Shoot abundances of host plant species (observed in 2018) were not significantly different between egg enclosures and the respective plots that accompanied egg enclosures during different years (2018 – target plots, 2019 – comparison plots) on the same dates (each date tested individually using a Welch two sample t-test or 95 % C. I.; each t-test $p > 0.05$; Table 2.5).

Table 2.5. The standardised number of shoots of host food plant species in 2018 and 2019 (mean ± SD).

Date	***A. gerardi***	***M. richardsonis***	***S. heterolepis***	***S. scoparium***
3 August 2018				
Egg enclosure	43.2 ± 20.2	17.0 ± 20.5	57.3 ± 53.3	5.0 ± 9.3
Target plot	35.7 ± 25.6	37.1 ± 49.0	82.8 ± 29.2	7.2 ± 12.3
9 May 2019				
Egg enclosure	0.8 ± 1.3	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Comparison plot	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
30 May 2019				
Egg enclosure	0.2 ± 0.4	10.8 ± 12.1	27.3 ± 18.5	0.0 ± 0.0
Comparison plot	0.0 ± 0.0	2.8 ± 2.5	16.1 ± 12.2	0.0 ± 0.0
11 June 2019				
Egg enclosure	9.0 ± 7.2	10.7 ± 13.8	37.2 ± 31.4	3.5 ± 7.2
Comparison plot	12.4 ± 6.6	2.1 ± 1.6	29.4 ± 18.9	1.1 ± 1.2
5 July 2019				
Egg enclosure	21.3 ± 11.3	2.8 ± 1.9	33.7 ± 35.0	7.3 ± 10.5
Comparison plot	41.2 ± 21.7	1.3 ± 1.9	30.7 ± 17.6	5.8 ± 11.9

The relative abundance of host plant shoots was also not significantly different in egg enclosures compared to the respective control plot types at any date (each Welch two sample t-test; $p > 0.05$; Fig. 2.5).

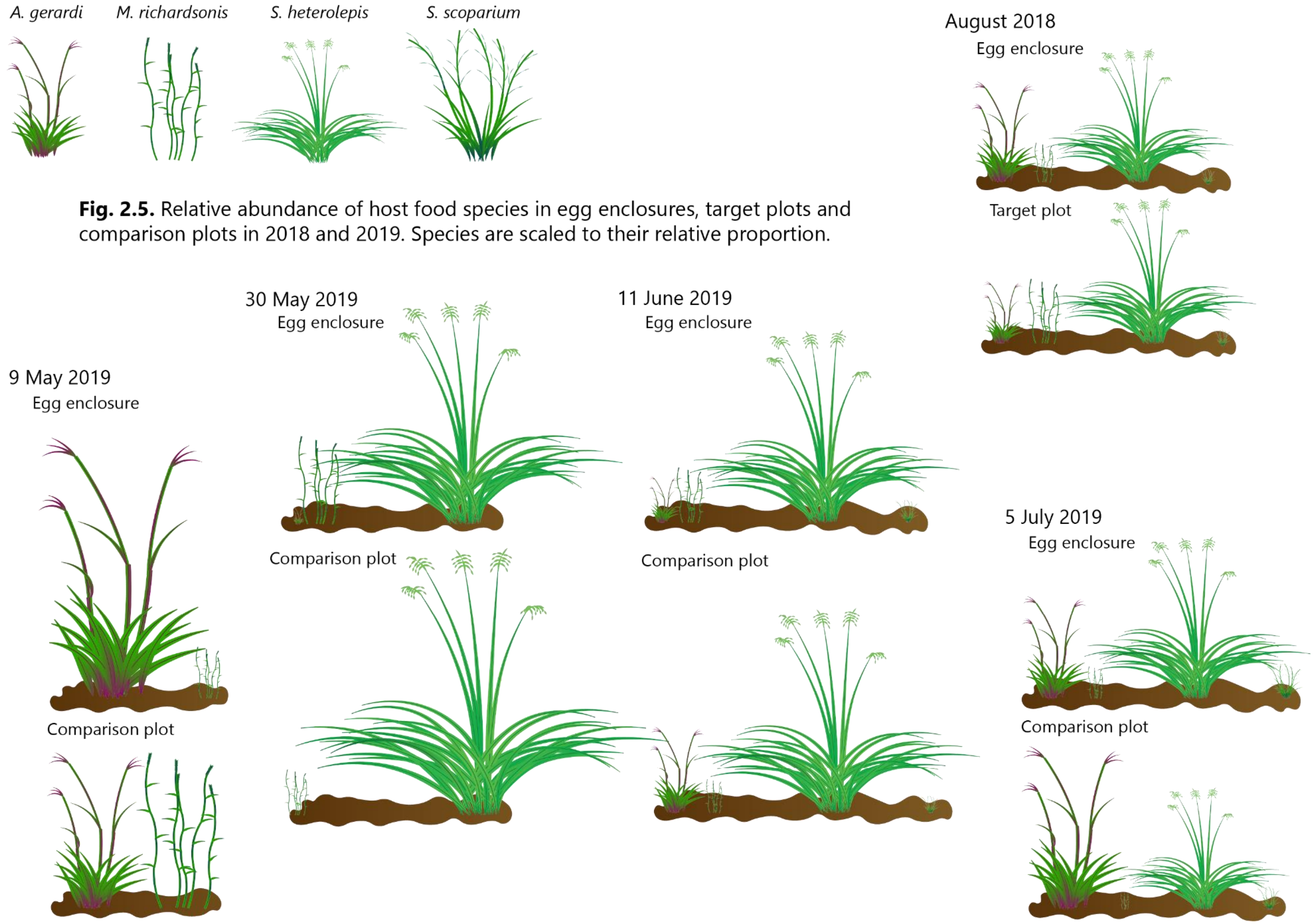

Fig. 2.5. Relative abundance of host food species in egg enclosures, target plots and comparison plots in 2018 and 2019. Species are scaled to their relative proportion.

Graminoids were shorter in egg enclosures (31.7 ± 3.8 cm; mean ± SD) than in target plots (36.5 ± 1.4 cm) ($t = -2.934$, $df = 6.355$, $p = 0.024$; Table 2.6). Duff depth in egg enclosures was not significantly different from target plots (egg enclosure: 4.0 ± 0.9 cm, target plot: 4.1 ± 0.7 cm; $t = -0.025$, $df = 9.604$, $p = 0.981$). The percent cover of duff was greater in egg enclosures (29.8 ± 6.3 %) compared to target plots (20.7 ± 5.5 %) ($t = 2.665$, $df = 9.815$, $p = 0.024$). The percent cover of shrubs was lower in egg enclosures (0.3 ± 0.5 %) compared to target plots (4.2 ± 4.7 %) ($t = -3.597$, $df = 7.468$, $p = 0.008$). The percent cover of graminoids, forbs and bare soil in egg enclosures was not significantly different than in target plots (percent cover of graminoids and percent cover of forbs – Welch two sample t-tests; $p > 0.05$; percent cover of soil – 95 % C. I.; confidence intervals overlapped).

Table 2.6. Vegetative and physical characteristics (mean ± SD) in egg enclosures (n = 6), target plots (n = 6) and comparison plots (n = 6). The duff (cm) at 18 July 2019 represents the thatch component - deepest point of dead vegetation layer - while the litter (cm) is the depth of fine decomposing plant material (please see methods). Measurements of each variable at each date followed by different letters are significantly different (Welch two samples t-tests; $p < 0.05$).

Date	**Graminoids (cm)**	**Duff (cm)**	**Litter (cm)**	**Duff (%)**	**Shrub (%)**
2, 3 August 2018					
Egg enclosure	31.7 ± 3.8 a	4.0 ± 0.9		29.8 ± 6.3 b	0.3 ± 0.5 a
Target plot	36.5 ± 1.4 b	4.1 ± 0.7		20.7 ± 5.5 a	4.2 ± 4.7 b
9 May 2019					
Egg enclosure	5.2 ± 1.1 a	3.9 ± 0.9 a		92.2 ± 2.9	0.7 ± 1.6
Comparison plot	6.9 ± 1.0 b	6.5 ± 1.9 b		93.2 ± 1.9	0.6 ± 0.5
30 May 2019					
Egg enclosure	6.2 ± 1.0	3.0 ± 0.9 a		87.0 ± 5.3	0.5 ± 0.8
Comparison plot	6.6 ± 1.0	4.4 ± 1.0 b		89.8 ± 3.1	0.8 ± 1.0
18 July 2019					
Egg enclosure		5.1 ± 1.2 a	1.7 ± 0.5 a		
Comparison plot		8.2 ± 2.4 b	2.3 ± 0.4 b		

Plant diversity within egg enclosures and comparison plots - 2019

Host plant species broke the surface of the soil at different dates during spring, but these dates were not significantly different between egg enclosures and comparison plots (Welch two sample t-tests; $p > 0.05$; Table 2.5). The estimated abundance of all host plant species (observed in summer 2018) in both egg enclosure and comparison plots between 30 May to 5 July 2019 was significantly greater than on 9 May 2019 (Kruskal-Wallis H; $H = 28.306$, $df = 3$, $p < 0.001$; the estimated abundance of host shoots in only egg enclosures is shown in Fig. 2.6). The percent host plant abundance in August 2018 and from 30 May and 5 July 2019 are approximately similar (did not compare directly because likely different weather growing conditions between years). The relative abundance of individual host plant species was not significantly different between egg enclosures and comparison plots, on individual sampling dates (30 May, 11 June and 5 July) or as a mean from 30 May through 5 July (Welch two sample t-tests; $p > 0.05$) (Fig. 2.5). Also, the abundance of host plant shoots as a group in egg enclosures was not significantly different than in comparison plots, on individual sampling dates 30 May, 11 June and 5 July (PerMANOVA tests; $p > 0.05$) or as a mean from 30 May through 5 July (PerMANOVA; $F = 0.337$, $df = 6$, $p = 0.799$).

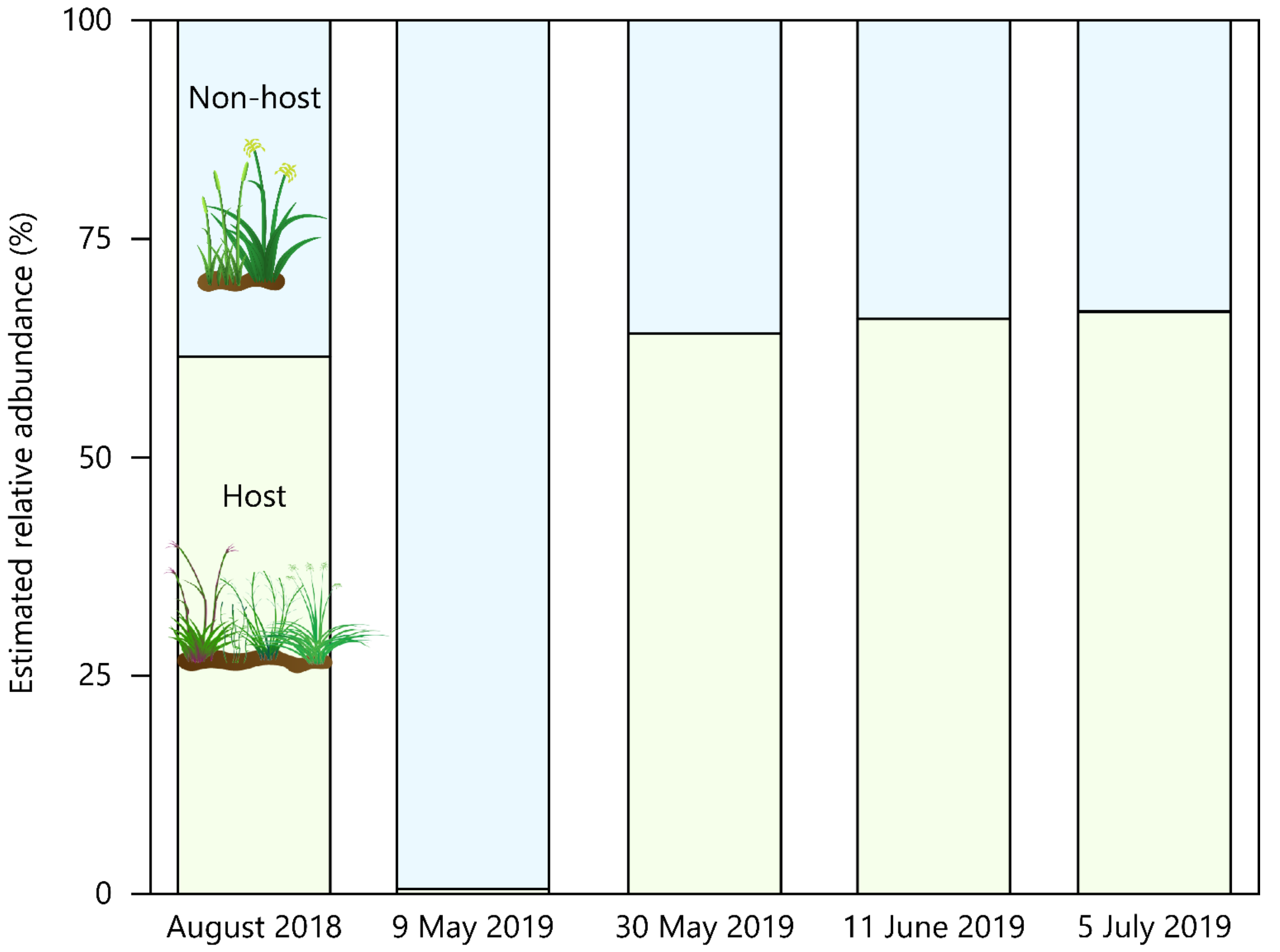

Fig. 2.6. Estimated relative abundance of shoots of host food species and non-host graminoids (graminoid species which we did not observe larvae consuming) in egg enclosures during sampling in 2018 and 2019. Since plants in 2018 and 2019 may have grown differently (weather, chance), we only compare approximately and not by using exact percentages. Observed host forage displayed in illustration, however graminoids in non-host species don’t represent particular species.

The graminoid height was significantly lower in egg enclosures (5.2 ± 1.1 cm; mean ± SD) than in comparison plots (6.9 ± 1.0 cm) on 9 May 2019 (t = -2.881, df = 9.937, p = 0.016) but not on any other date individually or combined (Welch two sample t-tests; p > 0.05; Table 2.6). The mean depth of duff was significantly less in egg enclosures than in comparison plots on 9 May (t = -2.925, df = 7.003, p = 0.022) and 30 May (t = -2.479, df = 9.731, p = 0.033). The mean depth of litter and mean depth of thatch was significantly less in egg enclosures compared to comparison plots on July 18, 2019 (litter; t = -2.410, df = 8.722, p = 0.040; thatch; t = -2.926, df = 7.261, p = 0.021). The percent cover of living vegetation, duff or soil were not significantly different between egg enclosures and comparison plots (Welch two sample t-tests; p > 0.05). The light intensity in egg enclosures (71.0 ± 10.9 %) was not significantly different than comparison plots (57.5 ± 11.9 %) (t = 2.047, df = 9.910, p = 0.068).

Soil moisture in egg enclosures was 28.5 ± 9.0 % VWC (n = 6) and electrical conductivity 0.15 ± 0.07 mS/cm in 2018 and 41.9 ± 6.5 % VWC and 0.19 ± 0.05 mS/cm in 2019. Soil moisture and electrical conductivity were not significantly different between egg enclosures and comparison plots (soil moisture; t = -0.241, df = 9.939, p = 0.814; electrical conductivity; t = -0.103, df = 9.407, p = 0.920).

The height of larval host plant species and height of graminoid species which we did not observe larvae consuming (non-hosts) was not different between egg enclosures and comparison plots on 5 July 2019 (host plants; t = -1.351, df = 8.371, p = 0.212; graminoids not consumed; t = -1.243, df = 9.548, p = 0.243).

All vegetation species enumerated in this study are reported in Supplementary Table 2.3.

Discussion

Larval host plants in Manitoba

Eggs were laid on grass species and a forb, following what appeared to be female inspections. In 2018, larvae consumed *A. gerardi* (big bluestem), *M. richardsonis* (mat muhly), *S. heterolepis* (prairie dropseed) and *S. scoparium* (little bluestem) in natural tall grass prairie habitats in Manitoba. Immature 2 varied its graminoid forage (*M. richardsonis*, *S. heterolepis* and *S. scoparium*) during different instars while the other two larvae consumed foliage of only one species during all instars observed (Immature 1 - *A. gerardi,* Immature 3 – *S. heterolepis*). Researchers have observed larvae in the United States using two hosts that we also observed (*S. heterolepis* in Wisconsin and *S. scoparium* in Minnesota; Borkin 1995; Dana 2020 unpublished). However, our direct observations of larvae feeding on *A. gerardi* and *M. richardsonis* are new. Our research provides evidence for prediction by Henault (2017) that larvae would vary their forage species and move amongst host shoots. *Euphydryas editha bayensis* (Sternitzky 1937) (Lepidoptera: Nymphalidae) larvae were observed to switch host plant species to access high quality food (Hellmann et al. 2002) and Karban et al. (2010) reported increased survival of *Arctia virginalis* (Boisduval, 1852) (Lepidoptera: Erebidae) larvae that ate multiple host species compared to a single host. Polyphagy by individual Poweshiek skipperling larvae may improve their fitness, however research focused on answering that question is required.

Vegetative and physical characteristics of microhabitats

The height of graminoids and duff, percent cover of shrubs and duff (Table 2.6), mean and minimum relative humidity (Table 2.4) was different in oviposition locations compared to areas where females did not lay eggs, however the host plant diversity was not different (Table 2.5, Fig. 2.5). The oviposition site in 2017 contained all the species that we observed larvae consuming throughout 2018. However, we had no comparison plot in that year to assess potential differences. In 2018 research, larval microhabitats appeared to be more open and contained drier microclimates during the entire life cycle than areas where eggs are not laid (based on vegetation height and relative humidity). We assume the taller graminoid height in egg enclosures may in part explain the lower relative humidity observed in egg enclosures than in comparison areas. The taller vegetation may potentially have trapped moisture that was evaporating from the soil and blocked the wind which may have dispersed moisture in the air. In grasslands in Europe, *Hipparchia fagi* (Scopoli, 1763) (Lepidoptera: Nymphalidae) larvae use isolated host grass microhabitats on the edges of physical gaps in grassland vegetation that potentially provided exposed and drier microclimates (Möllenbeck et al. 2009). The architecture of Poweshiek skipperling microhabitats may provide specific microclimates to immature stages, in the immediate vicinity of suitable host species.

Larval foraging behaviour

Females laid eggs relatively closer to the plant tip than the base. The female J. H. observed in 2017 also laid an egg close to the tip. Sparsely vegetated canopy was likely chosen to provide microclimatic temperatures for egg development, as observed in *Erebia epiphron* (Knoch, 1783) (Lepidoptera: Nymphalidae) electing to lay in locations likely with warmer temperatures (Ewing et al. 2020). We did not measure the temperatures where larvae were active on plants. However, we encourage further research to determine a potential range of microclimates used larvae for various behaviours.

Our observations are the first to track the activities of individual larvae during their development in natural habitats. All larvae moved from their natal plant to feed on a different species, and travelled between shoots, regardless of the species (Fig. 2.3). Each movement by larvae was substantial enough to change their location in the vegetation, and their activity appeared to increase at later instars (Table 2.2). Immature 2 consistently travelled in approximately the same direction while Immature 3 did not. We only saw one movement by Immature 1, after which it likely perished. We do not think enough evidence has been gathered to support directional or random movements. However, the fact that very newly hatched larvae moved from a suitable food source and that larvae switched seemingly suitable hosts (species) suggests that other factors in addition to host species characterise suitable larval feeding locations.

All larvae fed at the tips of leaves near the top of the canopy of vegetation, possibly taking advantage of warmer temperatures at least in part (as described for *Anthocharis cardamines* (Linnaeus, 1758) (Lepidoptera: Pieridae) (Dempster 1997) and *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) (Jugovic et al. 2017)). The plant tissue at the tips of newly emerged shoots might be less tough and thus easier for larvae to physically process using their mouthparts. However, the host plant species used by larvae grew new leaf tissue from the base and not the tip of the leaf. Thus while observing leaf blades which had grown for several weeks, most appeared to be softer near the proximal portions of the plant and the tips of some species had dried out turning physically tough and brown. We did not observed larvae exclusively eating leaves immediately as they emerged from the base of the plant. We imagine the toughness of leaves may at least partially influence larval foraging but the position at the top of the canopy appears to be more important (as suggested by our research).

After feeding, larvae rested closer to the ground (Fig. 2.4), potentially for vegetation shelter from predators (*Spilosoma congrua* Walker, 1855 (Lepidoptera: Erebidae) (Stamp 1992)). Feeding bouts of all three Immatures were shorter than resting bouts (95 % C. I. did not overlap). Perhaps, larvae briefly feed where the temperatures are suitable in the canopy but spend much more time in the relatively safer locations closer to the ground. Larvae appear to be navigating their microhabitat to optimize the local environment where they feed or rest. Additionally, Immature 3 positioned its vertically oriented body on the north side of shoots while feeding (approximately 307°) and resting (approx. 357°). Immature 2 may have positioned its body while feeding (approximately 0°; small sample size of three) but did not orient itself in a specific direction while resting. Given that the sun approaches from the south, larvae on the north side would potentially be shaded by the shoot. A grass-feeding moth (*Hemileuca oliviae* Cockerell, 1898 (Lepidoptera: Saturniidae)) has been shown to adjust its body temperature by shading itself with a vertical leaf blade by Capinera et al. (1980). Although we were unable to robustly analyse directionality, we think the idea that Poweshiek skipperling larvae orient their exposure to the sun to fine-tune their local microclimate would be an exciting focus for future research. We suggest to observe more larvae and use small data loggers to measure microclimates in mini-environments surrounding larvae during any future study.

Female Poweshiek skipperling may lay eggs in locations with suitable host plant species and microclimate characteristics for the development of immature stages, potentially compromising between host plants with optimal nutrients and areas with the neccessary temperature and humidity attributes as observed in *Pyrgus malvae* (Linnaeus, 1758) (Lepidoptera: Hesperiidae) (Krämer et al. 2012). Within microhabitats, we think it is conceivable that very small locations may exist where a suitable host food plant (*e.g.,* section of a host leaf) as well as suitable temperature and humidity intersect. Such an area would have specific food, climatic and shelter attributes like a microhabitat, but would be one scale smaller with many such areas fitting within a microhabitat. Potentially, this area may be termed a “nanohabitat”. We were unable to gather enough evidence during our study to support this concept. However, if such an area does exist we hope that future research would be able to discover it.

Larvae appear to navigate within microhabitats, using living and dead vegetation, to locate specific areas with particular microclimates to consume preferred hosts, rest and moult. Larvae of *Boloria aquilonaris* (Stichel, 1908) (Lepidoptera: Nymphalidae) have been reported to eat hosts exposed to the sun during periods of lower light intensities and move to cool and humid moss hummocks to rest (Turlure et al. 2010). Relocation movements by *O. poweshiek* may be timed by feeding-induced plant defenses, as reported in *Parnassius smintheus* Doubleday, 1847 (Lepidoptera: Papilionidae) by Roslin et al. (2008). Additionally, older instar Poweshiek skipperling larvae may move frequently because switching plants is less risky than eating an activated host (herbivory defenses primed), while the opposite may be likely for younger instars, as observed in *Manduca sexta* (Linnaeus, 1763) (Lepidoptera: Sphingidae) by van Dam et al. (2001). Larvae appeared to occupy areas within microhabitats regardless of their proximity to previous areas they used, as observed in *Chlosyne harrisii* (Scudder, 1863) (Lepidoptera: Nymphalidae) (Dethier 1959). We think this movement pattern suggests that the microhabitat enclosure size that we chose was suitable for Poweshiek skipperling.

We observed all larvae eating the tips of leaf blades. At later instars, larvae also ate notches or larger sections to the midvein. Tip and/or notch feeding marks were also made by larvae observed in natural habitat by Borkin (1995). In a captive setting, larvae were also observed eating the tips and consuming one side of blades (McAlpine 1972) which we also observed. Larvae that make brief excursions multiple times per day to leaf tips may reduce detection by predators. By feeding at the tips, thus only shortening the blade, larvae may also camouflage their feeding by leaving leaves that look do not strongly indicate its presence.

We also observed similar feeding marks made by other Lepidoptera (including Family Geometridae Stephens, 1829), suggesting that feeding marks observed on *M. richardsonis* in natural habitats by Pointon (2015) might also have been created by other Lepidopterans or Poweshiek skipperling. *Sporobolous heterolepis* and *S. scoparium* were observed as host food plant species in Minnesota and Wisconsin. We also observed larvae in Manitoba eating species from other jurisdictions, so think there is at least a moderate chance that larvae in Michigan eat *M. richardsonis* just like we observed in Canada. *Muhlenbergia richardsonis, S. heterolepis, S. scoparium* and potentially *A. gerardi* (and/or other species) appear to be ubiquitously used and important to larval Poweshiek skipperling throughout their range. However, to confirm the host plants eaten where *O. poweshiek* populations survive in the remaining parts of their range, we recommend direct observations of larval feeding.

Female Poweshiek skipperling may locate egg microhabitats by evaluating the diversity of suitable host species and the depth of duff (as suggested by our egg enclosures vs. comparison plots), then possibly by assessing the height of graminoids and percentage cover of shrubs and duff (as suggested by our egg enclosures vs. target plots) to lay an egg. We think *O. poweshiek* evaluate potential microhabitats in a sequential manner similar to *Iolana iolas* (Ochsenheimer, 1816) (Lepidoptera: Lycaenidae) observed by Rabasa et al. (2005). These potential cues may be evaluated by Poweshiek skipperling in flight, as observed in multiple Lepidopteran species by Wiklund (1984), or while on the plant as reported by Singer and McBride (2010) and Lund et al. (2019). We observed one flying female land in several areas, but immediately take off to lay eggs at new locations during one egg laying excursion (the investigated locations had tall vegetation, deep duff, relatively high *M. richardsonis* abundance but otherwise similar host diversity). The skipperling appeared to be initially attracted to the areas, but not stimulated adequately after landing to lay an egg. Identifying the microhabitat characteristics needed by Poweshiek skipperling larvae may help determine potential microhabitat location cues used by females, and possibly enable further research into microhabitat requirements. The microhabitat knowledge that we generated could also guide reintroduction programs to ensure potential reintroduction prairies contain microhabitats with the specific attributes to support larvae.

Euphydryas editha (Boisduval, 1852) (Lepidoptera: Lycaenidae) lay eggs once stimulated by the potential oviposition environment past a specific threshold, and this threshold reduces with each unsuccessful oviposition attempt (stimuli not adequate to initiate egg laying; Singer 1971). Are remnant *O. poweshiek* habitats providing microhabitats well within the stimulation threshold (resulting in many eggs laid, and potentially in suitable conditions)? Or are habitats oversaturated with under-stimulating microhabitats, increasing the likelihood of a female encountering a microhabitat that would lower its oviposition threshold? Eventually this may result in females laying eggs in poorly suited locations, possibly reducing larval fitness and subsequently reducing the population size. Due to random events in any given year, the threshold may be reduced unpredictably. Thus, microhabitats where eggs are laid would vary in quality and this potential mechanism may explain population fluctuations. *Euphydryas editha,* in Singer and Parmesan (2019), encountered adequate oviposition stimuli on a new host species, before switching back following negative climatic pressures. Have Poweshiek skipperlings adapted to maladaptive hosts requiring stochastic events to facilitate a return to their typical hosts? Alternatively, perhaps adults first evaluate the available microhabitats in the area in which they live (in general, not exact locations) and then return to lay eggs in the best available locations. Of course, all of these ideas require further research.

