

ARTICLE

Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada

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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 among tall grass prairie remnants. However, the host food plants eaten by Poweshiek skipperling larvae, the vegetative and microclimatic descriptions of immature and adult microhabitats, and *O. poweshiek* behaviour in Manitoba are unknown. We followed Poweshiek skipperling adults in their natural habitat to locate microhabitats where eggs were laid and to observe larval foraging behaviour and development. We measured vegetative, structural, and microclimatic characteristics of microhabitats used by immatures and documented the host species larvae consumed, their general behaviour (on plants; movement within microhabitats), and their developmental schedules. Larvae ate *Andropogon gerardi*, *Muhlenbergia richardsonis*, *Sporobolus heterolepis*, and *Schizachyrium scoparium* (all Poaceae) in natural tall grass prairie. Larvae appeared to navigate microhabitats to locate host food plants, alternating between shoots of various species throughout their development. Microhabitats seemed to be more open, with drier microclimates, than areas where eggs were not laid. This improved understanding of larval feeding patterns, adult behaviours, and microhabitat attributes may help local grassland stewards and researchers reduce the list of possible causes of decline and identify potential solutions to recover the Poweshiek skipperling.

Introduction

The Poweshiek skipperling, *Oarisma poweshiek* (Parker, 1870) (Lepidoptera: Hesperiiidae), 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 2014; Belitz *et al.* 2018). The number of colonies across their range declined (Committee on the Status of Endangered Wildlife in Canada 2014; Smith *et al.* 2016), before the number of adults in remnant colonies also decreased since approximately the 1990s to potentially a few hundred individuals across its range (Swengel and Swengel 1999; Committee on the Status of Endangered Wildlife in Canada 2014; Smith *et al.* 2016; Grantham *et al.* 2020). The destruction of 99% of tall grass prairie (Samson and Knopf 1994) likely contributed to the initial decline, but causes of the decrease in remnant colonies over the last few decades are unknown (Committee on the Status of Endangered Wildlife in Canada 2014; Smith *et al.* 2016). *Oarisma poweshiek* is now listed as endangered in both Canada and the United States of America (Committee on the Status of Endangered Wildlife in Canada 2014; United

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States Fish and Wildlife Service 2015; Canada Gazette 2019) and critically endangered across the planet (Royer 2020). A better understanding of *O. poweshiek* biology may facilitate successful recovery efforts.

Remaining *O. poweshiek* populations are found at the margins of the historical range in Manitoba, Canada (Committee on the Status of Endangered Wildlife in Canada 2014) and Michigan, United States of America (Belitz *et al.* 2018). In Manitoba, the Poweshiek skipperling occurs in wet to mesic tall grass prairies composed of convoluted prairie, forest, and wetland plant communities (Catling and Lafontaine 1986; Committee on the Status of Endangered Wildlife in Canada 2014). Typical species supported in these plant communities include *Sporobolus heterolepis* A. Gray (Poaceae), *Liatris ligulistylis* (A. Nelson) K. Schumann (Asteraceae), *Potentilla fruticosa* Linnaeus (Rosaceae) in the prairie areas, *Populus tremuloides* Michaux (Salicaceae) in the forest areas, and Juncaceae Jussieu spp. in the wetland areas, both permanent and ephemeral (Catling and Lafontaine 1986; Committee on the Status of Endangered Wildlife in Canada 2014). *Oarisma poweshiek* occurs most often in tall grass prairie complexes approximately 10 km² in area (Westwood *et al.* 2020) and at relatively higher abundances in sites that are comparatively less wet, as indicated by the presence of plants that prefer various soil moistures (Henault 2017).

The Poweshiek skipperling in Manitoba flies from the last week of June through early August (Semmler 2010; Committee on the Status of Endangered Wildlife in Canada 2014). Females lay eggs on plants, and hatched larvae feed until they enter diapause for winter. Larvae are thought to overwinter near the base of plants (McAlpine 1972; Borkin 1995; Committee on the Status of Endangered Wildlife in Canada 2014) and resume feeding in the spring, before pupating in mid- to late June (Layberry *et al.* 1998; Committee on the Status of Endangered Wildlife in Canada 2014).

The substrates these butterflies use for egg laying in Canada are unclear, comprising several plant families. Dupont-Morozoff (2013) observed ovipositions on *Andropogon gerardi* Vitman (Poaceae), *Melilotus* (Linnaeus) Miller spp. (Fabaceae), *Solidago* Linnaeus spp. (Asteraceae), and *Quercus macrocarpa* Michaux (Fagaceae). One of us (J.H., unpublished data) observed a female and male mating in the canopy of graminoids at a visually mesic location, after which the female immediately flew approximately 3 m to lay an egg on a *S. heterolepis* leaf (Supplementary material, Fig. S1; Henault 2021). Several oviposition substrates have been reported in the United States of America (Table 1). However, reports of direct observations of larval feeding in natural habitats are scarce. Borkin (1995) observed individual larval feeding bouts in natural prairie habitat (Wisconsin, United States of America), where larvae consumed *S. heterolepis* and *Schizachyrium scoparium* (Michaux) Nash (Poaceae). Larvae from ova of captured females were placed on small, potted clumps of prairie graminoids that had been dug from a road backslope (Minnesota, United States of America; Dana, personal communication); they consumed the species observed by Borkin (1995) and also *A. gerardi* (Dana, personal communication).

Butterflies lay eggs in microhabitats that contain host food plants and the microclimatic conditions (*e.g.*, temperature or humidity) required for immature stages to develop (Ashton *et al.* 2009; Krämer *et al.* 2012; Ewing *et al.* 2020). If eggs are 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, including host plants and plant chemical composition, to identify suitable microhabitats (Wiklund 1984; Lund *et al.* 2019).

The developmental rate of *O. poweshiek* in confirmed field microhabitats in Manitoba has not been reported. As a result, annual surveys of adults in Manitoba and the United States of America (Westwood *et al.* 2012; Grantham *et al.* 2020) must be synchronised by using estimated larval development rates to predict adult eclosion (Dearborn and Westwood 2014; Dearborn *et al.* 2014–2022). A better understanding of immature development, followed by a

Table 1. Taxa used by *Oarisma poweshiek* for oviposition (all in field conditions) and larval feeding in Canada and the United States of America.

Taxa		Oviposition substrate	Host food plant ¹	Jurisdiction ²	
<i>Andropogon gerardi</i> Vitman	Poaceae	Y	Y	Manitoba	Henault and Westwood current
				Minnesota (cr)	Dana, personal communication
				N/A	Manitoba
<i>Muhlenbergia richardsonis</i> (Trinius) Rydberg	Poaceae	Y	Y	Manitoba	Henault and Westwood current
				?	Michigan
<i>Schizachyrium scoparium</i> (Michaux) Nash	Poaceae	Y	Y	Manitoba	Henault and Westwood current
				Minnesota (cr)	Dana, personal communication
				Wisconsin	Borkin (1995)
<i>Sporobolus heterolepis</i> (A. Gray) A. Gray	Poaceae	Y	Y	Manitoba	Henault and Westwood current
				Minnesota (cr)	Dana, personal communication
				Wisconsin	Borkin (1995)
				N/A	Manitoba
<i>Hypoxis hirsuta</i> (Linnaeus) Coville	Hypoxidaceae	Y	Larva did not consume	Manitoba	Henault and Westwood current
<i>Carex</i> Linnaeus spp.	Cyperaceae	Y	Y	North Dakota (cr)	McCabe and Post (1977)
<i>Carex sterilis</i> Willdenow	Cyperaceae	Y	?	Michigan	Belitz <i>et al.</i> (2019)
<i>Eleocharis elliptica</i> Kunth	Cyperaceae	Y	N/A	Michigan	Holzman (1972)
<i>Eleocharis</i> R. Brown spp.	Cyperaceae	Y	N/A	Wisconsin	Borkin (1995)
<i>Hesperostipa spartea</i> (Trinius) Barkworth	Poaceae	Y	N/A	Minnesota	Dana, personal communication
<i>Melilotus</i> (Linnaeus) Miller spp.	Fabaceae	Y	N/A	Manitoba	Dupont-Morozoff (2013)
<i>Muhlenbergia glomerata</i> (Willdenow) Trinius	Poaceae	Y	N/A	Michigan	Belitz <i>et al.</i> (2019)
<i>Potentilla fruticosa</i> Linnaeus	Rosaceae	Y	N/A	Michigan	Belitz <i>et al.</i> (2019)
<i>Quercus macrocarpa</i> Michaux	Fagaceae	Y	N/A	Manitoba	Dupont-Morozoff (2013)
<i>Silphium terebinthinaceum</i> Jacquin	Asteraceae	Y	N/A	Wisconsin	Borkin (1995)

