The role of ambient temperature in the ontogeny of endangered *Oarisma poweshiek* and their relative *O. garita* reared *ex-situ* at Assiniboine Park Zoo, Manitoba

by

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ABSTRACT

Regionwide extirpations of Poweshiek skipperlings (*Oarisma poweshiek*) have prompted an international conservation effort to understand the causes of their decline and to recover the species. However, aspects of their basic biology remain unknown: in particular the role of temperature in their development and their sensitivity to climate change. I studied degree day (DD) accumulations in Poweshiek skipperling and its sister species, Garita skipperling (*O. garita*), from egg hatch to eclosure. I calculated the DDs accrued by both species reared from 2017 to 2020 at Assiniboine Park Zoo, Manitoba, and compared them between generations and species. I also compared the variability in their hatch dates, the start and end of overwintering, pupation dates, and eclosure dates for each generation and their ages (in days) at each stage. I used thermal upper and lower development thresholds of 32 °C and 6 °C, respectively, using the standard and double-sine method to calculate degree-days as well as using the double-sine method without an upper threshold.

I calculated similar DDs for both species, as well as similar averages within generations, hatch and eclosure dates and final ages at eclosure. However, Garita skipperlings exhibited substantially more DD accumulation variation, within and between generations, except for the pupal duration where Poweshiek skipperlings were more variable. Poweshiek and Garita skipperling DDs were more variable between generations than within generations, suggesting that variables other than temperature (such as photoperiod) may influence the synchronization of adult emergence. While Poweshiek and Garita skipperlings had similar eclosure dates, final ages, and DD accumulation across the generations, the larger variation observed in most developmental stages in Garita skipperling could suggest that they are more resilient to the effects of climate change.

I also reared Garita skipperling larvae in constant, elevated temperatures and compared their growth and survivorship to larvae reared in natural, diurnal temperatures. I measured head capsules widths with an ocular micrometer and determined the total number of instars for Garita skipperling that survived to eclosure. Larvae were grouped using Dyar's Values, without assuming Dyar's Rule, then analyzed with k-means clustering to estimate the instar at each measurement. Garita skipperlings eclosed after five, six, or seven instars, and did not maintain a consistent size ratio between instars as assumed by Dyar's Rule. While larvae with five, six, or

seven instars were observed surviving to eclosure in both diurnal and static temperature regimes, the affect of treatment on larval instar number was indeterminable. These results cast doubt on the generalizability of Dyar's Rule in instar determination for these species and reinforce previous authors' conclusions that using statistical analysis without applying Dyar's Rule may be more accurate.

Experimental temperature regimes were used to investigate the affect of elevated temperatures on growth of Garita skipperling. Larvae that were reared in these trials were placed into environmental chambers held at 28 °C, 21 °C, or reared outdoors in the control. Two groups of larvae were added to the 28 °C environmental chamber: neonates added by the beginning of August, and larvae added at the end of August (Late 28 °C, hereafter), after several weeks of development. Only neonates were placed into the 21 °C chamber and into the control group. I compared the survival, final ages (in days), and DD accumulation of the larvae that survived to eclosure in each treatment. The 21 °C-trial had 15% lower and the 28 °C trials 6% higher (or 57% higher for late inductees) survivorship versus larvae reared outdoors. However, neonate mortality could not be calculated for late inductees to the 28 °C-trial. Temperature appeared to influence the differences between my 28 °C trial and control. The results of the 21 °C treatment are difficult to interpret as potential equipment failure likely reduced the survival of this treatment group. Although there were two treatment groups, the phenology of almost all larvae in the 28 °C chamber was extremely accelerated and almost all survivors eclosed in the year they hatched. All other larvae that survived to adulthood, regardless of treatment, eclosed at the same time as the control group. Larvae that eclosed in a single season and larvae in the control had comparable DDs. Larvae in both treatments that eclosed after overwintering had substantially higher DDs at eclosure than the control group. Photoperiod may provide cues to prepare for hibernation, and when to eclose, thus may be responsible for the similar eclosure dates of larvae that overwintered in all treatments. However, its role was not estimable because photoperiod was matched in the treatments and control.

Poweshiek and Garita skipperlings currently have similar ontogenies in Manitoba, but the greater variability in the development of Garita skipperling seen in this study, and their longer wild flight period, suggests an increased resilience to climate change. My temperature manipulation experiments provide evidence that high static temperatures will accelerate most larvae exposed within several weeks of hatching, causing them to eclose before overwintering, and suggests that rapid climate change could be a factor in loss of Poweshiek skipperling populations as they have lower variability in their developmental rates. My results also suggest that overwintering may act to “reset” development so that individuals emerge in synchrony with conspecifics, also suggesting added resilience in Garita skipperling. Further research on the precise DD accumulation thresholds throughout development for both species is needed to determine the risk and severity of climate change induced phenological shifts in Poweshiek and Garita skipperlings.

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The populations used in this study were sourced from wild butterflies residing on Treaty 1 territory — traditional lands of the Anishinaabe, Cree Nation, Oji-Cree, Dene, and Dakota and homeland of the Métis Nation both before and since colonization — and was conducted in facilities built upon same. I recognize, and thank, these groups for their longstanding stewardship of the land.

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LIST OF ABBREVIATED TERMS

1. Lower thermal threshold – LTT
2. Upper thermal threshold – UTT
3. Degree-day – DD
4. Assiniboine Park Zoo – APZ
5. Confidence interval – CI
6. Nature Conservancy Canada – NCC
7. Tall grass prairies – TGP
8. Tall Grass Prairie Preserve – TGPP
9. Environmental control – EC
10. Dyar’s value – DV

CHAPTER 1: LITERATURE REVIEW

Poweshiek skipperling habitat, biology, and ecology

The Poweshiek skipperling, *Oarisma poweshiek* (Parker, 1970), is a critically endangered insect native to North America (Schweitzer and Opler, 2023). Following the loss of most of North America's tall-grass prairies (TGP), their populations were fragmented, but stable, across the remnants of TGP habitat (Henault & Westwood, 2022; Henault & Westwood, 2023). However, in recent decades they have become extirpated across most of their remaining range in the United States (Selby, 2005; Cuthrell and Slaughter, 2012; Pogue *et al.*, 2016). Historically, the TGP of North America formed a wide, mosaic, landscape stretching from south central Manitoba to the border of Mexico, and from Montana into North Dakota and Wisconsin (Sampson and Knopf, 1994; Harris and Foster, 2014). This once-expansive prairie ecosystem has been all but eradicated following centuries of agricultural expansion, urbanization, and other anthropogenic activities (Selby, 2005; Swengel, 2008; Cuthrell and Slaughter, 2012; Pogue *et al.*, 2016). Today, less than 1% of North America's TGP remain, with only isolated fragments still in existence, and it is considered one of the most imperiled habitats worldwide (Sampson and Knopf, 1994; Swengel, 2008).

Poweshiek skipperlings began to vanish across much of their remaining range in the north-central U.S. and Canada starting in the mid to late 1990's (Swengel, 2008; Poweshiek Working Group, 2011; Pogue *et al.*, 2016). From 1988–1997, approximately 2,400 Poweshiek skipperlings were observed across 20 sites in Iowa, Minnesota, and North Dakota (Swengel and Swengel, 1999), but the species is now considered extirpated in all three states (Runquist and Nordmeyer, 2018). In Manitoba (the only Canadian province they inhabit), 322 Poweshiek skipperlings were observed in 2008 and 79 in 2009 (Dupont-Morozoff *et al.,* 2022), increasing to 238 in 2018 (Belitz *et al.,* 2018). In Iowa, 352 Poweshiek skipperlings were observed across all sites, with 624 recorded in Minnesota, 56 in North Dakota, and populations in Illinois, Nebraska, Ohio, and Wisconsin totaling 112 prior to being extirpated from each state (Belitz *et al.,* 2018). The species is currently classified as endangered in Canada and the United States, and at the state or provincial level in all jurisdictions with known populations (Schweitzer and Opler, 2023).

Poweshiek skipperlings are small, orange-coloured skippers, approximately 25 mm in wingspan, with low mobility compared (Burke *et al.,* 2011) to most other Hesperiidae (Scudder, 1872). Poweshiek skipperlings are considered obligate dwellers of mesic TGP and prairie fens with comparable TGP components: i.e., wetland-rich grasslands with dry and mesic zones (Pogue *et al.*, 2016). Field studies indicate that adult Poweshiek skipperlings are highly specific in their choice of habitat; even within tall-grass prairie ecosystems (Summerville and Clampitt, 1999; Hooshmandi, 2016; Henault & Westwood, 2022; Henault & Westwood, 2023). In Michigan, they have only been documented within a small region of prairie fens, 10–15% of the overall habitat, with marly (carbonate-rich mudstone) substrate and are absent from the adjacent, *Carex*-dominated habitats, despite an abundance of nectar sources (Summerville and Clampitt, 1999). This may be attributable to tall forbs and/or shrub encroachment in the rest of the fen habitat producing undesirable habitats for adults or their offspring versus the areas with marly substrate. Irrespective, this emphasizes the high degree of habitat-specificity the Poweshiek skipperlings exhibits: they require wetland-dominated prairies on a suitable substrate with a heterogeneous assemblage of sedges, rushes, grasses, and short forbs (Summerville and Clampitt, 1999; Pogue *et al.,* 2016).

Female Poweshiek skipperlings have been observed *in-situ* ovipositing onto the stems and blades of Big Bluestem, *Andropogon gerardi* (Vitman, 1972), Prairie Dropseed, *Sporobolus heterolepis* (Gray, 1857), Mat Muhly, *Muhlenbergia richardsonis* (Rydb), and Little Bluestem, *Schizachyrium scoparium* (Michx.), in both Canada and the United States (Borkin, 1994; Pogue *et al.*, 2016; Henault & Westwood, 2022; Henault & Westwood, 2023). There is some evidence females' oviposition preferences are based upon the thread-like ends of some grass species tips rather than larval host-plant preferences (Dana, 1991). Females have also been observed depositing eggs onto plastic, rocks, flower stems, and synthetic meshes during *ex-situ* oviposition trials (Breiter and Burns, 2018). To date, Henault and Westwood (2022) is the only detailed study that has been conducted on *in-situ* larvae in Manitoba. They observed Poweshiek skipperling larvae move between multiple species of host plant over the course of development: focusing on Prairie Dropseed, Mat Muhly, Big Bluestem, and Little Bluestem (Henault & Westwood, 2022). While the specifics of their host plant requirements remain unclear, larvae have only been successfully reared *ex-situ* on Prairie Dropseed or a combination of Prairie Dropseed, Big Bluestem, and Little Bluestem (Breiter and Burns, 2018).

Poweshiek skipperlings are a univoltine species — having a single generation per year — that overwinter as larvae. Captively reared individuals are overwintered after approximately four months of development (Breiter and Burns, 2018) and it is thought that Poweshiek skipperling larvae undergo hibernation during their fourth larval instar (Henault & Westwood, 2022). Larval hibernation may help to synchronize the emergence of a particular population the following spring or summer by suspending development at a specific stage, and thus ensuring that they pupate and eclose at similar times the following year (Scott and Epstein, 1987). The flight period for Poweshiek skipperling's begins from late June to mid July, depending on degree-day accumulation (Holzman, 1972; Swengel and Swengel, 1999; Dearborn and Westwood, 2014). However, the first emergence of adults can be somewhat erratic between years: varying by as many as 24 days in Manitoba (Dearborn and Westwood, 2014).

As adults, Poweshiek skipperlings exhibit a much higher degree of specificity to nectar-sources than most skippers (Cuthrell and Slaughter, 2012). They have exclusively been documented feeding on native prairie-flowers, including False Sunflower, *Heliopsis helianthoides* (Rydb.), Black Samson, *Echinacea angustifolia* (de Candolle 1813), Blackeyed Susan, *Rudbeckia hirta* (Linnaeus, 1753), Shrubby Cinquefoil, *Dasiphora fruticosa* (Rydb.), Sticky False Asphodel, *Triantha glutinosa* (Michx.), and Northern Bedstraw, *Galium boreale* (Linnaeus, 1753), in the United States (Holzman, 1972; Swengel and Swengel, 1999; Cuthrell and Slaughter, 2012). Canadian populations have predominantly been recorded nectaring from Blackeyed Susan but have also been observed using Wild Liquorice, *Glycyrrhiza lepidota* (Nutt), Ragwort, *Packera* sp. (Á. Löve and D. Löve, 1976), Upland White Aster, *Solidago ptarmicoides* (B. Boivin), and Self-Heal, *Prunella vulgaris* (Linnaeus, 1753), as nectar-sources (Breiter and Burns, 2018; Dupont-Morozoff *et al.*, 2022; Henault & Westwood, 2023).

Poweshiek skipperling threats

Poweshiek skipperling are endangered globally, partly due to their single, short, flight-period (which limits their fecundity) and their habitat specificity within the imperiled TGP. They are considered very vulnerable to stochastic events such as extreme weather (USFWS, 2011; Harris and Foster, 2014, Pogue *et al.*, 2016). Other suspected threats are changes to and/or destruction of TGP habitats, and alterations to adjacent landscapes that affects hydrology and land cover (e.g., agriculture and roads) (Pogue *et al.*, 2016).

Threats also include invasive plants like Narrowleaf Cattail, *Typha angustifolia* (Linnaeus, 1753), and Common Reed, *Phragmites australis* (Cav. Trin. ex Steud, 1996). These invasives are gaining footholds due to agricultural runoff, seepage from drainage fields, and septic tanks adjacent to some habitats in Michigan (Spieles *et al.*, 1999; Poweshiek Recovery Group, 2018). Encroachment by woody species has also been documented in most sites in Canada and the United States (Poweshiek Recovery Group, 2018). Additionally, pesticides, improper land management techniques (e.g., haphazard burning), the spread and introduction of pathogens, and now climate change are all considered potential threats to the Poweshiek skipperling (Selby, 2005; Poweshiek Recovery Group, 2018).

Poweshiek skipperling are highly synchronous in their flight period, even compared to many other univoltine species, with all adults eclosing and dying within an approximately two-to-three-week span annually (Dearborn and Westwood, 2014; Dupont-Morozoff *et al.,* 2022). Currently, no example of climate-associated phenological advancements have been recorded in *Oarisma sp.*, but subarctic moths in Finland and Sweden have exhibited phenological responses to changes in climate (Valtonen *et al.*, 2011; Navarro-Cano *et al.,* 2015). Numerous insects in the Canadian Arctic similarly exhibit metabolic responses to increasing body temperature, with some regulating their ontogeny and phenology by seeking out cooler or warmer microclimates (Danks *et al.*, 1994). The desynchronization of host plants and insects (and other taxa) has been observed across a whole ecosystem in Kivach, Russia over the last four decades, as species-specific phenological shifts have occurred at different rates in response to rising temperatures (Ovaskainen *et al.,* 2013). Thus, climate change is increasingly being viewed as a potential, significant, threat to Poweshiek skipperlings (Poweshiek Recovery Group, 2018), as rising temperatures may impact their highly synchronized, univoltine, lifecycle: e.g. via phenological shifts.

Although habitat destruction has mostly abated, it is considered the primary culprit of its initial range decline (Selby, 2005; Poweshiek Working Group, 2011). Continued changes and habitat degradation are considered significant threats to the long-term survival of remaining populations (Selby, 2005; Poweshiek Working Group, 2011; Pogue *et al.*, 2016). Hydrological changes to groundwater, mostly due to agricultural and/or residential uses causing depletion of the water table, are considered a significant threat to the diverse matrices of supporting flora found in Michigan's prairie fens (Spieles *et al.*, 1999). Within Canada, all known occurrences of Poweshiek skipperlings are limited to TGP preserves maintained by Nature Conservancy Canada and other organizations (Breiter and Burns, 2018). While the habitat is protected by Nature Conservancy Canada in Manitoba, pesticide drift from agriculture is considered a possible threat for some populations (Poweshiek International Workshop, 2019; Dupont-Morozoff *et al.,* 2022).