We did not observe larval feeding following diapause. We observed host plant species (observed in 2018) become the dominant species emerging above the ground in egg enclosures at the end of May 2019 (Fig. 2.6). Initially, *S. heterolepis* and *M. richardsonis* made up the highest proportion of host species. By the middle of June, a mixture of host plants was observed (Table 2.5, Fig. 2.5). Assuming larvae eat the same species after diapause that they did before entering diapause, we hypothesize that larvae may consume *S. heterolepis* and *M. richardsonis* first then feed on a broader mixture of species (including *A. gerardi*) over spring until pupation. Potentially new host plant species may be used in spring, possibly ones with physiologies better suited to developing in cooler temperatures compared to warmer development traits in the observed host species (plant physiologies as described in Kindscher and Wells 1995). However, observations in natural habitats are required to determine the physiology type and species of plants larvae consume in spring. Poweshiek skipperling larvae likely consume species phenologically-timed (as reported in *A. cardamines* by Posledovich et al. 2015) to attain suitable sizes prior to pupation (*Epargyreus clarus* (Cramer, 1775) (Lepidoptera: Hesperiidae) in Rosenwald et al. 2017). Also, we observed that the relative plant species composition in July 2019 approached a similar composition in August of 2018, nearing more equal proportions of host food plants by the time that eggs hatch and early instar larva begin to feed (in late July and August). It appears that the proportion of individual graminoid species seasonally change throughout the year, with specific species becoming more dominant at certain times of the year and less at others (as suggested by our research in Fig. 2.5). Therefore, any univoltine graminoid-feeding skippers likely would need to be adapted to changing proportions of graminoids species over the year. It should be more difficult to find one specific graminoid species once the proportions start to change, so it seems reasonable that butterflies that could feed on multiple graminoid species could have a relatively higher fitness. If graminoid proportions seasonally change consistently amongst grassland ecosystems, then this hypothesis may contribute to explaining the commonly reported polyphagy in natural habitats of grass-feeding skippers (natural habitats: Wiklund 1984; Dana 1991; García-Barros and Fartmann 2009; lab-choice: Molleman et al. 2020; Nordmeyer et al. 2021). To the best of our knowledge, no other graminoid-feeding skipper larvae have been tracked as we have done. Therefore, more evidence is required to understand larval foraging adaptations to prairie habitats.

Potentially, *O. poweshiek* are limited generalists that prefer a small selection of species but may eat any of the species within this selection depending on availability and seasonal variation in quality. The nutritive and physical characteristics of the graminoid hosts are likely different and may result in different growth or survival rates (as described in *Hesperia dacotae* (Skinner, 1911) (Lepidoptera: Hesperiidae) by Nordmeyer et al. 2021). As environmental conditions change throughout the period when larvae are active, perhaps the responses of certain plant species plant tissue facilitate larval survival better than others (*e.g.*, if tissues of some plant species degrade in quality in terms of nutrients and physical toughness more readily during droughts or extreme temperatures). Subsequently, this pressure may partially cause larvae to locate the shoot of a different graminoid species to consume (host switching with changing environmental conditions by *E. e. bayensis* in Hellmann et al. 2002). Additionally, the changing relative proportions of specific host plant species (Fig. 2.5) may necessitate the capacity to begin to feed on a new species of host graminoid. We encourage additional studies of larval foraging in natural habitats during various environmental conditions as well as research focused on the differential survival of larval by feeding on individual species of host food plants and mixtures of host food species.

Larval development

The body lengths of larvae were consistent with measurements reported during the same instars in McAlpine (1972). Immatures in the current study required approximately 125 to 415 degree days (calculated using either model) to complete each immature stage observed, increasing substantially between the second and third instars (Table 2.3). All three larva completed the egg stage in the same number of calendar days and a very similar number of degree days. Also, Immatures 2 and 3 required the same number of degree days to complete the egg, first and second instar stages. Larvae that we observed developed at similar rates, although their life history could be determined with greater accuracy in the future by monitoring a larger number of eggs, caterpillars and hopefully pupae. Approximately 1700 degree days (calculated using the standard model) or 1720 degree days (double sine model) are required by larvae in natural habitats to complete their life cycle. Since these values are based on only one generation, we recommend that data for several generations be collected to acquire a more precise development estimation. Each model was calculated assuming a lower development threshold of 6 ℃, however please recall that the actual lower threshold of *O. poweshiek* may be different. Larvae were reported to molt to the fifth instar prior to diapause in McAlpine (1972), but Poweshiek skipperling in Manitoba likely undergo diapause in natural habitats in the fourth instar. After we last observed larvae in the fall, the remaining degree days available for development before winter were approximately 115 (standard model) and 145 (double sine model). Given that approximately 415 degree days (standard model) and 385 degree days (double sine model) were required to complete instar three, we do not think larvae could accumulate enough degree days to develop from instar four to five before entering diapause. Poweshiek skipperling in Manitoba (high latitude) may have fewer available degree days than in Michigan (lower latitude). Therefore *O. poweshiek* in Manitoba may have adapted to complete fewer instars in order to time their summer emergence as adults, as observed in *Lycaena hippothoe* (Linnaeus, 1761) (Lepidoptera: Lycaenidae) that completes fewer pre-diapause instars (Fischer and Fiedler 2001) in a region with less time to develop (Fischer and Fiedler 2002).

We calculated that approximately 210 degree days (double sine model) may be accumulated in egg enclosures during the period of dates which we assigned for diapause by immatures (approximately a few degrees on either side of 0° C which we also assumed would be unsuitable for larval development) (Supplementary Table 2.6). Also, that approximately 535 degree days (double sine model) may be accumulated during the period which occurs after the break of diapause through the date which the first adult emerges. In addition to the developmental stages which we accounted for (egg and instars 1 through 4), there may be enough degree days collectively during this period for immatures to perhaps complete two additional instar stages and the pupation stage. As we acknowledged, the start/end dates which we assigned to the diapause developmental period may be inaccurate. Perhaps, larvae develop through the beginning of the fifth instar before diapause before completing it after diapause. We also did not observe immatures in the spring season prior to eclosion. Direct observations of larvae diapausing in prairie habitats in Manitoba are required to more accurately determine *O.*

poweshiek's development, including the approximate dates which developmental periods begin and end, in this region. The degree day measurements we report contribute to a gap in the Poweshiek skipperling research community's understanding of this skipperling's developmental schedule in natural habitats in Manitoba. Laboratory studies of immatures from Canadian populations may help to determine their precise lower developmental threshold.

Egg microhabitats accumulated approximately 635.7 degree days (standard model) and 706.6 degree days (double sine model) between the biofix date (March 1) and the emergence of the first adult, compared to 602.8 degree days and 653.8 degree days respectively in microhabitats randomly placed in prairies that supported *O. poweshiek* in Dearborn and Westwood (2014). Annual predictions using the double-sine model prepared for international conservation partners appear to be more accurate than the standard (Henault and Westwood unpublished), so we focus more on the double-sine model. *Oarisma poweshiek* in the current study required more degree days or developed less with each degree day unit, compared to a hypothetical skipperling developing according to the data reported by Dearborn and Westwood (2014). The discrepancy may be caused by datalogger placement (egg/larval microhabitats - current study; locations in prairie with Poweshiek skipperling adult observations, but not necessarily egg/larval microhabitats - Dearborn and Westwood 2014) or annual weather fluctuations (one year: current study; 5-year mean: Dearborn and Westwood 2014) or a combination. During this time period used to synchronise surveys, data loggers in the current study accumulated calculated degree days at approximately the same rate as the Emerson weather station when we used the double sine model. However when we used the standard model, data loggers accumulated approximately 30 fewer calculated degree days than the weather station over the same period. Egg microhabitats accumulated from 0.0 degree days per calendar day to 18.9 degree days per calendar day during this period. Assuming that *O. poweshiek* microhabitats accumulate degree days at a rate somewhere intermediate these two values in any given year, surveys using the weather station could potentially miss adult emergence by a couple of calendar days. Therefore, we suggest future adult surveys to be timed using the double sine technique instead of the standard model. We also recommend that conservation partners continue to begin surveys a few days before the date which adults are predicted to emerge that year in order to observe adults which emerged earlier than predicted. Additionally, data loggers and the weather station accumulated a similar number of degree days from the date when the first egg was laid to when the first adult emerged when calculated using either model. We recorded microclimate data only during one year. Measurements in appropriate microclimates during the spring in several additional years could help to determine developmental schedules for post-diapause larvae and pupae.

The March 1 date was chosen to calibrate our study with the only previous research about its development rate in Manitoba (Dearborn and Westwood 2014). Plant species which we did not observe larvae consuming (mostly with the cool season physiology) dominated egg enclosures on 9 May before host plant species (all with the warm season physiology) dominated by 30 May (Fig. 2.6). The daily minimum temperature in egg enclosures returned to within a few degrees on either side of 0° C after the winter season on 22 May. An availability species which larvae consume and an ambient environment with temperatures which are possibly the absolute minimum for development, both occurred during the last two weeks of May. Therefore, we think that during approximately the last two weeks of May larvae resumed being active. Potentially, this approximate time period is when larvae resume their activities during most years in Manitoba. However, direct observations in natural habitats over several years are likely needed to further explore this idea. If larvae do resume eating before the last two weeks of May, based on the results suggested by our research larvae potentially eat species with the cool season physiology before switching to the confirmed host plants (warm season physiology) during the second half of May before pupating. Observations of larvae in natural habitats near the end of diapause through pupation would be helpful.

In our study, the light intensity underneath the thatch layer in egg enclosures and comparison plots was not significantly different. However, the corresponding p-value was very to the boundary suggesting significance and our small sample size may have falsely rejected an actual pattern. The light intensity recorded in egg enclosures was higher than that recorded in comparison plots (egg enclosures – approximately 71 %, comparison plots – approximately 58 %). Taking this into consideration, perhaps a higher intensity of light in larval microhabitats stimulates larval host plants to emerge earlier and enables larval feeding earlier in the spring. However, there was no difference in timing of plant growth based on our research and we did not observe larvae in the spring to determine their behaviour prior to emergence. We recommend further research to determine whether the intensity of light measured in a microhabitat may influence larval development.

Broader conclusions

After the initial presumed reduction in the number of *O. poweshiek* populations following habitat destruction across North America, the number of individuals of the Poweshiek skipperling has consistently declined in the last several decades. Given the small number of skipperlings and subsequent constraints on analysis, we may not be able to determine if changes at the landscape scale, microhabitat scale or within microhabitats have caused this latest observed decline. However, equipped with an improved understanding of specific larval feeding patterns, adult behaviours and microhabitat attributes we can hopefully narrow the list of possible causes of decline and facilitate the identification of potential solutions.

Few Poweshiek skipperling adults are observed every year in natural tall grass prairie habitats in Manitoba. However, we still think that we generated useful knowledge to help Poweshiek skipperlings recover. We are proud of the knowledge which our research has generated. While the small number of individuals limited our capacity to develop statistical inferences, we think the larval host plant feeding behaviour, immature stage microhabitat requirements and development schedules which we reported can spark further studies. Potential studies may include research about the foraging behaviours by immatures after diapause, increasing the precision of the length of development stages, increase the timing of adult population surveys to improve our monitoring accuracy and enhance essential habitat descriptions all to galvanise the successful recovery of Poweshiek skipperling. We think the design of our enclosures likely facilitated larval behaviour similar to natural habitats. The height of species consumed by larvae and those not eaten were not different while growing inside egg enclosures compared to comparison plots uninfluenced by the refuse pails. We interpret that plant growing conditions (potentially soil moisture, snow cover as well as wind and sun exposure) were not altered by egg enclosures. We encourage any similarly designed studies in the future, to incorporate control type enclosures to help determine more accurately the effects that such a structure may have on the vegetative and physical attributes of microhabitats. We think that the risk of influencing the behaviour of other wildlife by using these enclosures, might be acceptable after having considered the conservation status of other wildlife which may be affected by this activity.

Our research was likely the last opportunity to research the behaviour and biology of *O. poweshiek* which have not been directly influenced by humans. We assume that at some point during the last several thousand years when numerous Indigenous peoples lived prior to colonisation in this region, prairie habitats which supported *O. poweshiek* were likely intentionally burned by these peoples. Stewardship activities in the last few decades by conservation organisations were likely also generally indirect forms of influence. Recent reintroduction efforts however, are likely influencing Poweshiek skipperlings more directly via captive rearing and release activities. We think that the biology and behaviours of *O. poweshiek* may be different between the time periods before and after the initiation of direct influences. We only suggest this difference to help researchers to explain traits which may be lost and other traits which may be gained, as the Poweshiek skipperling community attempts to successfully recover this species. Hopefully by knowing the causes of these changes, we can adapt our strategies to continue to support this species. Since the planet is changing drastically, we think that the environmental pressures experienced by *O. poweshiek* might require even more direct intervention from humans. We look forward to contribute to the adaptive synthesis of knowledge from stewardship, reintroduction, recovery strategy, field studies and other efforts to ensure the survival of Poweshiek skipperling.

Disturbances such as haying and prescribed burns may be applied to sites to improve the suitability of habitats for butterflies (Swengel 1996; Thom and Daniels 2017). Sites intentionally burned approximately every three to ten years supported higher abundances of Poweshiek skipperlings in Manitoba (Dupont-Morozoff et al. submitted). However, disturbances ought to be compatible with the life history of these species to avoid the destruction of individuals (Swengel and Swengel 1999; 2015). Our current study may inform management strategies to improve the suitability of habitat for *O. poweshiek* using conservation disturbances that are compatible with these skipperlings. Immature pre-diapause development schedules reported in the current study may guide disturbance timing to reduce mortality to Poweshiek skipperling. For example, haying sites in late summer or fall when larvae are closer to the ground (Thesis, Chapter 2) may reduce mortality compared to mid-summer when eggs or larvae are higher in the vegetative canopy. We also suggest a goal for habitat stewards to maintain host and microclimate diversities required by Poweshiek skipperling. We are unaware of techniques to disturb habitats in a way to focus on creating or improving the quality of attributes at a microhabitat scale. However, we assume that by using a variety of approaches (such as haying and intentionally burning habitat, or potentially varying the percentage of a habitat to which disturbances are applied) habitats will become chaotic enough to potentially increase the likelihood of containing highly suitable microhabitats. Of course, the effects of conservation disturbances on desired plant growth and the requirements of other species at risk ought to be considered as well. Previous techniques may be suitable (Dupont-Morozoff et al. submitted), however additional research into the direct effects of disturbances on immature and adult stages is required.

Further research into larval observations in other regions and during spring, female host cues and conservation disturbances may lead to a more comprehensive knowledge about Poweshiek skipperling as a species to determine why this butterfly is declining and to generate solutions.

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Supplementary Material

Supplementary Figure 2.1. Observations of a female and male *O. poweshiek* during a mating event in 2015. In **A**, the female is situated with the abdomen curved on a graminoid leaf and the male is fluttering nearby. We displayed this image to provide perspective of their location in the vegetative canopy of the prairie plant community. The male lands to the left of the female and crawls alongside in **B**. In **C**, the female and male are shown just prior to mating. The claspers at the end of the male's abdomen appear to be visible. After this apparent mating event had completed, the female immediately flew to the eventual oviposition location (J. Henault unpublished 2015).

Supplementary Video 2.2. Observations of activities by a female and male prior to mating in 2017. Part **A** occurred immediately before part **B** within the same minute. In **A**, the female is situated on the leaf of a graminoid. The male lands nearby and rapidly swings the end of its abdomen in the vicinity of the female's abdomen. Perhaps, the male is using this motion combined with using its claspers, to reach out to the female's abdomen to mate. The female then crawls away further towards the tip of the leaf tip. In **B**, the female is situated on the leaf of a graminoid with its hindwings perpendicular to its body, in a similar position which we observed while adults basked and flew. The male crawls up the leaf blade to below the female and swings its abdomen in a similar way to that observed in part **A**. The female's wings appear to partially impede 1) the male from crawling closely alongside and 2) the tips of the abdomens from touching. Following these events, the male attempted to mate a few times but no mating occurred (not recorded in the video). Then, the female flew to land a few metres away and the male followed. This set of behaviours occurred two or three times before the skipperlings mated. While mating, the pair perched on the graminoid for approximately 30 – 60 sec before separating. Following this, the female immediately flew approximately three metres away and laid an egg on a Cyperaceae spp. (sedge). These behaviours were similarly observed during the mating observation in 2015 which was mentioned earlier.

Filename: Supl. Video 2.2. Adult activities prior to mating Henault, J. 2021.mp4.

Supplementary Table 2.3. Enumerated species ("+" = observed "-" = not observed) in egg enclosures, target plots and comparison plots during 2018 and 2019. Taxa with blank cells were not enumerated, thus we do not know whether those taxa were present or absent. The fonts of taxa used during egg laying activities and larval foraging behaviours are bolded. We use the most recent scientific names and authorities amongst Looman and Best (1987), Leighton and Harms (2014) and Tropicos.org (Missouri Botanical Garden 2021). Common names follow Tropicos.org (Missouri Botanical Garden 2021) where possible; for species not listed, we used names from the older references (Looman and Best 1987; Leighton and Harms 2014).

Scientific Name and Authority	Family	Common name	Egg enclosure	Target plot	Comparison plot
Achillea L. spp.[1]	Asteraceae	Yarrow	-	+	
Amelanchier alnifolia (Nutt.) Nutt. ex M. Roem.	Rosaceae	Saskatoon berry	-	+	
Andropogon gerardi **Vitman**	**Poaceae**	**Big bluestem**	+	+	+
Antennaria Gaertn. spp.	Asteraceae	Everlasting genus	-	+	
Asteraceae Bercht. and J. Presl spp.[2]		Aster family	+	+	
Beckmannia syzigachne (Steud.) Fernald	Poaceae	Slough grass	-	+	
Bromus L. spp.	Poaceae	Brome grass	+	+	
Caltha palustris L.	Ranunculaceae	Marsh-marigold	-	+	
Campanula rotundifolia L.	Campanulaceae	Harebell	-	+	
Cyperaceae Juss. spp.		Sedge family	+	+	
Dalea purpurea Vent.	Fabaceae	Purple prairie-clover	+	+	
Deschampsia cespitosa (L.) P. Beauv.	Poaceae	Tufted hairgrass	+	+	
Eleocharis R. Br. spp.	Cyperaceae	Spike rush genus	-	+	
Equisetum L. sp.[3]	Equisetaceae	Horse-tail sp. 1	+	+	
Equisetum L. sp.[4]	Equisetaceae	Horse-tail sp. 2	+	+	
Fragaria virginiana Mill.	Rosaceae	Strawberry	+	+	
Geum triflorum Pursh	Rosaceae	Three-flowered avens	+	+	
Glycyrrhiza lepidota Pursh	Fabaceae	Wild licorice	+	+	
Helianthus maximiliani Schrad.	Asteraceae	Maximillian's sunflower	+	+	
Hesperostipa spartea (Trin.) Barkworth	Poaceae	*H. spartea* grass	+	+	
Hypoxis hirsuta **(L.) Coville**	**Hypoxidaceae**	**Star-grass**	+	+	+
Juncaceae Juss. spp.		Rush family	+	+	
Lathyrus L. spp.	Fabaceae	Wild pea genus	-	+	
Liatris ligulistylis (A. Nelson) K. Schum.	Asteraceae	Meadow blazingstar	+	+	
Lithospermum canescens (Michx.) Lehm.	Boraginaceae	Hoary puccoon	+	+	
Lysimachia quadriflora Sims	Primulaceae	*L. quadriflora* forb	-	+	

Supplementary Table 2.3 continued.

Scientific Name and Authority	Family	Common name	Egg enclosure	Target plot	Comparison plot
***Muhlenbergia richardsonis* (Trin.) Rydb.**	**Poaceae**	**Mat muhly**	+	+	+
Muhlenbergia Schreb. spp.[5]	Poaceae	Muhly	+	+	+
Parnassia L. spp.	Celastraceae	Grass-of-Parnassus genus	+	+	
Poaceae Barnhart spp.		Grass family	-	+	
Populus tremuloides Michx.	Salicaceae	Trembling aspen	-	+	
Potentilla fruticosa L.	Rosaceae	Shrubby cinquefoil	+	+	
Rudbeckia hirta L.	Asteraceae	Black-eyed susan	+	+	
Salix L. spp.	Salicaceae	Willow genus	-	+	
***Schizachyrium scoparium* (Michx.) Nash**	**Poaceae**	**Little bluestem**	+	+	+
Solidago ptarmicoides (Torr. and A. Gray) B. Boivin	Asteraceae	Upland white goldenrod	+	+	
Solidago rigida L.	Asteraceae	Rigid goldenrod	+	+	
Solidago L. sp.	Asteraceae	*Solidago* L. sp. 1	+	+	
Solidago L. spp.[6]	Asteraceae	Goldenrod genus	+	+	
Sorghastrum nutans (L.) Nash	Poaceae	*S. nutans* grass	+	+	+
***Sporobolus heterolepis* (A. Gray) A. Gray**	Poaceae	**Prairie dropseed**	+	+	+
Sporobolus michauxianus (Hitchc.) P.M. Peterson and Saarela	Poaceae	Prairie cordgrass	+	+	
Thalictrum dasycarpum Fisch. and Avé-Lall.	Ranunculaceae	Tall meadow-rue	+	+	
Toxicodendron radicans (L.) Kuntze	Anacardiaceae	Poison-ivy	-	+	
Viola L. spp.	Violaceae	Violet	+	-	
Zizia W.D.J. Koch spp.[7]	Apiaceae	*Zizia* spp.	+	+	

1. Most likely *A. millefolium* L..
2. Not including other species in the Asteraceae family which were enumerated.
3. Most likely *E. laevigatum* A. Braun.
4. Most likely *E. pratense* Ehrh..
5. *Muhlenbergia* spp. other than *M. richardsonis* (Trin.) Rydb.. A mixture of most likely *M. glomerata* (Willd.) Trin. and *M. racemosa* (Michx.) Britton, Sterns and Poggenb..
6. *Solidago* L. spp. other than *S. ptarmicoides* (Torr. and A. Gray) B. Boivin, *S. rigida* L. and *Solidago* L. sp. 1.
7. Mixture of *Z. aptera* (A. Gray) Fernald and *Z. aurea* (L.) W.D.J. Koch.

Supplementary Table 2.4. Locations on shoots where Immatures (as larvae) were observed feeding and resting. We report the distance (cm) along graminoid shoots from the apex of the larval head to the leaf tip and the vertical distance to the ground where larvae ate or rested. To reduce the risk of displacing larvae, we did not record a measurement from larvae to the tip and to the ground during all events (please see methods). Thus, amongst all Immatures we observed feeding events 44 times and resting events 37 times during the development of immature stages of *O. poweshiek*.

Feeding

Stage	Distance to the leaf tip (mean (range))	Distance to the ground (mean (95 % C. I.))
Immature 1		
Instar 1	0.00 (0.00 to 0.00) (n = 3)	(n = 0)
Immature 2		
Instar 1	0.00 (0.00 to 0.00) (n = 6)	16.7 (16.4 to 17.0) (n = 3)
2	0.17 (0.00 to 0.85) (n = 5)	13.4 (7.1 to 19.8) (n = 3)
3	0.14 (0.00 to 0.86) (n = 8)	12.4 (9.3 to 15.4) (n = 7)
4	0.13 (0.00 to 0.54) (n = 8)	9.3 (7.5 to 11.1) (n =8)
Immature 3		
Instar 1	0.11 (0.00 to 0.35) (n = 6)	25.8 (21.6 to 30.0) (n = 6)
2	0.00 (0.00 to 0.00) (n = 3)	27.8 (25.3 to 30.3) (n = 4)
3	0.31 (0.00 to 0.61) (n = 2)	9.1 (8.5 to 9.7) (n = 2)

Resting

Stage	Distance to the leaf tip (mean (range)	Distance to the ground (mean (95 % C. I.)
Immature 1		
Instar 1	1.01 (0.93 to 1.09) (n = 2)	9.9 (3.8 to 16.0) (n = 2)
Immature 2		
Instar 1	1.28 (0.90 to 1.72) (n = 4)	15.8 (15.3 to 16.3) (n = 3)
2	5.56 (3.40 to 10.56) (n = 4)	11.1 (7.0 to 15.2) (n = 6)
3	7.15 (3.67 to 11.18) (n = 6)	8.4 (5.7 to 11.2) (n = 6)
4	15.29 (n = 1)	5.2 (n = 1)
Immature 3		
Instar 1	2.94 (2.29 to 5.10) (n = 5)	23.4 (21.8 to 25.0) (n = 5)
2	7.69 (1.26 to 17.52) (n = 5)	19.0 (12.1 to 25.8) (n = 5)
3	1.68 (1.39 to 1.95) (n = 3)	8.4 (8.2 to 8.6) (n = 3)

Supplementary Video 2.5. Immature 3 (second instar larva) consuming a leaf of *S. heterolepis*. Immature 3 has already eaten the notch on the right side of the leaf and now is leaving a notch while consuming the left side of the leaf. It is feeding near the tip of this leaf blade. After it finished feeding, Immature 3 turned around on the leaf and crawled down the leaf to a position closer to the ground where it rested (please see typical durations while resting in the results). Typically, a larvae would then crawl back up the stem to feed, alternating feeding and resting behaviour while we observed larvae.

Filename: Supl.Video 2.5. Larvae feeding on a leaf Henault, J. 2021.mp4

Supplementary Table 2.6. Degree day accumulations in egg enclosures and comparison plots calculated using two models (mean (95 % C. I.)). The values are based on four data loggers in egg enclosures and four data loggers in comparison plots.

Plot	Active larval	Diapause	Break of diapause to adult eclosion
Egg			
Standard	1050.1 (1011.7 to 1088.4)	107.3 (93.0 to 121.7)	542.6 (526.9 to 558.3)
Double Sine	976.7 (909.5 to 1043.8)	211.3 (202.1 to 220.4)	535.9 (522.1 to 549.8)
Comparison			
Standard	1017.5 (971.6 to 1063.3)	111.6 (70.8 to 152.4)	564.3 (540.2 to 588.4)
Double Sine	980.9 (954.0 to 1007.8)	205.3 (138.3 to 272.3)	535.7 (508.2 to 563.3)

Supplementary Table 2.7. The temperature (º C), relative humidity (%) and dewpoint (º C) during the developmental period from the date which the first egg hatched through the date which the first adult eclosed. We report the mean (95% C. I.)) of each variable recorded by data loggers in egg enclosures and comparison plots. We bolded the font of means (95% C. I.) recorded by data loggers containing confidence intervals that did not overlap with at least 3 data loggers from the other type (α = 0.05).

Plot	**Temperature$_{mean}$**	**Temperature$_{min}$**	**Temperature$_{max}$**
Egg			
1	5.2 (4.2 to 6.2)	-0.2 (-1.0 to 0.5)	11.0 (9.6 to 12.3)
2	5.2 (4.2 to 6.2)	-0.3 (-1.0 to 0.5)	10.8 (9.4 to 12.2)
3	5.4 (4.4 to 6.4)	-0.3 (-1.0 to 0.4)	11.5 (10.1 to 12.9)
4	5.9 (5.0 to 6.8)	1.0 (0.3 to 1.7)	11.3 (10.0 to 12.7)
Comparison			
5	5.7 (4.7 to 6.7)	0.3 (-0.5 to 1.0)	11.1 (9.8 to 12.5)
6	5.4 (4.4 to 6.4)	-0.2 (-1.0 to 0.6)	11.4 (9.9 to 12.8)
7	5.5 (4.6 to 6.5)	1.1 (0.4 to 1.8)	10.3 (9.0 to 11.6)
8	5.4 (4.5 to 6.4)	0.7 (0.0 to 1.5)	10.5 (9.1 to 11.8)

Plot	**Relative Humidity$_{mean}$**	**Relative Humidity$_{min}$**	**Relative Humidity$_{max}$**
Egg			
1	**80.2 (78.9 to 81.5)**	**61.8 (59.5 to 64.2)**	95.7 (95.1 to 96.4)
2	84.5 (83.2 to 85.7)	67.3 (64.9 to 69.8)	**98.1 (97.6 to 98.6)**
3	**79.5 (78.3 to 80.6)**	**62.7 (60.5 to 64.9)**	**92.6 (92.1 to 93.1)**
4	**80.0 (78.8 to 81.1)**	65.4 (63.2 to 67.6)	**91.5 (90.9 to 92.1)**
Comparison			
5	**83.1 (81.8 to 84.3)**	68.0 (65.8 to 70.2)	95.3 (94.8 to 95.8)
6	**83.5 (82.3 to 84.8)**	67.3 (65.0 to 69.6)	96.0 (95.5 to 96.4)
7	**85.1 (83.5 to 86.7)**	**71.8 (69.6 to 74.0)**	94.9 (93.5 to 96.2)
8	79.5 (78.6 to 80.5)	67.1 (65.3 to 68.8)	**88.8 (88.2 to 89.4)**

Plot	**Dewpoint$_{mean}$**	**Dewpoint$_{min}$**	**Dewpoint$_{max}$**
Egg			
1	1.5 (0.7 to 2.4)	-2.6 (-3.4 to -1.7)	4.8 (3.8 to 5.7)
2	2.2 (1.3 to 3.1)	-1.8 (-2.6 to -1.0)	5.4 (4.4 to 6.4)
3	1.8 (1.0 to 2.7)	-2.5 (-3.3 to -1.7)	5.1 (4.1 to 6.1)
4	2.5 (1.6 to 3.3)	-1.5 (-2.2 to -0.7)	5.5 (4.5 to 6.4)
Comparison			
5	2.2 (1.2 to 3.1)	-2.6 (-3.5 to -1.8)	6.0 (4.9 to 7.0)
6	2.0 (1.0 to 2.9)	-2.9 (-3.8 to -2.1)	5.9 (4.9 to 7.0)
7	2.8 (1.9 to 3.7)	-1.8 (-2.5 to -1.0)	6.5 (5.4 to 7.5)
8	1.7 (0.8 to 2.7)	-3.0 (-3.9 to -2.2)	5.6 (4.5 to 6.7)

Chapter 3: Adult habitat interactions of the endangered *Oarisma poweshiek* butterfly are associated along a soil moisture gradient in tall grass prairie in Manitoba, Canada.