(Continued)

Table 1. (Continued)

Taxa		Oviposition substrate	Host food plant ¹		Jurisdiction ²
<i>Solidago</i> Linnaeus spp.	Asteraceae	Y	N/A	Manitoba	Dupont-Morozoff (2013)
<i>Bouteloua curtipendula</i> (Michaux) Torrey	Poaceae	N/ A	Y	Minnesota	Dana, personal communication
<i>Carex pensylvanica</i> Lamarck	Cyperaceae	N/ A	Y	Minnesota (cr)	Smith <i>et al.</i> (2016)
<i>Carex inops</i> subspecies <i>heliophila</i> (Mackenzie) Crins	Cyperaceae	N/ A	?	Minnesota (cr)	Dana, personal communication
Unidentified plant/Poaceae/Cyperaceae spp.		Y	Y	Michigan (cr)	McAlpine (1972)
			?	Minnesota (cr)	Dana, personal communication
			N/ A	Wisconsin	Borkin (1995)

¹Y = direct observation, ? = feeding marks near eggs.

²cr = captive-reared larva(e). Manitoba, Minnesota, North Dakota and Wisconsin: tall grass prairies; Michigan: tall grass prairie fens.

comparison of field and weather station data, may increase the accuracy of predictions and subsequent timing of surveys.

Observations tracking the foraging behaviour and development of the same *O. poweshiek* individuals in natural habitats in North America have not been reported. The vegetation, physical structure, soil characteristics, and microclimate attributes of suitable microhabitats of immature skipperlings also have not been described. Identifying the larval host plants that Poweshiek skipperlings use in natural habitats is critical for prioritising future research directions and facilitating the recovery of this species. The objectives of this research were to: (1) identify larval host plants and foraging behaviour in natural tall grass prairie sites in Manitoba, (2) examine the vegetative, physical, and microclimatic characteristics of microhabitats where eggs are laid, and (3) document *O. poweshiek* developmental rates in natural habitats. We hypothesised that areas where females laid eggs may possess specific host plant, structural, or microclimatic attributes, which might be facilitated by soil characteristics, and that larvae may travel within microhabitats to feed.

Methods

Field research was carried out within the Manitoba Tall Grass Prairie Preserve in southeastern Manitoba, Canada (the Nature Conservancy of Canada interpretive centre, ~49.153° N, 96.729° W). We selected a site where *O. poweshiek* was consistently present during collaborative surveys, using meandering transect walks (Royer *et al.* 1998), from 2007 through 2017 (Westwood *et al.* 2012; Grantham *et al.* 2020), where at least one of us had observed egg laying and where individuals had not been reintroduced (Assiniboine Park Zoo 2018). We studied *O. poweshiek* at three prairie patches of approximately 0.20-km² area within a 2.6-km perimeter that were isolated from each other by wetlands and forests. This study area is stewarded with prescribed burns and mechanical and herbicide removal of encroaching vegetation by the Nature Conservancy of Canada (Grantham *et al.* 2021); the last site-scale disturbance was a wildfire in autumn 2011.

In 2018, from 25 June through 4 July and on 6 July, from approximately 10:00–17:00 local time, we searched for and followed adult Poweshiek skipperlings to observe female egg laying. We observed females from 27 June through 3 July 2018 and males and females from 26 June through 4 July 2018. The bases of shoots where females were observed laying eggs were marked with a metal stake and recorded with a geographic positioning system unit (Garmin Oregon 700; Garmin, Olathe, Kansas, United States of America).

Egg enclosures, July 2018 to July 2019

We centred plastic refuse pails that we previously had cut in half at each location where eggs were laid ($n = 6$), breaking the ground surface to a depth of 3 cm (base 49 cm wide, or 0.19 m²) and extending 28 cm above the ground surface (Fig. 1). Enclosures were removed after the first adults of the next generation were observed in the site (July 2019). Our primary goal was to mimic environmental conditions that are typically experienced by local larvae (*i.e.*, with no mesh and with potentially cooler white walls) to facilitate unaltered behaviour while limiting larval escape. We clipped vegetation approximately weekly, 3–5 cm along the pails' internal walls, and any plants that overlay the pails' rims. On sampling dates, plants were clipped after variables were recorded to ensure a consistent sampling arena. We hypothesised that this surface area would accommodate all movements during the development of these small larvae, which are approximately 2–24 mm long (McAlpine 1972). If we did not see a larva in an enclosure during two consecutive weeks, we ceased comprehensive observations. To avoid the chance that an egg or larva was present but not seen, we periodically checked the enclosures to confirm the absence of immatures until the end of the study.

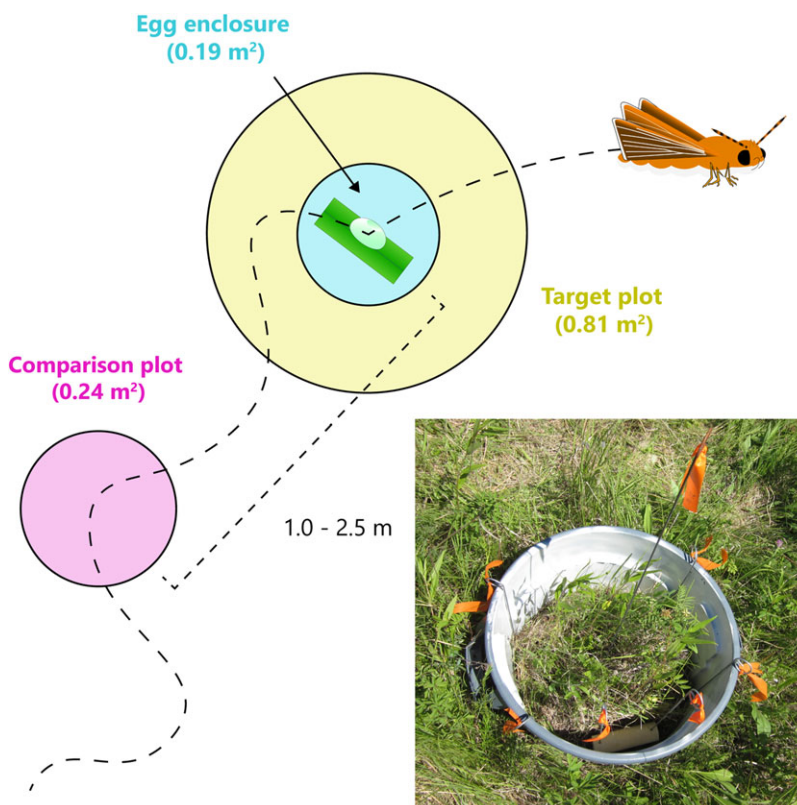


Fig. 1. Relative positions of egg enclosures, target plots, and comparison plots along an *Oarisma poweshiek* adult flight track. A microclimate data logger is shown in the shade of the egg enclosure.