To combat the Poweshiek skipperling decline, an international consortium of governmental, non-governmental, conservation, and research organizations (e.g. Nature Conservancy Canada, U.S. Fish and Wildlife Services, Assiniboine Park Zoo, Minnesota Zoo, John Ball Zoo, The University of Winnipeg, Central Michigan University, and others) have established a series of *in-situ* and *ex-situ* conservation and research initiatives and working groups (Poweshiek International Workshop, 2019). All known habitats with extant populations of Poweshiek skipperling in Manitoba are on actively managed lands under the guidance of the Manitoba Tall Grass Prairie Preserve management committee with representatives from governmental and non-profit conservation organizations. The U.S. state and federal organizations work with zoos, universities, and municipalities to manage known populations. *Ex-situ* initiatives, consisting of headstarting, captive rearing, and, most recently, captive breeding, are being conducted at Assiniboine Park Zoo and Minnesota Zoo (Poweshiek International Workshop, 2019).

Garita skipperling habitat, biology, and ecology

The Garita skipperling, *Oarisma garita* (Parker, 1970), occupies a much wider range across North America than the Poweshiek skipperling. It is found as far east as the Manitoulin District in Ontario and as far westerly as the grasslands of southeastern British Columbia, while in the United States its eastern range becomes restricted with decreasing latitude and extends as far south as New Mexico and Arizona (Wilson, 2023). This range is discontinuous but is larger than the Poweshiek skipperling's historic range (Schweitzer and Opler, 2023). Although Garita skipperling was previously thought to be common east of the Rocky Mountains, many of the records from its eastern range appear to be misidentified Poweshiek skipperling (Catling, 1977).

More recent research indicates that Garita skipperling's historic range is smaller than earlier estimates (Catling, 1977). Garita skipperlings are more widely dispersed in Canada but largely restricted to the grasslands of the Rocky Mountains and middle-western prairies of the United States (Catling, 1977; Harris and Foster, 2014). In contrast, Poweshiek skipperlings were historically common across the TGP of middle to eastern North America as far south as Mexico (Catling, 1977; Harris and Foster, 2014).

Garita skipperlings are small butterflies, with brown-orange wings approximately 20 mm in wingspan. While they were often misidentified as Poweshiek skipperlings, Garita skipperlings are distinguishable by the grey bands on the underside of their ventral hindwings, and the absence of silvery-white hairs that characteristically follow the veins on the underside of Poweshiek skipperling's ventral hindwings (Klassen *et al.*, 1989; Layberry *et al.*, 1998). Little is documented about Garita skipperling larvae, though it is stated in the *Butterflies of Canada* that Garita skipperlings overwinter in their fourth larval instar (Layberry *et al.,* 1998). However, the source of this information is not included.

Garita skipperlings are restricted to grasslands with moderately wet zones dominated by a variety of grasses like Elliptic Spikerush, *Eleocharis elliptica* (Kunth, 1837) and sedges, *Carex* sp., which are typically adjacent to higher elevation, moderately dry, zones dominated by Prairie Dropseed and Bluestems (Catling, 1977). Field surveys have shown that Garita skipperlings specialize in prairie habitats composed of many of the same species of grasses and nectar sources found in Poweshiek skipperling habitats. Both are typically dominated by a diverse assemblage of sedges, Elliptic Spikebrush, bluestems, *Andropogon* sp., and Prairie Dropseed, *S. heterolepis* (Holzman, 1972; Catling, 1977; Catling and Lafontaine, 1986). The frequency of wetland-dry prairie matrices appears to be the principal difference in their habitats: Poweshiek skipperlings require heterogeneous tallgrass prairies with a mixture of wet, dry, and mesic zones, while Garita skipperlings are found in drier areas with comparatively short vegetation and sparser, or more ephemeral, wet and mesic zones (Catling, 1977; Catling and Lafontaine, 1986; Pogue *et al.*, 2016).

Garita skipperling threats

While the species is ranked Secure (G5) overall and is thought to have a sufficient range and population to be insensitive to stochastic weather and other range-encompassing threats (Wilson, 2023), the potential effects of climate change are unknown as the species' sensitivity to phenological shifts, thermal tolerances, etc. have not been assessed. In Canada, Garita skipperling are common within the short, tall, and mixed-grass Prairie Zones of southern Alberta and Saskatchewan, and into southwestern Manitoba (Klassen *et al.*, 1989; Layberry *et al.*, 1998), and are considered Secure or Apparently Secure across this range (Wilson, 2023). While a sole population exists in Manitoulin District, Ontario, it is proposed by Catling (1977) that it either represents an artifact of its pre-glaciation range, or a possible eastern range expansion via anthropogenic corridors. It considered Critically Endangered in Ontario due to the size of this population (Wilson, 2023). The majority of Garita skipperlings range in the United States has not been assessed, aside from Washington (Vulnerable), Minnesota (Threatened), and Nebraska (Threatened) (Wilson. 2023).

Larval ontogeny and ambient temperature

Insect growth and development is not dictated by purely temporal factors (i.e., a set length of time required for development), though the phenology of many insects follow seasonal trends (Diamond *et al.,* 2011). Instead, insect ontogeny is variable and ultimately governed by the interaction of abiotic and biotic factors. The role of temperature in lepidopteran larval development (and insect growth in general) is well-established (Clench, 1966; Pivnick & McNeil, 1986; Kingsolver, 2000; Oliphant *et al.,* 2013). A lower temperature threshold (LTT) exists at each stage of development below which no development occurs. There is a positive association between the rate of development and temperature above their LTT until temperatures approach and/or exceed their upper thermal threshold (UTT), at which point growth is reduced and often mortality increases (Clench, 1966; Scott and Epstein, 1987; Diamond *et al.,* 2011).

Assuming their nutritional requirements are met, the ambient temperatures experienced over an insect's development dictates their developmental rate and the subsequent timing of their pupation and eclosure, along with any cues from photoperiod (Clench, 1966; Pogue *et al.,* 2016). Researchers employ degree-day (DD) models to predict the timing of an organism's life-stages, e.g., the start of their flight period, by treating a life stage's occurrence as a function of time and ambient temperature (Perkins, 2007). This relationship is used to determine the degree-days accrued by the species by totalling the intervals where daily temperatures rise above a lower thermal threshold and remain below an upper thermal threshold across development (Perkins 2007).

If the UTT or LTT of a species is unknown, a comparable species' can be used as a surrogate (Perkins, 2007; Dearborn and Westwood, 2014). The DD accrued between the developmental stages of interest (e.g. between hatching and eclosure) can then be used to predict the time between these stages for future generations or other populations based on weather data or forecasts (Perkins, 2007). Degree day modelling tends to produce more accurate predictions than date-based predictions for species where temperature dictates the timing of specific life-stages (Perkins, 2007). However, DD modelling can produce errors, depending on the quality of data available and the biology of the species in question (Perkins, 2007; Moore *et al.,* 2012; Cayton *et al.,* 2015).

Degree day modeling has some nuances based on the species of interest. Including a UTT to predict life-stage in regions is common where higher temperatures are frequent, while often only a LTT is used in cooler areas where insects' development is arrested seasonally at the onset of winter (Perkins, 2007). As temperatures rise globally, models which do not incorporate a UTT may become less accurate in areas where they have not been needed historically (Moore *et al.,* 2012). Temperature and development typically have a non-linear relationship towards the upper and lower thermal thresholds, where development begins to be increasingly negatively impacted (Perkins, 2007). Nonetheless, linear models are generally accurate when daily temperatures are between these extrema (Perkins, 2007). Some insects exhibit spikes in their growth and development at temperatures between their thermal thresholds, likely due to the activation energy requirements of metabolic enzymes, this may limit the accuracy of linear models for some species (Danks *et al.*, 1994; Moore *et al.,* 2012).

Researchers in multiple disciplines use DDs for specific purposes. Conservation scientists depend on the ability to predict the timing of various life stages of the Poweshiek skipperling to determine when adults will eclose, and thus when to conduct field-surveys or collect eggs for *ex-situ* rearing of the hatchlings. The majority of degree day modelling for

Lepidoptera has focused on pest species, i.e., to increase the precision of pesticide applications or map their potential distribution based on thermal tolerances (Cayton *et al.,* 2015). Degree day modelling has also been applied to a variety of skippers like *O. poweshiek*, Silver-spotted Skipper, *Epargyreus clarus* (Cramer, 1775)*,* Least Skipper *Ancyloxypha numitor* (Fabricus, 1793)*,* and Essex Skipper, *Thymelicus lineola* (Ochsenheimer, 1808), and been found to reliably predict their flight periods (Dearborn and Westwood, 2014; Cayton *et al.,* 2015).

While DD models have been useful for predicting life stages, models based on historic data may be less accurate as the climate rapidly warms and becomes more variable (Moore *et al.,* 2012). Biological and ecological traits can also affect the reliability of DD models. Multivoltine Lepidoptera with minimal host plant and/or ecological specialization, and/or mobile overwintering forms (i.e., adults or larvae) have been shown to deviate more from DD models' predictions than univoltine specialists which overwinter as eggs or pupae (Cayton *et al.,* 2015). Although it can be less reliable in some species, DD modelling often predicts emergence, or peak abundance, better than date-based predictions; even for species with biological traits that impair the accuracy of DD models like multivoltine generalists (Cayton *et al.,* 2015). Sex can also impact the accuracy of DD models in some organisms, possibly due to the earlier eclosure of males in some species (Pivnick and McNeil, 1986; Dearborn and Westwood, 2014), which can impact DD modeling's accuracy if it is not incorporated (Cayton *et al.,* 2015). Uncertainty in the LTT and total DD required for development can affect the reliability of predictions based on degree-days, particularly for species whose development is less intimately tied with temperature (Moore *et al.,* 2012). Thus, refining the role of temperature in larval growth is important for optimizing *ex-situ* conservation efforts like captive-rearing.

Poweshiek and Garita skipperlings are both specialists (ecologically and with respect to host plants) and univoltine. Predictably, DD modelling is an appropriate technique for examining their ontogeny. While DD modelling in both Michigan and Manitoba have proved highly effective for determining Poweshiek skipperlings flight periods (Dearborn and Westwood, 2014; Breiter and Burns, 2018; Poweshiek International Workshop, 2019), the thermal requirements for Poweshiek, and Garita skipperlings larval development before hibernation are not as well-researched. Adult Poweshiek skipperlings tolerate a wide temperature range while remaining active: from 14 – 32 °C (Swengel and Swengel, 1999). This is likely true for all life stages, as their historic range spanned multiple latitudes (Schweitzer and Opler, 2023). The thermal ranges for many insects are known to vary between life stages (i.e., between egg, larva, and pupa), however no temperature thresholds have been established experimentally for Poweshiek skipperlings (Perkins, 2007). Garita skipperlings likely tolerate a similar temperature range as adult Poweshiek skipperlings, given their geographic range (Wilson, 2023), but this has not been confirmed experimentally. Research on the development of these species would likely benefit from investigation of their degree-day requirements, thus a review of how degree days are calculated and used is warranted.

Calculating degree-days

The standard method to calculate daily DDs is by subtracting a LTT from an average of the day's temperature (simply calculated by subtracting the minimum temperature from the maximum) via the following formula:

$$DD = \frac{(T_{max} - T_{min})}{2} - LTT$$

More complex approaches involve fitting daily maxima and minima to a sine function then using the integral between a LTT and UTT to calculate the DDs for each day, such as the double-sine method (Dearborn and Westwood, 2014). Dearborn and Westwood (2014) determined the DD accrued by Poweshiek skipperlings in Manitoba using temperature data from the weather station nearest to known populations from the beginning of March until the first observed emergence of adult Poweshiek skipperlings.

Based on the known developmental thresholds of another local lepidopteran species, Spruce Budworm, *Choristoneuras fumiferana* (Clemens, 1865), which also overwinter as larvae Dearborn & Westwood used an LTT of 6 °C (Dearborn & Westwood, 2014) in calculating DDs for Poweshiek skipperling. Using the LTT of 6 °C, Dearborn and Westwood (2014) calculated found a mean DD accumulation of 602.8 (± 65.2 degree-days) when the standard method was used, increasing to 653.8 (± 67.1 degree-days) using the double-sine method, for Canadian Poweshiek skipperlings from the start of March to the beginning of eclosure in June or July. When using data logged by an on-site weather station, these values changed to 647.4 (± 70.5 degree-days) and 689.4 (± 69.5 degree-days) for the standard and double-sine models.

Despite some differences, both models successfully predict the emergence of Poweshiek skipperlings in Manitoba within 48 hours (Breiter and Burns, 2018). The method developed by Dearborn and Westwood (2014) has been used to predict adult emergence of multiple generations of Poweshiek skipperlings and has been applied by researchers at Assiniboine Park Zoo and Minnesota Zoo to inform the timings of their conservation initiatives for Poweshiek skipperlings (Breiter and Burns, 2018; Runquist and Nordmeyer, 2018). Henault and Westwood (2022) found a mean of 1700 degree days using the standard method and 1723.9 with the double-sine method were accrued between the first egg laid and first adult that eclosed, using data loggers placed into the enclosures surrounding *in-situ* Poweshiek skipperlings eggs. Similar values (1693.4 and 1722) were observed in comparison plots without eggs or larvae (Henault & Westwood, 2022).

Climate change and phenological shifts in butterflies

The effects of climate change on the survival and phenology of lepidopterans are the focus of numerous studies (Crozier, 2003; Hellmann *et al.,* 2008; Kearney *et al.,* 2010; Navarro-Cano *et al.,* 2015). In particular, the phenomena of phenological advancements in emergence dates, and extended seasons for northern and alpine butterflies have been observed, with climate change being implicated as the driver (Karlsson, 2014). The current effects of climate change, and those predicted under various climate change scenarios, vary among species depending on numerous variables including their: life-history traits (i.e., the number of generations per year, overwintering stage), range, and host plant-specificity (Kearney *et al.,* 2010; Valtonen *et al.,* 2011; Karlsson, 2014). Numerous butterfly species in temperate climes have displayed phenological advancement, however those with limited ranges, greater host plant specificity, and immobile overwintering stages are considered more susceptible to phenological advancement than other species (Diamond *et al.,* 2011; Diamond *et al.,* 2014). The effects of climate change are also dependent upon organisms' dispersal capacity, ecological breadth, and any pre-existing reductions in their populations' fitness (e.g. following a bottleneck), with phenological responses following climatic gradients (Buckley and Kingsolver, 2012).

In Manitoba, the average number of days with a mean temperature above 30 °C per annum has risen from 14.0 days between 1976–2005 to 25.0 by 2020 (Blair et al., 2019). Climatological forecasts indicate that number will increase over the span of 2021–2050 to 39.4, averaging 31.1 days across this interval, with an expected rise in the total number of degree-days above 5 °C of over 20%: from 1722 to 2105 (Blair et al., 2019). Between 2020 and 2050, the mean annual freezing degree-days (the sum of mean temperatures for days where temperatures stay below 0 °C) are predicted to decrease by over 20% from 1358 to 1054 (Blair et al., 2019). These changes from historical climatic norms could cause severe temporal asynchronies between native species with different upper and/or lower thermal thresholds, optima, and growth rates, such as host plants and larvae within Manitoba (Diamond *et al.,* 2011; Forrest and Thomson, 2011).

Research on the Poweshiek skipperling response to temperature is limited compared to studies on other Arctic and temperate Lepidoptera, but inferences drawn from such studies suggest that species like Poweshiek skipperlings are especially vulnerable to climate change. The limited range, habitat and host-specificity, and life-history traits of Poweshiek skipperlings have all been associated with greater phenological shifts in other lepidopterans than species lacking these traits (Diamond *et al.,* 2011; Valtonen *et al.,* 2011; Diamond *et al.,* 2014). It is thus crucial for conservationists to investigate the immediate and long-term effects of climate change on Poweshiek skipperlings.