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Abstract

The Poweshiek skipperling (*Oarisma poweshiek*) is an obligate tall grass prairie butterfly, and now critically endangered globally. Existing populations are scattered amongst tall grass prairie remnants. The vegetative and physical descriptions of locations which facilitate various adult activities, such as egg laying and resting, in Manitoba tall grass prairie are unknown. I followed adults in prairie patches to identify locations in which various activities, such as egg laying or nectar feeding, were facilitated by the habitat. I measured vegetative, structural and microclimatic attributes of these locations to determine potential characteristics which facilitate these adult interactions. Adults flew almost exclusively in the prairie plant community, rarely flew in shrub or ephemeral wetland communities and were never observed flying in wetland or forest communities. Adult activities appeared to be distributed along a soil moisture gradient, with egg laying associated with the mesic section of the moisture gradient, resting and/or basking associated with the drier section and nectar feeding generally associated with sections throughout the moisture gradient. Adults consumed nectar from *Rudbeckia hirta* most often, but nectared from a mixture of species during the flight period which also included *Packera* spp. and *Glycyrrhiza lepidota*. We described how adult skipperlings likely use various sections with different soil moistures in critical prairie habitat to lay eggs, rest, bask and consume nectar. Since the arrangement of critical habitat is not identical in every patch of prairie, this research can be used to guide researchers, stewards and recovery organisations in our efforts to facilitate the successful recovery of *O. poweshiek.*

Introduction

The Poweshiek skipperling (*Oarisma poweshiek*, (Parker, 1870), Lepidoptera: Hesperiidae) is a species endemic to the tall grass prairie in North America (Catling and Lafontaine 1986; Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2014, Belitz et al. 2018). Once occurring in suitable locations from southern Manitoba, Canada through several central states to Michigan, United States of America, this species is now endangered in Canada, the United States and critically endangered globally (COSEWIC 2014; United States Fish and Wildlife Service 2015; Canada Gazette 2019; Royer 2020). Approximately 99% of the tall grass prairie ecosystem has been destroyed (Samson and Knopf 1994), resulting in remnant Poweshiek skipperling populations only in southern Manitoba, Canada and Michigan, United States of America (COSEWIC 2014; United States Fish and Wildlife Service 2015). Up to several hundred individuals were observed in Manitoba during the 2000s, with approximately dozens of adults observed consistely in individual research sites (approximately 1 quarter section parcels) (COSEWIC 2014). Across its range, the abundance of adults in remnant colonies has declined since approximately the 1980s to potentially a few hundred individuals now (abundance estimates in late 1980s by P. Klassen and R. Westwood unpublished; COSEWIC 2014; Smith et al. 2016; Grantham et al. 2020).

Tall grass prairie habitat supporting *O. poweshiek* in Manitoba is contained within the Manitoba Tall Grass Prairie Preserve (MTGPP; interpretive centre approximately 49.153° N, 96.729° W). This tall grass prairie area contains mesic grassland openings, forest stands as well as ephemeral and permanent wetlands (Catling and Lafontaine 1986). Typical mesic prairies include the grasses *Andropogon gerardi* Vitman (Poaceae) (big bluestem) and *Sporobolous heterolepis* (A. Gray) A. Gray (Poaceae) (prairie dropseed); the forbs *Solidago rigida* L. (Asteraceae) (rigid goldenrod) and *Liatris ligulistylis* (A. Nelson) K. Schum. (Asteraceae) (meadow blazingstar); trees *Populus tremuloides* Michx. (Salicaceae) (trembling aspen) and *Quercus macrocarpa* Michx. (Fagaceae) (bur oak) with wetter areas supporting species including *Eleocharis elliptica* Kunth (Cyperaceae) and Juncaceae Juss. spp. (rush family) (Catling and Lafontaine 1986; COSEWIC 2014). Henault (2017) reported that sites with higher abundances of adult *O. poweshiek* were relatively drier than those with lower adult abundances, with *Hypoxis hirsuta* (Linnaeus) Coville (Hypoxidaceae) (star-grass) (in thesis erroneously reported as *Bouteloua curtipendula* (Michx.) Torr. (Poaceae), J. Henault correction 2021) indicative of high abundance sites, *Deschampsia cespitosa* (L.) P. Beauv. (Poaceae) indicative of medium abundance and Juncaceae spp. indicative of low abundance sites. *Rudbeckia hirta* L. (Asteraceae) occurred at relatively high densities in sites supporting more *O. poweshiek* adults (Henault 2017). *Rudbeckia hirta* was the nectar source used most often by Poweshiek skipperlings during observations in Semmler (2010). Prairie patches within a range of patch sizes might be suitable for *O. poweshiek* (Westwood et al. 2020).

Poweshiek skipperlings are consistently observed within the prairie type of plant community during annual adult surveys in the MTGPP (Grantham et al. 2020), but vegetative and physical characteristics supporting egg laying, nectar feeding and other adult interactions are unknown. Therefore, we aspired to determine how adults use prairie environments in Manitoba by evaluating whether locations used for specific behaviours were different (*e.g.*, are eggs laid in different locations than nectar is consumed?).

Plebejus samuelis (Nabokov, 1944) (Lepidoptera: Lycaenidae) butterflies predominantly laid eggs on host plants (lupines) less exposed to sunlight; this behaviour facilitated quicker larval growth (Grundel et al. 1998). *Carterocephalus palaemon* (Pallas, 1771) (Lepidoptera: Hesperiidae) butterfly males searched for mates in locations intermediate between drier and wetter habitat types, and females laid eggs in these transition areas and nectared in wetter locations (Ravenscroft 1994). Poweshiek skipperling also may be attracted to different locations for specific activities.

Tall grass prairies where we conducted research contain physical slopes (Catling and Lafontaine 1986), possibly providing a soil nutrient and moisture gradient (Liu et al. 2020) which creates a change of plant species (Zalatnai and Körmöczi 2004). We hypothesised that sections at middle elevations may provide a compromise in soil conditions that support host plant species typically more abundant where soils are drier, other species typically found where soils are wetter and species found in mesic soils simultaneously. The microhabitats supporting immature Poweshiek skipperlings contain specific host food plants and architecture (Thesis, Chapter 2). We hypothesise that middle elevations along slopes may provide suitable soil attributes to support the mixture of host plant species and physical characteristics that immature stages require. Part of this study was designed to determine where host plant species are typically found along a hypothetical soil moisture gradient. Locations transitional between drier and wetter areas are used as larval microhabitats by *C. palaemon*, likely because larval host plants are most abundant here (Ravenscroft 1994). Given egg laying microhabitats appear to have strict requirements (Thesis, Chapter 2), it is logical to wonder whether microhabitats facilitating nectar feeding, resting or other adult interactions are similarly regulated. The physical locations of microhabitats using by *O. poweshiek* relative to soil attributes in any given prairie habitat have not been determined in tall grass prairies in Manitoba.

Microhabitats supporting immature Poweshiek skipperlings contain a specific combination of host plant species and physical characteristics (Thesis, Chapter 2). We hypothesised that adults would conduct specific activities at certain locations along a soil moisture gradient, with each location providing specific requirements to facilitate each type of interaction (*e.g.*, host plants and microclimates providing for egg laying, and nectar sources providing for nectar feeding). Also, we predicted that adults would fly, subsequently interacting with the habitat in various ways, most often in habitats that facilitate any of these behaviours but not areas where activities were poorly supported. We predicted that adults would lay eggs in mesic sections of the soil moisture gradient because they provide the required host food plants and microclimates for larval development. Mesic areas may have a mixture of dry and wet-preferring species that create suitable food and microclimate opportunities for larvae. We hypothesised that adults would nectar feed in any accessible area containing usable nectar species. However, we predicted they would feed most often in higher elevation areas (potentially drier areas along the soil moisture gradient) where we thought we had observed the highest densities of *R. hirta* during previous surveys. We also predicted that resting, basking, mating and any other activity which we have not considered would be conducted at soil moisture locations containing specific vegetation and structural characteristics required to conduct each activity. The objectives of our research were first, to determine the locations along a soil moisture gradient in which Poweshiek skipperlings conduct various activities in tall grass prairies (especially interested in egg and larval microhabitats, but we put the same amount of effort into all activity types). We planned to achieve this objective by documenting the vegetative and physical attributes at multiple points along a soil moisture gradient and simultaneously at locations where adults interacted with their habitat (such as where adults consumed nectar). Then, we would compare the attributes at locations where activities were supported to locations along the moisture gradient to determine the approximate locations that each type of activity occurred. Our second objective was to determine the types of plant communities where adults fly in tall grass prairie by following adults in natural habitat in Manitoba. These observations would in part help to confirm whether adults flew in habitats where this hypothetical soil moisture gradient may exist. Finally, we wanted to record the species used by Poweshiek skipperlings to nectar feed. The exact adult activity microhabitats and flying locations will not be the same in different prairies obviously, so we determined characteristics to distinguish between microhabitat types to guide conservation disturbances and reintroduction planning in novel prairies.

Methods

We researched Poweshiek skipperling adult behaviours in southeastern Manitoba, Canada, at the MTGPP. This preserve is located in the Steinbach ecodistrict of the Boreal Plains ecozone (Agriculture and Agri-Food Canada 2013). Soils are generally composed of Dark Gray Chernozems with Peaty Gleysol and Mesisol components in wetter areas, and limestone glacial residue as well as bedrock (Ecological Stratification Working Group 1995). Temperatures in the warmest month of July average 18.8 °C and -16.6 °C in the coldest month January, and precipitation in the wettest month of June average 101.1 mm and 17.9 mm in the driest month February (average of 1981-2010 at Zhoda, MB: Government of Canada 2021).

We chose a site where adult Poweshiek skipperlings were consistently observed and egg laying events had also been recorded within the MTGPP, based on annual surveys. Adults have been counted every year since 2006 by conservation organisations (Westwood et al. 2012; Grantham et al. 2020). Either one or both of us have directly participated in these surveys since 2006. During surveys we recorded observations of types of adult behaviours such as egg laying and nectar feeding and their approximate locations in each site. To count adults, surveyors use a meandering walk technique (Royer et al. 1998) along established routes in each survey site to encounter adults. Established paths occur in habitats with various soil moistures dominated by graminoids. Then the number of adults counted is divided by the number of hours during which we surveyed to obtain a relative adult abundance for each tall grass prairie habitat site. Only the number of adults were recorded during surveys in 2006 and 2007, but not the duration of surveys

(R. Westwood unpublished). During this study, we researched within three adjacent but distinct prairie patches (totalling approximately 0.20 km^2, and a perimeter of 2.6 km) within a larger tall grass prairie habitat (prairie, wetland, forest components). These prairies were within one quarter section land parcel (0.64 km^2) in size. Encroaching *P. tremuloides* and other woody plant species are removed regularly (typically when needed) from prairie sites by the Nature Conservancy of Canada using prescribed fires, mowing equipment or by applying herbicides (Grantham et al. 2021). The last major disturbance in the research site occurred by a wildfire in autumn of 2011.

Poweshiek skipperlings appeared to consistently be observed in areas where prairie patches (areas which were potentially drier relative to the rest of the local tall grass prairie site) were adjacent to wetlands. Consequently, we wondered if this arrangement of plant communities was required for Poweshiek skipperling survival. We hypothesised adults may use the transitional zones between dry to wet areas to lay eggs, with these areas potentially supporting multiple host plant species preferring drier and wetter soil moistures. Additionally, we hypothesized adults would nectar feed, rest, bask and mate along specific sections of this soil moisture gradient. Therefore, we established transects in our research site to measure vegetation and specific environmental characteristics in prairie patches.

Soil moisture gradient

We established transects (n = 8) in 2017 on slopes in prairie patches from higher to lower elevations, in what appeared to by drier to wetter soil moistures respectively. The start of transects started at the highest elevated area and we ended the transects where water seeped out of the ground when standing on it. Transects varied in length (range 15 – 25 m) to ensure that they started and ended at approximately the same elevation and respective soil moisture. We sampled in seven plots at evenly spaced intervals along the transect. Therefore, plots at each rank position on the transects should have had similar relative soil moistures and consequentially plant species and physical attributes. We think that transects represented approximately the entire soil moisture gradient which was present in our research site. To measure the general topography along each transect and the elevational difference between the beginning and end of each transect, we used a levelled string. We tied a string onto wooden stakes at the start and end of each transect before levelling the string. Then, we measured the distance from the string to the ground at each sampling plot along the transects. We sampled the soil moisture and electrical conductivity (EC) using a soil probe, by pushing the metal sensor into the ground (soil rod depth of 10 cm) at two random locations in each sample plot and recording the electronic display (measured simultaneously using a Fieldscout TDR 150; Spectrum Technologies, Incorporated, Aurora, Illinois, United States of America). We measured at this depth because we wanted to measure the soil moisture that was potentially experienced by the roots of plants instead of the surface of the soil, and this was the length of the probe that we had. The EC (Natural Resources Conservation Services 2012) is a measure of the concentration of molecular ions (or degree of salinity) in the soil. Sampling was conducted on May 9 and 10, 2019 after at least 6 days without precipitation.

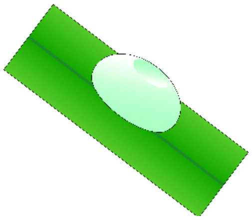
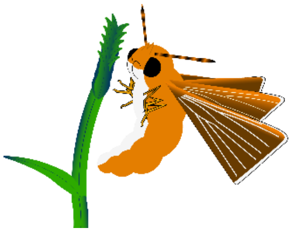
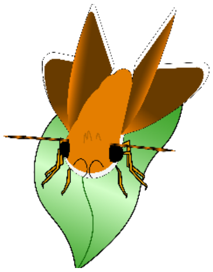
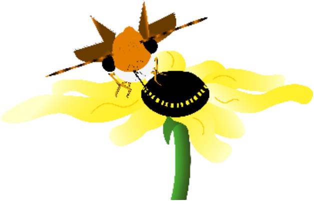
Our sampling areas along transects were square plots (0.25 m^2). Within each plot we counted the number of shoots of graminoid species (Poaceae Barnhart spp., Cyperaceae Juss. spp., Juncaceae spp. and Juncaginaceae Rich. spp.) and *H. hirsuta*. We also estimated the percent cover of graminoids, forbs, shrubs, duff (dead pieces of vegetation including larger and fine broken-down segments) and bare soil to the nearest 1 % by visually assessing each plot. We understand it was unlikely for us to be accurate to this degree, but thought that estimating to this degree would result in closer estimations than estimating to the nearest 10 %. The number of blooming *R. hirta* inflorescences within a 3 m radius of each plot were counted, a measure that was designed to indicate the relative location of the plots to high quality nectar habitat (*R. hirta* was used most often in the MTGPP; Semmler 2010). We measured the height of graminoids in each plot, by visually estimating the height representing 95% of the shoots before recording the corresponding height on a ruler. Duff depth was measured randomly in three places within each plot. We recorded the depth by randomly placing the ruler three times in every plot. We initially identified species by eye, based on our field experience, before confirming each species by using the dichotomous keys in Looman and Best (1987) and Leighton and Harms (2014). We matched the nomenclature to current revisions at Tropicos.org (Missouri Botanical Garden 2021), an online database of accepted plant nomenclature. Common names follow Tropicos.org (Missouri Botanical Garden 2021) where possible; for species not listed, we used names from the older references (Looman and Best 1987; Leighton and Harms 2014). We realised at the end of field season in 2017 that we had erroneously enumerated *Beckmannia syzigachne* (Steud.) Fernald and likely two *Triglochin* L. (Juncaginaeceae) spp. (*T. palustris* L. and *T. maritima* L.) as the same species. Therefore, we communicate them as *B. syg*. /*Tri*. spp. throughout this manuscript. All taxa grow almost exclusively in seasonal or permanent wetlands (Lichvar et al. 2012; United States Army Corps of Engineers 2018).

We sampled the soil at positions representing the start (position 1), middle (position 4/5) and end (position 7) along each transect and at the location where egg laying was observed (observation during 2017 in Chapter 2 of this thesis). Within each sample plot, we dug soil samples at two random points each containing the top 15 cm of soil. We collected these soil samples in all transects after at least 2 days of no precipitation in July 2017, and maintained them in a freezer at approximately -20 ℃ until analysis. We air-dried (7 days at 23 ℃) then homogenized the samples from each plot. We sieved (2 mm) each homogenised sample, then used the pipette method (Gee and Bauder 1986) to separate sand, silt and clay portions and determine soil particle size proportions. We removed transect position samples where we made errors while conducting analysis procedures (after removals; n = 16). We also sent approximately one-third of each homogenised sample to Farmers Edge Laboratories in Winnipeg, MB, Canada to analyse soil nutrients. Vegetation data and physical measurements were collected between July 17 to 27, 2017. The physiologies of some graminoid guilds make them suited to developing in cooler temperatures and others at warmer temperatures, among other guild types (as described in Kindscher and Wells 1995). The mature inflorescences of plants suited to cooler temperatures were still present while the inflorescences of plants suited to warmer temperatures were newly formed during this sampling period. We think that the timing of our sampling period made identifications more accurate than would have been possible in the spring or fall. We also walked on only one side of each transect throughout the sample period to minimize vegetation disturbance, and to reduce the risk of damaging or displacing Poweshiek skipperling larvae if present.

Adult activity locations

We followed adult Poweshiek skipperling every day of the flight period, marking locations they laid eggs and conducted other activities as described in Chapter 2 (Thesis). We initially planned to mark egg laying, nectar feeding, resting, basking and mating activity locations. However, once we started following adults we also observed attempted oviposition activities and never observed mating behaviours (Table 3.1). When females searched for oviposition locations, we sometimes observed what we thought to have been failed oviposition attempts (one female). Typically, this entailed fluttering at the location and always probing the surface of only one leaf blade but not followed by egg deposition. Nectar feeding behaviour involved and adult landing on a plant (typically directly on an inflorescence) and extending its proboscis into flower (s). Adults frequently moved across an inflorescence and probed multiple florets during one visit. We cannot confirm that nectar feeding occurred while an adult was at a flower. However, we assume that adults did consume nectar every flower which they visited. We marked the bases of these shoots. Each adult track was recorded with a GPS unit (Garmin Oregon 700; approximate accuracy 3 m) when possible, by programming the unit to record coordinates at every second to create paths. We did not create tracks for adults that we observed for less than 30 seconds. However, if we positively identified these individuals, we recorded any accompanying interactions which we observed. Justis Henault observed and classified all adult behaviours (except for two ovipositions and two nectar feeding activities by Richard Westwood), with note taking assistance from field helpers. The substrate which facilitated each activity was recorded (*e.g.*, rested on a graminoid, nectared from an *R. hirta*).

Table 3.1. Adult activity descriptions and accompanying images used throughout this manuscript. We grouped basking and resting together because we frequently saw adults alternating between these behaviours at the same spot. We did not observe mating during this research but J. Henault did in 2015 and 2017 (please see Chapter 2 of this thesis).

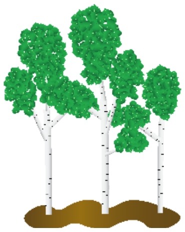
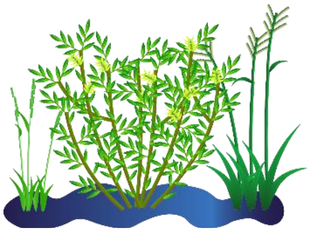
Activity	Attributes
Egg laying 	Flying closer to vegetation, hovering above a small area, occasionally landing on leaf blades and touching the leaf blade with the tip of the abdomen but not laying eggs, and finally landing and depositing an egg before elevating and departing.
Attempted oviposition 	Searching typically by fluttering and always probing the surface of a leaf blade as observed for "egg laying", but after touching a leaf (usually only once) the female departed and resumed a search at another location.
Resting/ Basking 	Resting: Positioned stationary on a substrate (living or dead vegetation; no observations on bare soil), with wings closed or opened briefly. Basking: Positioned stationary, but different from resting in that wings are generally open during the entire observation (forewings 45° angle and hindwings nearly parallel to the ground); may reposition their wings periodically.
Nectar feeding 	Landing on a plant (typically directly on an inflorescence) and extending proboscis into flower (s). Adults frequently moved across an inflorescence and probed multiple florets during one visit. We cannot confirm that nectar feeding occurred while an adult was at a flower. However, we assume that adults did consume nectar every flower which they visited.
Mating	Female and Male mating (at apparently first attempt or after several unsuccessful times) in the canopy of graminoid. After mating the male flew off and the female immediately flew to the eventual oviposition location during our observations.

At each location where an adult interacted with the habitat, we sampled in a circle plot of 0.25 m^2. We sampled egg laying plots of 0.19 m^2 due to the size of the enclosures (Thesis, Chapter 2). We counted the number of shoots of all species using the same methods as described above for transects, however also counted shoots of all forb and shrub species. We also estimated the approximate percent cover of the same groups (*e.g.*, forbs, duff), counted the number of blooming *R. hirta* inflorescences nearby plots and measured graminoid height and duff depth using the same references and methods as described above for transect samples. These vegetation and physical attributes were sampled between 2 to 14 August 2018, and 26 August 2018. We sampled the soil moisture and electrical conductivity using a soil probe using methods as described for transect samples. Samples were taken on 18 and 30 July 2018 and 17 August 2018 after a similar number of days without substantial rain showers (approximately 8 days; occasional light precipitation events which did not substantially increase the moisture of the soil surface occurred at some point). Soil plugs were not removed nor nutrients analysed in adult activity locations.

We think our soil moisture transects represent the complete moisture gradient within our site (please see below). We sampled along these transects in case our adult observations did not encompass the entire moisture spectrum, to sample the available soil moisture range in this tall grass prairie area.

While in the field, we noted vegetative and environmental attributes typical of plant communities in and near locations in which adults were observed in our research site. We noted the approximate boundaries of each plant community type, before using satellite imagery (in ArcGIS Pro (ESRI Inc. 2021) to augment our notes to delineate boundaries for each community type. Also while in the field, we noted the plant species typically observed in each plant community and the general height of approximately 95 % of the vegetation. We also described the elevation of plant communities relative to other community types in the patches, estimated the light intensity which we perceived in each type of plant community (*e.g.*, the forest type was subjectively less intense than the prairie type) and whether standing water was present in the plant community type. We report typical attributes of each plant community Table 3.2, and boundaries as part of data results in Figs. 3.3 and 3.4.

Table 3.2. Typical characteristics of plant communities in our research site. Please refer to Supplementary Table 3.1 for the family of each taxon.

Plant community	Attributes
Prairie 	Open grassland with vegetation less than 0.5 m tall; the most abundant grasses are *Sporobolous heterolepis* and *Andropogon gerardi*; most abundant forbs are *Helianthus maximiliani* Schrad. and *Dalea purpurea* Vent. and most abundant shrub is *Potentilla fruticosa* L.; at higher elevation; high light near ground level.
Forest 	Stands of trees over 5 m tall; most abundant species is *Populus tremuloides*; low light near ground level.
Tree colony	Forested stand with trees less than 5 m tall; most abundant species is *P. tremuloides*; low light near ground level.
Wetland 	Standing water most of the year; perennial vegetation less than 0.5 m tall; most abundant graminoids are *Sporobolus michauxianus* (Hitchc.) P.M. Peterson & Saarela, *B. syg. /Tri.* spp., and *Scirpus* L. spp.; abundant forbs are *Typha* L. spp.; at lower elevation; high light near ground level.
Shrub wetland 	Perennial vegetation more than 0.5 m tall; standing water only a portion of the year; most abundant graminoids include *S. michauxianus* and *B. syg. /Tri.* spp.; most abundant shrubs are *Salix* L. spp.; intermediate light near ground level.
Ephemeral wetland	Perennial vegetation less than 0.5 m tall; standing water only a portion of the year; most abundant graminoids include *S. michauxianus* and *B. syg. /Tri.* spp.; high light near ground level.

We did not know the host plant species used by larvae, as reported in Chapter 2 (Thesis), until the year after we established the transects and after we observed adult activity behaviour (larval feeding confirmed late July to September 2018; soil moisture transects in 2017; adult activities in early July 2018). Therefore, we conducted this current study based on our hypotheses at the time. After determining several host plants consumed by larvae (Thesis, Chapter 2), we focused on these plant species during analysis of this 2017-18 transect and activity research.

We updated Lepidopteran nomenclature to Pohl et al. (2018); for taxa absent from that document, we used nomenclature from GBIF.org (Global Biodiversity Information Facility 2021). Pohl et al. (2018) is the most recent classification of Lepidoptera in Canada and Alaska (United States of America). GBIF.org is an online database that contains classifications which have been developed based on academic literature.

Analyses

We collected a small number of adult observations, because endangered *O. poweshiek* are difficult to locate. Also, we did not observe individual adults conducting an equal number of each type of adult activities. Therefore, we searched for meaningful patterns within adult activity and transect data using means, ranges and ordination comparisons, declining statistical tests that would likely result in types 1 and 2 errors. Transects and activity observations were conducted during different years, so we could not directly compare these data. Thus, we analysed data collected during the adult activity components and data during the transect research components independently, before synthesising the results. The growth of plants and resulting plant architecture would likely differ between years, even though the perennial species likely create similar habitats in both years.

Soil moisture gradient

The beginning and ends of transects (highest and lowest elevations respectively) differed by a mean 16.3 cm (n = 7; range = 4.0 – 26.0 cm). We calculated the Spearman’s rho correlation coefficients of sample plots at postions along the transects (1 to 7 along each transect) with soil moisture and EC separately using RStudio (RStudio Team 2021). We then calculated the means of the correlation coefficients for soil moisture and EC. Sample plot position (1 to 7 along each transect) correlated with a change in soil moisture from the beginning to end of the transects (Spearman’s rho; n = 7, mean = 0.648, range = 0.312 – 0.878).

One transect appeared to support prairie grasses and forbs at the start as well as *Tyhpa* spp. where water seeped out of the ground at the end. We perceived this transect to start at highest elevation and end at the lowest as we had assessed by using the same techniques while establishing the other transects. However, our measurements of the topography of this transect indicated that it was shaped like a ridge which we did not perceive while in the field. The topography of all other transects undulated along a slope but all declined in general from the highest elevation to the lowest (positions 1 and 7 respectively). Additionally, the soil moisture along this suspect transect was highest in the middle portion and lowest at beginning and end portions instead of steadily increasing along the transect as we observed in the other sampling transects. By the time we had discovered these facts, we could not resample the soil and topography along this transect to determine if we made an error or set up a new transect in an area with a declining elevation along a slope. We had designed this research to evaluate the potential of a soil moisture gradient along slopes but not ridges. We were also unsure what how to accurately interpret data collected along seven slopes and one ridge simultaneously. Therefore, we decided to remove this transect from our research. For the rest of this manuscript we use seven transects (number of plots equals 49).