Larval foraging behaviour

The distance from eggs to the tip of the leaf blade and the height above the ground were recorded. One to three times per week, J.H. located a larva to watch for at least one hour. Based on these direct observations, the host species that larvae consumed and the locations on plants that larvae occupied relative to the tip of the leaf (from apex of head capsule along the leaf) and to the ground (vertical distance), as well as the horizontal distance between sequential larval activity locations, were recorded. Larvae were oriented vertically, except on one occasion, when they were oriented horizontally; therefore, we measured the direction that the dorsal side of the larva's body faced to determine a larva's degree of exposure to the sun. By chance, J.H. observed larvae immediately after each moult, allowing us to accurately identify the instars. Photographing dislodged Immature 1 from the plant while hatching; after replacement on its host, Immature 1 rested near the duff layer. Thereafter, to reduce disturbance, including during windy conditions or if larvae flinched, we measured only the larval position relative to the leaf tip or ground, in addition to the host species, during some observation events. During the only major measurement disturbance, Immature 2 left its host before returning the next day to the original location. In this way, larvae appear not to have been substantially influenced by our observations. Larvae were measured (total length) with a ruler while on plants from 1 to 3 days after moulting and were not handled in order to minimise risks of stress, displacement, and damage.

The thermal development thresholds of *O. poweshiek* are unknown; Dearborn and Westwood (2014) used 6 °C as the lower threshold, based on studies of other Lepidoptera that

overwinter as larvae in this region. The diapause-inducing conditions likely comprise a combination of temperature and photoperiod, as has been observed for other butterflies (Kim *et al.* 2014). Given this uncertainty, we assumed that larvae would be inactive approximately when the minimum daily temperature was below 0 °C and during a shorter photoperiod (late fall and early spring), with developmental conditions likely deteriorating from September to December. Field observations began 26 June 2018, before the predicted adult flight period, paused 14 September, with the first date with minimum daily temperature below 0 °C being 5 September, and resumed 8 May 2019, after the snow had melted but before minimum daily temperatures returned above 0 °C on 22 May.

Microclimate in microhabitats

We recorded temperature, relative humidity, and dew point at one-hour intervals (9 July 2018 to 18 July 2019, inclusive) using data loggers (HOBO® Pro V2 U23-001: Onset Computer Corporation, Bourne, Massachusetts, United States of America) in egg enclosures ($n = 4$) and their accompanying comparison plots (see below; $n = 4$). Data loggers were placed in open-ended plastic tubes at the boundary of the dead vegetation layer (Fig. 1) in shaded areas to equalise sun exposure (data loggers in egg enclosures: shade of walls; in comparison plots: shade of tall vegetation). Vegetation was flattened within approximately 5 cm of the tube openings to replicate air flow and the humidity of the clipped areas in the egg enclosures. We also acquired temperature data from the weather station used to synchronise surveys (Emerson, Manitoba; ~ 35 km to the southwest, or 254° from the study site) from an online repository (Environment Canada 2020).

Attributes of egg enclosures and target plots, 2018

In the egg enclosures, we counted the number of shoots of all plant species and estimated the percent cover of graminoids, including Poaceae spp., Cyperaceae spp., Juncaceae spp., and Juncaginaceae spp.; forbs; shrubs, including trees; duff, including both dead pieces of vegetation and fine decomposing material; and bare ground. We measured the height of graminoids (of approximately 95% of the plants) and, in three random locations, the depth of duff. Soil moisture and electrical conductivity (*i.e.*, concentration of molecular ions; Natural Resources Conservation Service 2014) were measured 10 cm below the surface to focus on roots instead of soil surface at two random locations using soil probes that were connected to an electronic display (Fieldscout TDR 150; Spectrum Technologies, Inc., Aurora, Illinois, United States of America). Measurements were conducted on 18 and 30 July 2018 after seven and eight days without substantial precipitation, respectively.

We also established “target plots” outside of egg enclosures (Fig. 1), which we sampled on 2 and 3 August 2018, using the same methods except that we took no soil measurements. By comparing prairie attributes at different radii from deposited eggs, we hoped to determine the size of prairie from which females receive oviposition stimuli. We identified plant species using dichotomous keys and visually using Looman and Best (1987) and Leighton and Harms (2014), before updating nomenclature to [Tropicos.org](https://tropicos.org) (Missouri Botanical Garden 2021).

Attributes of egg enclosures and comparison plots, 2019

While following adults in 2018, we staked a metal rod at locations where females flew over immediately before the eventual oviposition location. Apparently, females were not stimulated above the oviposition threshold, as Singer (1971) had described for other Lepidopteran taxa. We established one circular “comparison plot” in 2019 at these locations to accompany each oviposition location while prioritising locations with similar sun exposures (Fig. 1).

We sampled egg enclosures and comparison plots using the same methods as in 2018, with the following exceptions. Only graminoids were enumerated, and these were split into two groups: (1) confirmed and possible larval and egg hosts, as observed in 2018, and our hypothesised additional hosts, *Sorghastrum nutans* (Linnaeus) Nash and *Muhlenbergia* Schreber spp., and (2) all other graminoids. On 9 May, 30 May, 11 June, and 5 July, we counted only the number of shoots of species in the confirmed and potential host group, and we estimated the proportion of graminoid shoots belonging to host or nonhost graminoid groups. The heights of all combined graminoids and of the two groups were independently measured. On 18 July 2019, the duff depth was subdivided into thatch (upper, dead pieces) and litter (lower, fine decomposing material). Soil variables were measured, as in 2018, on 9 and 10 May 2019. The light intensity was measured once per plot (the upper plane of the duff and below; in immediate succession; Triple Range Light Meter, Model 217, General Electric Company, Boston, Massachusetts, United States of America) in the absence of clouds on 23 July 2019, within a 3.5-hour period.

Lepidopteran nomenclature follows Pohl *et al.* (2018) primarily and GBIF.org (Global Biodiversity Information Facility 2021) secondarily.

Analysis

We report the relatively shorter distances to the tip, as measured with a ruler along the leaves, with greater precision than we report distances to the ground. When calculating the duration of subsequent feeding and resting activities, we used only events where larvae alternated between feeding and resting at least once. The mean angle, directionality (r), and significance of direction (Rayleigh test, which must include at least five samples) of larval movements and orientations while on plants were calculated as in Batschelet (1981).

Microclimate data recorded by data loggers were standardised by adding or subtracting recorded values ($^{\circ}\text{C}$; %) relative to the bias of each data logger, which was determined by operating the loggers for one week in a building under equal conditions, before conducting analyses. Temperature data from the weather station (30 June–8 July 2018, inclusive) were used to supplement egg-period microclimate data from the data loggers. Degree-day accumulations were calculated following Dearborn and Westwood (2014) to enable comparisons: from the first date of a period (biofix date; calibration), the mean daily temperature was calculated before subtracting the hypothesised lower development threshold (6°C), using the standard model (standard), or accounting for degree-day accumulations between lower (6°C) and upper thresholds (32°C), using the double sine model (double sine).

We did not observe any larvae completing their entire life cycle. Because of this, we estimated date ranges of hypothetical developmental periods of *O. poweshiek* during any given year as follows: 30 June–27 September (inclusive) – first egg laid and active larvae; 28 September–21 May – diapause; 22 May–10 July – break of diapause through first adult eclosion; and 30 June 2018–10 July 2019 – first egg laid through first adult eclosion. We think that the dates when the minimum daily temperatures are consistently below 0°C may provide a biologically relevant calibration date for future research targeting similar taxa. The postdiapause period encompasses all dates during which degree-days could be accumulated following winter. We also assessed the period used by Dearborn and Westwood (2014) – 1 March through the first adult observation – to synchronise surveys with adult emergence. Because we did not operate field data loggers for the entire egg development period, we revised the following periods when analysing temperature, humidity, and dew point: active larvae – 11 July–27 September, and egg hatch (instead of egg laid) to adult eclosion – 11 July 2018–10 July 2019.