Larval instars and insect ontogeny

Instar refers to the stages between the shedding of an organism's exoskeleton (moults) in which larval growth and development occur. Although many insect larvae are soft-bodied and can expand some regions of their bodies between moults, all members of superphylum Ecdysozoa (Ruggiero *et al.*, 2015) must periodically shed any rigid chitin or calcified regions of their exoskeletons to increase their volume (Dyar, 1890; Hawes, 2019). The number of instars is an important feature of insect biology as it defines the number of discrete, ontogenic, stages that occur throughout larval development. This has a significant bearing on accurately modelling population dynamics and phenology in insects (Cazado *et al.,* 2014), since the number of instars required to reach adulthood and biotic and abiotic requirements of each instar dictate their ontogenic rate (Grunert *et al.,* 2015). In general, larval ontogenic rates and body size are highly correlated to their fitness, with reductions in developmental rate and size being associated with reduced fitness (Grunert *et al.,* 2015). Modelling ontogeny via instars is used in a variety of applications in entomology and arthropodology: e.g. to predict the timing and/or severity of agricultural pests (Castañeda-Vildózola *et al.,* 2016) or optimize the rearing and conservation of insects (Gold *et al.,* 1999; Diaz *et al.,* 2019). It has also been used in paleoentomology to classify trilobite fossils into instars (Hunt and Chapman, 2001).

For many insects, especially species with soft-bodied larvae, instar number is determined by measuring the width of their head capsules, which can only increase in size shortly after moulting when the chitin is soft (Cazado *et al.,* 2014; Grunert *et al.,* 2015; Castañeda-Vildózola *et al.,* 2016). The foundational research of Dyar (1890) states that head capsule widths increase via a geometric (i.e., also discontinuous) growth pattern. Furthermore, Dyar (1890) states that larvae maintain a consistent size ratio between instars (e.g., subsequent instars being 1.3 times the size of preceding instars). These assumptions are frequently referred to as Dyar's Rule (Safranek and Williams, 1984; Hunt and Chapman, 2001; Hawes, 2019). The assumptions of geometric growth with a consistent size ratio between instars have been applied to numerous butterfly and beetle species as a basis for modelling larval ontogeny (Dyar, 1890; Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016). However, it has never been universally accepted. The many species documented not following Dyar's Rule have led numerous researchers to challenge its generalizability over the last century (Gains and Campbell, 1935; Beck, 1950; Grunert *et al.,* 2015; Hawes, 2019).

The core assumptions behind Dyar's Rule (that insect growth is geometric and discontinuous) were not observed in early studies on the Corn Earworm, *Helicoverpa zea* (Boddie 1850.), or in more recent work on the Tobacco Hornworm, *Manduca sexta* (Linneaus, 1753), where semi-continuous growth following a sigmoid pattern was observed (Grunert *et al.*, 2015). This occurred in Tobacco Hornworms reared in diurnal and constant temperature regimens, irrespective of a natural or artificial food source (Grunert *et al.*, 2015). Hawes (2019), noted that Dyar's original paper did not propose a mathematical model to generalize his rule, rather it outlined his assumptions about larval growth and his reasoning with respect to the restrictions imposed on it by chitinized body-parts (Dyar, 1890). The research that has followed has not represented the assumptions of fixed, discontinuous, geometric growth consistently, or agreed upon a method for statistically verifying their results (Hawes, 2019). This has led researchers to develop alternative methods for identifying instars that do not assume Dyar's Rule (Hunt and Chapman, 2001; Grunert *et al.,* 2015; Hawes, 2019).

While there are a variety of methods that have been developed to determine instars, they all have limitations which make them impracticable for Poweshiek or Garita skipperling, though some of the methods could work if modified. Geometric head capsule growth can be transformed exponentially or logarithmically into a linear association that can be modelled by simple regression. However, multiple authors have cautioned that linear models of transformed data can generate better r^2 values for models that under calculate instar number (Gains and Campbell, 1935; Hawes, 2019) and are insensitive to the presence of supernumerary (more than normal) instars (Grunert *et al.*, 2015). When analyzing weevil pest species, researchers designed software (dubbed the hcap program) to determine instars rather than rely solely on regressions of transformed data (Cazado *et al*., 2014; Castañeda-Vildózola *et al.,* 2016). Since the hcap program was tailored for weevil larvae, it cannot be applied to Poweshiek or Garita skipperlings without reprogramming.

Relying purely on visual assessment by plotting head capsule widths in histograms or density plots and counting the number of distinct peaks or groupings (i.e., instars) that head capsule data fall into is another alternative for instar determination that does not assume Dyar's Rule (Capinera, 1984). Visual assessment refers to evaluating graphical outputs, such as histograms, based on set criteria (Capinera, 1984; Hunt and Chapman, 2001). However, histograms and density plots typically require large datasets to determine instars visually (Capinera, 1984; Hunt and Chapman, 2001; Hawes, 2019). Researchers' may also be influenced by confirmation bias and/or scaling errors due to the effects of different scales, bin widths, etc., on the number of distinguishable peaks in a histogram or density plot (Hunt and Chapman, 2001). Head capsule widths can also be substituted for larval weights and a critical size threshold of each instar can be determined by rearing larvae on nutritionally deficient diets, as has been done with Tobacco Hornworm (Grunert *et al.,* 2015). However, Tobacco Hornworm, which is a well-researched model organism where large populations can be readily maintained and manipulated to determine instars' critical weight thresholds, which is not feasible for Poweshiek or Garita skipperlings.

Hawes (2019) proposed expressing geometric growth by first representing the relationship between instar head capsule measurements. The values calculated at each stage of growth can then be generalized across development by averaging them across instars. Hawes (2019) used the original datasets of Dyar (1890) to validate their proposed formulas. While their method worked for the dataset used in the study, consisting of well-studied lepidopteran species that are known to follow the assumptions of Dyar's Rule, and independent research was available to cross-validate results (Hawes, 2019) it may not apply to all insects. This makes the method unsuitable for Poweshiek or Garita skipperling, where their adherence to Dyar's Rule is unknown.

Perhaps the most useful methods for determining Poweshiek and Garita skipperling come from paleoinvertebrate research on trilobites, which mirrors many of the restrictions on data collection that are endemic to conservation biology. Hunt and Chapman (2001) tested existing models that assumed or did not assume Dyar's Rule by applying the models' estimations of instar size ranges to newly obtained fossils using Maximum Likelihood Estimation (MLE) coupled with visual assessment. Maximum Likelihood Estimation tests the fitness of distributions to a dataset by calculating the probability of data points falling into specified distributions and selects the distribution with the highest probability (Hunt and Chapman, 2001). Using visual assessments of mixed distributions selected via MLE, Hunt and Chapman (2001) concluded that Dyar's Rule did not hold for their study-species (a trilobite).

Although MLE was useful for cross-validating analyses using visual fitness was effective for determining the size ranges of instars, despite the limited datasets from fossil records, the method employed by Hunt and Chapman (2001) cannot be applied directly to raw data. It requires pre-existing size ranges garnered from previous studies for each instar to compare when applied to a new dataset (Hunt and Chapman, 2001). While MLE cannot currently be applied to Poweshiek or Garita skipperlings, pairing a different form of statistical analyses (e.g., k-means clustering) with comparisons of the visual fitness of histograms is possible.

While the use of Dyar's Rule is contentious and has been repeatedly violated by some study-species, the basic assumption that head capsule width exhibit discontinuous growth has been challenged far less frequently (Gains and Campbell, 1935; Grunert *et al.,* 2015). The majority of studies, whether supporting or challenging Dyar's Rule, have not observed head capsule width follow a pattern of continuous growth (Dyar, 1890; Hunt and Chapman, 2001; Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016; Hawes, 2019). It is therefore likely that the head capsules of Poweshiek and Garita skipperling larvae grow discontinuously, rather than semi-continuously or continuously.

Conclusions

In addition to anthropogenic threats like habitat loss (Swengel, 2000; Selby, 2005; Harris and Foster, 2014; Pogue *et al.*, 2016), both Poweshiek and Garita skipperling may be especially vulnerable to climate change-induced phenological shifts due to their univoltine lifecycles and ecological specificity (Hodkinson and Bird, 1998; Diamond *et al.,* 2014). However, there is a need for more research into the ontogeny and phenology of both species, particularly with respect to the impact of higher temperatures on their development and the timing of their eclosure. It is essential to broaden our understanding of how temperature affects the development and eclosure dates of Poweshiek and Garita skipperling and develop a more detailed understanding of larval development to assess the risk that climate change poses to each species.

References

Beck SD. 1950. Nutrition of the European Corn Borer, *Pyrausta nubilalis* (HBN.). II. Some effects of diet on larval growth characteristics. Physiological Zoology. 23(4):353–361.

Belitz M, Hendrick L, Monfils M, Cuthrell D, Marshall C, Kawahara A, Cobb N, Zaspel J, Horton A, Huber S, Warren A, Forthaus G, Monfils A. Aggregated occurrence records of the federally endangered Poweshiek skipperling (*Oarisma poweshiek*). Biodiversity Data Journal 6(1): 1–15.

Blair D, Casson N, Allen C, Kreutzer M, Loxley M, Huson B, MacPherson E, Robak I, Ebhuoma E, Mauro I. 2019. The climate atlas of Canada. Winnipeg (MB): Prairie Climate Centre (Canada).

Borkin, S. 1994. Ecological studies of the Poweshiek skipper (*Oarisma poweshiek*) in Wisconsin, 1993. Madison (WI): Wisconsin Department of Natural Resources (US).

Breiter, C-J, Burns L. 2018. Grassland butterfly conservation program 2017 annual report. Winnipeg (MB): Assiniboine Park Zoo.

Buckley LB, Kingsolver JG. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate change. Annual Review of Ecology, Evolution, and Systematics. 43:205–226.

Burke R, Fitzsimmons J, Kerr J. 2011. A mobility index for Canadian butterfly species based on naturalists' knowledge. Biodiversity and Conservation. 20 (1): 22773–2295.

Capinera JL. 1984. Instar number in Range Caterpillar, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae). Journal of the Kansas Entomological Society. 57(2):344–347.

Castañeda-Vildózola Á, González-Hernández H, Equihua-Martínez A, Valdez-Carrasco J, Peña JE, Cazado LE, Franco-Mora O. 2016. Head capsule width is useful for determining larval instar in *Heilipus lauri* (Coleoptera: Curculionidae). The Florida Entomologist. 99(4):822–825.

Catling P. 1977. On the occurrence of *Oarisma garita* (Reakirt) (Lepidoptera: Hesperiidae) in Manitoulin District, Ontario. The Great Lakes Entomologist 10(2): 59–63

Catling P, and Lafontaine J. 1986. First documented record of *Oarisma poweshiek* (Lepidoptera: Hesperiidae) in Canada. The Great Lakes Entomologist 19(2): 63–66

Cayton HL, Haddad NM, Gross K, Diamond SE, Ries L. 2015. Do growing degree days predict phenology across butterfly species? Ecology. 96(6): 1473–1479.

Cazado LE, Van Nieuwenhove GA, O'brien CW, Gastaminza GA, Murúa MG. 2014. Determination of number of instars of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) based on head capsule widths. The Florida Entomologist. 97(2): 639–643.

Clench HK. 1966. Behavioral thermoregulation in butterflies. Ecology. 47(6): 1021–1034.

Cuthrell DL, Slaughter SL. 2012. Special animal abstract for *Oarisma poweshiek* (Poweshiek skipperling). Lansing (MI): Michigan Natural Features Inventory (US).

Dana, R.P. 1991. Conservation management of the prairie skippers *Hesperia dacotae* and *Hesperia ottoe*: basic biology and threat of mortality during prescribed burning in spring. St. Paul (MN): University of Minnesota.

Danks H, Kukal O, Ring R. 1994. Insect cold-hardiness: insights from the arctic. Arctic 47(4): 391–404.

Dearborn S, Westwood R. 2014. Predicting adult emergence of Dakota Skipper and Poweshiek skipperling (Lepidoptera: Hesperiidae) in Canada. Insect Conservation 18(1): 875–884.

Diamond SE, Cayton HL, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, Ries L. 2014. Unexpected phenological responses of butterflies to the interactions of urbanization and geographic temperature. Ecology. 95(1): 2613–2621.

Diamond SE, Frame AM, Martin RA, Buckley LB. 2011. Species' traits predict phenological responses to climate change in butterflies. Ecology. 92(5): 1005–1012.

Díaz S, Settele L, Brondízio ES, Ngo HT, Guèze M, Agard J, Arneth A, Balvanera P, Brauman KA, Butchart SHM, Chan KMA, *et al.* 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Bonn (DE): IPBES (International); 2019 Report.

Dupont-Morozoff J, Westwood R, Henault J. 2022. An assessment of prairie management practices for maintaining habitat quality for the endangered Poweshiek skipperling butterfly in Canada. The American Midland Naturalist 188(1): 74–100

Dyar HG. 1890. The number of molts of Lepidopterous larvae. Psyche: a Journal of Entomology. 5(1): 175–176.

Forrest JRK, Thomson JD. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecological Monographs. 81(3): 469–491.

Gains JC, Campbell FL. 1935. Dyar's Rule as related to the number of instars of the corn ear worm, *Heliothis obsolete* (Fab.), collected in the field. Annals of the Entomological Society of America. 28(1): 445–461.

Gold CS, Neyeme PS, Coe R. 1999. Recognition and duration of larval instars of banana weevil, *Cosmopolites sordidus* Germar, in Uganda. African Entomology 7(1): 49–62.

Grunert LW, Clarke JW, Ahuja C, Eswaran H, Nijhout HF. 2015. A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. PLoS ONE. 10(5): 1–23.

Harris A, Foster R. 2014. Assessment and status report on the Poweshiek skipperling *Oarisma poweshiek* in Canada. Ottawa (ON): Committee on the Status of Endangered Wildlife in Canada (Canada).

Hawes T. 2019. An equation for geometric progression in Brooks-Dyar growth ratios. Invertebrate Reproduction and Development. 64(1): 1–12.

Hellmann J, Pelini S, Prior K, Dzurisin J. 2008. The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. Oecologica. 157(4): 583–592.

Henault J, Westwood R. 2022. Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada. The Canadian Entomologist 154 (e49): 1–27.

Henault J, Westwood R. 2023. Adult activities of endangered *Oarisma poweshiek* butterflies are associated with a soil moisture gradient in tall grass prairie in Manitoba, Canada. Journal of Insect Conservation 27 (1): 825–839.

Holzman R. 1972. Eastern range extension for *Oarisma poweshiek* Parker (Lepidoptera: Hesperiidae). The Great Lakes Entomologist 5(4): 111–114

Hooshmandi M. 2016. A critical habitat suitability model for Poweshiek skipperling (*Oarisma poweshiek*): with a focus on micro-habitat characteristics [thesis]. [Winnipeg (MB)]: University of Winnipeg.

Hunt G, Chapman RE. 2001. Evaluating hypotheses of instar-grouping in arthropods: A Maximum Likelihood Approach. Paleobiology. 27(3): 466–484.

Karlsson B. 2014. Extended season for northern butterflies. International Journal of Biometeorology. 58(5): 691–701.

Kearney M, Briscoe N, Karoly D, Porter W, Norgate M, Sunnucks P. 2010. Early emergence in butterfly causally link to anthropogenic warming. Biology Letters. 6 (1): 674–677.

Kingsolver J. 2000. Feeding, growth, and the thermal environment of cabbage white caterpillars, *Pieris rapae* L. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches 73 (5): 621–628.

Klassen P, Westwood AR, Preston WB, and Mcillop WB. 1989. The butterflies of Manitoba. Winnipeg (MB): Manitoba Museum of Man and Nature.

Layberry R, Hall P, Lafontaine D. 1998. Butterflies of Canada- Garita skipperling. Toronto (ON): University of Toronto Press.

Moore JL, Liang S, Akullian A, Remais JV. 2012. Cautioning the use of degree-day models for climate change projections in the presence of parametric uncertainty. Ecological Applications. 22(8): 2237–2247.

Navarro-Cano J, Karlsson B, Posledovich D, Toftegaard T, Wiklund C, Ehrlén J, Gotthard K. Climate change, phenology, and butterfly host plant utilization. Ambio. 44(1): 78–88.

Oliphant A, Hauton C, Thatje S, Dam H. 2013. The implications of temperature-mediated plasticity in larval instar number for development within a marine invertebrate, the shrimp *Palaemonetes varians*. PLoS ONE 8(9): 1–9.

Ovaskainen O, Skorokhodova S, Yakovleva M, Sukhov A, Kutenkov A, Kutenkova N, Shcherbakov A, Meyke E, del Mar Delgado M. 2013. Community-level phenological response to climate change. Proceedings of the National Academy of Sciences (USA). 110(33): 13434–13439.