Transects were established in patches where adult activity was observed in the past. However adults were only observed in patches (two out of the three patches) where two of the transects had been placed. We risked analysing a wider range of prairie vegetation and environmental microhabitats than were available to adults if we used transects in different than where adult activities occurred. To address this, we compared the number of shoots of plant species counted along each transect (comparing plant data between transects instead of between positions along transects) using non-metric multidimensional scaling ordination (please see details about how to conduct this type of analysis below; Kruskal 1964; Mather 1976; McCune and Mefford 2011). Since data for the remaining transects (n = 7) from all three prairie patches appeared to overlap, the total collection of transects seems to represent the microhabitats available to adults where they flew (in patches near the two transects). Therefore, we used transects from all of the three prairie patches in our analysis.

We screened vegetation data using the same criteria as for activity samples. For Fig. 3.1, shoot counts of each species were divided into the maximum number of each species counted in any plot (McCune and Mefford 2011). We did this to compare the trends in the number of stems within individual species, to compare to the number of shoots at locations where adults conducted various activities to identify the approximate soil moistures where they occur.

Adult activity locations

We did not mark individuals thus do not know the number of adults that we observed, or which activities can be attributed to individual adults. Considering this limitation, egg laying, nectar feeding and resting/ basking activities appeared to be distributed amongst several adults whereas attempted oviposition activities were observed in one adult. The microhabitat data at locations where attempted oviposition was observed may be biased. However, we still thought that exploring the relationship of attempted oviposition activities to the others would still be worthwhile.

Adults were observed resting and basking alternately at the same locations, so we combined these locations. We uploaded locations for adult activities and tracks into ArcGIS Pro (ESRI Inc. 2021). ArcGIS Pro was used to measure the length of each track and then RStudio (RStudio Team 2021) was used to calculate mean ± SD distance travelled by each butterfly and the total distance of adult track data which we gathered. We calculated 95 % confidence intervals (C. I.) of the geospatial location where each type of adult activity may occur in ArcGIS Pro. Two tracked adults travelled one metre and then rested for at least the remainder of the day. Since these observations were of extremely limited movements, we excluded these observations when we calculated the mean distance travelled by adults to ensure we evaluated travelling movement within habitats. For Figs. 3.3 and 3.4, we focused on one prairie patch, of the three patches, where the majority of adult observations occurred (45 out of 56) to ensure all observations could be displayed in a single figure. We used the vegetative, physical and soil data recorded at all 56 locations during further analyses. Nectar source proportions in Fig. 3.5 were created using Adobe Illustrator (Adobe Inc. 2021).

We standardised the number of shoots counted at locations where attempted oviposition, nectar feeding and resting/ basking were observed (0.25 m^2) to the area sampled at locations where egg laying was observed (0.19 m^2). We thought that reducing the number of shoots by standardising from 0.25 m^2 to 0.19 m^2 made more sense than adding shoots, we which did not count, if we were to standardise from 0.19 m^2 to 0.25 m^2. During our analyses, we used all species which were counted in at least one sample location. Generally, only using species with at least several observations would be suitable for some analyses. Crucial to our analysis however was to identify any species important to Poweshiek skipperling. Therefore, we did not want to exclude infrequently observed species which actually helped to describe the ecology and critical habitat of *O. poweshiek*.

Recall that while following adults, we placed metals stakes into the ground at locations where we observed adults interacting with the habitat as noted above. The accuracy of our placements varied; some were exactly where we observed the observation and others were up to approximately 15 cm away. Therefore, we separated our locations into high (within approximately 5 cm of the location where the interaction was observed) and low (6 – 15 cm from location) confidence subsets. In the "high" subset, we measured all variables as described earlier. We think that imprecision of up to 15 cm may affect accurate counts of the number of shoots of species substantially, but would less effect estimations of the approximate percent cover of plant groups and means of measurements of vegetation or duff heights as well as soil moisture. Thus, we counted the number of shoots of plant species at only the "high" confidence locations. The egg laying and attempted oviposition locations were unaffected. However, 5 out of 14 resting/ basking observations were classified as "high" confidence and 23 out of 31 nectar feeding observations were assessed as "high" confidence. This reduced the 56 observations of adult activities to the sample sizes observed in Table 3.5.

Associating activity types with positions along the moisture gradient

We calculated the mean, minimum and maximum abundance for shoots of each species and for environmental characteristics in RStudio (RStudio Team 2021). The means of the recordings of duff depth in each plot were calculated using RStudio.

We compared vegetation and physical attributes between transect positions along the soil moisture gradient and adult activities using multivariate analyses; we analysed transect positions and activity locations separately, then visually compared similarities between these parts. We investigated plant species that are occur most frequently and abundantly in transect positions and adult activity locations using indicator species analysis (ISA) (Dufrêne and Legendre 1997). Indicator species analysis identifies species highly associated with any plot sample grouping, producing an indicator value (IV) for each species. Indicator values were tested for significance using Monte Carlo randomisation with 1000 permutations using PC-ORD (McCune and Mefford 2011). The Dufrêne and Legendre (1997) analysis is suitable for presence and absence data or quantitative data (McCune and Mefford 2011). Shoot counts of species in sample plots were divided into the maximum shoot count of that species across all plots prior to analysis (relativize by maximum; McCune and Mefford 2011).

We assessed species composition and abundance amongst transect positions and adult activity types using multi-response permutation procedures (MRPP) (Berry et al. 1983; Mielke 1984; Mielke and Berry 2001; McCune and Mefford 2011). Prior the analysis of the abundances of shoots of host plant species enumerated, we removed samples for which no species were counted. Three samples were removed from the analysis of host food plant species at positions along transects (one sample which represented position 6 and two samples which represented position 7) resulting in 46 samples being used in the analysis. No samples needed to be removed when we analysed all plant species enumerated at positions along the transects (n = 49). Also, no samples required removal when analysing all plant species enumerated and when analysing only host food species at locations where adult activities were observed (for both analyses n = 39). We conducted the MRPP test for each respective focus: 1) positions along the transects and 2) types of adult activities. The positions along transects and types of adult activities were defined as groups during the respective analyses. Multi-response permutation procedures analysis calculates the relative observed difference between groups (producing **T** statistic) and runs a permutation test between the observed values and random expected values to assess if groups are significantly different from each other (p-value) and to provide a measure of the degree of homogeneity within groups (producing **A** statistic; completely homogenous A = 1; McCune and Mefford 2011). For example, these values may be used to indicate that plant communities at positions along transects may be significantly different from one another. This test does not require equal sample sizes or homogeneous variances of residuals (McCune and Mefford 2011), therefore is most appropriate for our data. We used the Sorenson (Bray-Curtis) distance measure in MRPP analyses (Bray and Curtis 1957; McCune and Mefford 2011). This distance measure calculates similarity between samples based on weighing each species equally and integrating the abundance of each species, an important criterion for this study where we wanted to identify any species important to Poweshiek skipperling (Bray and Curtis 1957; Magurran 1988). It is suitable for analysis of presence and absence or continuous data and is compatible with heterogenous data sets with numerous zeroes typical in community datasets (Magurran 1988, McCune and Mefford 2011), both of which characterise our data. This MRPP analysis also conducts pair-wise tests when using this analysis in PC-ORD (*e.g.*, plant data sampled at locations where egg laying was observed compared to where nectar feeding was observed; plant data between position 1 and position 6). However, these tests do not correct the produced p-values to compensate for conducting multiple comparisons (McCune and Mefford 2011). Thus, by making inferences using these unadjusted values we would risk type 1 errors by rejecting null hypotheses which are in fact true (*e.g.*, inferring that the plant species and abundance associated with egg laying locations were different than that associated with nectar feeding locations, when the plant species and abundance were in fact not different between the activity types). We were unable to locate a technique to adjust the p-values which we thought appropriately balanced the risks of conducting types 1 and 2 errors (type 2 errors: if we were to not reject null hypotheses which were false). Therefore, we used the MRPP test relevant to the groups in general (without using the pair-wise tests) and relied on the other various analyses which we conducted to determine which species and abundances of plants which were associated with groups within each respective focus (transect positions and adult activities) may be different.

We visually assessed vegetation diversity between plots using non-metric multidimensional scaling (NMDS). Non-metric multidimensional scaling analysis projects species and plots as points in ordination space (Kruskal 1964; Mather 1976; McCune and Mefford 2011). The distance between all points in ordination space represents the similarities observed and recorded in plots. In an NMDS solution that accurately reflects the similarities in the data set, the species and plots which are more closely associated in the real world should hypothetically be shown closer together in the ordination output. The physical distances between species and plots along axes explaining greater percentages of variation explain a relatively greater proportion of variation in the data than along axes with lower percentages of variation. We used Sorenson distance measure to produce the ordination and calculate correlations or axis loadings within the plot, orthogonal principal axes rotation to visualise the ordination and didn’t adjust tied scores. We chose this rotation technique to ensure axes were independent of one another (McCune and Mefford 2011), potentially making interpretation more straightforward using the moisture gradient as a likely main explainer. We used the cumulative r^2 button to make sure the axes explaining the most variance were displayed on NMDS 1 and 2 respectively (Peck 2011).

Please recall that we analysed activities that may be biased by differences of adults due to the small sample size. Also, that these samples are not truly independent. Since we did not identify the individuals in any way (we did not mark them) we do not know how many adults we saw; all data could be from several individuals or perhaps three dozen. We think assessing these locations of activities along a soil moisture gradient, even if biased, will generate knowledge of Poweshiek skipperling in Manitoba that may increase the likelihood of successful recovery efforts.

We conducted tests in RStudio (RStudio Team 2021) using R version 4.1.2 as the programming base (R Development Core Team 2021). We used the following R packages and thank several authors for wonderful vignettes using these packages: “ggforce” (Pedersen 2021; “super-ellipse” example by Kindt (2020)), “ggplot2” (Wickham et al. 2020; “convex hull points” example Chizinski (2014)) and “svglite” (Wickham et al. 2021). We used ArcGIS Pro version 2.9.0 (ESRI Inc. 2021) to create standard deviation ellipses for activity locations and visualise activity locations and tracks. We used PC-ORD (McCune and Mefford 2011) for multivariate analyses. Plant and butterfly stage illustrations were illustrated (J. Henault) using Adobe Illustrator (Adobe Inc. 2021). We conducted NMDS analyses in PC-ORD, exported scores to create plots in RStudio, then imported plots into Adobe Illustrator to improve the resolution, colours and add plant or *O. poweshiek* illustrations. We used multiple software programs because we were most confident that we knew the consequences of our NMDS analysis settings in PC-ORD, were able to add convex ellipses in RStudio and to increase the visual quality of the illustrations in Adobe Illustrator. The figure that displays relative shoot abundance on transects was created in RStudio and exported to Adobe Illustrator.

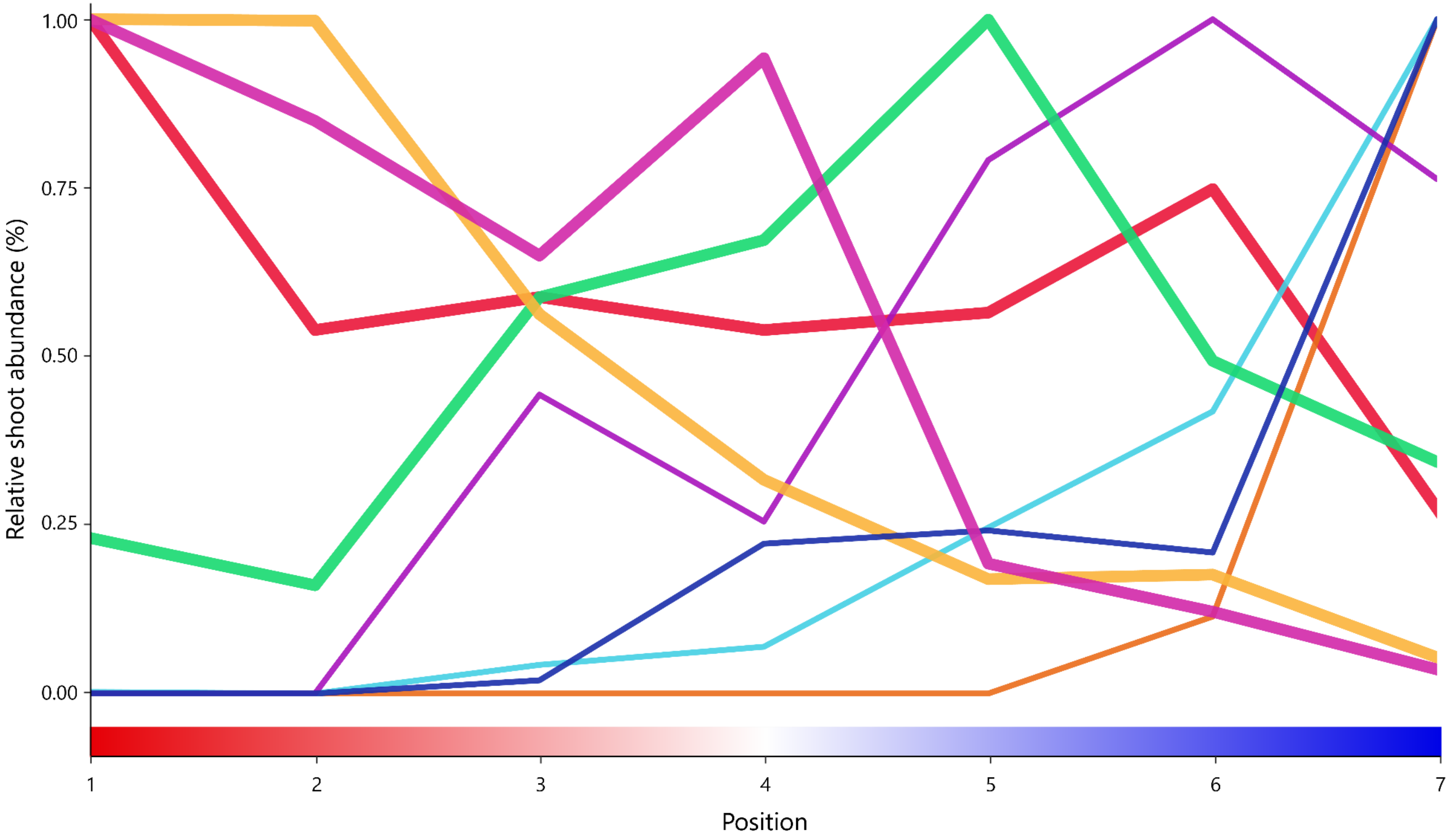
Results

Attributes of positions along the soil moisture gradient

Like soil moisture, soil electrical conductivity (mS/cm) correlated with sample plot position from the beginning to the end of the transects (elevation: high to low; soil moisture; dry to wet) (Spearman's rho; n = 7, mean 0.452, range 0.070 – 0.796). Soil nutrients and texture in positions 1 (top), 4 (middle) or 7 (bottom) along transects had overlapping 95 % C. I. thus were not significantly different (Supplementary Table 3.2).

We display relative shoot abundance at different positions along transects corresponding to soil moisture values (Fig. 3.1). *Andropogon gerardi* was the most abundant at position 1 in the dry part of the transects, was moderate-high in abundance along positions 2 to 6 and low at position 7. *Schizachyrium scoparium* was generally abundant at positions 1 and 4, but reduced in abundance from positions 5 to 7 in the wetter end. *Muhlenbergia richardsonis* was moderate-highly abundant at positions 3 to 6, but not as abundant in the drier end (positions 1 and 2) and wettest end (position 7) of the transects. *Sporobolus heterolepis* was most abundant at positions 1 and 2, moderately abundant at positions 3 and 4, and declined throughout positions 5 to 7. *Hypoxis hirsuta* is not shown in Fig. 3.1; it was only present at position 1 of the transects (also an indicator of position 1 as reported in Table 3.3). *Sporobolous michauxianus* was most abundant at position 7 (the wettest end), occurred at a low abundance from positions 4 to 6 but was almost absent at transect positions 1 to 3. Juncaceae spp. were most abundant at positions 5 to 7, present at positions 3 and 4, but not present at positions 1 and 2. *B. syg. /Tri.* spp. were most abundant at position 7, present at position 6, but were not observed at any other position along the transects. *Eleocharis* spp. abundance peaked at position 7 of the transects, were moderately abundant at positions 5 and 6, present at positions 3 and 4, but were not observed at positions 1 and 2. The relative abundances of all species which we enumerated are displayed in Supplementary Fig. 3.3 and the stem counts of all enumerated species are reported in Supplementary Table 3.4. *Hesperostipa spartea* was most abundant at positions 1 and 2, moderately abundant at position 3, but was not observed at positions 4 to 7, in the mesic to wetter portions of the transects. The abundance of *Deschampsia cespitosa* peaked at position 3 of the transects, was high at position 2, and low at position 1 (driest) as well as at positions 4 to 7 (mesic-wet to wettest sections).

Fig. 3.1. Relative abundance of graminoid shoots (%) along transects. Shoot counts were relativized by the maximum number for each species. Lines representing graminoid species which we observed larvae consuming are wider than those of species which we did not observe larvae eating. Transect positions are shown along the x-axis accompanied by their relative soil moisture on the y-axis.



The indicator species analysis showed that *S. heterolepis* and *H. hirsuta* are indicators of position 1 of transects (Table 3.3). The MRPP analysis showed that the transect positions contained different abundances of shoots of all plant species enumerated (n = 49, T = -3.785, A = 0.067, p = 0.001) and abundances of only plant species consumed by larvae (n = 46; T = -2.240, A= 0.057, p = 0.024).

Table 3.3. Indicator species of adult activity locations and positions along the soil moisture gradient (generated using ISA). For analysis of plant data along transects: all transect positions, n = 7. The sample sizes for the analysis of adult activities were: attempted oviposition, n = 5 (all one adult); egg laying, n = 6; nectar feeding, n = 23; resting/ basking, n = 5. Position 1 was at the driest end of the moisture gradient and position 7 at the wettest. Indicator value is abbreviated as "IV".

Moisture gradient positions			**Adult activity locations**		
1	*S. heterolepis*	IV = 30.5, p = 0.046	Resting/ Basking	*Rosa* spp.	IV = 40.0, p = 0.031
	H. hirsuta	IV = 42.9, p = 0.012			
7	*S. michauxianus*	IV = 50.6, p = 0.003	Attempted oviposition	*M. richardsonis*	IV = 75.1, p = 0.002
	Eleocharis spp.	IV = 40.2, p = 0.012		*Eleocharis* spp.	IV = 37.6, p = 0.037
				Cyperaceae spp.	IV = 38.0, p = 0.029

We evaluated an NMDS for plant species, and overlaid environmental attributes (Fig. 3.2). Please recall the physical attributes do not constrain the samples associated with locations where various adult activities were observed or plant species at the same locations in this ordination. We used the lines representing the physical attributes as guides to compare in combination with the plant species, characteristics of locations associated with various adult activities in the three prairie patches. The strength of the associations corresponding to various physical attributes (red lines) were scaled to 250 % for straightforward interpretation. The NMDS had 3 axes, with mean stress of 15.0 (p = 0.012). *B. syg. /Tri.* spp., *Eleocharis* spp., Juncaceae spp. and *S. michauxianus* were most associated with the lower end of the transects; *H. spartea*, *H. hirsuta*, *S. heterolepis* and *A. gerardi* were associated with the higher end and *M.*

richardsonis was most associated with the middle. The positions appeared to strongly follow the soil moisture gradient designed into the experiment, with the soil moisture being more highly associated with plots 7, 6 and 5 at the lower end of the transects. The graminoid height was also positively associated with the wetter end of the transects. The direction and magnitude of the line representing soil EC (not displayed) approximately matched that of the line representing graminoid height. The graminoid percent cover and the number of blooming *R. hirta* inflorescences within a 3 m radius were positively associated to a high degree with the dry section of transects, and the forb percent cover moderately associated with the dry section of transects. Duff (cm) was positively and weakly associated with positions 4 and 1 of the transects.

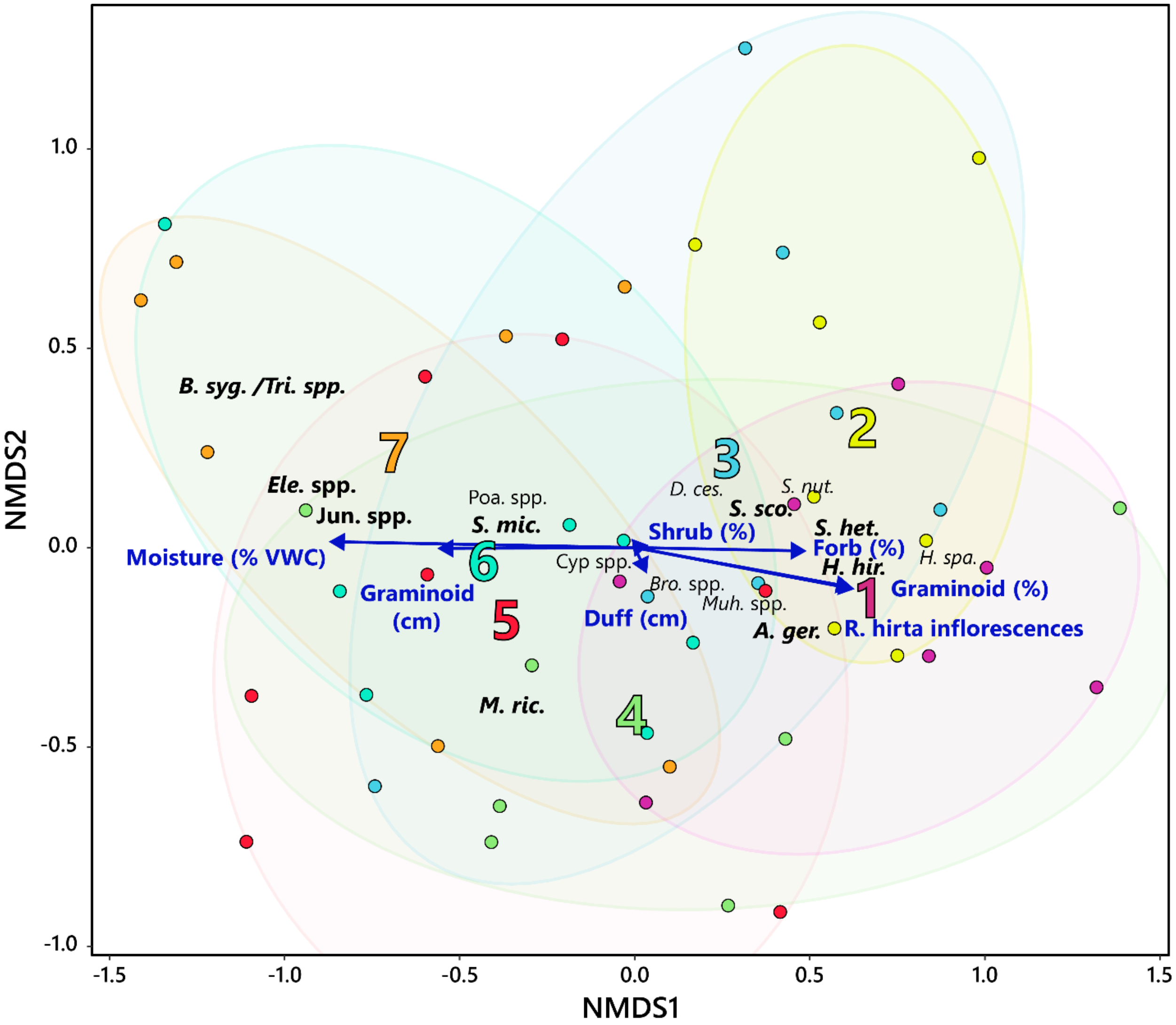


Fig. 3.2. An NMDS ordination of transect positions, plant species enumerated and environmental attributes measured. The positions of samples along the transect (1 = highest elevation, 7 = lowest) are reported as circles of various colours. We display centroids and convex ellipses of respective transect positions in the corresponding colours of each position. Species names are reported the first letter of the genus and the first three letters of species and unknown species are reported as the first three letters of the respective genus or family (species that are discussed in manuscript are bolded). We overlay environmental attributes using blue arrows and blue font (scaled to 250%).

Adult activities

Movement and habitat use. While adults were flying, we observed them travelling at approximately a consistent elevation approximately 0.3 m above the vegetation while travelling in a sinusoidal path. Their hindwings were most often held parallel to the ground with little flapping movement, and their forewings were vigorous flapping throughout their flight. When changing direction, adults flapped their hindwings generally one time before returning them to the parallel position. During this brief flap, the white-silver markings on their hindwings were most easily observed. Adults flew at a speed which was slightly slower than a comfortable walking pace by J. H.. Based on our design, we were unable to precisely record the velocity at which adults flew.

Adults were observed flying almost exclusively in prairie plant communities (Fig. 3.3), were rarely observed in shrub or ephemeral wetland communities and never observed in wetland or forest communities (communities as we described in Table 3.2). The mean distance we followed an adult was 86.5 ± 65.4 m (n = 22) (mean ± SD). The total distance of the tracks was 1.9 km. Adults were observed flying 1696.7 m in the prairie plant community (89.2% of the total distance flown), 166.2 m in the shrub wetland community and 39.1 m in the ephemeral wetland community. Adults also appeared to navigate within prairie patches, not flying into the treed colony community on one side and the small ephemeral wetland community on the other. Locations where adults were observed conducting various activities in the focal prairie patch are displayed in Fig. 3.4. Including locations from all prairie patches, 49 tracks were within the prairie plant community, 3 on the border of the prairie and shrub wetland plant communities (1 egg laying, 2 resting/ basking) and 4 within the shrub wetland community (nectar feeding).

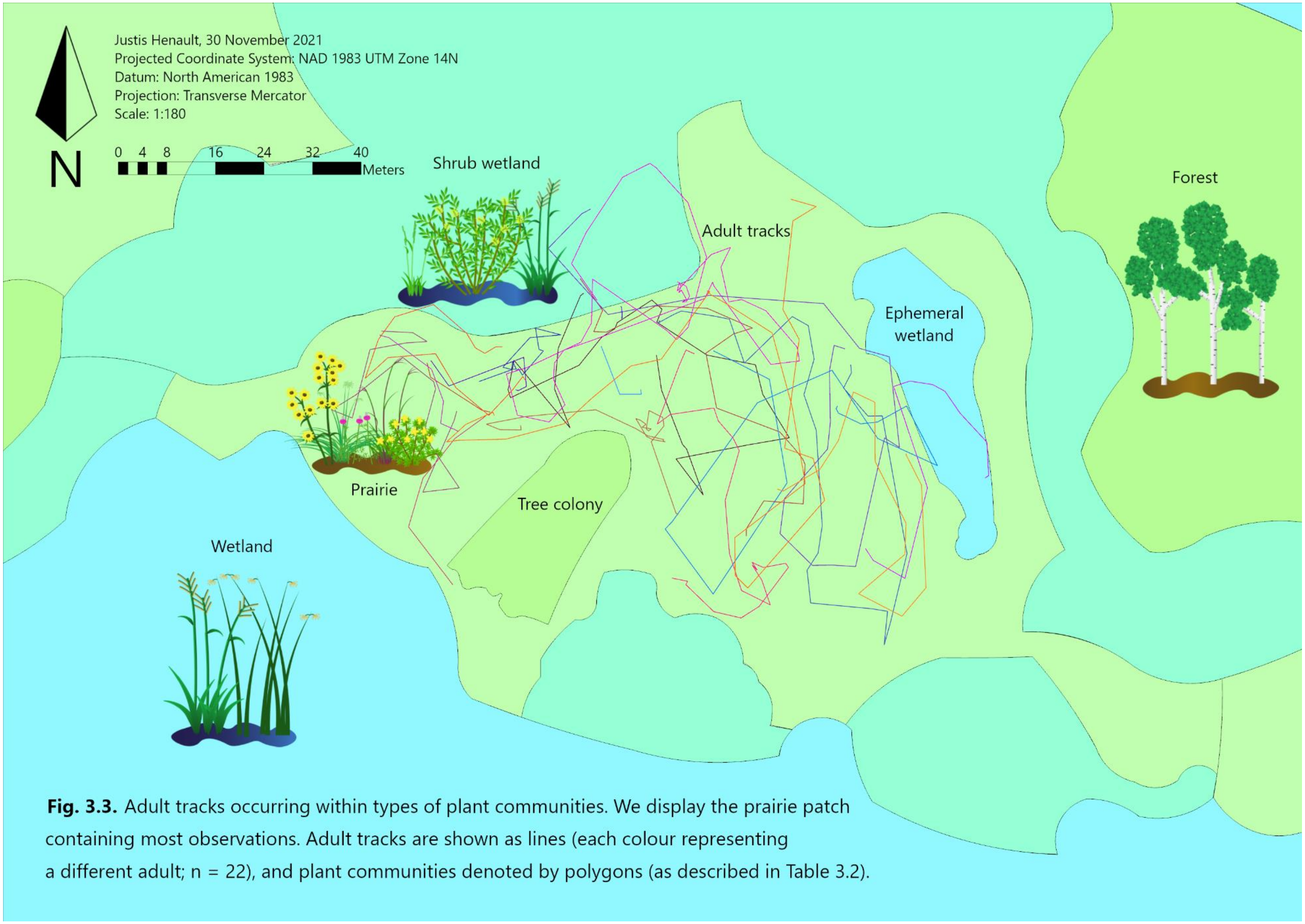


Fig. 3.3. Adult tracks occurring within types of plant communities. We display the prairie patch containing most observations. Adult tracks are shown as lines (each colour representing a different adult; n = 22), and plant communities denoted by polygons (as described in Table 3.2).