We standardised shoot counts in all plots to 0.19 m^2 , which was the area of egg enclosures, before analyses. Data were transformed so that accompanying residuals met the assumptions

of normality before tested using Welch's two-sample *t*-tests; if the assumptions continued to fail, we evaluated 95% confidence intervals instead. We subtracted the percent of *S. nutans*, *Muhlenbergia* spp., and *Hypoxis hirsuta* (Linnaeus) Coville (Hypoxidaceae), which were counted as shoots, from the estimated proportion of host plants before generating Figure 6. Plant composition and abundance were compared using permutational multivariate analysis of variance, with observed values compared to random expected values, and then were assessed by a permutation test (Anderson 2001; McCune and Mefford 2011). We used the Sorensen (Bray–Curtis) distance measure, with species weighed equally while integrating respective abundances, which is suitable for continuous and heterogeneous data sets (Bray and Curtis 1957; Magurran 1988; McCune and Mefford 2011). We calculated the means of the duff or thatch and litter depths from samples within each plot, as well as soil moisture and electrical conductivity from samples within each plot across sample days. Measured footcandles were converted to metric lux, and light intensity was derived using the following formula:

$$\text{light intensity (\%)} = (\text{lux}_{\text{below plane}} / \text{lux}_{\text{above plane}}) \times 100$$

We used RStudio (RStudio Team 2021) to calculate summary statistics, conduct tests, and create Figure 6 (base R, version 4.1.2; R Development Core Team 2021; “ggplot2”, Wickham *et al.* 2020; “svglite”, Wickham *et al.* 2021). We improved the resolution of figures in Adobe Illustrator (<https://www.adobe.com/products/illustrator.html>) while also creating illustrations. Permutational multivariate analysis of variance was conducted using PC-ORD (McCune and Mefford 2011). We used Adobe Premiere Pro (<https://www.adobe.com/products/premiere.html>) to stabilise the video.

Results

Oviposition

Poweshiek skippering females were observed laying six eggs during five separate oviposition episodes – that is, up to five different females. All egg laying occurred on separate plants: grasses *A. gerardi* – four eggs; *S. heterolepis* – one egg; and the forb *H. hirsuta* – one egg. Eggs were laid between 30 June and 3 July 2018.

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 ovipositing before elevating and departing. Before 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 probed at three separate possible oviposition locations, laid an egg at a fourth location, and then immediately repeated this alternating sequence once before our observations of this excursion ended. This meant that the female searched eight locations and laid two eggs over the entire observation. One female was observed nectar feeding immediately before initiating flight patterns that are typical before oviposition.

In 2017, before formal observations, J.H. observed a female and male attempting to mate near the top of the vegetative canopy at a visually mesic location (Supplementary material, Video S1; Henault 2021). After several mating attempts, during some of which the female might have blocked mating by using her wings, similarly to what Borkin (1995) reported, the skipperlings mated perched on a graminoid for approximately 30–60 seconds before separating. Following this, the female immediately flew approximately 3 m away and laid an egg on a Cyperaceae sp. These behaviours were similarly observed during the mating observation in 2015.

Table 2. The number of feeding, resting, and movement observations among all *Oarisma poweshiek* instars, and of days during which immatures were observed during the study period.

Immature	Feeding	Resting	Movement	Span of days
1	3	2	1	12
2	27	19	8	70
3	14	14	5	36
Total	44	35	14	71

Observations of immatures

The changing egg colour during maturation and the larval colour pattern and body shape were as described in McAlpine (1972). However, in that study's 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. Not including our visits to monitor eggs, we observed larvae for 48.1 hours during 2018 (Table 2). The following sections describe our observations of immatures, as eggs and larvae, by type of foraging behaviour and developmental aspect.

Oviposition location and hatching. The egg of Immature 1 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, behaviour that was also observed in Immatures 2 and 3. Immature 2's egg was laid on a *S. heterolepis* leaf (dorsal surface; 7.0 cm from tip, 15.0 cm above ground), and the egg of Immature 3 was laid on *A. gerardi* (dorsal surface of the leaf; 2.5 cm from the leaf tip, 36.8 cm above the ground).

Immatures 4, 5, and 6 were laid as eggs on *A. gerardi*. Immature 5 was laid 4.9 cm from the leaf tip and 29.6 cm from the ground. The locations where the eggs of Immatures 4, 5, and 6 were laid were searched on 17, 16, and 17 subsequent visits, respectively, over the entire study period, but no larvae were found.

Feeding and resting patterns: locations and durations. Immature 1 was next observed one day later, on a shoot of *A. gerardi* that was 12.4 cm to the east (90°), to begin feeding on the youngest developing leaf of the plant. Immature 2 moved from its natal plant to *Muhlenbergia richardsonis* (Trinius) Rydberg (Poaceae), and Immature 3 moved to a *S. heterolepis*. During these initial stages in an immature's development, feeding occurred at the tip of a leaf (Fig. 2).

Whereas Immature 1 was observed only feeding at the tip, Immatures 2 and 3 ate at the tips of host plants during instar 1 and at the sides of blades in addition to the tip during instars 2–4. In addition to consuming the point of a leaf tip in a rotary movement around the tip, Immature 3 also ate the tip down to wider portions of the blade by chewing from one edge to the opposite edge in one feeding pass. The feeding patterns of Immature 2 on the sides of blades produced two staggered notches on alternate edges of a leaf (mean length of two adjacent notches = 0.7 cm, range = 0.2–1.1 cm). These notches that were made by Immature 3 sometimes occurred on the same side of the leaf blade. Immature 2 also ate longer stretches of one side of a leaf (mean = 0.9 cm, range = 0.5–1.3 cm). To eat the long sections, Immature 2 chewed from the edge of a blade to the midvein before restarting from the edge (one feeding pass = width of its head capsule). On one occasion, Immature 3 consumed a relatively longer section of leaf (approximately half the length of this larva) on both sides of a leaf to the midvein at the same location (Supplementary material, Video S2).

Immature 2's dorsal surface while feeding appeared directional ($r = 0.846$), but a small sample size prevented testing, and its orientation while resting was not towards a specific direction



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

(Supplementary material, Table S1). The dorsal surface of Immature 3 while feeding and resting was significantly directed northwest.

Immatures 1, 2, and 3 fed at or near the tip, crawled closer to the ground, and rested on a leaf blade, and then repeated the sequence by crawling to or near the tip to feed (Fig. 3; Supplementary material, Table S2). The durations of all immatures' feeding bouts (Immature 1: $n = 3$, mean = 10 minutes, 95% confidence interval = 9–12 minutes; Immature 2: $n = 4$, mean = 21 minutes, 95% confidence interval = 15–27 minutes; Immature 3: $n = 7$, mean = 25 minutes, 95% confidence interval = 11–39 minutes) were significantly shorter than their resting bouts (Immature 1: $n = 2$, mean = 54 minutes, 95% confidence interval = 45–62 minutes; Immature 2: $n = 11$, mean = 95 minutes, 95% confidence interval = 54–136 minutes; Immature 3: $n = 7$, mean = 87 minutes, 95% confidence interval = 50–124 minutes). In general, Immatures 1, 2, and 3 faced the tip of the leaf while feeding and the base while resting, except for one instance when Immature 2 rested facing the leaf tip.

Relocations between hosts. Immatures 1, 2, and 3 travelled both between shoots of the same species and between shoots of different species to feed, rest, and moult (Fig. 4). We observed Immature 2 using living and dead vegetation to travel, but we did not observe the substrates of Immatures 1 and 3. Immature 2 appeared to move more frequently during later instars (Table 3), and its movement was directional.

Immature 1 consumed only *A. gerardi*, and Immature 3 ate only *S. heterolepis* during our observations. 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 4).

Immatures 1, 2, and 3 were always observed on host plants, whether feeding, resting, or moulting – including after accidental disturbance by observers. After all larvae finished feeding, they rested on the same shoot which they had previously fed on.