Perkins O. 2007. Using degree-day modelling to ascertain early flight periods of Michigan butterflies and Skippers. The Great Lakes Entomologist 40(3,4): 103–119.

Pivnick KA, McNeil JN. 1986. Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). Ecology. 67(4): 1024–1035.

Pogue C, Monfils M, Cuthrell D, Heumann B, Monfils A. 2017. Habitat suitability modeling of the federally endangered Poweshiek skipperling in Michigan. Journal of Fish and Wildlife Management 7(2): 359–368.

Poweshiek Working Group. 2011. Minutes from the Poweshiek skipperling workshop, March 24 and 25. Winnipeg (MB): International Poweshiek Working Group (Canada).

Poweshiek Recovery Group. 2018. Minutes from the Poweshiek skipperling Recovery Group annual meeting, November 28, Winnipeg, Manitoba, Canada.

Poweshiek International Workshop. 2019. Poweshiek skipperling conservation partnership: proceedings and priorities. Apple Valley (MN): International Poweshiek Working Group (US).

Safranek L, Williams CM. 1984. Determinants of larval molt initiation in the Tobacco Hornworm, *Manduca sexta*. Biological Bulletin. 167(3): 568–578.

Sampson F, Knopf F. 1994. Prairie conservation in North America. Other Publications in Wildlife Management 6(1): 418–421.

Schweitzer DF, Opler PA. 2023. Comprehensive report: species – *Oarisma poweshiek*. Arlington (VI): NatureServe (US).

Scott J, Epstein M. 1987. Factors affecting phenology in a temperate insect community. The American Midland Naturalist 117(1): p 103–118.

Selby G. 2005. Status assessment and conservation guidelines: Poweshiek skipperling (*Oarisma poweshiek* (Parker) (Lepidoptera: Hesperiidae). Bloomington (MN): U.S. Fish and Wildlife Service (US).

Spieles JB, Comer PJ, Albert DA, Kost MA. 1999. Natural community abstract for prairie fen. Lansing (MI): Michigan Natural Features Inventory (US).

Summerville K, Clampitt C. 1999. Habitat characterization of five rare insects in Michigan (Lepidoptera: Hesperiidae, Riodinidae, Satyridae; Homoptera: Cercopidae). The Great Lakes Entomologist 32(3): 225–238.

Swengel A. 2008. Poweshiek paradise lost. American Butterflies 16(4): 16–33.

Swengel A, Swengel S. 1999. Observations of prairie Skippers (*Oarisma poweshiek, Hesperia dacotae, H. ottoe, H. leonardus pawnee*, and *Atrytone argos iowa* [Lepidoptera: Hesperiidae]) in Iowa, Minnesota, and North Dakota During 1988–1997. The Great Lakes Entomologist 32(4): 267–292.

USFWS. 2011. US Fish and Wildlife Service species assessment and listing priority assignment form. Washington (DC): Department of the Interior (US).

Valtonen A, Ayres M, Roininen H, Pöyry J, Leinonen R. 2011. Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. Oecologia 165(1): 237–248.

Wilson K. 2023. Comprehensive Report: Species – *Oarisma garita*. Arlington (VI): NatureServe (US).

CHAPTER 2: DEGREE-DAY MODELLING OF *EX-SITU* POWESHIEK AND GARITA SKIPPERLINGS AT ASSINIBOINE PARK ZOO, MANITOBA.

Abstract

The Poweshiek skipperling (*Oarisma poweshiek*) is the focus of a large conservation effort to repopulate sites in southern Manitoba and Michigan where it was once common. However, the role of temperature in larval development needs further study. I developed degree-day (DD) models with upper and lower thermal thresholds of 32 °C and 6 °C, respectively, via the standard and double-sine method and using the double-sine method without an upper threshold, for *O. poweshiek* and *O. garita* reared at Assiniboine Park Zoo, Manitoba, from 2017–2020. I determined the DDs accrued before and after hibernation, during pupation, and overall (from hatching to eclosure), then compared them across generations for both species. Poweshiek and Garita skipperlings had similar DDs post hibernation across generations, with comparable eclosure dates and ages, but with substantially more variability observed in Garita skipperlings. Degree day modelling appears a promising technique for modelling the phenology of Genus *Oarisma*, which may be further improved by integrating abiotic variables like photoperiod with degree-days. Despite comparable phenologies across the generations I studied, my results indicate that Poweshiek skipperling and Garita skipperling could have distinct responses to climate change.

Introduction

Climate-change induced phenological shifts are increasingly common in the Anthropocene (Valtonen *et al.,* 2011; Moore *et al.,* 2012; Diamond *et al.,* 2014). In an ecosystem-wide analysis of plants, vertebrates, and insects in Kivach, Russia, have exhibited distinct changes in the timings of various stages of their lifecycles over the last four decades: putting taxa at risk of phenological desynchronization (Ovaskainen *et al.,* 2013). Phenological responses tend to follow geographic and temporal climatic gradients (Buckley and Kingsolver, 2012). Modelling and observational studies have demonstrated that early or delayed maturation from phenological shifts can reduce the co-occurrence of species (e.g., larvae and host plants) and desynchronize developmental stages from the correct season (Buckley and Kingsolver, 2012).

Due to their short lifespans and rapid development, insects have exhibited greater phenological shifts in response to shorter-term temperature changes than most flora or vertebrates (Ovaskainen *et al.*, 2013). This has the potential to disrupt ecologically significant species interactions, like insect pollination and predation, and emphasizes the need to research and mitigate the effects of climate change (Diamond *et al.,* 2011; Ovaskainen *et al.*, 2013). The life-history traits of insects (e.g., the number of generations per year, overwintering stage, range, ecological specificity) can influence the severity of climate-change induced phenological responses (Hodkinson and Bird, 1998; Diamond *et al.,* 2014). Although insects' responses vary, the close interplay between insect phenology and temperature makes them susceptible to phenological shifts and confers high predictive power to temperature-based modelling for estimating the phenological effects of climate change on entomofauna (Valtonen *et al.,* 2011).

Researchers commonly use degree-days (DD) to represent the relationship between temperature and development by treating development as a function of time and ambient temperature. The duration and extent that daily temperatures exceed a lower thermal threshold (LTT) and remain below an upper thermal threshold (UTT) are used to calculate the DDs a species needs to reach a specific life stage, which can be used to predict its occurrence in future generations or other populations (Perkins, 2007). DD-based predictions tend to be highly reliable for species that are ecological specialists with restricted ranges, a single generation per year (univoltine), and which overwinter at immobile stages of their lifecycle (Diamond *et al.,* 2011). Butterflies with these traits have also exhibited greater phenological advancements than other species in response to increasing temperatures (Diamond *et al.,* 2011; Diamond *et al.,* 2014). Changes in the duration and intensity of seasons could impact their survivorship in hibernation by causing larvae to develop beyond their overwintering stage, and/or cause adults to eclose when nectar sources are not available (Scott and Epstein, 1987; Pogue *et al.,* 2016).

The Poweshiek skipperling, *Oarisma poweshiek* (Parker, 1870), is a globally endangered, univoltine, ecological specialist of North America's tallgrass prairies (Harris and Forster, 2014; Dupont-Morozoff *et al.,* 2022). Climate change within the range of Poweshiek skipperling in Canada is changing some important aspects of important climatic variables. For example, in 2022 there were more than 30 days with a mean air temperature above 30 °C in southern Manitoba. In comparison from 1976–2005 there was on average less than 17 days exceeding 30 °C (Blair *et al.*, 2019). Climatological forecasts, assuming little to no action is taken to reduce emissions, predict that Poweshiek skipperling in Canada will experience over 40 days per year with a mean temperature above 30 °C in the next 25 years. It is predicted that there will be over a 20% increase in annual degree-days with a 5 °C lower thermal threshold in this interval (Blair *et al.*, 2019). The number of days with a mean temperature below -30 °C is also expected decrease by half, and annual freezing degree-days (the sum of mean daily temperatures below 0 °C) are predicted to decrease by over 20% (Blair *et al.*, 2019). These temperature changes have the potential to impact local Poweshiek skipperling populations: possibly through climate-change induced phenological shifts that may reduce survivorship while overwintering, and/or disrupting the timing and/or synchrony of adult eclosure.

Dissimilar responses to climate change between taxa, as observed in Ovaskainen *et al.,* (2013), indicate that rising temperatures can cause temporal asynchronies between native species with different upper and/or lower thermal thresholds, optima, and growth rates (Forrest and Thomson, 2011; Diamond *et al.,* 2011). These effects are highly contingent upon an organisms' dispersal capacity, ecological niche, and any pre-existing reductions in the population's fitness (Buckley and Kingsolver, 2012). However, most reliable DD models for insects yield higher DDs — and therefore higher thermal requirements — than their supporting plant communities: emphasizing the risk of climate change-induced plant-pollinator asynchronies for species like Poweshiek skipperling (Forrest and Thomson, 2011).

Garita skipperling, *Oarisma garita* (Parker, 1970), is a close relative of Poweshiek skipperling (Harris & Foster, 2014; Wilson, 2023). Garita skipperlings populations are still considered stable across much of its remaining range, but in the U.S. are classified as Vulnerable in Washington, Threatened in Minnesota and Nebraska, and Critically Endangered in Ontario (Wilson, 2023). While Garita skipperlings are also univoltine, their wider range (both historic and current), and occurrence within short to medium grass prairies and alpine meadows (Catling, 1977; Wilson, 2023) suggests that they are less ecologically specialized than Poweshiek skipperling. Both their larger range and ability to inhabit other, less imperiled, prairie habitats than tallgrass prairies may mitigate the impacts of climate change on Garita skipperling relative to Poweshiek skipperling. Similarly, Garita skipperlings longer flight period, approximately one month compared to two or three weeks (Harris and Foster, 2014; Wilson, 2023), may reduce the risk of climate change desynchronizing populations' eclosure.

Uncertainty can meaningfully affect the reliability of climatological forecasts and thus modelling, particularly for species whose development is less intimately tied with temperature (Moore *et al.,* 2012). Despite this drawback, the limited range of Poweshiek skipperling, its habitat and host-specificity and highly synchronous eclosure, make it especially vulnerable to climate change. It is important for researchers to investigate the immediate and long-term effects of climate change on Poweshiek and Garita skipperlings in order to understand its effects on the species, and further our understanding of insect responses to climate change. (Ovaskainen *et al.,* 2013; Pogue *et al.,* 2016)

The purpose of this study is to determine the degree-days accrued by *Oarisma poweshiek* and *O. garita* before and after overwintering, during pupation, and from hatching to eclosure using the larvae reared at Assiniboine Park Zoo (APZ) from 2017–2020. I will also compare the variability at these stages and differences between DDs calculated using the standard method, and the double-sine method with and without an upper thermal threshold that larvae accrued before and after overwintering, during pupation and throughout the life cycle. While preliminary, these results will provide the foundation for my further research on Garita skipperlings (See Chapters 3 and 4) and provide some insight into the comparative resilience of both species with respect to phenological shifts to temperature changes at different stages of development.

Methods

Egg collection

Since both species are univoltine (Harris & Foster, 2014; Wilson 2023), generations were defined by the year that larvae hatched: e.g., the 2017 generation hatched in 2017 and eclosed in 2018. All Poweshiek and Garita skipperling eggs used in this study were sourced from the *ex-situ* populations maintained by the Assiniboine Park Zoo Conservation and Research team as part of the broader recovery effort for Poweshiek skipperling. This recovery effort was conducted in partnership with Nature Conservancy Canada (NCC), and other organizations. Adult Poweshiek and Garita skipperlings were collected annually from field sites in late June or early July after the first observed flight in field surveys. Poweshiek skipperling were collected from the Tall Grass Prairie Preserve (TGPP), land managed by the TGPP committee, and Garita skipperling were collected from grasslands adjacent to the Hogsback Trail in Spruce Woods Provincial Park

(Breiter and Burns, 2018; Burns *et al.,* 2022). Only female Poweshiek skipperlings were collected for egg laying, while male and female Garita skipperlings were netted for mating trials and egg laying.

Poweshiek skipperling females were held in oviposition chambers in the laboratory for 72 hours before being released back into the wild, while Garita skipperlings remained at Assiniboine Park Zoo (Breiter and Burns, 2018). All activities and collections were permitted by the Government of Manitoba, Spruce Woods Provincial Park, and the TGPP management board. Adults were housed individually in mesh enclosures in the same hoop-houses used to rear larvae. Eggs from ovipositing females were placed in separate petri dishes to track their oviposition dates, hatch dates, and genealogy. A subset of Garita skipperlings reared at APZ in 2018 and 2019, and Poweshiek skipperlings reared at APZ in 2019, were used for mating trials and only unrelated adults were mated. Eggs produced by mating trials were collected using the same method as eggs collected from wild-caught females. Eggs were placed inside incubators held at 25 °C and 55% relative humidity to maximize hatch-rates and neonate survivorship (APZ, 2024). Egg development was not considered in calculating DDs, which focused on the interval between hatching and eclosure.

Larval rearing and hibernation

Larvae were reared on host plants kept in outdoor hoop-houses after hatching in early to mid July. They were cared for by members of the Conservation and Research Department of APZ and me. Each plant pot contained a single host plant covered by a frame fitted with fine mesh: allowing for light penetration, watering, and gas exchange while excluding predators and parasitoids. Only individually reared larvae (one per host plant) were used to prevent intraspecific competition from effecting larval growth. Larvae were reared on Prairie Dropseed, *Sporobolus heterolepis*, sourced from the Living Prairie Museum, Winnipeg, Manitoba. Larvae and host plants were checked weekly, and any mortality were recorded. Host plants were watered depending on the temperature and frequency of natural rainfall. Their health was assessed weekly based on the presence and severity of leaf wilt, yellowing, and dieback, and were replaced if needed.

Larvae were removed from the hoop-houses in the fall when daily low temperatures fell to 0 °C, or if frost warnings were issued, and placed into hibernation chambers. These were paper cones in Eppendorf tubes set in water saturated Hydrostone® to prevent desiccation while overwintering (Breiter and Burns, 2018). Hibernation chambers were held in an environmental control (EC) chamber to mimic the temperatures under the snow. The temperature of the EC chamber was gradually lowered from 4 °C to -4 °C over a two-week period to imitate the onset of winter and prevent sudden drops in air temperature from affecting survivorship. Larvae were brought out of hibernation the following spring, once outside temperatures exceeded a daily mean above 10 °C, host plants had begun regrowing, and the risk of frost was minimal: mid-April or early May. Larvae were removed from their chambers, the overwintering survivorship was recorded, and rearing procedures were resumed until pupation was observed (Breiter and Burns, 2018). Pupa were then checked daily until eclosure whereupon the sex of each adult was determined. Eclosure was defined as the end of development, thus adults were not considered in subsequent DD calculations.

Degree-Day modelling

I adapted the methods of Dearborn and Westwood (2014) to determine the DD accumulation from egg hatch to eclosure for Poweshiek and Garita skipperlings reared at APZ from 2017–2020. Daily temperatures were sourced from Richardson International Airport, Winnipeg, Manitoba (the nearest Environment Canada weather station). I used the 'weathercan' package (Lazerte & Albers, 2018) available in R (R Core Team, 2024) to download the temperature data recorded by Environment Canada from May 2017 to August 2020, then extracted the maximum and minimum temperatures recorded each day. I used the standard and double-sine methods to calculate DDs. I used a lower thermal threshold of 6 °C and upper thermal thresholds of 32 °C for both methods, and additionally used the double-sine method without an upper thermal threshold. Thresholds were selected based on previous work by Dearborn and Westwood (2014). Standard DDs for each day were calculated using the following formula:

$$DD = ((T_{max} + T_{min})/2) - 6\ °C$$

where T_{max} and T_{min} represent the highest and lowest daily temperatures, and 6 °C the LTT, respectively. Degree day accumulations using the double-sine method were calculated using the

DD calculator provided by the University of California Agriculture and Natural Resources Integrated Pest Management Program (University of California, 2016).

I linked the hatch date, start and end of hibernation, and the beginning and end of pupation of Garita and Poweshiek Skippering larvae to the DDs accrued by individual larvae for each interval. These values were summed to calculate their cumulative DDs for each generation, and across generations, for both species. The final ages of larvae (in days) were calculated using their respective hatching and eclosure dates. In total, 24 sets of cumulative DDs were generated: three per generation for each species (18) and three multigenerational values for both species (6). Larvae that died before eclosure were omitted.