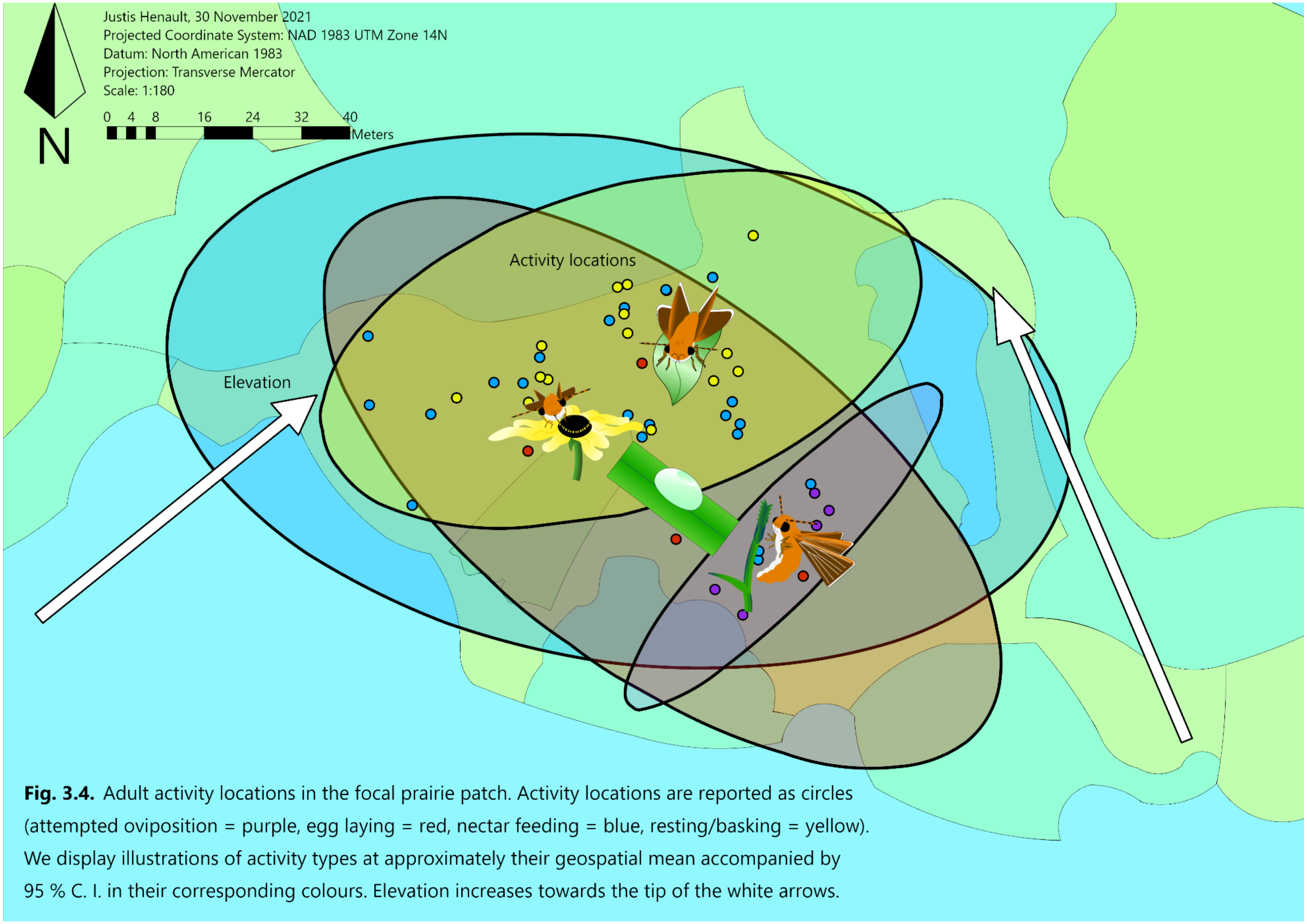


Fig. 3.4. Adult activity locations in the focal prairie patch. Activity locations are reported as circles (attempted oviposition = purple, egg laying = red, nectar feeding = blue, resting/basking = yellow). We display illustrations of activity types at approximately their geospatial mean accompanied by 95 % C. I. in their corresponding colours. Elevation increases towards the tip of the white arrows.

Attempted oviposition locations were at the lower elevations, but concentrated in one strip (almost certainly because they are from a single adult observed in one track). Egg locations were at intermediate elevations in the prairie community, ranging closer to the higher elevations and to the lower elevations. Oviposition was observed by up to 5 different individuals (please see lack of marking). The egg 95% C. I. is within prairie patches, adjacent to the larger wetland to the south. Resting/ basking locations range from middle to high elevations at the border between prairie and shrub wetland, and the locations appear the most concentrated of all activity types given the number of resting/ basking samples. Finally, nectar feeding locations occurred in prairies, ranging between dry and wetter areas at higher and lower elevations respectively. The nectar feeding 95% C. I. demarcation is large, encompassing adjacent shrub wetland and ephemeral wetland plant communities, however it seems they were concentrated from middle to high elevations.

Using observations from all three prairie patches, adults were observed 34 times (13 were identified as females and 9 as males) in 2018. One male was observed with worn off scales in a straight line from the apex of the hindwing to the costa at the body; this male was observed on two days (26 June and 28 June 2018). Adults otherwise were not marked, so we do not know how many unique individuals were observed. During the 34 observations of adults, 24 individual adult flights were tracked. Six adults flew without conducting one of the various activities; thus 28 adults were observed creating a total of 56 activity events (egg laying: n = 6, attempted oviposition: n = 5, resting/ basking: n = 14, nectar feeding: n = 31).

Nectar feeding and activity substrates. Adults used several different substrate species for each activity, and consumed nectar from different proportions of nectar species (Table 3.4; we report species names and authorities as well as the most likely unknown species in each respective genus in Supplementary Table 3.1). *R. hirta* was fed on most often, but several other nectar species made up portions of their diet. Adults rested on graminoids on the forb *G. lepidota* most frequently but also rested on the forbs *Packera* L. (Asteraceae) spp. and the shrub *Amorpha canescens* Pursh (Fabaceae). Please see Chapter 2 (Thesis) for a detailed discussion of adult oviposition behaviour.

Table 3.4. Relative proportion of substrates used during adult activity types (% (number of observations)).

Attempted oviposition		**Egg laying**		**Nectar feeding**		**Resting/ Basking**	
Graminoid	100% (n = 5)	*A. gerardi*	67% (n=4)	*R. hirta*	58% (n =18)	Graminoid	43% (n=6)
		H. hirsuta	17% (n=1)	*Packera* spp.	19% (n=6)	*G. lepidota*	36% (n=5)
		S. heterolepis	17% (n =1)	*G. lepidota*	13% (n=4)	*Packera* spp.	14% (n=2)
				Apocynum spp.	3% (n=1)	*A. canescens*	7% (n=1)
				P. fruticosa	3% (n=1)		
				Unidentified	3% (n=1)		

Nectar feeding was observed 26 June to 3 Jul 2018. *Packera* spp., *R. hirta*, *Glycyrrhiza lepidota* Pursh (Fabaceae), and *Apocynum* L. (Apocynaceae) spp. were used at the beginning of the flight period, a mixture of species (*Packera* spp., *G. lepidota* and *Potentilla fruticosa* L. (Rosaceae)) which also incorporated a greater proportion of *R. hirta* midway through the flight period, and mostly *R. hirta* at the end (Fig. 3.5). On 26 June and 30 June, all nectar feeding was on *R. hirta* however each date had only one observation of nectar feeding on any species.

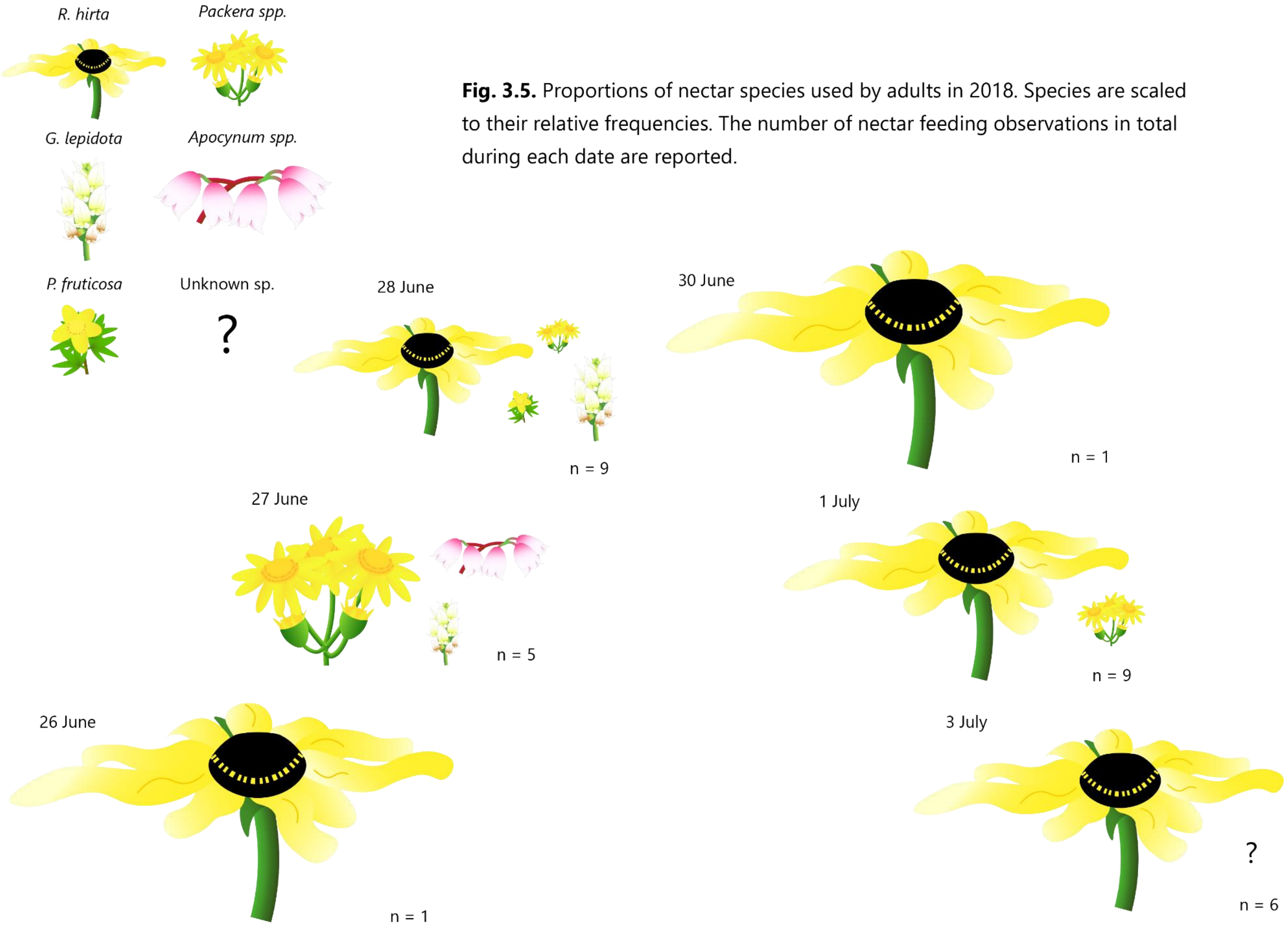


Fig. 3.5. Proportions of nectar species used by adults in 2018. Species are scaled to their relative frequencies. The number of nectar feeding observations in total during each date are reported.

Microhabitat attributes. In Chapter 2 (Thesis), larva consumed *A. gerardi*, *S. scoparium, M. richardsonis* and *S. heterolepis.* During the current research, the mean and ranges of host food plants and *R. hirta* counted in locations that supported all types of adult activities are reported in Table 3.5. The majority (81%) of *R. hirta* shoots were counted in locations where adults consumed nectar.

Table 3.5. The number of shoots of host plants and the most frequently used nectar species (mean (range)) in locations where adults conducted various activities. For all species, sample sizes were: attempted oviposition, n = 5 (all one adult); egg laying, n = 6; nectar feeding, n = 23 and resting/ basking, n = 5.

Plant species	Attempted oviposition	Egg laying	Nectar feeding	Resting/ Basking
A. gerardi	31.6 (6.1 to 65.4)	43.2 (16.0 to 76.0)	21.3 (0.0 to 76.0)	27.5 (22.0 to 44.1)
M. richardsonis	129.0 (28.1 to 305.5)	17.0 (0.0 to 45.0)	18.5 (0.0 to 147.4)	7.3 (0.0 to 30.4)
S. heterolepis	55.9 (10.6 to 107.2)	57.3 (4.0 to 133.0)	42.8 (0.0 to 106.4)	44.5 (9.1 to 66.9)
S. scoparium	10.9 (3.0 to 25.1)	5.0 (0.0 to 23.0)	18.7 (0.0 to 66.1)	17.9 (0.8 to 47.1)
R. hirta	0.2 (0.0 to 0.8)	0.7 (0.0 to 4.0)	1.0 (0.0 to 3.0)	0.2 (0.0 to 0.8)

The indicator species analysis showed *M. richardsonis* was an indicator of attempted oviposition locations and *Rosa* L. (Rosaceae) spp. were indicators of resting/ basking locations (Table 3.3). *Hypoxis hirsuta* was nearly indicative of egg laying locations (IV = 38.0, p = 0.052). The MRPP analysis showed that the locations in which various adult activities were supported (all three prairie patches in total) contained different abundances of shoots of all plant species enumerated (n = 39, T = -3.132, A = 0.041, p = 0.005) and abundances of only plant species consumed by larvae (n = 39, T = -2.476, A= 0.046, p = 0.017). We report the unadjusted numerical values for all MRPP analyses in Supplementary Table 3.5.

The environmental characteristics associated with adult activity are shown in Table 3.6. Soil moisture was highest in locations where egg laying and attempted oviposition activities were observed and lower in locations where resting/ basking and nectar feeding were observed. Graminoid height was highest in locations where attempted oviposition was observed, moderate in locations where nectar feeding and egg laying were observed and lowest in locations where resting/ basking was observed. Locations associated with egg laying and attempted oviposition were almost devoid of shrub cover compared to locations associated with resting/ basking and nectar feeding (albeit still contained a low cover). Forb cover was highest in locations where nectar feeding was observed and decreased to its minimum recorded value at locations where attempted oviposition was observed. Duff depth was highest where attempted oviposition was observed, moderate in locations where nectar feeding was observed and lowest in locations where egg laying and resting/ basking were observed.

Table 3.6. The environmental characteristics associated with various adult activities (mean (minimum and maximum)). For Duff (cm) and Graminoid (cm), sample sizes were: attempted oviposition, n = 5 (all one adult); egg laying, n = 6; nectar feeding, n = 30 and resting/ basking, n = 14. For the remaining characteristics, sample sizes were the same except that nectar feeding had 31 samples instead of 30. A higher soil moisture value means it is wetter and a higher soil EC value means that it is more saline.

Environmental characteristic	**Attempted oviposition**	**Egg laying**	**Nectar feeding**	**Resting/ Basking**
Duff (cm)	6.33 (5.2 to 7.6)	4.0 (2.5 to 4.9)	4.9 (2.4 to 8.4)	4.1 (2.2 to 7.6)
Graminoid (cm)	40.6 (38.5 to 42.5)	31.7 (24.7 to 35.3)	34.0 (17.0 to 53.4)	28.9 (13.4 to 41.5)
Bare soil (%)	1.0 (1.0 to 1.0)	1.3 (1.0 to 2.5)	1.6 (1.0 to 4.0)	1.4 (1.0 to 3.0)
Duff (%)	31.6 (28.0 to 36.0)	29.8 (19.0 to 37.5)	29.3 (20.0 to 39.0)	29.1 (15.0 to 38.0)
Graminoid (%)	59.2 (55.0 to 62.0)	60.8 (50.0 to 70.0)	55.6 (42.0 to 75.0)	57.4 (40.0 to 80.0)
Forb (%)	7.4 (3.0 to 15.0)	7.8 (3.0 to 15.8)	9.2 (2.0 to 20.0)	8.2 (1.9 to 14.0)
Shrub (%)	0.8 (0.0 to 2.0)	0.3 (0.0 to 1.1)	4.4 (0.0 to 28.5)	3.9 (0.0 to 25.0)
Number of *R. hirta* flowers	2.0 (1.0 to 4.0)	3.2 (0.0 to 9.0)	2.7 (0.0. to 7.0)	1.9 (0.0 to 4.0)
Soil moisture (% VWC)	29.5 (22.5 to 41.7)	28.5 (16.5 to 41.4)	20.8 (9.0 to 38.3)	21.2 (12.7 to 30.8)
Soil EC (mS/cm)	0.17 (0.10 to 0.30)	0.15 (0.06 to 0.25)	0.09 (0.00 to 0.24)	0.10 (0.04 to 0.18)

We visually assessed vegetation in activity locations using an NMDS (Fig. 3.6). We programmed 3 dimensions and the ordination had a mean stress of 12.4, p = 0.004. We ran the program several times to ensure the placement of species and samples in the output were consistent (Peck 2011). *Muhlenbergia richardsonis*, *Eleocharis* spp. and *Sporobolus michauxianus* (Hitchc.) P.M. Peterson & Saarela were associated with attempted oviposition samples and *Rosa* spp. were most highly associated with resting/ basking samples. The various activity types appear to be arranged along NMDS 1, with some separation between the nectar feeding type and both the egg laying and resting/ basking types on NMDS 2.

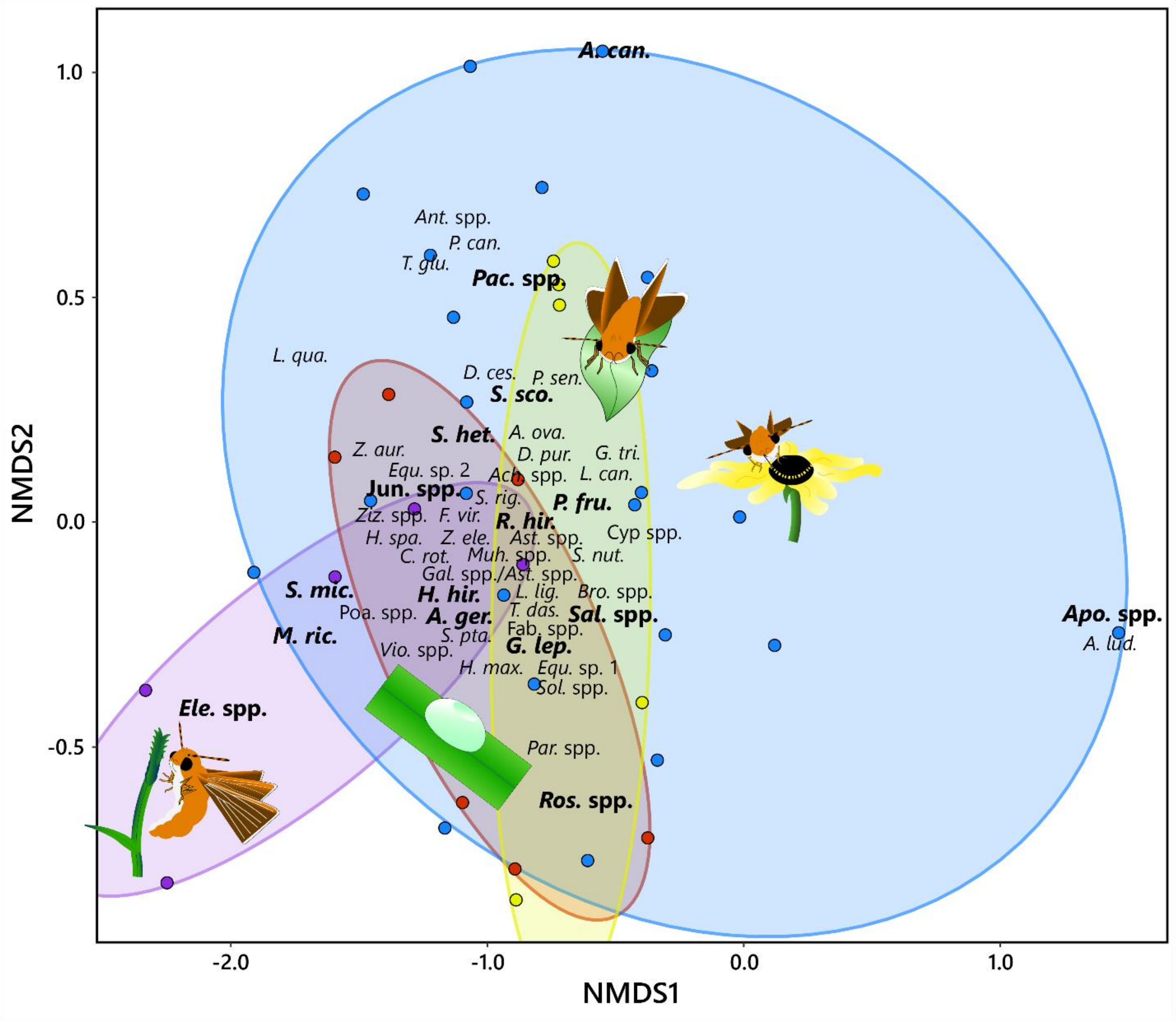


Fig. 3.6. An NMDS ordination of adult activity locations and plant species enumerated. Activity locations are reported as circles (attempted egg laying = purple, egg laying = red, nectar feeding = blue, resting/ basking = yellow). We display convex ellipses of activity types in their corresponding colours accompanied by illustrations within each ellipse. Species names are reported the first letter of the genus and the first three letters of species and unknown species are reported as the first three letters of the respective genus or family (species that are discussed in manuscript are bolded).

Discussion

Adult activity behaviours

Nectar feeding. Adults nectared at a mixture of potential nectar sources at the beginning of the flight period including *R. hirta*, incorporated relatively more *R. hirta* inflorescences into their diet midway in the flight period and consumed nectar almost exclusively from *R. hirta* by the end. On 26 June and 30 June, all nectar feeding was on *R. hirta* however each date had only one sample, so these observations contributed very little to the overall pattern. While we do not know whether adults consumed nectar whenever they visited a flower, we are assuming that adults did consume nectar at every flower we observed them land on during our study and during research by the studies which we cite. Thus, adults appeared to incorporate various species into their diet as the flight period progressed. These observations represent the phenological timing between nectar sources and butterfly emergence during only one year, thus the composition of their diet may not be the same every year. Adults did not consume nectar from *Solidago ptarmicoides* (Torr. & A. Gray) B. Boivin (Asteraceae) (upland white goldenrod), or *Prunella vulgaris* L. (Lamiaceae), in contrast to observations in Semmler (2010) and Dupont Morozoff (2013) respectively. Semmler (2010) observed nectar feeding most often from *R. hirta*, as we also reported, but never from *P. fruticosa*, in contrast to our observations. *Solidago ptarmicoides* blooms later in the season and there were few mature inflorescences blooming while the butterflies during our study were flying (although we did not count the number of blooming *S. ptarmicoides* inflorescences). The low abundance of *S. ptarmicoides* inflorescences during our study, likely reduced the chance that *O. poweshiek* would feed on nectar from this species. We think that *P. fruticosa* was blooming when *O. poweshiek* needed nectar sources at the same time during which very few *R. hirta* inflorescences had matured. During the study period of Semmler (2010), perhaps *O. poweshiek* emerged when both *P. fruticosa* and *R. hirta* were blooming, resulting in adults feeding from a preferred nectar source in *R. hirta* while avoiding *P. fruticosa* entirely. Climate-induced changes in nectar availability may lead to a mistiming of butterflies and plants, and potentially cause butterfly mortality (Hindle et al. 2015; Donoso et al. 2016; Patterson et al. 2020). However, we think that early emerging *O. poweshiek* feed from several species such as *Packera* spp. and *G. lepidota*, use *R. hirta* during the majority of the flight period, and if they fly later may use *S. ptarmicoides*, supplementing their diet with several species throughout the period. Feeding somewhat opportunistically may improve the fitness of individuals, reducing the impact of nectar shortages, and thus this trait may result in local *O. poweshiek* populations with higher abundances of individuals. A larger local population may be more resilient to stochastic events, and thus may reduce the likelihood that the population becomes extirpated. *Parnassius mnemosyne* (Linnaeus, 1758) (Lepidoptera: Papilionidae) also feeds on nectar species available during its flight period, even though it prefers relatively few species (Szigeti et al. 2018). It appears *O. poweshiek* are able to locate nectar sources even if the emergence is mistimed, so likely less-than-drastic changes in blooming and butterfly phenology would not increase skipperling mortality from our perspective. To know for sure, we would need to research the following question: Can the previous years' adult diet be a predictor of the following years' population numbers, leading to more/less eggs laid or more/less fit offspring produced? We think this question seems worthwhile to research, by observing adult feeding (including confirming that nectar was actually consumed from flowers), butterfly population sizes and floral abundance in natural habitats during consecutive years. If survival is still high regardless of the species and *O. poweshiek* feeds indiscriminately on any species in bloom but consistently feeds on some more than others, then we suppose *O. poweshiek* may be a generalist feeder of a subset of prairie forbs. We observed Poweshiek skipperling flying most often in prairie types of plant communities (Figs. 3.3 and 3.4). Adults may have chosen to spend time in the prairie communities because these areas contain the adult nectar sources and larval host plants Poweshiek skipperling require.

Lobelia spicata Lam. was reported as very common in sites by Catling and Lafontaine (1986). While we have walked through the MTGPP during various research projects, we have seen *Lobelia* spp. in several sites. However, *Lobelia* spp. are typically abundant in small areas within a site instead of being common throughout. During this study, *Lobelia* spp. occurred within the same quarter section which the three research prairie patches were situated in. However, we did not see any of these forbs in patches where *O. poweshiek* were flying. We did see a few *Lobelia* spp. plants along a strip of a prairie plant community separated from the study patches by a shrub wetland plant community. We do not know where Catling and Lafontaine (1986) surveyed, so perhaps their site had many *Lobelia* spp. and at least some *O. poweshiek* and might not have been where we researched during this study. If *Lobelia* spp. was in a patch where

O. poweshiek fly, we assume it would likely be used as a nectar source by adults depending on the other available nectaring plants as suggested by our research.