Growth. Immature 1 was 0.36 cm long, with measurements taken during instar 1, Immature 2 was 0.85 cm long when measured during instar 3, and Immature 3 was 0.61 cm and 0.62 cm six

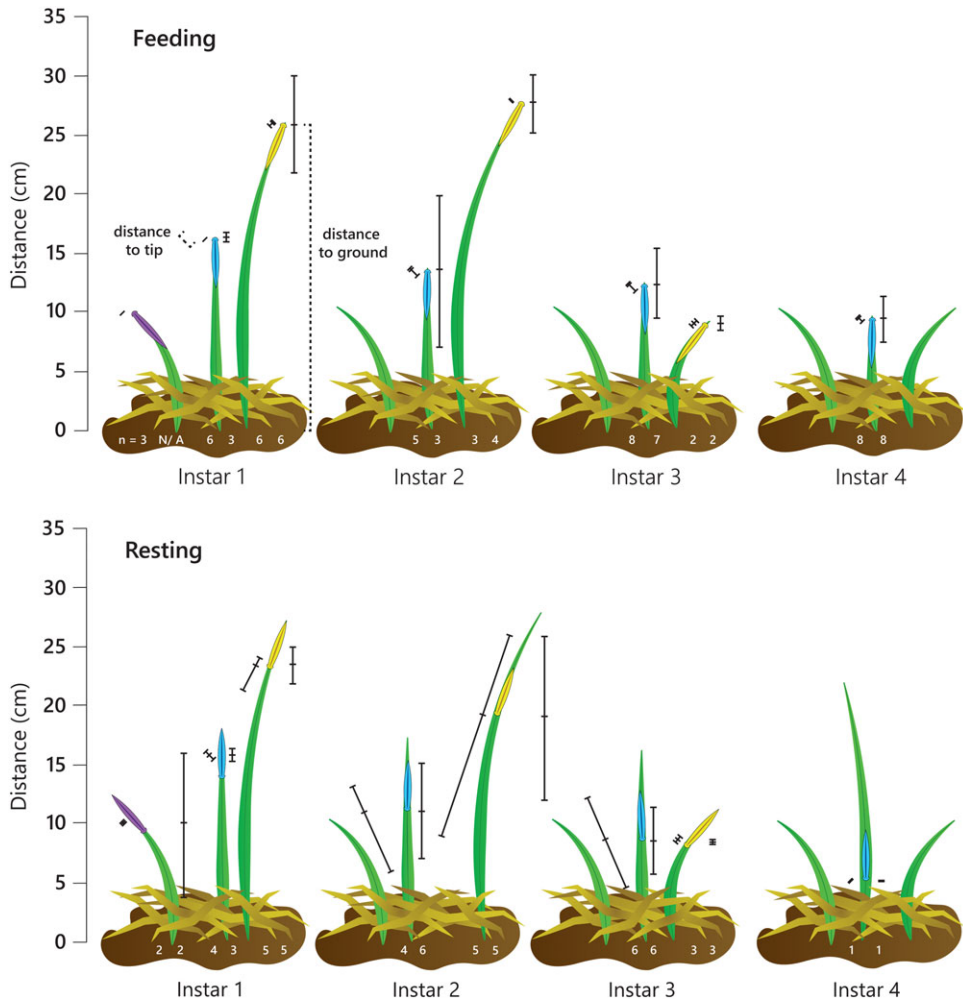


Fig. 3. *Oarisma poweshiek* larval host plant feeding and resting locations on shoots (bars on left, mean and range; bars on right, mean and 95 % confidence interval). We display larvae during each instar in which they were observed (Immature 1, purple; Immature 2, blue; Immature 3, yellow; approximately 7× size, to scale). Given the absence of measurements, we display Immature 1 feeding at the approximate distance it rested from the ground. Duff height is represented to scale.

days after the first measurement, during instar 2. We did not observe the cause of death for any immatures. Immature 2 was last observed resting at the base of a host plant approximately 5.2 cm above the ground on 9 September 2018.

Other species. Other Lepidopterans (e.g., Geometridae) were also observed feeding, and their feeding marks were similar to those of Poweshiek skipperling larvae. Thus, we did not record marks that we were not certain were made by *O. poweshiek*. Ants and jumping spiders were observed in the enclosures; however, no direct predation was observed.

Larval development

Larvae were observed on shoots as their exoskeletons hardened and adjacent to a shrivelled exuvia after moulting. Immatures 2 and 3 completed each stage in the same number of

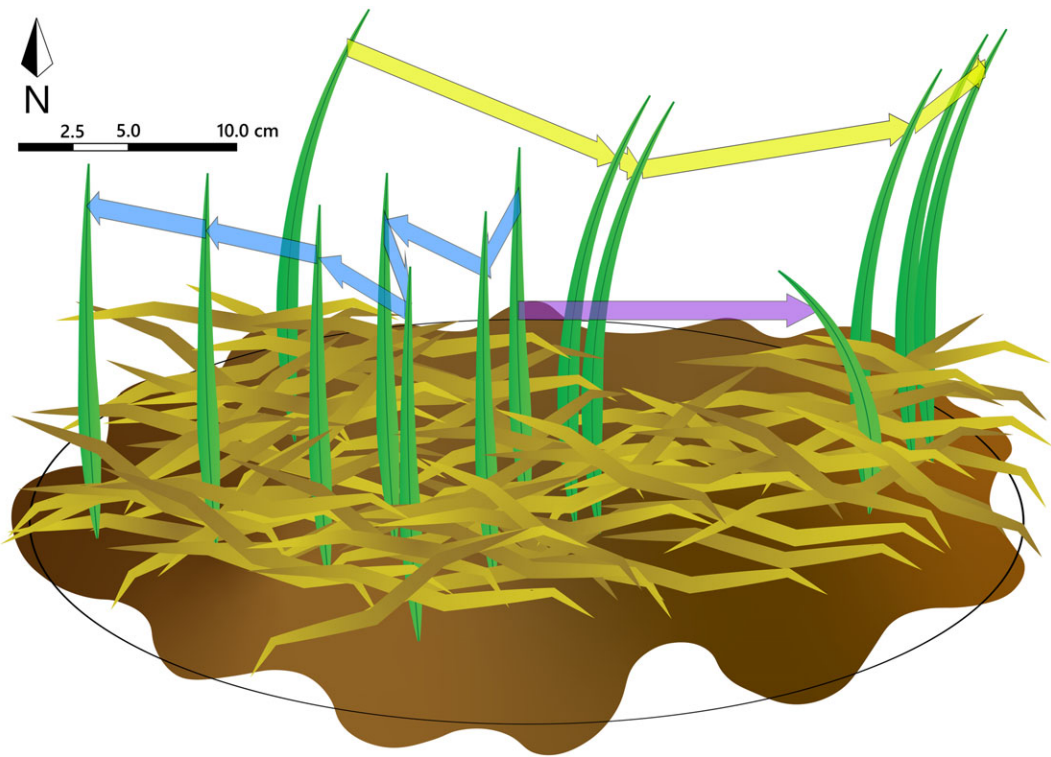


Fig. 4. *Oarisma poweshiek* larval tracks in egg enclosures displayed as arrows (Immature 1, purple; Immature 2, blue; Immature 3, yellow). Immatures 1 and 2 are shown starting from same blade for illustrative purposes only.

degree-days and calendar days (Table 5). Using data recorded by data loggers in the egg enclosures, we calculated that larvae required a mean of 1700.0 degree-days (standard model; $n = 4$, 95% confidence interval = 1644.5–1755.5 degree-days) and 1723.9 degree-days (double sine; $n = 4$, 95% confidence interval = 1654.3–1793.5 degree-days) to develop from the first egg laid to the first adult that emerged (30 June 2018–10 July 2019, inclusive). 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% confidence interval = 1590.7–1796.0 degree-days) and 1722.0 degree-days (double sine; $n = 4$, 95% confidence interval = 1655.9–1788.0 degree-days; degree-day accumulated during constituent periods in Supplementary material, Table S3). The degree-days calculated for egg enclosure and comparison plot locations using each model did not significantly differ, based on overlapping confidence intervals. Calculation of degree-days using data from the nearest Environment Canada weather station located at Emerson, Manitoba (Environment Canada 2020) during the same period resulted in 1715.1 degree-days (standard model) and 1737.0 degree-days (double sine).

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

Table 3. The distance that *Oarisma poweshiek* larvae moved during each relocation (mean and range; total accumulated during larval stages), frequency at which larvae travelled to different host shoots between observed activity bouts, and direction during relocations (mean angle \pm angular deviation) by immatures (number of movements).