Results

Dates and ages of Poweshiek and Garita skipperlings

In total, 128 of the 229 Garita skipperlings and 41 of 93 Poweshiek skipperlings that hatched survived to eclosure. The timespan from hatching to eclosure varied across generations from 330–360 days for both species. For Poweshiek skipperling the average hatch date across all generations was July 21st (± 7 days), with most neonates within a generation hatching within four to five days of one another (within 14 days across generations). Garita skipperlings hatched an average of seven days earlier than Poweshiek skipperlings, although their hatch dates were more variable: with most hatch dates within a generation spanning 7–14 days and by 32 days across all generations. Hibernation was induced between Mid-October to Mid-November for both species, depending on the year, with a later start for Poweshiek skipperlings in two of three generations. The initiation of hibernation was performed more consistently for Poweshiek skipperlings within generations than Garita skipperlings, with a maximum standard deviation of 5 versus 12 days, respectively, and was approximately 1.3 times more variable across generations than Garita skipperlings (Table 1.1).

Hibernation was broken by APZ staff between April 27th and May 12th, depending on annual temperature trends. However, the end of hibernation was kept consistent within individual generations: all Poweshiek skipperling larvae in each generation were removed from hibernation on the same day, and all Garita skipperling larvae within a generation were removed from hibernation within 3–5 days of one another, depending on the generation. Pupation dates were similar for both species within generations, and both species averaged the same pupation date (June 22nd) across generations with comparable standard deviations. The average date that pupation began was more variable for Poweshiek skipperlings than either hatching or eclosure within generations. The length of the pupal stage was similar for both species, lasting between 12 and 15 days, with an average eclosure date of July 4th ± 6 days for Poweshiek skipperlings and July 5th ± 7 days for Garita skipperlings across the generations of each species (Table 1.1).

Table 1.1. Average hatch, pupation, and eclosure dates for each generation, and overall. Table shows the average hatch dates, pupation dates, and eclosure dates of Poweshiek skipperling (*O. poweshiek*) and Garita skipperling (*O. garita*) reared at Assiniboine Park Zoo, Winnipeg, from summer 2017 to summer 2020, and averaged across generations. Generations defined by the year that larvae hatched. Standard deviation values (±) in days listed in parentheses.

Generation	Species	Hatch dates	Hibernation start dates	Hibernation end dates	Pupation dates	Eclosure dates
2017	Poweshiek	Jul-30 (± 1.11)	Oct-31 (± 5.16)	May-01 (± 0.00)	Jun-16 (± 1.83)	Jun-28 (± 1.11)
	Garita	Jul-25 (± 6.37)	Oct-27 (± 1.18)	May-11 (± 2.64)	Jun-13 (± 4.01)	Jun-25 (± 2.89)
2018	Poweshiek	Jul-11 (± 1.49)	Nov-12 (± 0.00)	May-06 (± 0.00)	Jun-29 (± 4.92)	Jul-12 (± 3.63)
	Garita	Jun-29 (± 6.98)	Oct-07 (12.10)	May-12 (1.56)	Jun-28 (4.7)	Jul-11 (4.21)
2019	Poweshiek	Jul-24 (± 1.69)	Oct-09 (± 0.43)	Apr-27 (± 0.00)	Jun-20 (± 4.37)	Jul-03 (± 2.01)
	Garita	Jul-30 (± 3.41)	Oct-16 (± 1.25)	May-02 (± 1.96)	Jun-17 (± 3.48)	Jun-30 (± 2.8)
Total	Poweshiek	Jul-21 (± 7.37)	Oct-23 (± 15.25)	Apr-30 (± 4.01)	Jun-22 (± 6.37)	Jul-05 (± 5.57)
	Garita	Jul-14 (± 16.4)	Oct-14 (± 11.47)	May-07 (± 5.60)	Jun-22 (± 7.12)	Jul-04 (± 6.69)

Poweshiek skipperling reached an average age of 75.9 ± 1.6 days to 98.3 ± 1.2 days from neonates to the initiation of diapause. Generations had similar standard deviation values: from ± 1.2 days to ± 1.9 days. Across generations, Poweshiek skipperlings had a mean age of 79.6 ± 8.1 days old when placed into hibernation. When hibernation was broken, Poweshiek larvae were on average 274.3 ± 1.2 days to 298.9 ± 1.6 days old, depending on the generation (a difference of 24.6 days), averaging 283.8 ± 10.6 days overall. The average age of Poweshiek skipperlings at pupation varied by up to 32.7 days, from 320.3 ± 2.1 days to 353.0 ± 5.6 days (Table 1.2). Poweshiek skipperlings took an average of 336.9 ± 12.8 days from first instar larva to the onset of pupation across generations. There was a 33.5-day difference between the highest and lowest average age of Poweshiek skipperlings at eclosure, depending on the generation, ranging from 333 ± 2.1 days to 366.5 ± 4.2 days. The average age across generations was 350.0 ± 12.5 days (Table 1.2). The average number of days Garita skipperling developed before hibernation was initiated varied from 71.8–103.8 days between generations (32 days), with a mean development time of 89 ± 15 days, for all generations combined. Their average age when larvae were removed from hibernation was 295.8 ± 20.3 days for all generations (Table 1.2).

Standard deviations differed notably between generations, ranging from ± 3.5 days to ± 10.6 days. Garita skipperlings that survived to eclosure averaged form 277.2 ± 3.1 to 318.4 ± 3.6 days: a 42-day difference. The average age of Garita skipperling at pupation differed by up to 44 days between generations from 321.9 ± 8.9 days to 365.9 ± 5.9 days, averaging 341.4 ± 21.3 days across generations. The average age at eclosure varied by 43.2 days, from 334.6 ± 8.3 days to 377.8 ± 5.9 days. The mean period to eclosure that Garita skipperling averaged across generations was 354.2 ± 21.2 days (Table 1.2).

Table 1.2. Average ages (in days) at the start of hibernation, end of hibernation, start of pupation, and at eclosure for each generation, and overall. Table shows the average ages (in days) of Poweshiek skipperlings (*O. poweshiek*) and Garita skipperlings (*O. garita*) reared to eclosure from 2017–2020 at Assiniboine Park Zoo, Winnipeg, at the beginning and end of hibernation, at pupation, and at eclosure. Generations defined by the year that larvae hatched. Standard deviations (±) given in parentheses in days.

Generation	Species	Ages at hibernation	Ages after hibernation	Ages at pupation	Ages at eclosure
2017	Poweshiek	98.3 (± 1.2)	274.3 (± 1.2)	320.3 (± 2.1)	333.0 (± 2.1)
	Garita	71.8 (± 7.1)	289.0 (± 7.3)	321.9 (± 8.9)	334.6 (± 8.3)
2018	Poweshiek	75.9 (± 1.6)	298.9 (± 1.6)	353.0 (± 5.6)	366.5 (± 4.2)
	Garita	103.8 (± 10.6)	318.4 (± 3.6)	365.9 (± 5.9)	377.8 (± 5.9)
2019	Poweshiek	76.6 (± 1.9)	277.4 (± 1.7)	331.8 (± 5.1)	345.0 (± 2.9)
	Garita	78.7 (± 3.5)	277.2 (± 3.1)	323.8 (± 4.6)	336.7 (± 3.9)
Total	Poweshiek	79.6 (± 8.1)	283.8 (± 10.6)	336.9 (± 12.8)	350.0 (± 12.5)
	Garita	89.0 (± 15.0)	295.8 (± 20.3)	341.4(± 21.3)	354.2 (± 21.2)

Degree-Day accumulation by Poweshiek and Garita skipperlings

Poweshiek skipperlings accumulated an average of 694 ± 19.0 degree-days to 864 ± 26.0 degree-days before the start of hibernation. Standard deviation values for DD accumulation before hibernation ranged from ± 18.6 to ± 26.0 degree-days, while pre-hibernation DD accumulation ranged from ± 71.7 to ± 75.0 degree-days across generations depending on the method used to calculate DDs (Table 1.3). Across generations, DD accumulation post-hibernation was very similar for Poweshiek skipperlings: between 436 and 463 using the standard and double-sine methods. Standard deviation values were high using both standard and double-sine methods (with and without a UTT): ranging from ± 62.0 to ± 63.4 degree-days, respectively. Generational averages and standard deviations were less consistent, ranging from 408 to 484 degree-days and from ± 27.2 to ± 76.8 degree-days, respectively. Degree day accumulation during pupation was very consistent between and across generations, with all averages falling between 208 and 220 degree-days. However, pupation also had the highest standard deviations of any life stage relative to the number of DD accrued: from ± 28.3 to ± 40.6 degree-days (Table 1.3).

Averages across generations for Poweshiek skipperling's total DD accumulation ranged from 1407 to 1443 using the double-sine method with and without an upper thermal threshold, respectively, with the lowest standard deviation (± 79.5 degree-days) associated with the standard method. Cumulative DD averages for individual generations were less consistent, ranging from 1309 to 1558, with a wide range in standard deviation values: from ± 32.2 to ± 65.5 degree-days. Within generations, standard deviations were comparable using the standard and double-sine method with and without a UTT. The double-sine method without an upper thermal threshold generated the highest values across generations and life stages, while the lowest values were produced alternately using the standard or double-sine method with an upper thermal threshold of 32 °C (Table 1.3).

Table 1.3. Degree-days averaged by Poweshiek skipperlings generations, and across generations, at the start/end of hibernation, during pupation, and overall. Table shows the average degree-day values and standard deviations (±) calculated for Poweshiek skipperlings (*Oarisma poweshiek*) larvae from hatching until the start of hibernation, between the end of hibernation and start of pupation, during pupation, and overall reared at Assiniboine Park Zoo, Winnipeg, from 2017 to 2020. Degre days (DD) were calculated using the standard method[1], double-sine method with an upper thermal threshold[2], and double-sine method with no upper thermal threshold[3], respectively. Generations defined by year that larvae hatched. Standard deviations (±) listed in parentheses in degree days.

Generation	DD accumulated pre-hibernation	DD accumulated post-hibernation	DD accumulated pupation	DD accumulated total
2017	706 (± 18.6)[1]	423 (± 27.2)[1]	208 (± 40.4)[1]	1337 (± 32.3)[1]
	694 (± 19.0)[2]	408 (± 27.7)[2]	207 (± 40.6)[2]	1309 (± 32.3)[2]
	716 (± 18.6)[3]	426 (± 27.2)[3]	208 (± 40.4)[3]	1350 (± 32.3)[3]
2018	817 (± 25.0)[1]	476 (± 73.8)[1]	210 (± 28.3)[1]	1502 (± 62.8)[1]
	860 (± 26.0)[2]	453 (± 76.6)[2]	210 (± 29.6)[2]	1522 (± 65.5)[2]
	864 (± 26.0)[3]	484 (± 76.8)[3]	210 (± 29.5)[3]	1558 (± 65.3)[3]
2019	713 (± 22.0)[1]	454 (± 58.3)[1]	218 (± 27.0)[1]	1385 (± 44.3)[1]
	713 (± 22.3)[2]	434 (± 58.2)[2]	219 (± 28.5)[2]	1366 (± 43.8)[2]
	720 (± 22.0)[3]	460 (± 58.7)[3]	220 (± 28.6)[3]	1400 (± 43.6)[3]
Total	745 (± 74.3)[1]	456 (± 62.7)[1]	214 (± 29.5)[1]	1415 (± 79.5)[1]
	757 (± 75.0)[2]	436 (± 62.0)[2]	215 (± 30.4)[2]	1407 (± 95.4)[2]
	765 (± 71.7)[3]	463 (± 63.4)[3]	215 (± 30.4)[3]	1443 (± 95.0)[3]

The average DD accumulation of Garita skipperlings before hibernation varied from 647 to 1059 between generations. Standard deviations similarly varied from ± 34.0 to ± 85.8 degree-days within generations. Averaged across generations, pre-hibernation DD accumulation had the highest standard deviations (regardless of the method): from ± 182.8 degree-days to 204.5 degree-days. Generational averages for larval DD accumulation post-hibernation varied from 308 to 449 degree-days, with standard deviations between 43.0 and 76.2 degree-days (Table 1.4). Averages across generations and standard deviations post-hibernation were consistent between the standard and double-sine methods (with and without a UTT): ranging from 394 to 416 ± 62.4 to 64.1 degree-days. Generational averages and averages across generations for DD accumulation during pupation were very consistent, with all averages falling between 197 and 203 (less than a 3% difference). Standard deviations were less consistent, falling between ± 14.8 DD to ± 22.7 degree-days (Table 1.4).

Averaged across generations, Garita skipperlings total DD accumulation ranged from 1417 to 1448 using the standard method and double-sine method without an upper thermal threshold, respectively, with the lowest standard deviation (± 217.6 degree-days) produced using the double-sine method with a UTT of 32 °C. Cumulative DD averages for individual generations were less consistent, ranging from 1202 to 1707, with a wide range in standard deviation values: from ± 60.0 to ± 117.3 degree-days. Generational standard deviations of the total DDs accrued were similar using the standard and double-sine method (with and without a UTT). The double-sine method without an upper thermal threshold generated the highest values across generations and life stages (where averages were not equal), while the lowest values were produced alternately using the standard or double-sine method with an upper thermal threshold of 32 °C (Table 1.4).

Table 1.4. Degree-days (DD) averaged by Garita skipperlings generations, and across generations, at the start/end of hibernation, during pupation, and overall. Table shows the average degree-day values and standard deviations (±) calculated for Garita skipperlings (*Oarisma garita*) larvae from hatching until the start of hibernation, between the end of hibernation and start of pupation, during pupation, and overall reared at Assiniboine Park Zoo, Winnipeg, from 2017 to 2020. DDs were calculated using the standard method[1], double-sine method with an upper thermal threshold[2], and double-sine method with no upper thermal threshold[3], respectively. Generations defined by year that larvae hatched. Standard deviations (±) listed in parentheses in degree-days.

Generation	DD accumulated pre-hibernation	DD accumulated post-hibernation	DD accumulated pupation	DD accumulated total
2017	691 (± 85.8)[1]	308 (± 72.0)[1]	203 (± 15.1)[1]	1202 (± 115.3)[1]
	691 (± 85.4)[2]	315 (± 76.2)[2]	203 (± 14.8)[2]	1208 (± 117.3)[2]
	701 (± 85.4)[3]	318 (± 76.2)[3]	203 (± 14.8)[3]	1221 (± 117.3)[3]
2018	1051 (± 52.1)[1]	426 (± 59.1)[1]	199 (± 15.3)[1]	1676 (± 87.3)[1]
	1008 (± 51.1)[2]	440 (± 58.8)[2]	199 (± 15.4)[2]	1647 (± 86.3)[2]
	1059 (± 52.3)[3]	449 (± 58.8)[3]	199 (± 15.4)[3]	1707 (± 87.2)[3]
2019	646 (± 34.0)[1]	379 (± 44.7)[1]	200 (± 21.8)[1]	1225 (± 60.0)[1]
	647 (± 34.1)[2]	397 (± 43.0)[2]	197 (± 22.7)[2]	1242 (± 60.3)[2]
	654 (± 34.2)[3]	401 (± 45.1)[3]	201 (± 22.1)[3]	1257 (± 60.0)[3]
Total	823 (± 204.5)[1]	394 (± 62.4)[1]	200 (± 18.8)[1]	1417 (± 238.4)[1]
	805 (± 182.8)[2]	409 (± 62.2)[2]	199 (± 19.3)[2]	1413 (± 217.6)[2]
	831 (± 204.0)[3]	416 (± 64.1)[3]	201 (± 18.9)[3]	1448 (± 238.9)[3]

Discussion

The number of Garita skipperlings reared to adulthood was greater than the number of Poweshiek skipperlings in every generation. Although this may have influenced the disparities observed between the species, the differences at each life stage were not consistent; suggesting that the variability at each life stage is not simply attributable to sample size. The variability observed in each species' eclosure dates were also consistent with other research on Manitoban populations of both species. The flight period of Garita skipperling is roughly twice the length of the Poweshiek skipperling: a month versus two to three weeks (Dearborn and Westwood, 2014; Harris and Foster, 2014). Eclosure is also known to vary between generations by up to a month for both Poweshiek and Garita skipperlings (Dearborn & Westwood, 2014; Breiter and Burns, 2018). This further suggests that the increased variability observed in Garita skipperlings is not solely due the higher sample sizes for Garita skipperling but is related to a biological difference between the species.