Resting/ basking. *Oarisma poweshiek*, of the subfamily Hesperiinae Latreille, rested or basked on vegetation instead of on the ground, as observed in *Erynnis martialis* (Scudder, [1870]) (Lepidoptera: Hesperiidae) (Mottled duskywing) of the subfamily Pyrginae Burmeister (Henault and Westwood accepted 2021). Perhaps behaviour tendencies of each subfamily explain part of this difference (Hesperiinae – forewings held at approximately 45° while hind winds parallel to ground, Pyrginae – both forewings and hindwings positioned parallel to the ground) or possibly the vegetative structure of plant communities in which these species occur (*O. poweshiek*: prairies, high proportion of ground covered by vegetation; *E. martialis* in Manitoba: clearings in sand-pine forests, high proportion of bare soil and/or vegetation less than 5 cm tall) may explain some of their behavioural differences. However, further observations of habitat interactions by each species are required.

Adults activities along a soil moisture gradient

The prairie plant community appears to be the most used by adults (Figs. 3.3 and 3.4), the wetland community occasionally used and the forest community avoided based on this study. The tracks collectively show that adults generally fly within the prairie type of plant community and adults turn while travelling, with the tracks of individual adults overlapping each other. Flights within the shrub wetland and ephemeral wetland plant communities were infrequent and provide evidence against adults using the wettest plant communities in general (shrub wetlands, ephemeral wetlands and wetlands). Adults seem to actively choose prairie plant communities, leaving shrub wetland and ephemeral wetland communities quickly if they fly into them. *Populus tremuloides* shoots, a species we used to describe forest and tree colony plant communities, absent from locations which facilitated various adult activities. We also never observed adults flying in the small stand of *P. tremuloides* in the study site (Figs. 3.3 and 3.4). Therefore, plant communities with *P. tremuloides* appear to be unsuitable for *O. poweshiek.* Perhaps, these types of plant communities create shaded habitats which are not conducive to adult and immature survival. Grundel et al. (1998) reported *P. samuelis* larvae benefit from shaded host plants on which they develop more quickly, but perhaps larval *O. poweshiek* survival may be reduced on host food plants with less sun exposure and or that they require host graminoids situated in sun-exposed locations. Simultaneously, potentially small stands of trees or large shrubs may result in wind and snow being blocked (or snow accumulating) within the prairie patch contributing to the suitability/ lack of suitability of locations to facilitate various adult activities. We encourage further research that focuses on how Poweshiek skipperlings navigate suitable habitats, and potentially unsuitable habitats to find patches which are more appropriate, as well as whether the arrangement of adjacent plant communities influences the environmental conditions where *O. poweshiek* live.

Locations where attempted oviposition and egg laying activities were observed appeared to have a higher soil moisture compared to locations where resting/ basking and nectar feeding were observed (Table 3.6). The soil EC appeared to be similarly higher at locations where attempted oviposition and egg laying activities were observed compared to locations where resting/ basking and nectar feeding were observed. Ellipses for the various activities in Fig. 3.6 seem to be arranged along NMDS 1 in generally the same order as soil moisture in Table 3.6, with attempted oviposition and egg laying (the wettest) on the left and resting/ basking and nectar feeding (the driest) on the right. Relatively high *M. richardsonis* shoot counts associated with attempted oviposition activities (Table 3.5, Fig. 3.6) and its indicator status of attempted oviposition locations (Table 3.3) suggest that attempted oviposition locations are associated with mesic sections to wet along soil moisture gradients. *Eleocharis* spp. and *S. michauxianus* relative shoot abundances are higher in wetter sections of the soil moisture gradient (Fig. 3.1), are more strongly associated with wetter soil (Fig. 3.2) and are indicator species of wetter sections (Table 3.3). These species are also strongly positively associated with attempted oviposition locations in the same analyses (also including Fig. 3.6). We think that the associations of these species with wetter sections of the gradient and simultaneously with attempted oviposition locations, provides evidence that the attempted oviposition activity type is in wetter sections at least during this study. Locations where attempted oviposition occurred also have a tall graminoid height characteristic of wetter positions along the moisture gradient, a low density of *R. hirta* inflorescences suggesting wetter sections (Table 3.5, Table 3.6, Fig. 3.2), and are spatially situated closer to wetter plant communities (Fig. 3.4). In contrast, the high graminoid cover suggests that attempted locations are associated with the drier section of the moisture gradient. *B. syzigachne* and/or *Triglochin* spp. appeared to be a consistent component of the wettest soil moistures existing in the research area (wetlands and ephemeral wetlands in Table 3.2, Fig. 3.3

and Fig. 3.4, as well as position 7 in Fig. 3.1), growing in similar seasonally or permanently wet habitats as in Lichvar et al. (2012). The presence of either species seems to indicate the wettest portions of the tall grass prairie soil gradient. However, *B. syzigachne* and/or *Triglochin* spp. were not counted at any location where an adult interacted with the habitat. Adults were not active in these wettest areas of the tall grass prairie moisture gradient which we measured (Fig. 3.4), and rarely flew through them (tracks in shrub wetlands and ephemeral wetlands; Fig. 3.3). Therefore, locations with *B. syzigachne* and/or *Triglochin* spp. likely do not support adult activities. The close proximity of attempted oviposition locations to the wetland plant community also provides evidence that these are likely closer to the wetter end of the moisture gradient – at least for this female. We think this area within the prairie patch in general was marginally suitable; the female alternately attempted to oviposit and actually lay eggs at locations within several metres. Locations where attempted oviposition was observed contained high graminoid cover, thus potentially attracting the female to an area with abundant plants which larvae may consume. We think that once the adult arrived, the specific host plant diversity or perhaps physical structure of the microclimate may not have stimulated the female to lay an egg (oviposition threshold as in Singer 1971). For the various adult activities, soil moisture appears to support species and potentially a habitat structure required for *O. poweshiek* to conduct specific behaviours. In Chapter 2 (Thesis), we counted two shoots of *B. syzigachne* close to an egg plot (target plot) but since it was outside of the sample size used in the rest of this study, we do not think it makes sense to compare. However, we interpret these results that potentially egg microhabitats are close to the wettest areas, but not within them.

Plant species and abundances were different amongst various types of adult activities (as suggested by our MRPP analyses). Recall that locations where egg laying was observed had high soil moisture compared to locations where resting/ basking and nectar feeding were observed. Egg laying locations appeared to be less associated with *Eleocharis* spp. (Fig. 3.6) which appears to be characteristic of attempted oviposition locations at the wettest section of the moisture gradient. Larvae were observed foraging amongst *A. gerardi*, *M. richardsonis*, *S. heterolepis* and *S. scoparium* in microhabitats in Henault (2021, Chapter 2). We assume that the collective density of these species within a microhabitat likely influences how easily larvae can access food and subsequently may influence a larva's likelihood of survival. During the current research, locations where egg laying was observed had the highest number of shoots of *A. gerardi* as well as *S. heterolepis* among the various activities, a relatively intermediate number of shoots of *M. richardsonis*, and the relatively lowest number of shoots of *S. scoparium* (Table 3.5). It appears that eggs are not laid at locations where all host food species individually occur at the highest relative densities within a prairie patch. These data do not suggest that access to multiple host food species is not necessary for larval survival. Therefore, perhaps locations where all four species are present but their shoots are not at the highest relative abundances facilitate adult egg laying and larval foraging behaviours. We think that the position along transects where larval diet would be best supported is Position 3 along the transects, and in mesic sections of the soil moisture gradient, where all confirmed host food species are present and at approximately intermediate abundances (Fig. 3.1). These vegetative data suggest that factors in addition to the abundance of host plant species, such as physical attributes, may characterise a microhabitat which facilitates larval development. The high graminoid percent cover, low graminoid height and high density of nearby *R. hirta* inflorescences at locations where egg laying was observed (Tables 3.5 and 3.6), suggest that egg laying activities are associated with drier sections of the moisture gradient (Fig. 3.2). Locations where egg laying was observed had higher densities of nearby *R. hirta* inflorescences than locations where nectar feeding was observed. We observed adults nectar feeding at several species (presumably feeding; please see above) and we did not count the number of inflorescences of other nectar sources which they visited. We interpret these data that *O. poweshiek* feed on a variety of nectar sources, thus might not necessarily feed where the density of nearby *R. hirta* inflorescences is the highest in the patch. Locations where egg laying was observed were spatially situated at moderate elevations where the mesic section of the soil gradient occurred (Fig. 3.4). Although most egg laying locations were in transitional elevation sections, they ranged from higher to lower portions of this intermediate section of relative elevation. To summarise, egg laying activities appear to be associated with wetter sections of the soil moisture gradient (as shown using soil moisture probes), mesic sections (as shown with species which are indicative of mesic sections, the abundance of shoots and species of plants which larvae consume, and geospatial locations in the research site), and drier sections (as shown with graminoid percent cover as well as height and the density of nearby inflorescences of *R. hirta*) simultaneously. Realistically, we think that *O. poweshiek* likely lay eggs at locations wherever these necessary characteristics occur. Also, that these locations most likely occur most often within the mesic sections of the soil moisture gradient.

Shrub cover may further distinguish resting/ basking and nectar feeding locations from egg laying and attempted oviposition locations. Resting and basking locations contained albeit small shrub cover compared to almost no shrubs in actual and potential microhabitats for immature Poweshiek skipperlings (Table 3.6). The maximum percent cover estimated at locations where resting/ basking (25.0 %) and nectar feeding (28.5 %) were observed were substantially higher than at locations where egg laying (1.1 %) and attempted oviposition (2.0 %) were observed. *Rosa* spp. shrubs were an indicator species of resting/ basking locations (Table 3.3), and *Salix* spp. shrubs appeared to be more highly associated with the nectar feeding and resting/ basking ellipses in Fig. 3.6 compared to those of attempted oviposition and egg laying. Although the shrub (all species) percent cover did not seem relevant in the analysis of transect data using the NMDS ordination (Fig. 3.2), it appears that shrubs are most often present at locations where adults rested/basked and consumed nectar but are rare at locations where females attempted to oviposit and where females laid eggs. Although locations where resting/ basking was observed had relatively high abundances of some host food species and relatively low abundances of other host species like locations where egg laying was observed, we have no reason to think these species consumed by larvae would benefit adults. Adults rested/basked on several plants representing graminoids, forbs and a shrub thus do not appear to have displayed a preference for graminoids or individual species of graminoids. The lowest relative graminoid height of resting/ basking locations and high graminoid percent cover suggest that resting/ basking locations are associated with in drier sections. The moderate forb percent cover suggests that these locations are associated with intermediate sections (Table 3.6) and the low number of nearby *R. hirta* inflorescences (Table 3.6) suggests wetter sections along the soil moisture gradient (Fig. 3.2). At locations where adults lay eggs, a lack of shrubs and lower graminoid height may create microhabitat-structures which facilitate the larval movement that was observed in my thesis (Chapter 2) and/or specific microclimates that facilitate larval development (Ashton et al. 2009).

Locations where resting and/or basking were observed appeared to be concentrated in an area of higher elevation (a drier area in general as suggested by our research) between two unused plant communities (shrub wetland and tree colony; Figs. 3.3 and 3.4). Perhaps, Poweshiek skipperlings were funnelled into this general area by their avoidance of these unsuitable plant communities. As a result of this funnelling effect, perhaps locations where resting and/or basking were observed were used by Poweshiek skipperlings as a better vantage point to see potential mates. This association would then be similar to *C. palaemon* selecting habitats with specific physical characteristics that facilitate encounters with mates (Ravenscroft 1994). We did not see mating or courtship during this study. During previous research, J. Henault observed mating (2015 and 2017; two observations in total) but these events occurred at locations that visually appeared mesic (Thesis, Chapter 2) instead of at locations in drier areas where resting and/or basking interactions occurred. Potentially, *O. poweshiek* locate mates by using hilltopping behaviour (perching near the highest elevations to locate females in flight) as described for several grass skippers including *Hesperia dacotae* (Skinner, 1911) (Lepidoptera: Hesperiidae) (McCabe 1981). Direct observations of mating activities during several instances are required, accompanied by the required attributes identified in our research, to determine the relative section along a soil moisture gradient where *O. poweshiek* in Manitoba mate. Additionally, perhaps the shorter graminoid height where resting and/or basking are observed also facilitate heating by basking skippers compared to other locations in this prairie research site.

Nectar feeding appeared to be associated with a relatively low soil moisture as well as a relatively higher cover of forbs and shrubs compared to egg laying and attempted oviposition activities (Table 3.6). Locations with a relatively higher cover of forbs will likely contain a species that is attractive to Poweshiek skipperlings. The moderate density of nearby *R. hirta* inflorescences where nectar feeding was observed (Table 3.6) suggests that *O. poweshiek* consume nectar at mesic locations along the soil moisture gradient (Fig. 3.2). Locations where nectar feeding was observed had the lowest percent cover of graminoids and an intermediate height of graminoids, which were also associated with the wetter to mesic sections of the moisture gradient. The geophysical locations where nectar feeding was observed seemed to occur most often at relatively higher elevations (lower soil moistures) but also occasionally took place at lower elevations (higher soil moistures) in the prairie patch, shown in Fig. 3.4. The relative abundances of all host food plants counted at locations where nectar feeding was observed varied similar to egg laying locations (Table 3.5). However, we have not found evidence that the graminoid species consumed by larvae benefit the nectar feeding activities of adults. In summary, nectar feeding appears to occur most often in drier section of the moisture gradient but occasionally occur in the mesic to wetter sections. Perhaps, adults take advantage of diverse nectar sources while they fly in prairie communities.

We explored the relative elevations where each nectar species was used to evaluate whether adults used specific sources at sections along the moisture gradient. *Rudbeckia hirta* (the most frequently used nectar species in this study), *G. lepidota* and *P. fruticosa* were nectar sources at relatively higher elevations (drier sections of the soil moisture gradient); *R. hirta*, *Packera* spp. and *Apocynum* spp. at intermediate elevations (mesic soils) and only *Packera* spp. at lower elevations (wetter soils). Furthermore, relatively higher and intermediate elevations (dry and mesic sections along the soil moisture gradient) contained the highest numbers of nectar plant species. It is difficult to determine whether adults fly to locations with certain soil moistures to feed on specific plants (*e.g.*, fly to drier sections of the soil moisture gradient) or opportunistically use nectar sources depending on where they are along the soil moisture gradient while flying within their environment. Henault (2017) found high abundances of *R. hirta* in sites supporting relatively higher abundances of adult *O. poweshiek*. We found that adult Poweshiek skipperlings feed on several floral species. However, apparently not relying exclusively on *R. hirta*. Since *Rudbeckia hirta* was used most often, adults may specialise on this species and consume nectar from other floral species depending on the degree of synchrony between when adults emerge and plant inflorescences are mature.

Resting, basking and nectar feeding locations appeared to be more highly associated with *Salix* spp. (Fig. 3.6); a group of species which we used to describe the shrub wetland plant community in the local prairie patch (Table 3.2, Figs. 3.3 and 3.4). Shrub wetland plant communities border the northwestern side of the prairie plant community in Figs. 3.3 and 3.4, separated by a ridge after which the elevation drops to the north. *Salix* spp. may sporadically disperse from shrub wetland communities into the prairie type of community where nectar feeding occurs, thus such dispersal may partially explain the apparent association of resting/ basking and nectar feeding with *Salix* spp..

The percent cover of bare soil and percent cover of duff at locations where the various activities were observed was approximately equal and consistently low. However, the relative percent cover of graminoids, forbs and shrubs did vary amongst the types of activities (Table 3.6, Fig. 3.2). Perhaps, adults may be active in areas with a minimum percent cover of soil and duff within prairie plant communities, and choose between locations with specific combinations of graminoid, forb and shrub percent cover to lay eggs, visit flowers to consume nectar and conduct other behaviours. Ovipositing *O. poweshiek* once initially located a generally suitable microhabitat location, appeared to deposit eggs at locations with relatively greater cover of duff and lower cover of shrubs compared to immediately adjacent locations (Thesis, Chapter 2). Further observations of adult egg laying and descriptions of the locations at which these behaviours occurred would be helpful to understand this relationship.

Locations where eggs were laid had the lowest relative duff depth amongst the various activities (Table 3.6). The duff depth in Fig. 3.2 appeared to be positively associated with the mesic to dry sections of the soil moisture gradient; approximately the section which we think may facilitate egg laying most often. Since the variable's response to transect position was weak, we do not think that relying solely on this ordination analysis makes sense. Based on Table 3.6, attempted oviposition locations had the highest duff depth. In Henault (2021, Chapter 2), locations where eggs were laid had a lower duff depth compared to locations where adults flew over but did not lay an egg. We acknowledge that these apparent preferences may be influenced by the biases of individuals in each study. Henault (2021, Chapter 2) suggested this shallower duff layer may influence microclimates in a larva's local environment. If the duff layer does contribute to certain microclimates which facilitate larval development, then the stimuli associated with the duff depth may be more influential to a female during a decision to lay eggs compared to during a decision to rest, bask or consume nectar (by females or males) (oviposition threshold as in Singer 1971). Measurements of the temperature, humidity and other relevant microclimatic factors immediately surrounding adults while conducting various activities and immatures during various stages of development or specific behaviours (*e.g.*, feeding, diapausing, pupating) would likely help to address this concept. Also, research into how microhabitat factors, including duff depth, stimulate females and all adults during various interactions with the habitat.

Adults were not active in the wettest sections of the plant community (based on our observations of adult flights and the absence of *B. syg.* /*Tri.* spp.), but flew most often in the prairie plant community. Thus, the locations where we established transects do appear to have encompassed the type of plant community and soil moisture which facilitate adult interactions with tall grass prairie patches.

In summary, we think that attempted oviposition by *O. poweshiek* may be associated with the wetter section of the soil moisture gradient in prairie plant communities, egg laying may be associated with the mesic section, resting and/or basking with drier sections and nectar feeding with mostly the dry section but occasionally with mesic and wetter sections (Fig. 3.7).

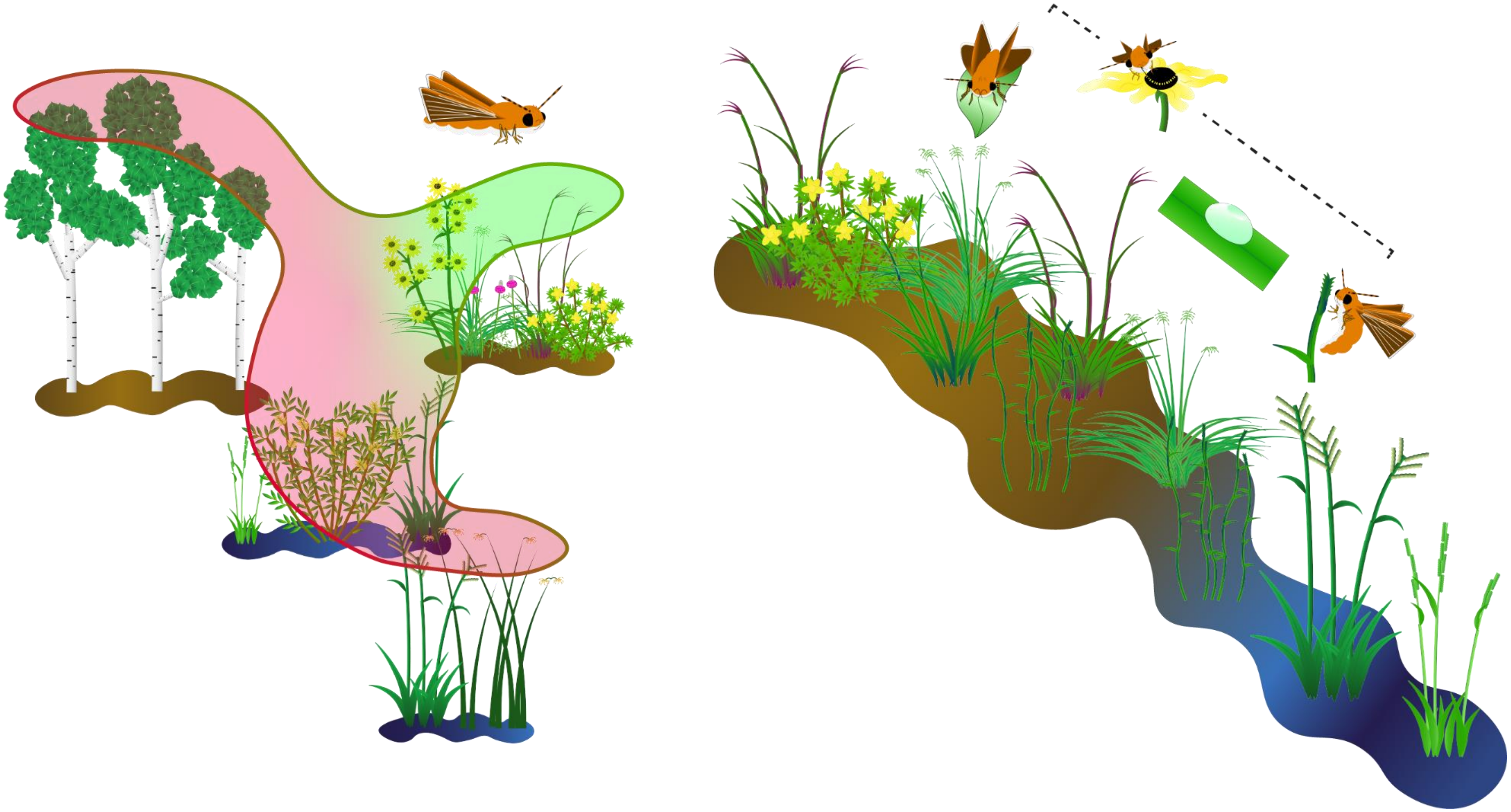


Fig. 3.7. Plant communities most often used by adults (left) and approximate relative locations of adult activities along the soil moisture gradient (right). On the left, we overlay a coloured polygon corresponding to plant communities in which adults flew (green) and where they flew occasionally or not at all (red). On the right, we display species typical of positions along the soil moisture gradient (brown soil = dry, blue soil = wet).

We report soil nutrients and texture along moisture gradients in our study to contribute to current literature describing edaphic components of tall grass prairie habitats (Supplementary Table 3.2). We hope these soil data can be useful to future research of species-at-risk and soil components in tall grass prairie habitats locally and elsewhere in North America!

Poweshiek skipperlings conducted activities at locations along a soil moisture gradient, similar to observations reported by Ravenscroft (1994). Ravenscroft (1994) reported that *C. palaemon* laid eggs and located mates in relatively mesic areas and nectared in the wettest areas. Coincidentally *C. palaemon* laid eggs in mesic areas, but possibly to avoid male harassment (Ravenscroft 1994) instead of to obtain a clear fitness benefit to larvae as we postulate in this study as well as in Henault (2021, Chapter 2). We think it is unlikely that female *O. poweshiek* in the study site are avoiding males while locating microhabitats in which to lay eggs. Since the density of their species is so low, adults likely are not constrained to specific plant communities or sections along the soil moisture gradient. When abundances within local populations were more dense with dozens of individuals in each prairie site (abundance estimations in 1980's by P. Klassen and R. Westwood unpublished; Westwood et al. 2012; COSEWIC 2014; Grantham et al. 2020), the effects of male harassment may have prompted females to fly to mesic sections of the moisture gradient to lay eggs. However, further research including additional observations are required to determine the likelihood that this hypothesis is accurate.

Henault (2017) hypothesized that adults may conduct various activity types in areas within close proximity. Our 2017 and 2018 research of adults interacting with various sections along the soil moisture gradient provides evidence that supports this hypothesis. Additionally, the indicator species in Henault (2017) may be usable to identify sites (*e.g.*, one quarter section of land) containing the entire spectrum of soil moisture required by adult *O. poweshiek*. In Henault (2017), the presence of *H. hirsuta* was indicative of prairie sites that support relatively high abundances of *O. poweshiek* and in our 2017 and 2018 research, the presence of *H. hirsuta* was indicative of the driest section of the soil moisture gradient within the prairie research site (Table 3.3). The presence of *D. cespitosa* was indicative of relatively medium abundances of adults in Henault (2017) and the presence of Juncaceae spp. was indicative of relatively low abundances. Although these species were not significant during the ISA, the number of *D. cespitosa* shoots peaked in mesic sections along the soil moisture gradient and the number of

Juncaceae spp. shoots were the highest in the wettest sections along the moisture gradient. We hypothesise that the presence of *H. hirsuta* in a site, may indicate that the dry section of the moisture gradient is present. Potentially *D. cespitosa* and Juncaceae spp. could be used similarly to *H. hirsuta*; the presence of *D. cespitosa* may be used to identify that the mesic section of the moisture gradient is present at a prairie site and the presence of Juncaceae spp. may be used to identify that the wetter section in present.

Collectively the presence of *H. hirsuta*, *D. cespitosa* and Juncaceae spp. may be used to indicate the sections along the soil moisture gradient which are present at a site, and subsequently the various adult activities which may be facilitated. For example, imagine that the vegetation at a research site has been sampled and *D. cespitosa* and Juncaceae spp. were observed but *H. hirsuta* is not. The presence of *D. cespitosa* may indicate that the mesic section of the soil moisture gradient is present but the absence of *H. hirsuta* may indicate that the dry section is absent. Furthermore, that egg laying activities would likely be facilitated at this site by mesic sections of the soil moisture gradient (as suggested by our observations of egg laying in mesic sections of the moisture gradient) but that resting and basking activities would likely not be facilitated at this site (as suggested by our observations of resting/ basking in drier sections of the moisture gradient). If Poweshiek skipperlings were present at this site and adults could not rest and/or bask due to an absence of facilitative drier sections of the moisture gradient, would they have a lower fitness? We assume that in reality adults would find lower quality locations which facilitate resting and/or basking, but perhaps this reduction in microhabitat quality would result in a smaller population of Poweshiek skipperlings. In Henault (2017), *H. hirsuta, D. cespitosa* and Juncaceae spp. were present in sites with a relatively high abundance of adults, *D. cespitosa* and Juncaceae spp. in sites with a relatively medium abundance of adults, and only Juncaceae spp. (of the three species) was present in sites with a relatively low abundance of adults. Potentially, the sites where the dry, mesic and wet sections of moisture gradient were present (as indicated by the presence of *H. hirsuta, D. cespitosa* and Juncaceae spp.) may have supported all adult activities which we studied. Whereas, the sites where only the mesic and wet sections were present (as indicated by the presence of *D. cespitosa* and Juncaceae spp.) may have been capable of supporting relatively fewer adult activities and thus lead to a relatively lower abundance of adults. Thus, the presence/absence of sections along the moisture gradient in a site may constrain the activities which adults may conduct and thereby the abundance of *O.*

poweshiek. We are eager for further research to confirm the consistency of adult activities along specific sections of the soil moisture gradient, and potential mechanisms by which the availability of high quality locations which facilitate adult activities may influence the fitness of individual Poweshiek skipperlings and subsequently impact the size of the local population.

Remnant populations of *O. poweshiek* have declined over the last few decades. If adults are actually constrained by the sections of the soil moisture gradient which are present/absent, then perhaps the integrity of the soil moisture gradient in remnant Poweshiek skipperling colonies has changed during this time span. Remnant colonies with the MTGPP are situated within a generally agricultural area. Potentially, sites supporting remnant colonies as well as the entire soil moisture spectrum have been converted for agricultural activities. The ditches along roads in the area are cleared and at least some proportion of groundwater is pumped. Perhaps these activities have directly or indirectly changed the soil moisture gradients which occur in sites with remnant colonies of *O. poweshiek*. The consistency and volume of precipitation that falls in the area may have increased/decreased due to climate change. As a result, individual tall grass prairie sites might have lost or gained specific sections of the soil moisture gradient (*e.g.*, a site containing the entire spectrum may have lost the wettest sections of the moisture gradient; another site with only the dry soil section may have gained the mesic soil moisture section). Due to these hydrologic changes, perhaps the fitness of individuals has decreased and resulted in a decline in the abundance of adults in populations. Since these concepts are hypotheses (J. Henault), we need to determine if climate change has reduced/increased precipitation in prairies leading to the loss/gain of specific sections of the soil moisture gradient and if these changes have caused the decline of *O. poweshiek*. Justis Henault will be pursuing this research concept further. For example, a researcher could first replicate our observational experiment to determine if our observations of *O. poweshiek* conducting various activities along a moisture gradient would be confirmed/revised. Then if Poweshiek skipperlings do interact with the habitat similarly, researchers could experimentally manipulate the soil moisture in small areas within a prairie patch which contain the entire moisture gradient to determine whether the addition/subtraction of sections along the soil moisture gradient influences how/ if *O. poweshiek* uses them. The changes in the soil moisture may take years to be reflected in the plant distributions in these small experimental areas. However if the prairie plant communities and Poweshiek skipperlings do respond at any point to these changes in the soil moisture gradient, then researchers might be able to discover techniques to apply to entire prairie patches to help this species recover. These strategies might be used to remediate habitat where sections of the moisture gradient have been added/ removed, improve existing habitat to respond to potential changes in the climate and create new habitats which facilitate the various adult interactions and immature developmental requirements of *O. poweshiek*.