Stage	Distance (cm)	Frequency (%)	Direction (°)
Immature 1		90	$n = 1$, not tested
Instar 1 (1)	12.4	100	
Total: 12.4/12 days			
Immature 2			286 ± 46 $n = 7$; $r = 0.677$; $P = 0.032$
Instar 1 (1)	5.6	25	
Instar 2 (1)	6.4	33	
Instar 3 (3)	7.2 (6.1–9.0)	60	
Instar 4 (3)	6.5 (5.2–8.9)	100	
Total:	60.6/60 days		
Immature 3			112 ± 59 $n = 5$; $r = 0.471$; $P = 0.338$
Instar 1 (3)	9.7 (1.3–15.8)	60	
Instar 2 (2)	3.7 (1.2–6.2)	100	
Instar 3 (0)			
Total:	36.5/26 days		

Table 4. Proportion of feeding observations on host plant species among all *Oarisma poweshiek* instars, percent (number of observations).

Immature	<i>Andropogon gerardi</i>	<i>Muhlenbergia richardsonis</i>	<i>Sporobolus heterolepis</i>	<i>Schizachyrium scoparium</i>
1	100 (3)			
2		29 (8)	67 (18)	4 (1)
3			100 (14)	

Microclimate in microhabitats

During the entire *O. poweshiek* life cycle, the relative humidity levels (mean and minimum) were recorded by most data loggers as lower in egg enclosures than in comparison plots (Table 6). The differences recorded by the data loggers occurred most frequently during the active larval period (Supplementary material, Table S4). The temperature and dew point recorded in the egg enclosures typically did not differ from that in the comparison plots during individual development periods or the entire life cycle.

Attributes of egg enclosures and target plots, 2018

The relative abundance (Fig. 5) and counted number (Supplementary material, Table S5) of host plant shoots did not significantly differ between egg enclosures and target plots (Welch's two-sample *t*-tests, $P > 0.05$; 95% confidence interval, confidence interval overlapped). All vegetation species enumerated are reported in Supplementary material, Table S6.

Table 5. Degree-day accumulations in egg enclosures calculated using two models (mean \pm standard deviation) and the number of calendar days required for individual *Oarisma poweshieks* to complete each development stage; only completed stages are displayed. Data from data loggers in all egg enclosures were used ($n = 4$).

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

Graminoid height was shorter, duff percent cover was higher, and shrub percent cover was lower in the egg enclosures than in the target plots (Table 7). Duff depth and the percent cover of graminoids, forbs, and bare soil in the egg enclosures did not significantly differ from those in the target plots (duff depth, percent covers of graminoids and forbs, Welch's two-sample t -tests; $P > 0.05$; percent cover of soil – 95% confidence interval; confidence interval overlapped).

Attributes of egg enclosures and comparison plots, 2019

The estimated proportion of shoots of consumed host species in both the egg enclosures and the comparison plots between 30 May and 5 July 2019 was greater than on 9 May 2019 (Fig. 6). The relative abundance and counted number of host plant species, individually and as a group, also did not differ significantly between plot types on individual sampling dates (30 May, 11 June, and 5 July) nor as a mean from 30 May through 5 July (individually: Welch's two-sample t -tests, $P > 0.05$; 95% confidence interval, confidence interval overlapped; as a group: permutational multivariate analysis of variance tests; $P > 0.05$; Fig. 5; Supplementary material, Table S5).

The graminoid height was significantly lower in the egg enclosures than in the comparison plots on 9 May 2019, whereas the mean depths of duff and litter were significantly shallower in the egg enclosures on all 2019 sampling dates (Table 7). The percent covers of duff, shrubs, living vegetation, and bare soil did not significantly differ between plot types (Welch's two-sample t -tests; $P > 0.05$). The light intensity in the egg enclosures ($n = 6$, mean 71.0% \pm standard deviation 10.9%) did not significantly differ from that in the comparison plots (mean 57.5% \pm standard deviation 11.9%; $t = 2.047$, $df = 9.910$, $P = 0.068$). Soil moisture in egg enclosures was 28.5% \pm standard deviation 9.0% volumetric water content, and electrical conductivity was 0.15 mS/cm \pm standard deviation 0.07 mS/cm in 2018 and 41.9% \pm 6.5% volumetric water content and 0.19 mS/cm \pm standard deviation 0.05 mS/cm in 2019. Soil moisture and electrical conductivity between plot types (2019) did not significantly differ (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 the height of graminoid species we did not observe larvae consuming (*i.e.*, nonhosts) in the egg enclosures did not differ from those in the comparison plots on 5 July 2019 (host plants: $t = -1.351$, $df = 8.371$, $P = 0.212$; nonhosts: $t = -1.243$, $df = 9.548$, $P = 0.243$).

Table 6. The temperature (° C), relative humidity (%), and dew point (° C) during the entire *O. poweshiek* life cycle (mean (95% confidence interval)). Bolded values were significantly different from the values of at least three data loggers of the other plot type.

Plot	Temperature _{mean}	Temperature _{min}	Temperature _{max}
Egg enclosure			
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 enclosure			
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	Dew point _{mean}	Dew point _{min}	Dew point _{max}
Egg enclosure			
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)

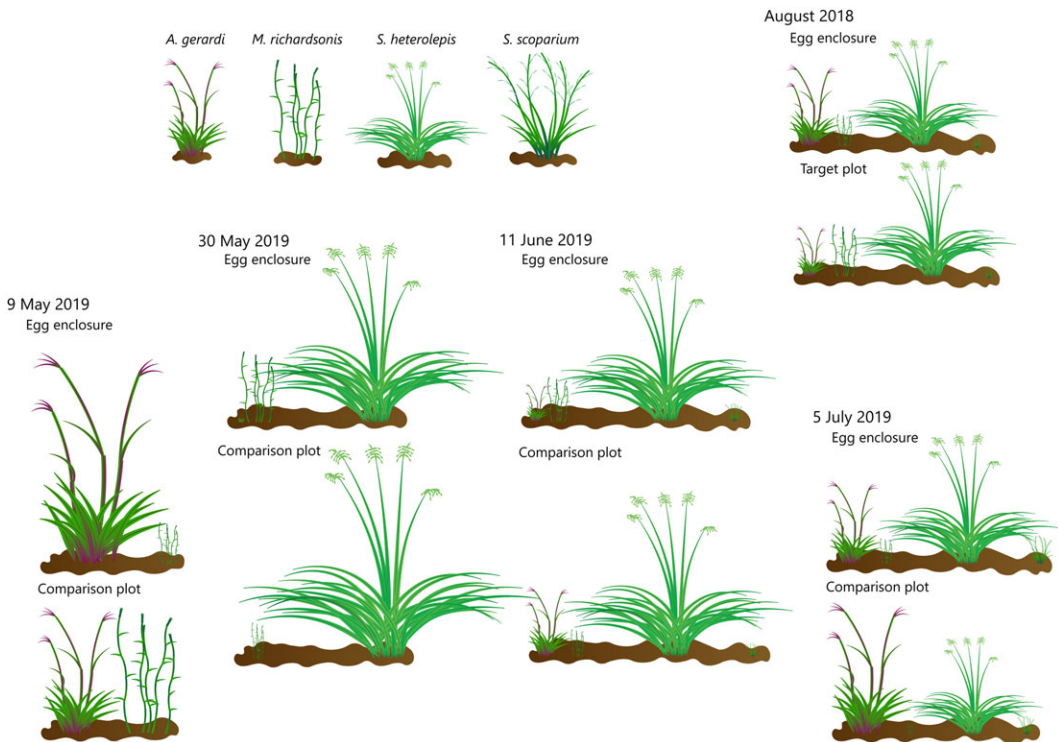


Fig. 5. Relative abundance of host food species in 2018 and 2019. Species are scaled to their respective proportions.

Discussion

Egg laying

Females appeared to receive oviposition stimuli (Singer 1971) on at least three levels. First, while in flight, the presence of suitable host food species for larvae and relatively shallower duff, as suggested by our findings from the egg enclosures *versus* comparison plots, might have attracted females to a general area. Within those initial general areas, smaller areas containing shorter graminoids, sparser shrub cover, and comprehensive duff cover may have then stimulated probing behaviour, as suggested by the egg enclosures *versus* target plots, followed finally by egg deposition or resumption of flight. In other Lepidopterans, sequential stimulations lead to oviposition (Wiklund 1984; Rabasa *et al.* 2005), with egg deposition prompted by chemical stimuli (*e.g.*, nitrogen concentrations) while the insect is in contact with the plant (Singer and McBride 2010; Lund *et al.* 2019).