Across generations, Garita skipperlings were an average of 10 days older at the start of hibernation than Poweshiek skipperlings. Their less variable age at the onset of hibernation is partly attributable to the more consistent hatch dates of Poweshiek skipperlings within generations compared to Garita skipperlings. However, hibernation was also induced for all Poweshiek skipperling larvae consistently within generations (within 5 days) while Garita skipperlings were placed into hibernation within 12 days of one another; contributing to their increased variability. Since larvae were also removed from hibernation by APZ staff, this also impacted the variability of larval ages at the end of hibernation: within generations and overall.

Both species had notably more consistent ages at pupation than they had at start of hibernation. Generally, the number of days needed to complete the life stages was similar for Poweshiek and Garita skipperlings, as indicated by their hatch, pupation, and eclosure dates. However, Garita skipperlings had more variable ages at every stage of development. They also typically hatched and eclosed slightly earlier than Poweshiek skipperlings, at a somewhat higher final age. While Garita skipperlings were more variable at both stages, the reduced variability (overall and within generations) at the onset of pupation for both species suggests that the span of larval development post-hibernation is more consistent than the onset of hibernation in both species. Another North American Skipper, *Hesperia juba* (Scudder, 1872) were observed halting development before the onset of pupation for almost a month, which was attributed to later instars' responses to temperature and photoperiod cues (James, 2009). It is possible that the final instars of Poweshiek and Garita skipperlings are similarly sensitive to cues from photoperiod to slow the developmental rate of larvae that reach their final instars prematurely, resulting in a consistent age at pupation.

Degree-day accumulation at the onset of hibernation was more consistent, both within and across generations, for Poweshiek skipperlings versus Garita skipperlings. Average DDs at the start of hibernation differed by a maximum of 20% for Poweshiek skipperlings, but up to 38.9% for Garita skipperlings between the generations studied. Overall, Garita skipperlings averaged approximately 50–75 more degree-days than Poweshiek skipperlings before hibernation was induced, but averages for individual generations ranged from 60 DD lower to 200 DD higher than Poweshiek skipperling in the same year. Garita skipperlings also varied more within generations than Poweshiek skipperlings.

The head capsules of Poweshiek and Garita skipperlings have been observed to stop growing between the end of September and start of October; well before hibernation is induced (Breiter and Burns, 2018). Thus, variation in DDs before hibernation may have limited impact on the development and/or timing of eclosure of Poweshiek and/or Garita skipperlings, as larvae appear to stop developing once they receive cues in the mid to late Fall to begin preparing for hibernation. Larvae that overwinter need to commit to entering hibernation before the onset of winter, in order to cease growing and make any physiological changes that are necessary for overwintering (Friberg *et al.,* 2011). While this can be reversed by prolonged, high temperatures, larvae tend to resist developing further once they have received the cues from temperature and/or photoperiod to prepare for overwintering (Friberg *et al.,* 2011).

Overall, larval DDs post-hibernation was very consistent across methods for both species, with only a 5.8% and 5.3% difference between the lowest and highest averages for Poweshiek and Garita skipperlings. Hibernation has been proposed to synchronize larval development in a variety of temperate butterflies (Scott and Epstein, 1987). This appears to be true of Poweshiek and Garita skipperlings, which have previously been shown to stop growing towards the mid to late fall (Breiter and Burns, 2018). Degree-days accrued after hibernation are also strongly associated with eclosion in Poweshiek skipperlings and are used as a predictor of adult emergence in both field and lab populations (Dearborn and Westwood, 2014; Breiter and Burns, 2018). It is therefore likely the degree-days accrued before hibernation have less of an effect on the eclosure dates of Poweshiek skipperlings, as larvae appear to halt development before the onset of hibernation (Breiter and Burns, 2018). It is likely the case for Garita skipperling, given their close relatedness to Poweshiek skipperling (Harris and Foster, 2014) and the high variability observed before hibernation.

Pupation was the only stage where Poweshiek skipperlings were more variable, both within generations and cross-generationally, than Garita skipperlings. While DD accumulation during pupation was very consistent across generations of Poweshiek skipperling, generational standard deviation values were higher (relative to DDs) than any other life stage: ± 13.5% to ± 19.6% of the DDs accumulated. In contrast, Garita skipperlings averages fell between 197 and 203 (a difference of 3.0%) with much lower standard deviations: between ± 7.2% to 11.5% of the DDs accumulated during pupation.

High variability in DD accumulation is commonly associated with photoperiod-sensitive stages of development for temperate insects (James, 2009; Friberg *et al*., 2011; Grevstad and Coop, 2015). Conversely, less variable response can be attributed to temperature playing a dominant role in an insects' developmental rate (Friberg *et al.,* 2011; Diamond *et al.,* 2014). The notably higher variation in pupal DD accumulation exhibited by Poweshiek skipperlings compared to Garita skipperlings may similarly relate to a difference between the species' responses to temperature and photoperiod during pupation. Poweshiek skipperling pupae may rely on a combination of temperature and photoperiod cues to help synchronize their eclosure, i.e. by delaying and/or accelerating pupation in response to photoperiod cues, while Garita skipperling pupae appear to be more consistent in their response to temperature than they were as larvae (as indicated by their comparatively low variation in pupal DD accumulation). Poweshiek skipperlings are known to pupate and eclose within a one to two-week period annually (Dearborn and Westwood, 2014; Breiter and Burns, 2018), while Garita skipperlings eclose over the course of three or more weeks (Harris and Foster, 2014; Breiter and Burns, 2018). It is possible that this difference is attributable to Poweshiek skipperling pupae being sensitive to photoperiod cues, and Garita skipperling pupae being less sensitive or insensitive to photoperiod.

The cumulative degree-days averaged cross-generationally were similar for both species. However, the associated standard deviations differed substantially between species (and somewhat between methods), ranging from ± 5.6% to ± 6.8% of the for Poweshiek skipperlings and from ± 15.4% ± 16.5% in Garita skipperlings. Cumulative DD averages for individual generations were notably less consistent for Garita skipperlings, differing by 29.5% between generations versus a 16% difference between Poweshiek skipperling's generational averages. Generations of Garita skipperlings also exhibited a wider range in standard deviation values, from ± 4.8% to ± 9.7% of the total DDs averaged by a generation, which were also substantially higher than those calculated for Poweshiek skipperlings that ranged from ± 2.3% to ± 4.3%. This is consistent with previous observations of both species' phenology, which indicate that Garita skipperling's eclosure is more variable (Breiter and Burns, 2018).

Microclimatic variations between the conditions experienced by individual larva can result in more variable development and emergence in butterflies (Kearney et al., 2010; Dupont-Morozoff *et al.,* 2022). However, larvae were reared in one of two adjacent hoop-houses with the same setup of a single host plant exposed to natural sunlight through fine mesh, which minimized the effects of microclimatic variation compared to field conditions (Breiter and Burns, 2018). However, it is possible that the temperatures recorded by the weather station at Richard International Airport, Winnipeg, Manitoba differed from the actual temperatures that larvae were exposed to at Assiniboine Park Zoo. While the two locations are within 4 km of one another, their different proximities to the Assiniboine River, tree coverage, and differences in albedo could impact the accuracy of DDs calculated using this data. Similar disparities were observed between DDs based on temperatures recorded by the nearest weather stations versus data-loggers placed in the habitats of *in-situ* populations of Poweshiek skipperling, although they did not have a substantial effect on their predictive power for eclosure dates (Dearborn and Westwood, 2014).

The double-sine method which did not include a UTT performed similarly to the double-sine and standard method that used a UTT of 32 °C. This was expected, as the daily high temperatures from 2017-2020 rarely exceeded the UTT of 32 °C in Winnipeg between the dates that larvae hatched and eclosure. Where average DD differed between standard and double-sine methods, using the double-sine method without an upper thermal threshold generated the highest degree-day averages for both species for all life stages, while the lowest values were produced alternately by the standard or double-sine method with an upper thermal threshold of 32 °C. Standard deviation values were also largely comparable across species and generations, though they were more variable between methods when applied cross-generationally. Given their similar outputs for both species, it is likely that all three methods are suitable. However, the lack of an upper thermal threshold in two of the three methods may become more significant as climate change worsens (Moore *et al.,* 2012; Diamond *et al*., 2014).

Generations of both species varied more in their final ages and DD accumulation compared to one another than individuals within generations, suggesting that other factors may influence their phenology. While differences between annual temperatures likely contributed to variation in DD accumulation, variation within generations may be also be attributable to phenotypic and/or genotypic differences between larvae, as well as any nutritional differences between host plants (Grunert *et al*., 2015; Nordmeyer *et al.,* 2021). It is possible that constraints on the age and/or DD accumulation necessary to reach an overwintering state, survive overwintering, pupate, and eclosure may play a role in Poweshiek skipperling's highly synchronized flight period, and perhaps to a lesser extent Garita skipperling's. Future studies should examine the significance of age before and after overwintering, at pupation, etc. compared to temperature in the ontogeny of both species. Males of both species have also been observed eclosing several days earlier than females: suggesting that sexual dimorphism may influence the DDs that larvae accrue by eclosure (Dearborn and Westwood, 2014; Breiter and Burns, 2018).

Cues from photoperiods before and after hibernation may also play a role the timing of both species' eclosure and flight periods and may explain the relatively consistent average eclosure dates of both species within generations and overall compared to their variable DD accumulation between generations. While preparing for (and/or undergoing) hibernation may play a role in synchronizing flight periods (Scott and Epstein, 1987), the results of this study suggest that the higher synchrony in the eclosure of Poweshiek skipperlings compared to Garita skipperlings may also stem from the interaction of temperature and photoperiod cues during pupation, such that individuals accelerate or delay their pupation rate to synchronize eclosure. However, the role of photoperiod was not investigated in this study. Further studies, such as using artificial and natural photoperiods to compare the responses of larvae to the photoperiod cues before versus after overwintering, as done by Friberg *et al.* (2011), are needed to examine the role of photoperiod in skipperling phenology.

Given the likelihood of stochastic weather events in the spring and fall, and the 20% decrease in freezing degree-days predicted by 2100 (Blair *et al.*, 2019), there is the potential for overwintering to be impacted by reducing the time spent below freezing temperatures when conditions are still unfavorable for development: increasing their metabolic demands while overwintering. Although hibernation's effects on wild populations' survivorship are not estimable, these results reinforce previous findings that Poweshiek skipperling are sensitive to temperature throughout development (Dearborn and Westwood, 2014; Breiter and Burns, 2018). Due to the geographic isolation of wild Poweshiek skipperling populations (Harris and Foster, 2014; USFWS, 2014), the impact of climate change likely depends on their tolerance to temperature changes, and the extent that the timing and severity of these events differ for wild populations (Harris and Foster, 2014; USFWS 2014). Garita skipperling may be less disrupted by temperature changes than Poweshiek skipperlings, given the consistently higher variation in development times they exhibited in this study.

Further research is needed to determine the threat that climate change poses to each species. Researchers have successfully trialed *ex-situ* rearing strategies on Garita skipperlings before implementing them on Poweshiek skipperlings (Breiter and Burns, 2018). While these results indicate that the phenology of both species is similar in Manitoba, the wider variability in DDs and final ages at eclosure exhibited by Garita skipperling may relate to higher flexibility on the DD accumulation and ages required for larvae to overwinter and survive to eclosion. This may limit the utility of Garita skipperling as a true surrogate for research on the effects that climate change on Poweshiek skipperling, and the role of temperature in their development warrants further research.

Though preliminary, this study can inform future *ex-situ* rearing practices by allowing conservationists to estimate the effects of unseasonal weather and/or extreme temperatures, such as heat waves, on larvae's phenology, as well as the effects of mitigation strategies like rearing larvae in climate-controlled chambers. The DDs obtained in this study support previous findings that DD accumulation is relatively consistent for Poweshiek skipperling across generations (Dearborn & Westwood, 2014; Breiter and Burns, 2018; Burns *et al.,* 2022). The mean annual number of days with averages temperatures above 30 ℃ in Southern Manitoba has nearly doubled since 1976, with climate models predicting almost 40 such days annually, by 2050 (Blair *et al.*, 2019). Assuming a 5 ℃ lower thermal threshold, degree days are expected to rise by over 20% of their historic values by 2100 (Blair *et al.*, 2019). Given the well-established relationship between ambient temperature and larval development (Diamond *et al.,* 2011) and the low variability in DD accumulation exhibited by Poweshiek skipperling, the risk of climate change-induced phenological shifts appears high for the species; even compared to its close relative.

References

APZ. 2024. Grassland Butterfly Conservation Program: Animal Care and Research Protocols for Poweshiek skipperling and Dakota skipper (2024 update). Assiniboine Park Conservancy, Winnipeg, MB. 61pp.

Blair D, Casson N, Allen C, Kreutzer M, Loxley M, Huson B, MacPherson E, Robak I, Ebhuoma E, Mauro I. 2019. The climate atlas of Canada. Winnipeg (MB): Prairie Climate Centre (Canada).

Breiter C-J, Burns L. 2018. Grassland butterfly conservation program 2017 annual report. Winnipeg (MB): Assiniboine Park Zoo.

Buckley LB, Kingsolver JG. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate change. Annual Review of Ecology, Evolution, and Systematics. 43:205–226.

Burns L, Grottoli A, Papineau A, Petersen SD. 2022. Grassland Butterfly Conservation Program: 2021 Annual Report. Assiniboine Park Conservancy, Winnipeg (MB): 1–45.

Cayton HL, Haddad NM, Gross K, Diamond SE, Ries L. 2015. Do growing degree days predict phenology across butterfly species? Ecology. 96(6): 1473–1479.

Dearborn S, Westwood R. 2014. Predicting adult emergence of Dakota Skipper and Poweshiek skipperling (Lepidoptera: Hesperiidae) in Canada. Insect Conservation 18(1): 875–884.

Diamond SE, Cayton HL, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, Ries L. 2014. Unexpected phenological responses of butterflies to the interactions of urbanization and geographic temperature. Ecology. 95(1): 2613–2621.

Diamond SE, Frame AM, Martin RA, Buckley LB. 2011. Species' traits predict phenological responses to climate change in butterflies. Ecology. 92(5): 1005–1012.

Dupont-Morozoff J, Westwood R, Henault J. 2022. An assessment of prairie management practices for maintaining habitat quality for the endangered Poweshiek skipperling butterfly in Canada. The American Midland Naturalist 188(1): 74–100

Forrest JRK, Thomson JD. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecological Monographs. 81(3): 469–491.

Friberg M, Haugen IMA, Dahlerus J, Gotthard K, Wiklund C. 2011. Asymmetric life-history decision-making in butterfly larvae. Oecologia. 165(2): 301–310.

Grevstad FS, Coop LB. 2015. The consequences of photoperiodism for organisms in new climates. Ecological Applications. 25(6): 1506–1517.

Grunert LW, Clarke JW, Ahuja C, Eswaran H, Nijhout HF. 2015. A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. PLoS ONE. 10(5): 1–23.

Harris A, Foster R. 2014. Assessment and status report on the Poweshiek skipperling *Oarisma poweshiek* in Canada. Ottawa (ON): Committee on the Status of Endangered Wildlife in Canada (Canada).

Henault J, Westwood R. 2022. Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada. The Canadian Entomologist 154 (e49): 1–27.

Henault J, Westwood R. 2023. Adult activities of endangered *Oarisma poweshiek* butterflies are associated with a soil moisture gradient in tall grass prairie in Manitoba, Canada. Journal of Insect Conservation 27 (1): 825–839.

Hodkinson ID, Bird J. 1998. Host-specific insect herbivores as sensors of climate change in arctic and alpine environments. Arctic and Alpine Research. 30(1): 78–83.

James, D. 2009. Comparative studies on the immature stages and biology of *Hesperia colorado idaho* and *Hesperia juba* (Hesperiidae). Journal of the Lepidopterists' Society 63(3): 129–136.