I predicted that steeper prairie slopes with corresponding soil moisture gradients that have a narrow spatial distribution may provide growth conditions for various species of host food plants to live in close proximity, making microhabitats easier for larvae to navigate to find host food plants (Henault 2017). For example, a total gradient encompassing 100 m may have a less abrupt change in species than approximately 20 m (as in this study). The gradual transition in species along the shallower slope would mean that host plant species associated with various growing conditions (dry, mesic and wet sections of the moisture gradient) would not occur as close in proximity. In my 2017-2019 research (Thesis, Chapter 2), *O. poweshiek* larvae moved amongst shoots in microhabitats and consumed several host plant species. These species were associated the most with drier, mesic and wetter sections of the soil moisture gradient in this current study. Potentially, at locations along a gradual transition (*e.g.*, 100 m) the increased distance which larvae would need to travel to reach shoots of various host plant species growing further away from one another would reduce the fitness of these larvae compared to larvae at locations along an abrupt transition (20 m; in this study). The resulting structure of living and dead vegetation at locations along a more abrupt slope may potentially be more conducive to larval movements (*e.g.*, possibly with a lower degree of irregularity) than a structure formed along a gradual transition. We did not compare species turnover and vegetation structure changes between relatively steep and shallow slopes. However, we think that larvae may not have been able to access all species if the slope was shallower. Further research into how plant species and microhabitat structures change between prairies having different degrees of slopes is required.

It appears likely the elevational differences which result in prairie and wetland plant communities occurring adjacent to each other provide the wide range of soil moistures required by adults, and later in the season the immatures. A prairie patch on a shallow slope would contain all of these facilitative sections of the moisture gradient but may be too far away from one another for adults to use efficiently. Adults which fly greater distances to reach oviposition, resting/ basking and nectar feeding locations would use more energy or increase their risk of predation and conceivably have a lower fitness compared to adults which fly shorter distances. At one of the prairie patches where we researched, adults could fly upslope to rest and locate nearby nectar sources, then navigate to mesic areas to lay eggs, all within a habitat transition of approximately 20 m in length. Observations of adults at sites with slopes of various inclines followed by comparisons of their relative fitness would be required to address this concept. Although all of the remaining prairie remnants ought to be stewarded, our study suggests that perhaps the prairies with steeper slopes should be prioritised as critical habitat for Poweshiek skipperling.

Egg microhabitats

Egg laying appears to occur most frequently at locations in the intermediate section of the soil moisture gradient, which seem to facilitate the development of immatures (Thesis, Chapter 2). Adults are likely stimulated by vegetative, physical and microclimatic criteria to lay an egg (potentially conspecific cues as well?). Females likely lay eggs wherever the necessary vegetative and physical components of a microhabitat occur, whether these locations are on a physical slope or within a generally level area of prairie. We observed some eggs being laid in high elevation areas in the focal prairie (displayed in Figs. 3.3 and 3.4) and in the two nearby patches. The entire research site contains numerous shallow depressions approximately one metre in diameter, and the bottom or along the sides of these depressions could potentially support mesic vegetative communities. These mini moisture gradients may provide the host plant species and microclimate characteristics for immature stages reported to be required in Chapter 2 (Thesis). Additionally, along a physical slope with a corresponding soil moisture gradient we assume that differential soil conditions and stochastic distributions of vegetation may create pockets which contain remarkable conditions for immatures to develop and stand out amongst other locations in the local area. We assume a similar logic may explain locations which facilitate the other various adult activities. It appears that microhabitats in which eggs are laid are complex and that the required conditions may be more precise than those suitable for the other adult activities. We think that having strict oviposition criteria makes sense because areas in which eggs are laid will be occupied for 11 months by developing immatures instead of occupied for several minutes by nectar feeding adults. We suggest that anyone searching for an immature microhabitat for research, should start their search in mesic areas along a slope however potentially explore depressions within relatively higher elevations of prairies.

Soil moisture gradient

We found that as soil moisture increased from the relatively higher elevations to the lowest elevations along physical slopes, the plant species which were supported also changed. These findings are consistent with research about soils reported by Liu et al. (2020) and about plant species reported by Zalatnai and Körmöczi (2004) when considered simultaneously. Our observations report the soil moisture attributes of a typical tall grass prairie patch in Manitoba, building on previously reported knowledge about tall grass prairie plant communities in the region by Catling and Lafontaine (1986). In addition to providing conditions for life requirements of the Poweshiek skipperling, this soil moisture configuration likely supports the survival of several taxa such as insects, plants and mammals in tall grass prairie ecosystems. We encourage research that explores how other taxa interact with varying soil moistures at relatively different elevations in tall grass prairie ecosystems in Manitoba.

Conclusion

In summary, adult activities appear to be distributed along a soil moisture gradient in Manitoba's tall grass prairies. Attempted oviposition appeared to be associated with the wetter section of the soil moisture gradient, egg laying with the mesic section of the gradient, resting and/or basking with the drier section and nectar feeding appeared to be associated most often with the drier section but occasionally was associated with mesic and wetter sections. Even though our sample size was small, we think the observations we did record are enough to make a reasonable suggestion of how adults interact with their tall grass prairie habitat. Given the scarcity of the Poweshiek skipperling and our extensive observation efforts we are proud of our research. We encourage further studies that approximately repeat our research to determine if they do/do not replicate our findings. This research contributes to our knowledge about habitat utilisation by the Poweshiek skipperling. Although likely to be most helpful for butterflies in Manitoba, the generated knowledge will hopefully be somewhat transferable to be used during *O. poweshiek* recovery efforts in the United States of America.

We described critical habitat for this population in remnant tall grass prairie patches in Manitoba and how adult skipperlings likely use various sections with different soil moistures in these prairies to lay eggs, rest, bask, consume nectar and fly. Since the arrangement of critical habitat is not identical in every patch of prairie, this research can be used to guide researchers in their search for habitats which facilitate various interactions of *O. poweshiek.* We display a suggestion of how an individual *O. poweshiek* might interact with the prairie plant community in a prairie patch (Fig. 3.8). We suggest that a researcher first locates – using a combination of field sampling and GIS analysis techniques – the boundaries of various plant communities (such as the prairie and wetland communities) and then the sections within the prairie plant community which are most likely to facilitate egg laying (mesic sections of the soil moisture gradient), resting and/or basking (drier sections) and nectar feeding (drier through occasionally wetter sections) activities. An adult flying in a prairie plant community may consume nectar at a flower in a drier section of the soil moisture gradient, lay an egg in mesic section, rest and/or bask in a drier section before continuing to interact with this habitat. The sequence of activities which we display are just an example; observations of adults behaving in the field are required to determined if adults do interact in a sequence.

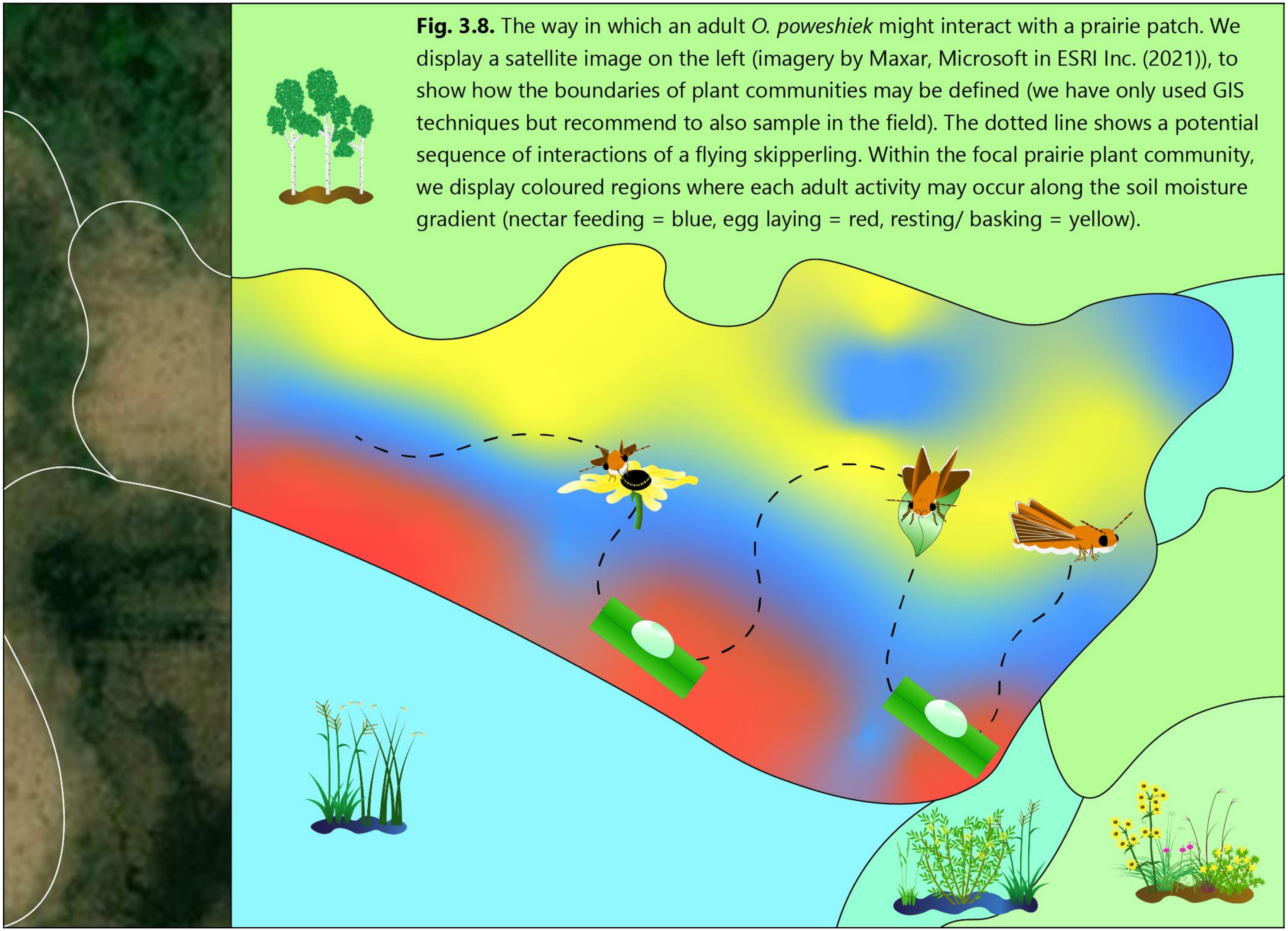


Fig. 3.8. The way in which an adult *O. poweshiek* might interact with a prairie patch. We display a satellite image on the left (imagery by Maxar, Microsoft in ESRI Inc. (2021)), to show how the boundaries of plant communities may be defined (we have only used GIS techniques but recommend to also sample in the field). The dotted line shows a potential sequence of interactions of a flying skipperling. Within the focal prairie plant community, we display coloured regions where each adult activity may occur along the soil moisture gradient (nectar feeding = blue, egg laying = red, resting/ basking = yellow).

The complexity of Poweshiek skipperling behaviour and habitat requirements that we have reported indicates that detailed research must be conducted to identifying suitable prairie areas which may support *O. poweshiek* populations. Furthermore, that investigations are required to locate the appropriate sections within individual prairie patches in which to apply conservation disturbances. Tall grass prairie stewards will likely need to sample candidate prairies, to determine the how *O. poweshiek* will likely use it to conduct various activities. For example, imagine we are at a prairie patch supporting a vibrant *O. poweshiek* population and we want to manage the patch to improve or maintain the quality of the habitat contained within it (Fig. 3.9). We suggest to first using plant sampling and GIS techniques to locate plant communities within a prairie patch as well as potential sections along the moisture gradient where each adult activity may occur. Next, we recommend to manage half of the site by using a conservation disturbance such as haying. Even if the conservation disturbance happens to decrease the suitability of the hayed area temporarily, *O. poweshiek* from the rest of the patch may be able to recolonize this managed area during the next year (assuming that haying is conducted in late summer or fall when larvae are closer to the ground; Thesis, Chapter 2). Conservation disturbances themselves may reduce Poweshiek skipperling populations initially if the technique risks destroying individuals (fire in Swengel and Swengel (1999; 2015)). However, disturbances that are compatible with the life history of butterflies (fire during some scenarios, haying during most cases) may support survival by providing higher quality vegetative environments (haying - *O. poweshiek* in Swengel (1996), fire - *Callophyrus irus* (Godart, [1824]) (Lepidoptera: Lycaenidae) in Thom and Daniels (2017)). Prairie sites in Manitoba which experienced a fire at intervals of approximately every three to ten years were shown to support higher abundances of *O. poweshiek* (Dupont-Morozoff et al. submitted). We are unaware of techniques that effect prairies at a small enough scale to focus on creating or maintaining microhabitats which facilitate specific activities (*e.g.,* nectar feeding). We assume that a prairie patch which contains a variety of small areas each with different combinations of vegetative and physical characteristics would likely contain at least some microhabitats which may facilitate the various adult activities (as well as larval behaviours and development). Thus, we think that stewardship strategies which use a variety of disturbance techniques (applying haying or burning techniques) as well as at different spatial scales and configurations (applying a disturbance technique to different percentages of the total prairie patch) will potentially help to generate such a prairie habitat which is suitable for Poweshiek skipperlings. We think it is important to also evaluate how conservation disturbances might simultaneously affect the desired plant growth and physical requirements of other species-at-risk. By increasing the diversity of vegetative and physical characteristics, we think that stewards might increase the diversity of species which these habitats may support. Simultaneously, these actions might reduce the number of individuals of some endemic species by decreasing the abundance of microhabitats which are specifically suitable to these species. Research which focuses on the creation and maintenance of habitats at the microhabitat-scale which facilitate the various activities of *O. poweshiek* – as well as the development of other prairie wildlife – will likely support the development of even more compatible stewardship strategies than are currently used. Stewards may use our research during habitat regenerations to ensure that sections along the moisture gradient which facilitate all adult activities are disturbed, while potentially providing a sanctuary for adults and developing larvae that may recolonise the habitats post-disturbance.

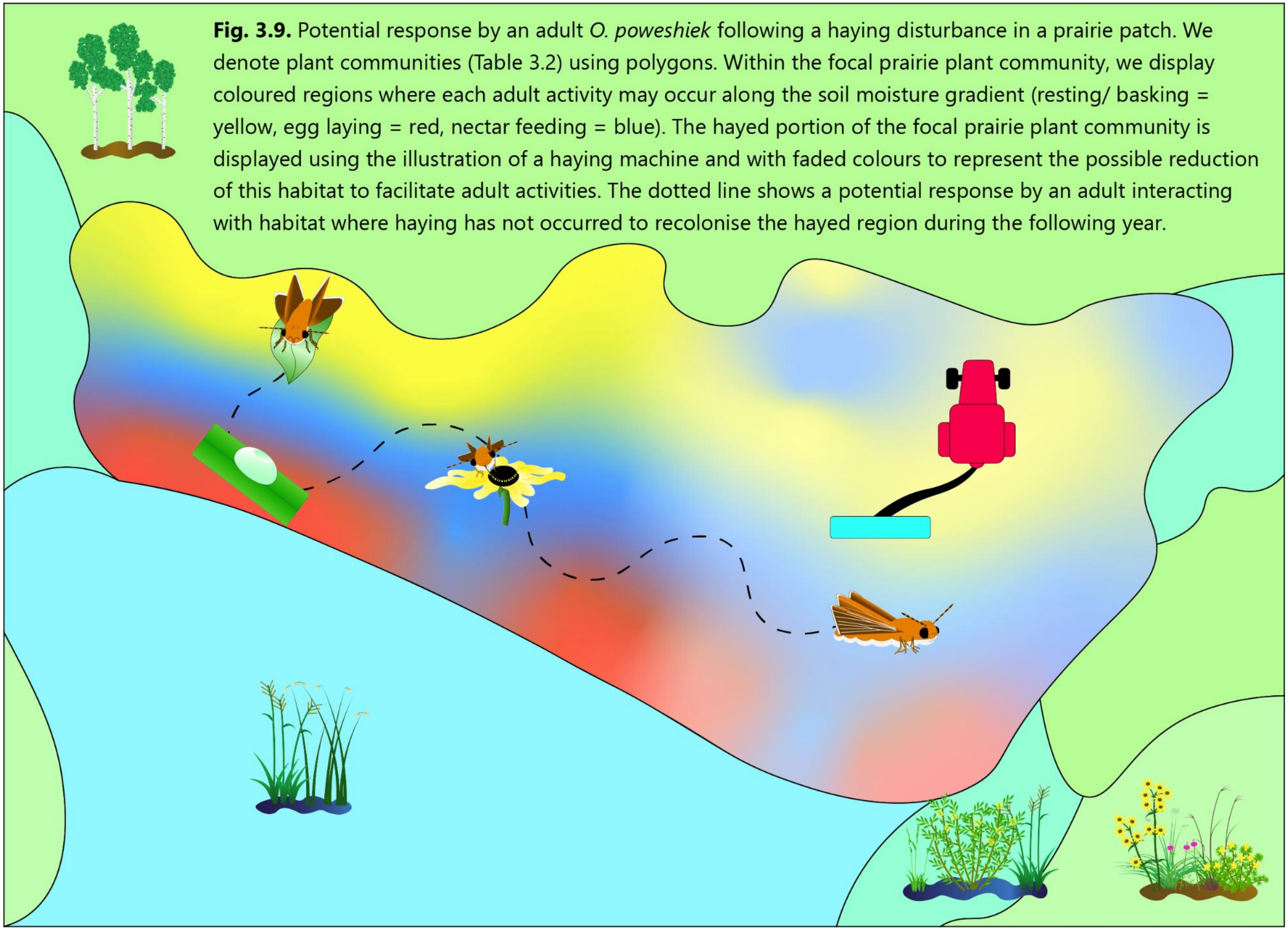


Fig. 3.9. Potential response by an adult *O. poweshiek* following a haying disturbance in a prairie patch. We denote plant communities (Table 3.2) using polygons. Within the focal prairie plant community, we display coloured regions where each adult activity may occur along the soil moisture gradient (resting/ basking = yellow, egg laying = red, nectar feeding = blue). The hayed portion of the focal prairie plant community is displayed using the illustration of a haying machine and with faded colours to represent the possible reduction of this habitat to facilitate adult activities. The dotted line shows a potential response by an adult interacting with habitat where haying has not occurred to recolonise the hayed region during the following year.

Using the same approach, organisations involved in butterfly reintroductions can evaluate the capacity of potential prairies to support new *O. poweshiek* populations in Manitoba. Researchers would have first evaluated satellite images displaying vegetation and identified a potential prairie site in which to release skipperlings (Fig. 3.10). We suggest to use plant sampling and GIS techniques to locate plant communities within a prairie patch as well as potential sections along the moisture gradient where each adult activity may occur as described earlier. Next, evaluate whether all components regarding plant communities and moisture sections are present and are arranged in a way that may be conducive to facilitate adult activities. Although we did not research the proximity in which sections along the moisture gradient must occur to facilitate adult activities (please see complementary discussions in Chapter 2 of this thesis), potentially the overall size of the prairie patch and the topographical characteristics (degree of incline along physical slopes between prairie and wetland plant communities, and degree of irregularity in generally level areas which may indicate the frequency of small depressions that may provide microhabitats) ought to be considered. The prairie patch in Fig. 3.10 appears to contain sections along the moisture gradient which will likely facilitate egg laying, resting and/or basking and nectar feeding activities. Therefore following sampling in the field, we think this patch could support *O. poweshiek* if skipperlings were to be released. This strategy to evaluate the suitability of sites to support Poweshiek skipperling, might create a better chance that reintroduced butterflies will survive in these locations for several decades!

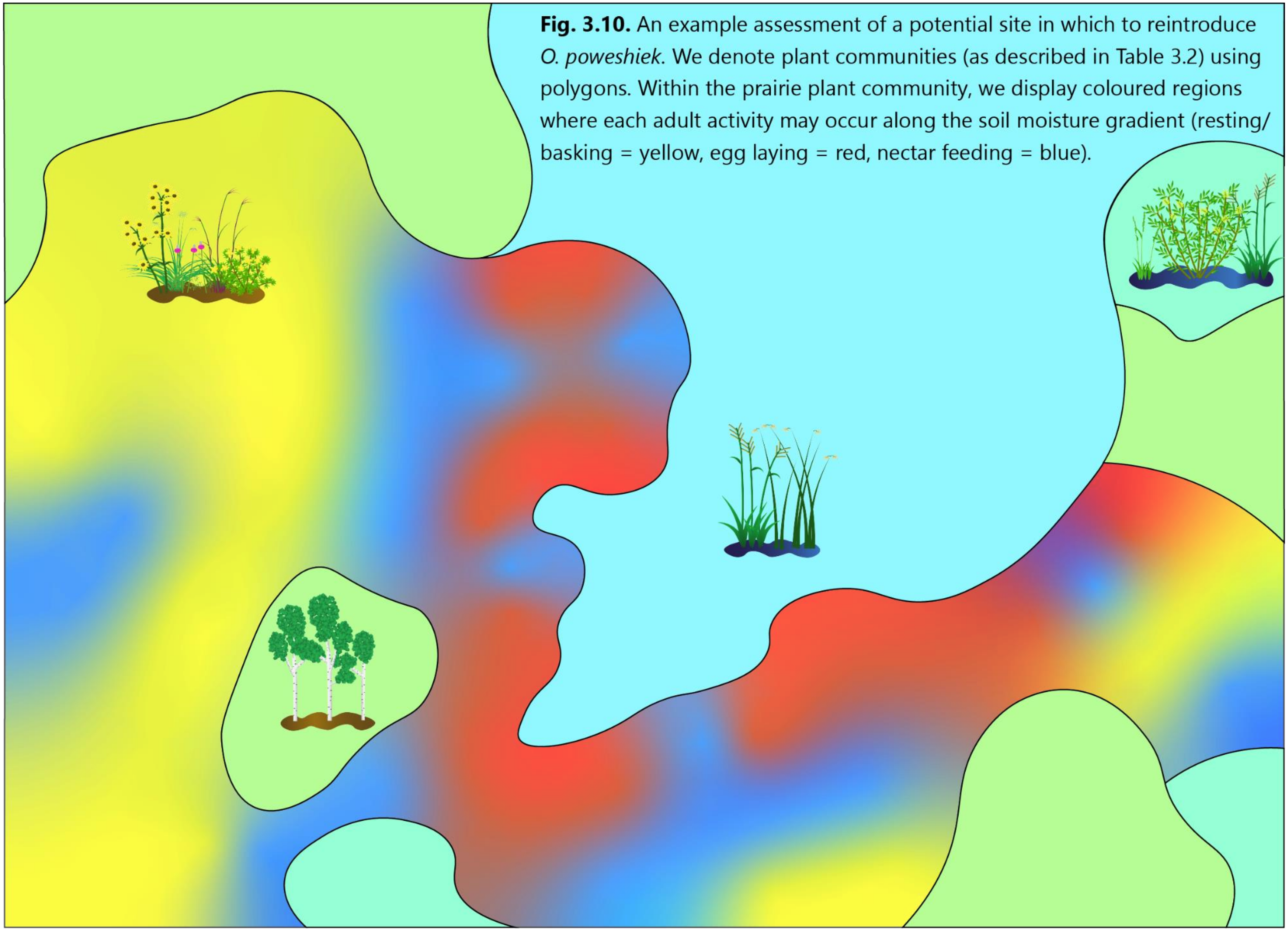


Fig. 3.10. An example assessment of a potential site in which to reintroduce *O. poweshiek*. We denote plant communities (as described in Table 3.2) using polygons. Within the prairie plant community, we display coloured regions where each adult activity may occur along the soil moisture gradient (resting/basking = yellow, egg laying = red, nectar feeding = blue).

In addition to guiding the management of prairie habitats and informing the reintroduction of skipperlings, our research may contribute to make the knowledge used in recovery strategies even more comprehensive (for example, strategies produced by Environment and Climate Change Canada). The plant communities used by adults and the sections along the soil moisture gradient which facilitate various activities in the prairie plant community may be incorporated into descriptions of critical habitat required for Poweshiek skipperling to survive.

We hope that our research will help to fuel the efforts of the International Poweshiek skipperling Partnership and inspire other researchers to strive to recover endangered species.

Contributions. J. H. and R. W. conceived the idea of the study as part of J. H.'s M. Sc. thesis, J. H. lead fieldwork, data analysis and drafting of the manuscript, J. H. and R. W. both contributed to the drafts and final version of the manuscript.

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Supplementary Table 3.1. Enumerated species during this research. The background of taxa used as substrates during various adult activities or larval foraging are coloured to match the types of activities (egg laying/ larval foraging = red, nectar feeding = blue, resting/ basking = yellow; *G. lepidota* also used during nectar feeding activities and *Packera* spp. also used during resting/ basking). We use the most recent scientific names and authorities amongst Looman and Best (1987), Leighton and Harms (2014) and Tropicos.org (Missouri Botanical Garden 2021). Common names follow Tropicos.org (Missouri Botanical Garden 2021) where possible; for species not listed, we used names from the older references (Looman and Best 1987; Leighton and Harms 2014).

Scientific Name and Authority	Family	Common name
Achillea L. spp.[1]	Asteraceae	Yarrow
***Amorpha canescens* Pursh**	**Fabaceae**	**Lead-plant**
***Andropogon gerardi* Vitman**	**Poaceae**	**Big bluestem**
Antennaria Gaertn. spp.	Asteraceae	Everlasting genus
***Apocynum* L. spp.[2]**	**Apocynaceae**	**Dogbane**
Artemisia ludoviciana Nutt.	Asteraceae	Sage
Asclepias ovalifolia Decne.	Apocynaceae	Dwarf milkweed
Asteraceae Bercht. and J. Presl spp.[3]		Aster family
Beckmannia syzigachne (Steud.) Fernald	Poaceae	Slough grass
Bromus L. spp.	Poaceae	Brome grass
Campanula rotundifolia L.	Campanulaceae	Harebell
Cyperaceae Juss. spp.		Sedge family
Dalea purpurea Vent.	Fabaceae	Purple prairie-clover
Deschampsia cespitosa (L.) P. Beauv.	Poaceae	Tufted hairgrass
Eleocharis R. Br. spp.	Cyperaceae	Spike rush genus
Equisetum L. spp.[4]	Equisetaceae	Horse-tail sp. 1 and sp. 2
Fabaceae Lindl.[5]		Bean family
Fragaria virginiana Mill.	Rosaceae	Strawberry
Galium L. spp. /Asteraceae spp.[6]	Rubiaceae/Asteraceae	Bedstaw and Asters
Geum triflorum Pursh	Rosaceae	Three-flowered avens
***Glycyrrhiza lepidota* Pursh**	**Fabaceae**	**Wild licorice**
Helianthus maximiliani Schrad.	Asteraceae	Maximillian's sunflower
Hesperostipa spartea (Trin.) Barkworth	Poaceae	*H. spartea* grass
***Hypoxis hirsuta* (L.) Coville**	**Hypoxidaceae**	**Star-grass**
Juncaceae Juss. spp.		Rush family
Liatris ligulistylis (A. Nelson) K. Schum.	Asteraceae	Meadow blazingstar
Lithospermum canescens (Michx.) Lehm.	Boraginaceae	Hoary puccoon
Lysimachia quadriflora Sims	Primulaceae	*L. quadriflora* forb
***Muhlenbergia richardsonis* (Trin.) Rydb.**	**Poaceae**	**Mat muhly**
Muhlenbergia Schreb. spp.[7]	Poaceae	Muhly
***Packera* L. spp.[8]**	**Asteraceae**	**Groundsel genus**
Parnassia L. spp.	Celastraceae	Grass-of-Parnassus genus
Pedicularis canadensis L.	Orobanchaceae	Wood-betony
Poaceae Barnhart spp.		Grass family
Polygala senega L.	Polygalaceae	*P. senega* forb
Populus tremuloides Michx.	Salicaceae	Trembling aspen
***Potentilla fruticosa* L.**	**Rosaceae**	**Shrubby cinquefoil**
Rosa L. spp.	Rosaceae	Rose genus

Supplementary Table 3.1 continued.