In the present study, eggs were laid on leaves that resembled grasses, at canopy positions that were relatively closer to leaf tips than to the bases. This was also observed in the female in 2017. As with *Anthocharis cardamines* (Linnaeus) (Lepidoptera: Pieridae) (Dempster 1997), *O. poweshiek* may have been attracted to potentially warmer temperatures in the vegetative canopy that might support egg development.

Attributes of microhabitats

In 2018 and 2019, the host plant diversity did not differ between oviposition locations compared to areas where females did not lay eggs (Fig. 5); however, several physical characteristics did differ (Tables 6–7). *Oarisma poweshiek* microhabitats appeared to be

Table 7. Vegetative and physical characteristics (mean \pm standard deviation) in *Oarisma poweshiek* egg enclosures (each plot type: $n = 6$), target plots, and comparison plots. The duff (cm) at 18 July 2019 represents the thatch component (see [Methods](#)). Measurements at each date followed by different letters are significantly different.

Date	Graminoids (cm)	Duff (cm)	Litter (cm)	Duff (%)	Shrub (%)
2, 3 August 2018					
Egg enclosure	31.7 \pm 3.8 ^a	4.0 \pm 0.9		30 \pm 6 ^b	0 \pm 1 ^a
Target plot	36.5 \pm 1.4 ^b	4.1 \pm 0.7		21 \pm 6 ^a	4 \pm 5 ^b
9 May 2019					
Egg enclosure	5.2 \pm 1.1 ^a	3.9 \pm 0.9 ^a		92 \pm 3	1 \pm 2
Comparison plot	6.9 \pm 1.0 ^b	6.5 \pm 1.9 ^b		93 \pm 2	1 \pm 1
30 May 2019					
Egg enclosure	6.2 \pm 1.0	3.0 \pm 0.9 ^a		87 \pm 5	1 \pm 1
Comparison plot	6.6 \pm 1.0	4.4 \pm 1.0 ^b		90 \pm 3	1 \pm 1
18 July 2019					
Egg enclosure		5.1 \pm 1.2 ^a	1.7 \pm 0.5 ^a		
Comparison plot		8.2 \pm 2.4 ^b	2.3 \pm 0.4 ^b		

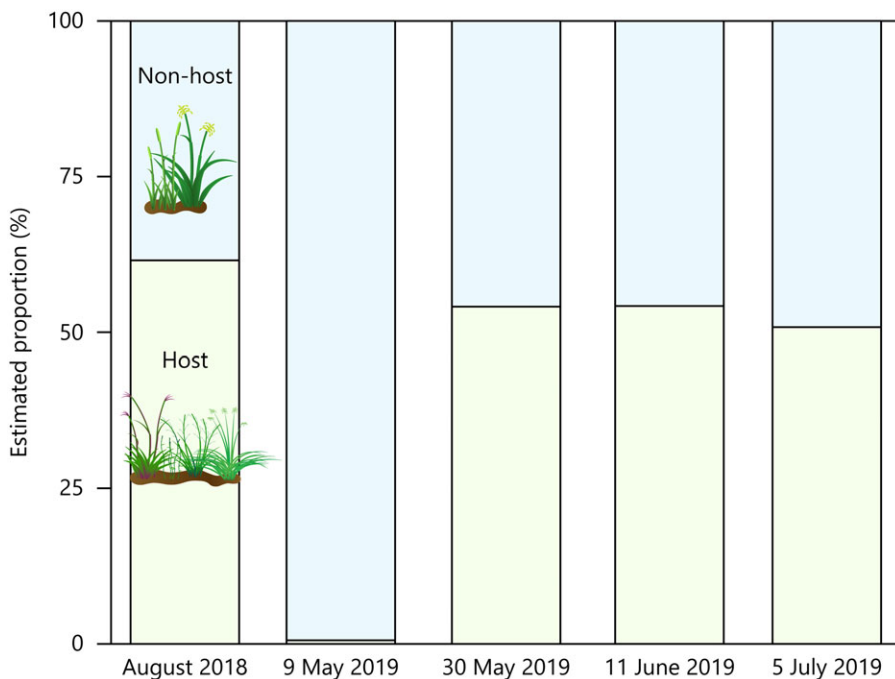


Fig. 6. Estimated proportion of shoots of consumed host species and all other graminoids (nonhost) in only egg enclosures during sampling. Data for August 2018 are calculated from shoot counts.

relatively more open and generally drier (based on shorter vegetation height and lower relative humidity). We cannot determine the degree to which the consequences of clipping vegetation, compared to flattening it, might have caused humidity differences. In grasslands in Europe,

Hipparchia fagi (Scopoli) (Lepidoptera: Nymphalidae) larvae use host grasses isolated on the edges of gaps among vegetation that, overall, may provide drier microclimates for immature development (Möllenbeck *et al.* 2009). In the present study, the light intensity was higher in the egg enclosures (approximately 71%; test not statistically significant) than in the comparison plots (approximately 58%). However, the recorded temperatures did not differ, and the timing of plant growth was not accelerated. We recommend further research to determine whether the intensity of light may influence larval development.

Larval foraging behaviour

Our observations are the first to track the activities of individual *O. poweshiek* larvae in natural habitats. All larvae moved from their natal plant to feed on a different species. Immature 2 varied its graminoid forage (*M. richardsonis*, *S. heterolepis*, and *S. scoparium*) during different instars, but Immatures 1 and 3 each consumed only one species (*A. gerardi* and *S. heterolepis*, respectively) during our observations. Although captive-reared larvae have been observed consuming *S. heterolepis* and *S. scoparium* in the United States of America (Borkin 1995; Dana, personal communication), our direct observations of larvae in natural habitats eating *A. gerardi* and *M. richardsonis* are new across *O. poweshiek*'s range. Although captive-reared larvae fed only one species may still survive (Assiniboine Park Zoo 2018), polyphagy in natural habitats might vary the nutrition, defensive chemicals, and parasites ingested by larvae and thereby confer fitness benefits (Erebidae; Karban *et al.* 2010). These four grasses are consistently associated with *O. poweshiek* across its range (oviposition and larval feeding; Table 1), suggesting a relatively higher facilitation of survival, possibly with additional regionally preferred hosts (*e.g.*, Nymphalidae; Meister *et al.* 2015).

Larvae also relocated over substantial distances to shoots of the same host plant species on which to live, suggesting that factors in addition to host species attract foraging larvae. Relocations appeared to occur more frequently at later instars, and accumulated movements were inconsistently directional (Table 4). Hellmann (2002) reported *Euphydryas editha bayensis* (Sternitzky) (Lepidoptera: Nymphalidae) larvae switching host plants following leaf desiccation. Feeding-induced plant defenses (Roslin *et al.* 2008) may prompt *O. poweshiek* larvae to relocate once the risk of movement outweighs that of eating an activated host as larger, later-instar larvae (*e.g.*, Sphingidae; van Dam *et al.* 2001).

As larvae matured, we observed feeding patterns changing to include notch feeding of various lengths and arrangements, in addition to tip feeding – a combination of patterns that is similar to that of wild larvae in Borkin (1995) and captive-reared larvae in McAlpine (1972). The leaf blade instead of the tip might provide a less exposed and safer location for larger, more visible larvae to feed. We also observed other Lepidoptera (including Geometridae) creating visually similar feeding marks; therefore, we recommend acquiring more direct observations of larval *O. poweshiek* feeding in natural habitats to determine or confirm the species consumed by larvae in extant skipperling populations (including, *e.g.*, *M. richardsonis* in Michigan; Pointon 2015; Belitz *et al.* 2019).