Lazerte S, Albers S. 2018. Weathercan: download and format weather data from environment and climate change Canada. The Journal of Open Source Software 3(22): 571.

Moore JL, Liang S, Akullian A, Remais JV. 2012. Cautioning the use of degree-day models for climate change projections in the presence of parametric uncertainty. Ecological Applications. 22(8): 2237–2247.

Nordmeyer C, E. Runquist, and S. Stapleton. 2021. Invasive grass negatively affects growth and survival of an imperiled butterfly. Endangered Species Research. 45: 301–314.

Ovaskainen O, Skorokhodova S, Yakovleva M, Sukhov A, Kutenkov A, Kutenkova N, Shcherbakov A, Meyke E, del Mar Delgado M. 2013. Community-level phenological response to climate change. Proceedings of the National Academy of Sciences (USA). 110(33): 13434–13439.

Perkins O. 2007. Using degree-day modelling to ascertain early flight periods of Michigan butterflies and Skippers. The Great Lakes Entomologist 40(3,4): 103–119.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Scott J, Epstein M. 1987. Factors affecting phenology in a temperate insect community. The American Midland Naturalist 117(1): p 103–118.

USFWS. 2011. US Fish and Wildlife Service species assessment and listing priority assignment form. Washington (DC): Department of the Interior (US).

Valtonen A, Ayres M, Roininen H, Pöyry J, Leinonen R. 2011. Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. Oecologia 165(1): 237–248.

CHAPTER 3: INSTAR DETERMINATION OF GARITA SKIPPERLING LARVAE REARED AT ASSINIBOINE PARK ZOO, MANITOBA

Abstract

The head capsule widths and lengths of captively-reared Garita skipperling larvae were measured weekly with an ocular micrometer from hatching to pupation to determine larvals instars across development. Larvae were reared in either natural or static temperatures of 21 ℃ or 28 ℃ as part of a larger study on the effects of temperature on Garita skipperling phenology and survivorship. Only larvae that survived to pupation were used. Measurements were divided by preceding measurements to express them as multiples of previous measurements (Dyar's Values). Larvae were grouped by the number of Dyar's Values ≥ 1.1 and analyzed with k-means clustering. Garita skipperlings were observed undergoing five to seven instars between hatching and pupation. This did not appear to be influenced by ambient temperature. This reinforces other studies casting doubt on the generalizability of Dyar's Rule's in instar determination and indicate that corroborating findings with statistical analysis may be more effective. Using k-means clustering to corroborate groupings based on Dyar's values warrants further study, as it appears to be resistant to small sample size and can distinguish larvae with different numbers of instars without invasive or destructive sampling.

Introduction

The Garita skipperling, *Oarisma garita*, is found from the Manitoulin District in Ontario to as far west as British Columbia, though their range is discontinuous (Wilson, 2023). Historically, it was considered common east of the Rocky Mountains, but many records from their apparent eastern range were misidentified Poweshiek skipperling, *O. poweshiek*; a close relative (Catling, 1977). It is now widely accepted that Garita skipperlings range was similar in size to that of the Poweshiek's before the latter's widespread decline, and recent estimates indicate that *O. garita* is mostly found in the grasslands of the Rocky Mountains and western prairies of North America. The Manitoulin population is considered a pre-glacial remnant (Catling, 1977). Garita skipperlings are Critically Endangered in Ontario, with other Canadian populations classified as Secure or Apparently Secure (Wilson, 2023). In the United States, Garita skipperling in Washington is considered Vulnerable, in Minnesota and Nebraska it is

Threatened, while the remaining U.S. range is unassessed (Wilson, 2023). The Garita skipperling's closest relative, the Poweshiek skipperling, used to be abundant in the Mideastern United States and Canada (Catling, 1977), but have been extirpated across the majority of their historical range and are now endangered globally (Harris & Forster, 2014). While conservation initiatives and research on both species is ongoing, the threat that climate change poses to either the Poweshiek or Garita skipperling is not well understood.

Ontogeny in insects is an essential aspect of their biology that can impacted by climate change (Pogue *et al.,* 2016). Larval ontogeny occurs in discrete periods of development between shedding the exoskeleton (moulting) known as instars. While soft-bodied larvae can increase their body size between moults, insects can only increase the volume of chitinous or calcified body parts (like head capsules) immediately after moulting; also known as ecdysis (Dyar, 1890; Hawes, 2019). This results in the staggered growth of chitinous regions during development that researchers can be modelled to determine an insects' age, its stage of larval development, or to examine the effects of biotic and/or abiotic variables on ontogeny (Dyar, 1890; Grunert *et al.,* 2015; Hawes, 2019). The number of instars larvae undergo is also crucial to understanding population dynamics and ecology (Cazado *et al.,* 2014). Thus, determining instar number is foundational for detailed studies on insect survival and development, i.e., mortality, survivorship via life tables, population modelling, etc*.,* in conservation, research, and pest management (Gold *et al.,* 1999).

Due to the limited interval that the exoskeleton can expand before hardening, it is typically assumed that caterpillars (and some other taxa) follow a geometric growth pattern with a consistent ratio between the size of consecutive moults, e.g. 130% or 1.3× the width of the preceding head capsule, called Dyar's Rule (Dyar, 1890; Beck, 1950; Hunt and Chapman, 2001; Hawes, 2019). Based on these assumptions, researchers have determined the number of instars, the critical size thresholds required to moult, and created detailed models of many insect ontogenies (Cazado *et al.,* 2014; Grunert *et al.,* 2015; Castañeda-Vildózola *et al.,* 2016). Although Dyar's Rule has been applied by numerous researchers in pest management, conservation, and invertebrate paleontology, many insect species have been observed violating one or more of its assumptions (Gains and Campbell, 1935; Beck, 1950; Hunt and Chapman, 2001; Grunert *et al.,* 2015).

As noted by Hawes (2019), Dyar's foundational paper describes the conceptual and biological basis for this rule but does not suggest a mathematical expression for a fixed geometric growth. Nor has subsequent research used a consistent formula for determining Dyar's values, how they relate to the size of subsequent instars, or what form of statistical analysis should be used to verify them (Hawes, 2019). One attempt to generalize this relationship uses logarithmic or exponential transformations of growth data to generate linear models via regression (Gains and Campbell, 1935). However, linear models are only a partial fit for discontinuous, geometric, data and are susceptible to producing higher r^2 values for models that underestimate the total number of instars (Hawes, 2019). Applying Dyar's Rule via exponential or logarithmic modelling to approximate geometric growth is another common approach (Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016), but it is typically paired with other methods to prevent underestimating the number of instars: e.g., destructive sampling of numerous individuals, or methods like the *hcap* program which are tailored to their study-species (Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016; Hawes, 2019). However, these methods are still prone to underestimating the number of instars in smaller datasets (Hawes, 2019).

Dyar's Rule is a foundational concept of insect ontogeny, but its assumption of a fixed size ratio between instars is invalid for many insects (Gains and Campbell; Gold *et al.,* 1999; Hunt and Chapman, 2001; Grunert *et al.,* 2015). The core assumption behind Dyar's Rule — that insect growth is geometric and discontinuous — has also been contradicted by studies on Tobacco Hornworm, *Manduca sexta*, where growth followed a sigmoidal curve regardless of larvae's diet or ambient temperature (Grunert *et al.,* 2015). Static temperatures can also produce supernumerary instars in the larvae of some species; further complicating instar determination (Grunert *et al.,* 2015). Subsequently, many researchers have also developed their own methods or paired linear models with other forms of analysis: such as Maximum Likelihood Estimation to test alternate model outputs when re-mapped to raw data (Hunt and Chapman, 2001), created purpose-built software like the *hcap* program for large-scale studies on granivorous weevils (Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016), or relied on visual representations like histograms or density plots (Capinera, 1984).

Head capsule widths can be used to determine the number of instars visually by plotting them in histograms or density plots and counting the number of distinct groupings, i.e., peaks (Capinera, 1984). While they can be applied without assuming Dyar's Rule or transforming data, using histograms and density plots can be influenced by confirmation bias and interpreting the results may be affected by scaling factors (e.g., bin widths) if they are not paired with another form of analysis (Hunt and Chapman, 2001). Coupling visual determination of instars with the outputs from Maximum Likelihood Estimation has been effective in evaluating competing hypotheses on the instars of fossilized trilobites (Hunt and Chapman, 2001), where limited sample availability mirrors the restrictions on data collection on endangered species. However, applying Maximum Likelihood Estimation to instar determination relies on competing, well-defined, hypotheses from pre-existing research (Hunt and Chapman, 2001).

As research on Poweshiek skipperlings must be as risk averse as possible, and the potential effects of handling larvae while measuring head capsules were unknown, only Garita skipperlings were used in this study. Since the North America population of Garita skipperlings are considered stable but are closely related and ecologically similar to the globally imperiled *O. poweshiek*, researchers have used *O. garita* as a surrogate before applying new conservation or experimental methods to Poweshiek skipperlings (Breiter and Burns, 2018).

While the generalizability of Dyar's Rule has been challenged by researchers, its assumption of discontinuous head capsule growth is less commonly violated. Most studies have reported head capsule widths grow sporadically, only after moulting (Dyar, 1890; Hunt and Chapman, 2001; Cazado *et al*., 2014; Castañeda-Vildózola *et al*., 2016; Hawes, 2019); although some species have exhibited continuous, sinusoidal, growth in experimental conditions (Gains and Campbell, 1935; Grunert *et al*., 2015). I expect Garita larvae to follow a discontinuous growth pattern but will need to determine whether their growth is continuous or discontinuous. The methods described in literature for measuring head capsules and determining the number of instars and their size ranges (i.e., within an instar) use destructive sampling or other methods that are currently impracticable on Poweshiek skipperlings or Garita skipperlings. A new method will need to be devised for this experiment.

Methods

Head capsule imaging and measurement

All Garita skipperling larvae were reared from 2019–2020 at Assiniboine Park Zoo, Winnipeg as part of my incubation trials (See Chapter 4). However, treatments were not considered during instar determination, which was applied to all larvae that were photographed from neonates until eclosure. I photographed each larva weekly through an Olympus™ Model WH10X ocular micrometer at 30x magnification. Any evidence of recent moulting, or the onset of moulting was recorded, and larvae found in the process of moulting were measured the next day. Head capsule measurements were made for the purpose of instar determination for larvae in both the experimental treatments and outdoor control-groups (Table 2.1).

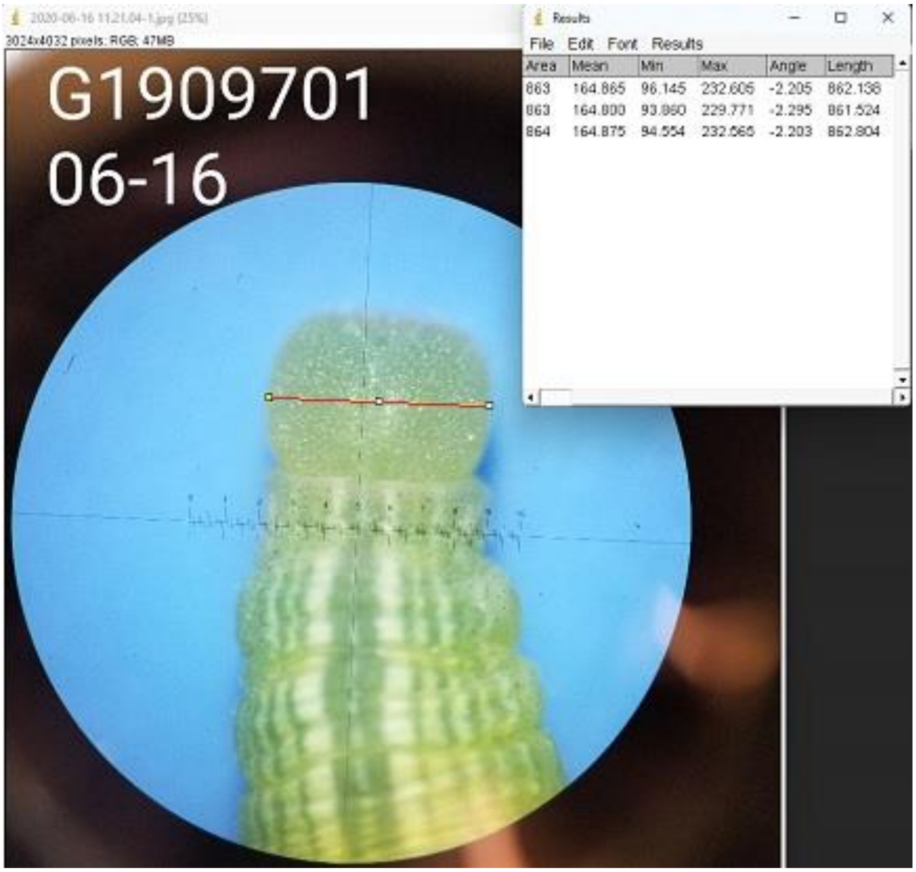
Table 2.1. Garita skipperling (*O. garita*) larvae used in instar determination by treatment. Table shows the start dates, final addition dates, and end dates of the static temperature trials that larvae were sourced from for instar determination. All larvae were sourced from the *ex-situ* population of Garita skipperlings (*O. garita*) reared at Assiniboine Park Zoo from 2019–2020. Only larvae which survived to pupation were used in instar determination. Larvae from all treatments were pooled for instar determination.

Treatment temperatures	Treatment start dates	Final larvae added	Treatment end date	Number of larvae added	Number of survivors
28C	July 15, 2019	August 2, 2019	September 28, 2019	76	18
21C	July 19, 2019	July 30, 2019	June 29, 2020	64	2
Control	July 17, 2019	August 7, 2019	July 2, 2020	62	11
Total	N/A	N/A	N/A	202	31

I removed larvae from their host plants using fine paintbrushes and placed them into plastic weighing dishes, imaged them, and returned them to their host plant. Since they typically responded to external stimuli by curling up (making them impossible to image at the correct orientation), they were left undisturbed in the dish until they uncurled before being measured. This slowed imaging and therefore limited the frequency that data could be collected from each larva to weekly measurements. Multiple images were taken of each individual to limit the differences in head capsule orientation, effects of motion blur, and variable focus, and only the highest quality photo of the dorsal view of the head capsule was used: approximately 1000 of the 3800 images recorded were measured. All images were recorded with the same Samsung Galaxy™ equipped with a 12MP digital camera.

Head capsule measurements were made using the ImageJ 1.53a program's ruler feature (Rasband, 2022). First the scale was set for an image using the reticle of the ocular micrometer. Since image quality varied, i.e. due to imperfect focus, scaled values were compared between images to ensure consistency by examining their per unit pixel count (e.g., the number of pixels between the 0 and 1 indicators of the ocular micrometer). While variable, it consistently fell within a range of 130 ± 15 pixels per unit regardless of image quality (Fig 2.1). When scales deviated from this range (e.g., 150 pixels per unit), they were reset and rechecked to ensure this was due to human error when initially setting the scale: not image quality. An alternate image (taken during the same weekly imaging) was used if the per pixel per unit continued to deviate from the 130 ± 15-pixel range.