Scientific Name and Authority	Family	Common name
***Rudbeckia hirta* L.**	**Asteraceae**	**Black-eyed susan**
Salix L. spp.	Salicaceae	Willow genus
***Schizachyrium scoparium* (Michx.) Nash**	**Poaceae**	**Little bluestem**
Scirpus L. spp.	Cyperaceae	Bulrush
Solidago ptarmicoides (Torr. and A. Gray) B. Boivin	Asteraceae	Upland white goldenrod
Solidago rigida L.	Asteraceae	Rigid goldenrod
Solidago L. spp.[9]	Asteraceae	Goldenrod genus
Sorghastrum nutans (L.) Nash	Poaceae	*S. nutans* grass
***Sporobolus heterolepis* (A. Gray) A. Gray**	**Poaceae**	**Prairie dropseed**
Sporobolus michauxianus (Hitchc.) P.M. Peterson and Saarela	Poaceae	Prairie cordgrass
Thalictrum dasycarpum Fisch. and Avé-Lall.	Ranunculaceae	Tall meadow-rue
Triantha glutinosa (Michx.) Baker	Tofieldiaceae	Sticky asphodel
Triglochin L.[10]	Juncaginaeceae	Arrow-grass
Typha L. spp.	Typhaceae	Cattail
Viola L. spp.	Violaceae	Violet
Zigadenus elegans Pursh	Melanthiaceae	White camas
Zizia aurea (L.) W.D.J. Koch	Apiaceae	*Z. aurea* forb
Zizia W.D.J. Koch spp.[11]	Apiaceae	*Zizia* spp.

1. Most likely *A. millefolium* L..
2. Either *A. cannabium* L., *A. androsaemifolium* L. or *A. medium* Greene.
3. Not including other species in the *Asteraceae* family which were enumerated.
4. *Equisetum* sp. 1 most likely *E. laevigatum* A. Braun and *Equisetum* sp. 2 most likely *E. pratense* Ehrh..
5. Most likely *Vicia* L. spp. and *Lathryus* L. spp..
6. *Galium* spp.. (including *G. boreale* L. and other species in the *Galium* genus) and more than one species in the Asteraceae family which all had narrow leaves similar in shape and arrangment to the *Galium* spp. we observed.
7. *Muhlenbergia* spp. other than *M. richardsonis* (Trin.) Rydb.. A mixture of most likely *M. glomerata* (Willd.) Trin. and *M. racemosa* (Michx.) Britton, Sterns and Poggenb..
8. Most likely *P. paupercula* (Michx.) Á. Löve and/or D. Löve or *P. aurea* (L.) Á. Löve and D. Löve.
9. *Solidago* L. spp. other than those already counted such as *S. ptarmicoides* and *S. rigida* .
10. Most likely *T. palustris* L. and *T. maritima* L.
11. Mixture of *Z. aurea* and *Z. aptera* (A. Gray) Fernald.

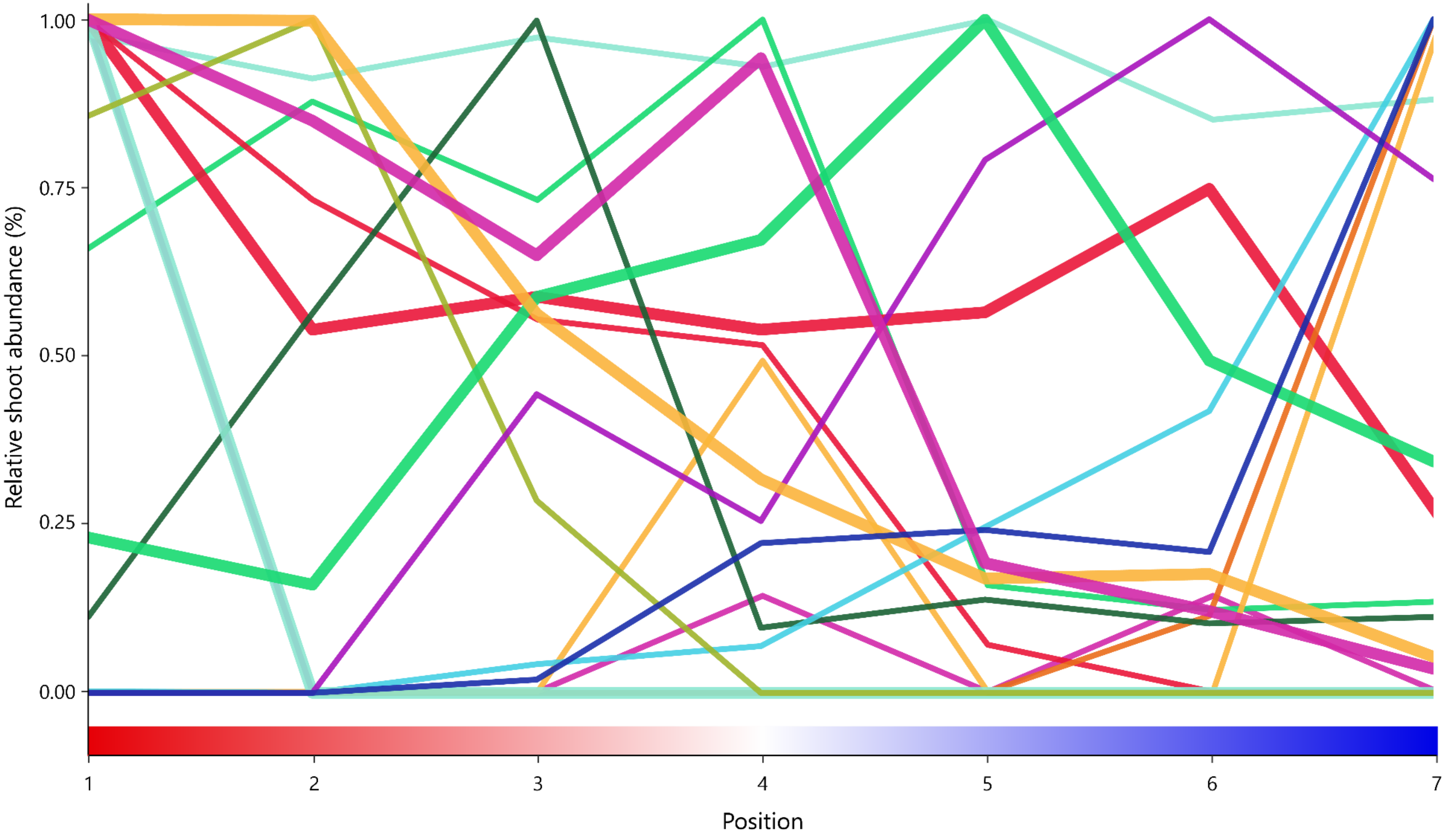
Supplementary Table 3.2. Soil nutrients and texture at positions along transects (mean (95% confidence interval)). Position 1 represents the beginning portion of the transects, positions 4 and/or 5 represent the middle portion and position 7 represents the end portion. The position number is followed by the corresponding number of samples in that portion of the transects. The respective number of samples for Sand (%), Silt (%) and Clay (%) are shown to the left of this group of variables. We report the analysed soil nutrients to the same degree of precision provided to us by Farmers Edge Laboratories.

Position	**NO_3-N (mg/kg)**	**PO_4-P (Olsen) (mg/kg)**	**SO_4-S (mg/kg)**	**Ca (mg/kg)**	**K (mg/kg)**	**Mg (mg/kg)**
1 (7)	4.0 (2.5 to 5.5)	10.2 (7.9 to 12.4)	7.2 (6.3 to 8.0)	5414 (4840 to 5989)	203 (59 to 346)	1120 (796 to 1444)
4/5 (7)	3.4 (2.7 to 4.1)	11.9 (9.1 to 14.6)	7.3 (5.7 to 8.9)	5386 (5073 to 5698)	96 (75 to 117)	777 (491 to 1063)
7 (7)	2.6 (1.2 to 4.1)	13.0 (9.3 to 16.6)	6.9 (5.5 to 8.2)	5257 (4538 to 5976)	98 (73 to 123)	970 (676 to 1264)

Position	**Na (mg/kg)**	**CEC (meq/100 g)**	**pH**	**OM (%)**	**BS Ca (%)**	**BS K (%)**
1 (7)	23 (11 to 34)	36.6 (32.1 to 41.1)	8.0 (7.9 to 8.2)	20 (16 to 24)	74.4 (68.9 to 80.0)	0.4 (0.1 to 0.7)
4/5 (7)	18 (15 to 21)	33.7 (30.8 to 36.6)	8.1 (8.0 to 8.1)	21 (19 to 24)	80.4 (74.8 to 86.1)	0.6 (0.4 to 0.8)
7 (7)	19 (13 to 24)	34.5 (29.4 to 39.6)	7.9 (7.7 to 8.2)	23 (16 to 29)	76.4 (72.0 to 80.8)	0.7 (0.6 to 0.9)

Position	**BS Mg (%)**	**BS Na (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**
1 (7)	24.0 (19.0 to 29.0)	0.3 (0.1 to 0.4)	(5) 44.6 (13.6 to 75.5)	22.2 (1.7 to 42.7)	33.3 (-2.5 to 69.0)
4/5 (7)	18.7 (13.0 to 24.4)	0.2 (0.2 to 0.3)	(6) 44.8 (13.9 to 75.6)	16.2 (7.1 to 25.3)	39.1 (8.4 to 69.7)
7 (7)	22.6 (18.2 to 27.0)	0.3 (0.1 to 0.4)	(5) 49.8 (5.5 to 94.2)	14.0 (1.8 to 26.3)	36.1 (4.0 to 68.3)

Supplementary Fig. 3.3. Relative abundance of graminoid shoots (%) along transects. Shoot counts were relativized by the maximum number for each species. Lines representing graminoid species which we observed larvae consuming are wider than those of species which we did not observe larvae eating. Transect positions are shown along the x-axis accompanied by their relative soil moisture on the y-axis.



Supplementary Table 3.4. Number of graminoid shoots counted (0.25 m^2) at positions along transects (mean (range)). The fonts of taxa which were used during egg laying activities and larval foraging behaviours are bolded. The number of samples for each position was seven.

Position	***A. gerardi***	*B. syz.* /*Tri.* spp.	*Bromus* spp.	Cyperaceae spp.	*D. cespitosa*	*Eleocharis* spp.	***H. hirsuta***	*H. spartea*
1	**105.1 (0 to 214)**	0.0 (0 to 0)	1.0 (0 to 6)	64.0 (5 to 158)	5.0 (0 to 17)	0.1 (0 to 1)	**0.6 (0 to 2)**	0.9 (0 to 4)
2	**56.7 (17 to 120)**	0.0 (0 to 0)	0.0 (0 to 0)	59.7 (14 to 130)	25.0 (0 to 139)	0.0 (0 to 0)	**0.0 (0 to 0)**	1.0 (0 to 7)
3	**61.9 (17 to 111)**	0.0 (0 to 0)	0.0 (0 to 0)	63.7 (10 to 136)	44.3 (0 to 207)	2.7 (0 to 18)	**0.0 (0 to 0)**	0.3 (0 to 2)
4	**56.7 (3 to 132)**	0.0 (0 to 0)	0.1 (0 to 1)	60.9 (8 to 133)	4.3 (0 to 28)	4.4 (0 to 17)	**0.0 (0 to 0)**	0.0 (0 to 0)
5	**59.4 (3 to 247)**	0.0 (0 to 0)	0.0 (0 to 0)	65.4 (6 to 126)	6.1 (0 to 13)	15.7 (0 to 71)	**0.0 (0 to 0)**	0.0 (0 to 0)
6	**78.7 (0 to 194)**	0.6 (0 to 4)	0.1 (0 to 1)	55.7 (13 to 84)	4.6 (0 to 20)	26.7 (0 to 87)	**0.0 (0 to 0)**	0.0 (0 to 0)
7	**28.9 (0 to 96)**	5.0 (0 to 20)	0.0 (0 to 0)	57.7 (29 to 91)	5.0 (0 to 18)	63.9 (0 to 144)	**0.0 (0 to 0)**	0.0 (0 to 0)

Position	Juncaceae spp.	***M. richardsonis***	*Muhly* spp.	Poaceae spp.	***S. heterolepis***	*S. michauxianus*	*S. nutans*	***S. scoparium***
1	0.0 (0 to 0)	**33.9 (0 to 104)**	7.7 (0 to 24)	0.0 (0 to 0)	**64.0 (20 to 145)**	0.0 (0 to 0)	22.4 (0 to 122)	**20.0 (0 to 65)**
2	0.0 (0 to 0)	**23.6 (1 to 59)**	10.3 (0 to 27)	0.0 (0 to 0)	**63.9 (4 to 136)**	0.0 (0 to 0)	16.4 (0 to 69)	**17.0 (0 to 82)**
3	24.6 (0 to 148)	**86.3 (0 to 408)**	8.6 (0 to 13)	0.0 (0 to 0)	**36.0 (5 to 113)**	0.4 (0 to 3)	12.4 (0 to 49)	**13.0 (0 to 40)**
4	14.1 (0 to 91)	**98.7 (0 to 231)**	11.7 (0 to 70)	0.1 (0 to 1)	**20.3 (0 to 100)**	4.9 (0 to 34)	11.6 (0 to 46)	**18.9 (0 to 125)**
5	43.9 (0 to 93)	**146.7 (27 to 457)**	1.9 (0 to 13)	0.0 (0 to 0)	**10.9 (0 to 42)**	5.3 (0 to 16)	1.6 (0 to 11)	**3.9 (0 to 23)**
6	55.4 (0 to 116)	**72.4 (0 to 129)**	1.4 (0 to 6)	0.0 (0 to 0)	**11.3 (0 to 31)**	4.6 (0 to 20)	0.0 (0 to 0)	**2.4 (0 to 14)**
7	42.3 (0 to 97)	**50.4 (0 to 157)**	1.6 (0 to 6)	0.3 (0 to 1)	**3.4 (0 to 14)**	21.9 (0 to 62)	0.0 (0 to 0)	**0.7 (0 to 5)**

Supplementary Table 3.5. The unadjusted numerical values produced during MRPP analyses of vegetation enumerated at **A**, positions along transects and **B**, locations where adult activities were observed. The sample sizes for transect positions during the respective analyses were: all plant species enumerated; all positions, n = 7; host plant species enumerated; positions 1 through 5, n = 7; position 6, n = 6; position 7, n = 5. For both analyses of plant data at locations where adult activities were observed sample sizes were: Attempted oviposition, n = 5 (all one adult); Egg laying, n = 6; Nectar feeding, n = 23; Resting/ Basking, n = 5.

A

All plant species

Positions compared	T	A	p
1 through 7	-3.785	0.067	0.001
1 - 2	0.546	-0.013	0.675
1 - 3	-0.142	0.003	0.374
1 - 4	-1.101	0.035	0.126
1 - 5	-2.197	0.067	0.037
1 - 6	-3.038	0.089	0.011
1 - 7	-4.602	0.137	0.002
2 - 3	1.263	-0.027	0.911
2 - 4	-2.028	0.049	0.042
2 - 5	-3.567	0.083	0.005
2 - 6	-5.181	0.123	0.000
2 - 7	-4.907	0.133	0.001
3 - 4	-0.099	0.002	0.396
3 - 5	-0.820	0.021	0.179
3 - 6	-2.251	0.051	0.028
3 - 7	-2.939	0.075	0.012
4 - 5	0.930	-0.024	0.838
4 - 6	-0.050	0.001	0.409
4 - 7	-1.730	0.048	0.063
5 - 6	0.926	-0.024	0.832
5 - 7	-0.315	0.009	0.313
6 - 7	0.022	-0.001	0.426

Host food plant species

Positions compared	T	A	p
1 through 7	-2.240	0.057	0.024
1 - 2	-0.049	0.001	0.412
1 - 3	-0.159	0.005	0.360
1 - 4	-1.562	0.073	0.077
1 - 5	-2.043	0.070	0.045
1 - 6	-1.215	0.060	0.112
1 - 7	-2.422	0.100	0.030
2 - 3	0.972	-0.027	0.839
2 - 4	-3.270	0.120	0.012
2 - 5	-3.766	0.093	0.002
2 - 6	-4.270	0.137	0.001
2 - 7	-2.550	0.088	0.014
3 - 4	-1.204	0.044	0.116
3 - 5	-0.652	0.020	0.226
3 - 6	-1.470	0.050	0.086
3 - 7	-0.498	0.017	0.266
4 - 5	0.961	-0.030	0.850
4 - 6	0.803	-0.041	0.848
4 - 7	0.685	-0.028	0.724
5 - 6	0.356	-0.014	0.561
5 - 7	1.170	-0.041	0.906
6 - 7	0.432	-0.020	0.598

Supplementary Table 3.5 continued. For both analyses of plant data at locations where adult activities were observed sample sizes were: Attempted oviposition, n = 5 (all one adult); Egg laying, n = 6; Nectar feeding, n = 23; Resting/ Basking, n = 5.

B

All plant species

Activities compared	T	A	p
Nectar feeding, Resting/ Basking, Egg laying and Attempted oviposition	-3.132	0.041	0.005
Nectar feeding - Resting/ Basking	-1.408	0.015	0.092
Nectar feeding - Egg laying	-1.576	0.016	0.074
Nectar feeding - Attempted oviposition	-2.355	0.026	0.026
Resting/ Basking - Egg laying	-1.068	0.031	0.141
Resting/ Basking - Attempted oviposition	-2.675	0.103	0.015
Egg laying - Attempted oviposition	-1.292	0.038	0.104

Host food plant species

Activities compared	T	A	p
Nectar feeding, Resting/ Basking, Egg laying and Attempted oviposition	-2.476	0.046	0.017
Nectar feeding - Resting/ Basking	-0.534	0.008	0.254
Nectar feeding - Egg laying	-1.763	0.026	0.059
Nectar feeding - Attempted oviposition	-2.366	0.034	0.025
Resting/ Basking - Egg laying	-0.002	0.000	0.428
Resting/ Basking - Attempted oviposition	-1.939	0.096	0.044
Egg laying - Attempted oviposition	-1.263	0.059	0.110

Chapter 4: Conclusion

I made discoveries about larval *O. poweshiek* foraging and adult habitat interactions in prairies in Manitoba, Canada. In the first section I identified larval host food plants and foraging behaviour, examined vegetative and physical characteristics of microhabitats and documented developmental rates of immature stages in natural tall grass prairie habitat. Then, I determined the sections along a soil moisture gradient in which *O. poweshiek* conduct various activities in tall grass prairies, by comparing the vegetative and physical attributes of locations associated with the various types of activities. I identified the plant communities where adults fly and the accompanying floral species which they consumed nectar from by following adults in natural habitat in Manitoba. These discoveries will likely guide further research into *O. poweshiek*'s biology and help captive-rearing programs be even more successful with immature skipperlings. They will also likely guide conservation disturbances (*e.g.*, haying, prescribed fires) to improve and maintain habitat with attributes required by Poweshiek skipperlings as well as inform reintroduction efforts while prioritising sites to release *O. poweshiek.* My research will also contribute to updates to federal recovery strategies all in an effort to increase the likelihood that Poweshiek skipperling will survive into the future.

During Chapter 2, I observed adult Poweshiek skipperlings appearing to locate areas with suitable host food species and relatively shallower duff, then laid eggs within these areas more precisely in microhabitats with relatively shorter graminoids and lower percent covers of duff and shrubs than in areas which they avoided. Larvae ate *A. gerardi* (big bluestem), *M. richardsonis* (mat muhly), *S. heterolepis* (prairie dropseed) and *S. scoparium* (little bluestem) in natural tall grass prairie, alternating between shoots of different species during their summer development period. Larvae appeared to navigate within microhabitats, using living and dead vegetation, to locate smaller locations where there may be a suitable host food plant and microclimate to then consume host species. Microhabitats appear to be more open and have drier microclimates than areas where eggs are not laid. Equipped with an improved understanding of specific larval feeding patterns, adult behaviours and microhabitat attributes we can hopefully narrow the list of possible causes of decline and facilitate the identification of potential solutions.

I observed adults flying almost exclusively in prairie plant community, observed rarely flying in shrub or ephemeral wetland communities and never observed in wetland or forest communities during Chapter 3. Adults consumed nectar from *Rudbeckia hirta* most often, but nectared from a mixture of species during the flight period which also included *Packera* spp. and *Glycyrrhiza lepidota*. In general, I think that attempted oviposition by *O. poweshiek* is associated with wetter sections of the soil moisture gradient in prairie plant communities, egg laying is associated with mesic sections, resting and/or basking is associated with drier sections and nectar feeding with mostly dry to mesic sections but occasionally with wetter sections. The sections which facilitated the various activities appeared to have different soil moistures; shrub, graminoid and forb percent cover; abundances of plant species (including host food plants); graminoid height; and relative elevation. We encourage further studies to determine if they replicate and/or revise our findings. Since the arrangement of critical habitat is not identical in every patch of prairie, this research can be used to guide researchers, stewards and recovery organisations in their search for habitats which facilitate various interactions of *O. poweshiek.*

Egg microhabitats in Chapter 2 contained shorter vegetation and shallower duff compared to plots over which adults flew but did not lay eggs. In Chapter 3, locations where egg laying was observed had shorter vegetation and shallower duff than at locations where attempted oviposition was observed. The height of vegetation likely influences microclimates of microhabitats facilitating the development of immature stages of other butterfly species (Dempster 1997; Möllenbeck et al. 2009). The repeated distinction observed in my research (along with generally lower relative humidity measured in egg microhabitats) provides evidence that open microhabitats are required by immature Poweshiek skipperlings and that these areas may be relatively drier than nearby locations. These attributes of immature microhabitats combined with the characteristics identified at locations which supported egg laying in Chapter 3, seem to be describing the critical components required by immatures stages of *O. poweshiek* in Manitoba, Canada.

In my honours thesis (Henault 2017), I found that higher adult *O. poweshiek* abundance was associated with drier sites. Building on those findings, I found in my MSc thesis that adults conduct various activities along a soil moisture gradient within prairie communities in a prairie site. The presence/absence of sections along the moisture gradient in a site may constrain the activities which adults may conduct and thereby the fitness of *O. poweshiek*. Potentially, a reduced ability by adults to engage in all necessary activities, may lead to population reductions such as the one observed over the past few decades. Hydrological changes at *O. poweshiek* sites (via conversion, channelling, groundwater extraction or climate change) may be the cause of this recently observed decline. I will be pursuing this research concept in the future.

The knowledge about larval foraging and adult habitat interactions which I generated will likely help recovery efforts in Manitoba, but will hopefully be somewhat transferable to recovery efforts in the United States of America. Direct larval foraging observations in natural habitats in the United States of America are required to determine host food species that are consumed. Individuals of the same butterfly species may use different host plants in specific parts of their range (as reported for *Euphydryas aurinia* by Meister et al. (2015)). I am willing to risk suggesting that *O. poweshiek*'s larval foraging behaviour of alternating between shoots of different host food species is expressed in individuals of Poweshiek skipperling throughout its range. The assemblages of plant species, soil moisture gradients and arrangements of plant communities in prairies in the central United States which historically supported *O. poweshiek* and fens in Michigan which currently support the Poweshiek skipperling are likely somewhat different than *O. poweshiek* habitat in Manitoba. However, perhaps the tendency of adults to conduct certain activities along a soil moisture gradient in plant communities similar to the "prairie" type of community in our study is similar. Hopefully, researchers can apply some of my findings to these locations in the United States during recovery efforts. I look forward to discoveries about larval foraging behaviour and the interactions of adults with their habitat in the United States.

This research has generated biological knowledge of the Poweshiek skipperling habitat requirements in Manitoba. Empowered with this information, habitat stewardship organisations (including non-profit, provincial, federal, university and independent research groups) may conduct conservation disturbances to improve the quality of existing habitat to support this endangered species. In sites which currently support *O. poweshiek*, I suggest that tall grass prairie stewards locate sections within prairie patches which are most likely to facilitate each type of adult activity (by using the characteristics of locations along the soil moisture gradient which facilitated various activities during my research) and manage a portion (*e.g.*, half) of the sections with a conservation disturbance such as haying. Even if the conservation disturbance happens to decrease the suitability of the hayed area temporarily, *O. poweshiek* from the rest of the patch may be able to recolonize this managed area. Management efforts guided by my results potentially may be conducted during dates that minimise the risk to developing larvae. For example, haying sites in late summer or fall when larvae are closer to the ground may reduce mortality compared to mid-summer when eggs or larvae are higher in the vegetative canopy (based on my findings in Chapter 2). We are unaware of techniques to disturb habitats in a way to focus on creating or improving the quality of attributes at a microhabitat scale. However, we assume that by using a variety of approaches (such as haying and prescribed burning habitat, or potentially varying the percentage of a habitat to which disturbances are applied) habitats will become chaotic enough to potentially increase the likelihood of containing highly suitable microhabitats. Of course, the effects of conservation disturbances on desired plant growth and the requirements of other species at risk ought to be considered as well. Strategies which stimulate the habitat to maintain attributes required by Poweshiek skipperling can be implemented, informed my findings. My research may help habitat stewards make informed decisions, instead of making educated guesses based on approaches used to manage habitat of endangered butterfly species which are evolutionarily similar.

My novel findings about larval foraging behaviours may guide reintroduction efforts to increase the abundance of *O. poweshiek* in Manitoba. The proportion of several plant species consumed by larvae in the natural habitats which I identified in this research may facilitate the creation of diets for larvae in rearing facilities that accurately reflect the host food plants consumed in natural habitats. An appropriate diet will likely increase their chances of survival, resulting in more butterflies that may be reintroduced into tall grass prairie sites. Additionally, my findings about where adults fly and how they interact with their habitat may inform the prioritisation of prairie locations at which to release *O. poweshiek.* I recommend that researchers locate sections along the hypothetical soil moisture gradient which are most likely to facilitate various adult activities in the prairie site. Prairie patches that contain sections along the moisture gradient which will likely facilitate egg laying, resting and/or basking and nectar feeding activities are locations (following confirmation in the field) where I think released *O. poweshiek* would be supported. Now with a more precise description of critical habitat in mind, partner groups (non-profits, zoos, provincial and federal governments, universities and independent researchers) will hopefully be more likely to locate and prioritize suitable areas in which to reintroduce the Poweshiek skipperling. Hopefully these efforts will lead to an increased abundance of this species!

Novel descriptions of locations which facilitate larval development and various adult activities may inform provincial and federal recovery strategies to increase the chances of *O. poweshiek*'s survival. The plant communities used by adults and the sections along the soil moisture gradient which facilitate various activities in the prairie plant community may be incorporated into descriptions of critical habitat required for Poweshiek skipperling to survive. My findings provide timely information to revise the recovery approach of the Canadian Wildlife Service (Environment and Climate Change Canada). Discoveries about areas which facilitate various adult activities may lead to prioritization of habitats to protect. Knowledge of Manitoba-specific requirements may facilitate the success of strategies to recover skipperlings in Manitoba. Additionally, the findings in this research may support federal recovery approaches in the United States of America, thereby improving the overall chances that this species survives in the long-term of the future.

After their initial decline following range-wide habitat destruction across North America, *O. poweshiek* have also consistently declined during the last several decades. Now with a greater understanding of larval foraging behaviour and adult interactions, we may be better able to generate novel ideas which explain *O. poweshiek*'s precarious situation and identify possible solutions. Observations of larval foraging may result in the identification of risks at the scale of individual larvae which may also be a threat to the species in general. I think my discoveries may guide disturbance actions to maintain suitable microhabitats, inform the development of nutrition programs for rearing larvae in captivity, provide criteria to choose reintroduction prairies and fuel updates of recovery approaches to facilitate the survival of the endangered Poweshiek skipperling. My research has generated numerous exciting streams that myself, or others, may research!

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