All larvae fed at the tips of leaves near the top of the vegetative canopy (Fig. 3), possibly to take advantage of warmer temperatures at these positions (Pieridae; Jugovic *et al.* 2017). After feeding, larvae rested closer to the ground, possibly to shelter from predators among vegetation (Erebidae; Stamp 1992). Turlure *et al.* (2010) reported larvae of *Boloria aquilonaris* (Stichel) (Lepidoptera: Nymphalidae) occupying sun-exposed hosts to feed and travelling to positions within cool and humid moss hummocks to rest. In the present study, Immature 3, which consistently occupied the north and shaded side of leaves while feeding and resting (south-approaching sun), might have been adjusting its body temperature, as Capinera *et al.* (1980) had observed in a grass-feeding moth. The durations of sequential feeding and resting bouts among Immatures differed, with feeding periods being shorter than resting periods. This suggests that the degree of larval

attraction to a certain location might change as they eat and rest, perhaps influenced by the amount of plant material ingested or, while resting, the degree of digestion.

Ovipositing *Pyrgus malvae* (Linnaeus) (Lepidoptera: Hesperidae) are influenced by both the microclimates and host plant species that are required by larvae (Krämer *et al.* 2012). We think a similar mechanism in *O. poweshiek*, which may instead be influenced by plant architectures, microclimates, and the presence of larval host plants, might result in females laying eggs at locations suitable for the development of eggs but not of larvae. As a result, larvae may reposition to very small areas where suitable host species and microclimates for activities (*e.g.*, feeding and resting) intersect. A “microhabitat” in this case would consist of a fuzzy radius from the location of oviposition, encompassing all suitable intersections that larvae could potentially navigate to. Following local measurements at observed locations of adult oviposition and of larvae, as well as hypothetical three-dimensional mapping and modelling of host plants and microclimates in microhabitats, such a suitable area for each respective life stage within a “microhabitat” may be termed a “nanohabitat”.

Given that we observed that host species emerge and potential larval development threshold resumes above 0 °C at the end of May, we predict that through the last two weeks of May to first week of June, larvae resumed feeding (if postdiapause larvae were to eat the same species). Poweshiek skipperling larvae likely consume species that are phenologically timed (*e.g.*, Pieridae spp.; Posledovich *et al.* 2015) to attain suitable sizes before pupation (*e.g.*, Hesperidae; Rosenwald *et al.* 2017). As environmental conditions, including extreme temperatures and precipitation, change from spring through fall while larvae are active, certain plant species may facilitate larval survival better than others. For example, the nutrients or the physical toughness of their tissues may degrade less readily or the timing and degree of maturation, or cool- or warm-season physiology (Kindscher and Wells 1995), may better support larvae at that time. In addition, we observed the relative shoot proportion of individual plant species changes throughout the year (Fig. 5). Hellmann (2002) reported that environmental changes led to degradation of plant tissue resulting in host switching by *Euphydryas editha bayensis*. Based on this, larval adaptations to navigate microhabitats to locate ephemerally suitable shoots and to digest multiple species would likely confer fitness advantages to *O. poweshiek*. This pattern may explain the polyphagy of graminoid-feeding skippers in natural habitats that have been observed globally (natural habitats: Wiklund 1984; Dana 1991; García-Barros and Fartmann 2009; lab choice: Molleman *et al.* 2020; Nordmeyer *et al.* 2021). The Poweshiek skipperling appears to be attracted to any species that meet its survival needs, including for ovipositing and larval feeding, possibly influenced by availability and seasonal variation in plant quality. This skipperling may be defined most accurately as a varied specialist or limited generalist. Further research that focuses on the larvae’s survival on single host species *versus* a mixture of host species as environmental conditions change could help determine and explain foraging adaptations to prairie habitats.

Because moving larvae in the present study did not circle along the plastic walls of the enclosures and the heights of respective species of both confirmed and potential host and nonhost groups did not differ between insides and outsides of the enclosures, we interpret that the egg enclosures neither limited larval relocations nor detectably altered the growing conditions of plants. However, we suggest that future studies use control enclosures to more accurately determine the effects of microclimates. While we do not encourage disturbing the habitat more than necessary, we think that information that could be generated by installing additional enclosures is worth the risk of minor disturbance.

Larval development

All three immatures completed developmental stages in a similar number of degree-days and calendar days, appearing to substantially increase in amount from the second to third instars

(Table 5). Their development, measured as body length, was consistent with McAlpine (1972). The combined record and estimate of the number of degree-days required by *O. poweshiek* to complete their life cycle in the present study of approximately 1700 degree-days (standard model) or 1725 degree-days (double sine) is reported here for the first time, based on estimated development periods and thermal thresholds and for only one generation.

Immatures required approximately 415 degree-days (standard model) and 385 (double sine) to complete instar 3. Once Immature 2 moulted to instar 4, only approximately 115 degree-days (standard model) and 145 (double sine) were available to be accumulated before winter. McAlpine (1972) reported that captive-reared larvae in Michigan, which is at a relatively lower latitude, diapause in the fifth instar. We think that *O. poweshiek* in Manitoba may have evolved fewer pre-diapause instars – that is, diapausing in instar 4 – or shorter instar durations – that is, moulting to instar 5 before diapause – to compensate for the shorter available season to develop while appropriately timing adult emergence (e.g., Lycaenidae: Fischer and Fiedler 2001, 2002). To accurately determine the developmental schedules, including factors that induce or release diapause, and both the lower and upper thresholds, including degree-day accumulations, of immatures, we recommend similar direct observations during spring and physiological studies of multiple generations of *O. poweshiek*.

Because the degree-day accumulations estimated to complete the *O. poweshiek* life cycle that were calculated using only the double sine model did not significantly differ between data loggers and the weather station, we recommend that conservation partners focus on the double sine model that uses data from confirmed microhabitats or the Emerson station to accurately synchronise adult surveys. Microhabitats of immatures during the survey-synchronisation period accumulated more degree-days than did microhabitats in Dearborn and Westwood (2014; our study: standard model = 635.7 degree-days, double sine = 706.6; Dearborn and Westwood's (2014) study: standard model = 602.8 degree days, double sine = 653.8). This was likely caused by: data logger placements, which in our study were in verified microhabitats and in Dearborn and Westwood's (2014) study were in random locations in prairies where *O. poweshiek* adults had been observed; annual weather fluctuations, which in our study were based on one year and in the 2014 study were a five-year mean; or an unknown factor.

Broader conclusions

Sites in Manitoba that had been intentionally burned approximately three to six years earlier supported relatively higher abundances of Poweshiek skipperlings than did sites with more recent and older burns and sites where grazing took place (Dupont-Morozoff *et al.* 2022). Disturbances such as a cutting action – haying – and prescribed burns may improve the suitability of habitats for butterflies (Swengel 1996; Thom and Daniels 2017) but ought to be compatible with the life history of these same butterflies to reduce mortality (Swengel and Swengel 1999, 2015). Our findings regarding seasonal larval positioning of Poweshiek skipperlings suggest that haying in late summer or fall, when larvae are relatively closer to the ground, instead of in mid-summer, when the eggs or larvae are located in the vegetative canopy, may positively influence the vegetative components of habitats without directly displacing or destroying larvae (e.g., grazing for Pieridae; Nippen *et al.* 2021). To determine conservation disturbances that create or maintain the intersection of microhabitat attributes that are suggested by our study and are compatible with other species at risk, we encourage future research.

Our findings suggest foraging by *O. poweshiek*, and possibly other species, has multiple components that likely increase fitness. These components are likely: nutrition or diverse diet; sensing quality hosts if or when conditions change; and the ability to move and navigate over distance, at different frequencies, over substrates. It is possible that, over the last few decades, factors, such as habitat management, climate, or other wildlife, that facilitate these foraging

traits have changed, resulting at least temporarily in reduced larval fitness and leading to population decline. Although the small number of individuals in the present study limited our capacity to develop statistical inferences, our findings could be used to inform habitat stewardship and reintroduction *via* captive-rearing and site-assessment efforts. With the findings from the present study providing an improved understanding of adult oviposition behaviours, larval foraging patterns, and microhabitat attributes, we can continue narrowing the list of possible causes of decline of *O. poweshiek* and generating potential solutions.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.4039/tce.2022.34>.

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