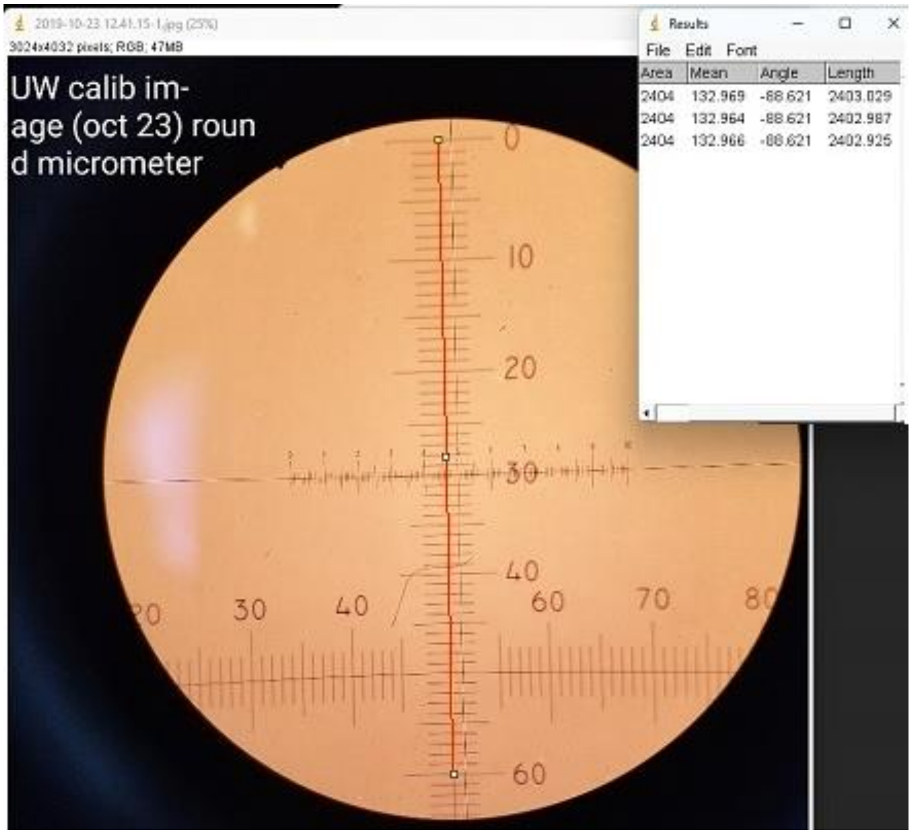
The broadest point of the dorsal surface of head capsules varies between larvae and instars but generally occurs at the midpoint of the head capsule, slightly towards the frons (Fig 2.1). The widest point was then measured three times, which were then averaged to limit the effects of human error. Each measurement was started on alternating sides of the head capsule to minimize confirmation bias. The symmetric patterning of the cuticle and arrangement of hairs were used as guides to ensure that measurements were as perpendicular to the dorsal-ventral midline as possible. Measuring head capsule length followed a similar procedure, except for the visual cues used. Larval head capsules are bilobed with a prominent midline (Fig 2.1), which was measured three times, starting at alternating ends, and averaged.



Area	Mean	Min	Max	Angle	Length
863	164.865	96.145	232.605	-2.205	862.138
863	164.800	93.860	229.771	-2.295	861.524
864	164.875	94.554	232.565	-2.203	862.804

Figure 2.1 Example of measuring head capsule width. Garita skipperling (*O. garita*) larvae were reared in incubation trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg and head capsule lengths and widths were measured weekly. Larvae were photographed through an ocular micrometer and measured via ImageJ (Rasband, 2022). Measurement line indicated in red.

To correct discrepancies between dissection scopes, adjust for the 30× magnification, and calculate the true size of head capsules, I calculated correction factors (1 unit = 0.3049 mm and 1 unit = 0.3352 mm) for each set of equipment used for imaging. These were determined by referencing each set of equipment to the same MICROYN™ MT-C7 stage micrometer. Images of the stage micrometer were taken through the same ocular micrometres and dissection scopes used to measure larvae then measured using the same process as head capsules to determine the correction factors for each set of equipment used to image larvae. The head capsule measurements obtained using each set of equipment were then multiplied by the corresponding correction factor, and the head capsule lengths and widths (in micrometres) were obtained (Fig 2.2).



Area	Mean	Angle	Length
2404	132.969	-88.621	2403.029
2404	132.964	-88.621	2402.987
2404	132.966	-88.621	2402.925

Figure 2.2 Example of calibration image used to calculate correction factor. Correction factors were applied to image data of Garita skipperlings (*O. garita*) reared in incubation trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg. Correction factors were calculated separately for each set of equipment used to image larvae by imaging the same stage micrometer with each set of equipment, and measuring them in ImageJ (Rasband, 2022). Measurement line indicated in red.

Instar determination

In order to determine whether larvae moulted between measurements, I divided the weekly head capsule measurements of each larva by the measurement from the preceding week to express them as multiples of previous measurements, termed Dyar's values (DV). I did this for both head capsule widths and lengths. When DVs were plotted into histograms, their values fell into 2 broad groups: DVs from 0.95 to 1.05 or from 1.1 to 1.5 with several DVs >1.5 (Fig 2.3). Measurements with DVs ≥ 1.1, were considered a moult. Larvae which survived to pupation were grouped by the number of total instars (five, six, or seven) based on the number of DVs ≥ 1.1 calculated for each larva.

Due to it's limited parametric assumptions and flexibility (Cordeiro de Amorim and Henning, 2015), I used K-means clustering to corroborate instar determination via Dyar's Values. Datapoints at different k-values (the total number of clusters) are assigned to clusters randomly, clusters' averages and sum of square residuals (SSR) are calculated, and this process is repeated until the clusters with the lowest SSR are determined at each k-value (Cordeiro de Amorim and Henning, 2015). A scree plot of SSR values at each k-value is then generated, and the optimal k-value is found where SSR values start to approach an asymptote as k increases, known as the 'elbow' of the scree plot (Everitt and Torsten, 2010).

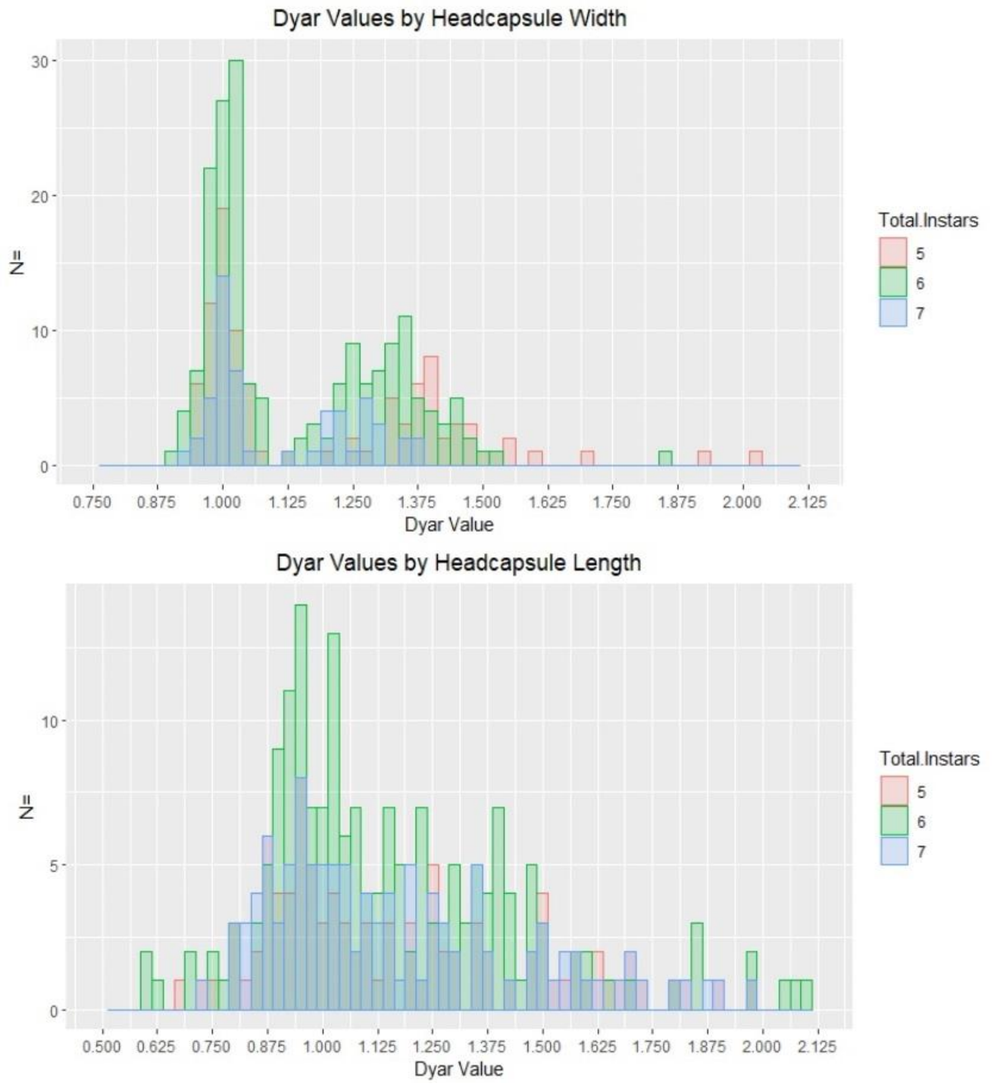
Instar determination was only applied to larvae that were measured from hatching to pupation. I made scree plots of SSR at different k-values (i.e., total instars) and determined the 'elbow' of each scree plot (Everitt and Torsten, 2010). Scree plots were generated for head capsule measurements by treatments, all larvae pooled together, and for larvae grouped by the number of Dyar's Values ≥1.1, and the optimal k-values were estimated. I clustered head capsule widths and lengths at the optimal k-values, and at 1 and 2 k-values above and below them, as determined by each scree plot. Overlaps between the maxima or minima of adjacent clusters, clustering that indicated moults below a 10% size increase, and/or that grouped measurements after an apparent moult (based on DVs or observed moults) was interpreted as inaccurate. I also plotted clusters via histograms to compare their outputs visually, i.e., whether clusters visually aligned with the distribution of the raw data (Capinera, 1984; Hunt and Chapman, 2001). Clustering which did not meet these criteria were rejected and omitted from the results. Finally, clusters that were not rejected were used to determine the number of instars each larva underwent from hatching to pupation, and to determine the size range of each instar.

This mirrored the instar determination of Hunt and Chapman (2001): which visually assessed histograms generated via Maximum Likelihood Estimation. However, k-means clustering can be applied to head capsule data without pre-defined size ranges for instars (which are not known for Garita skipperling) while still using statistics to corroborate visual assessments. K-means clustering similarly limited the influence of scaling factors and confirmation bias compared to visual assessment alone (Hunt and Chapman, 2001; Hawes, 2019). Males and females were not analyzed separately for instar determination, because some were mistakenly not sexed.

Results

Dyar's Values

When DVs from head capsule widths were grouped by the total number of Dyar's Values ≥ 1.1 calculated for each larvae, they fell into two groupings: from 0.88–1.095, centered around 1, and DVs between 1.1–1.5 centered around 1.35, with a small proportion (n = 8) above 1.5. Dyar's Values below 1.1 were tightly grouped around 1 regardless of the number of instars, with roughly 95% falling between 0.95–1.05. DVs around 1 were largely distributed equally above and below 1, but a slight skew above 1 was recorded for larvae with 6 instars. DVs ≥ 1.1 had a notably wider distribution across larva with five, six, or seven instars: roughly 2.5–3× the range of Dyar's Values around 1. Distinct peaks at 1.25 and 1.375 were observable for larvae with 6 instars, and a single, smaller, peak at approximately 1.4 was recorded for larvae with 5 instars. Dyar's values ≥ 1.1 exhibited a saw-toothed distribution for larvae with 7 instars: with most datapoints occurring around 1.2, 1.3, and 1.375 (Fig 2.3). Similar groupings were observed for DVs calculated using head capsule lengths: both of which violated Dyar's Rule. However, the groupings for head capsule lengths had much larger, overlapping, ranges with less defined peaks and more values occurring outside of these ranges (Fig 2.3).



Dyar Values by Headcapsule Width

Dyar Values by Headcapsule Length

Figure 2.3. Dyar's Values for head capsule widths and lengths of survivors grouped by total instar number. Instar number based on the Dyar's Values ≥ 1.1 calculated for each larva. Garita skipperling (*O. garita*) larvae were reared as part of static temperature trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg, and the University of Winnipeg, Manitoba. Only larvae which survived to pupation were used in instar determination. Treatments were not considered in instar determination.

Selecting k-values via scree plots

All scree plots loosely followed the expected pattern of exponential decay. Higher decay factors were mostly associated with larger sample sizes but were also higher when using head capsule lengths versus widths. All SSR values fell until they reached their asymptote or began oscillating around it. The "elbow" of screes, i.e., the optimal k-value, was defined by the point before the horizontal asymptotes. Elbows aligned with larvae with five, six, or seven instars based on DVs calculated from head capsule widths. Only the five and seven instar groups had k-values that aligned with their instar number for head capsule lengths (Fig 2.4).

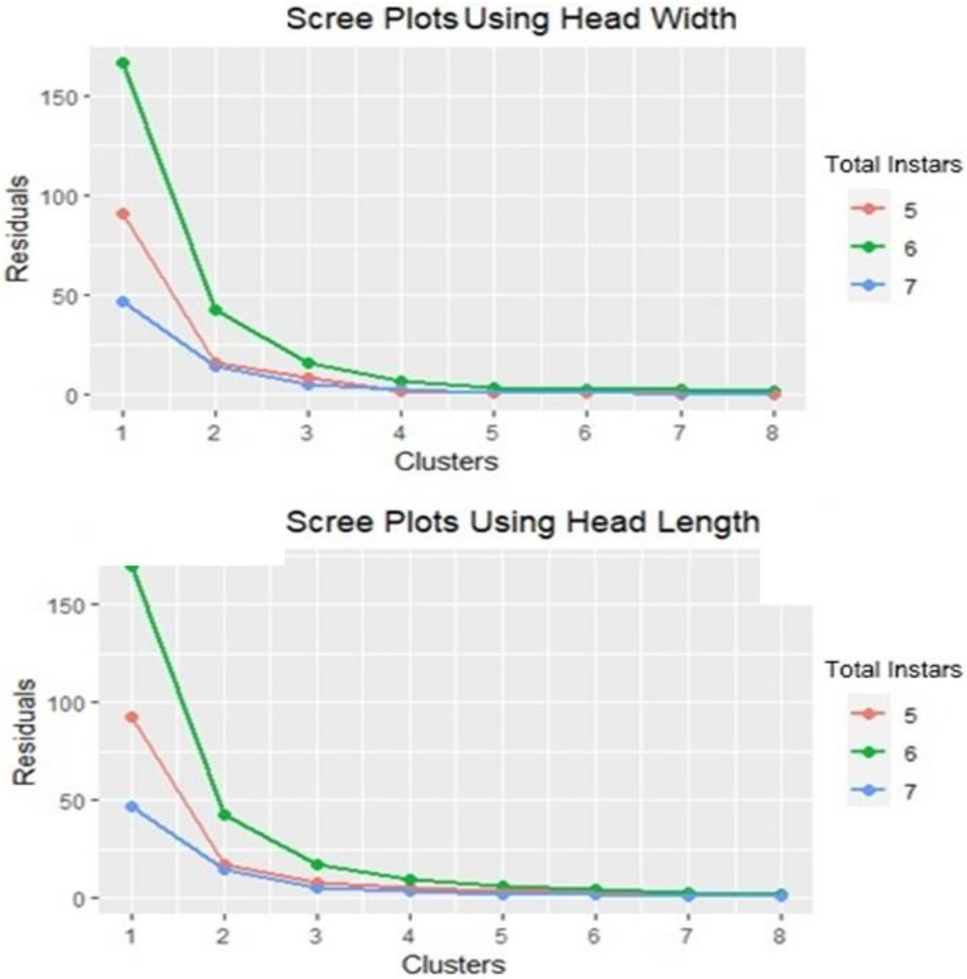


Figure 2.4. Scree plots of Garita skipperlings' (*O. garita*) head capsule widths and lengths based on sum of squared residuals at each k-value. All larvae were reared in incubation trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg (See Chapter 4). Larvae were grouped by the number of instars based on the Dyar's values ≥ 1.1 calculated for each larva that survived to pupation. Treatments were not considered in instar determination.

K-Means clustering and instar determination

K-means clustering produced histograms that visually fit the distribution of raw data, but only at k-values that equalled the number of instars (five, six, or seven). These groupings also had distinct size ranges for each instar, grouped measurements with DVs below 1.1 into the same clusters as preceding measurements, and grouped measurements with DVs ≥ 1.1 into successive clusters relative to their preceding measurements. The majority of head capsule widths fell in the middle of their respective ranges. Head capsules grew an average of 3.5–5× in width and 3.5–6× in length overall. K-means clustering was more consistent when applied to head capsule widths: datapoints were concentrated into higher peaks, with more separation between adjacent clusters. There were also discrepancies between head capsule lengths and widths: head capsule lengths placed some larvae's measurements into different instars than head capsule widths when clustered at the same k-value. (Fig 2.5).

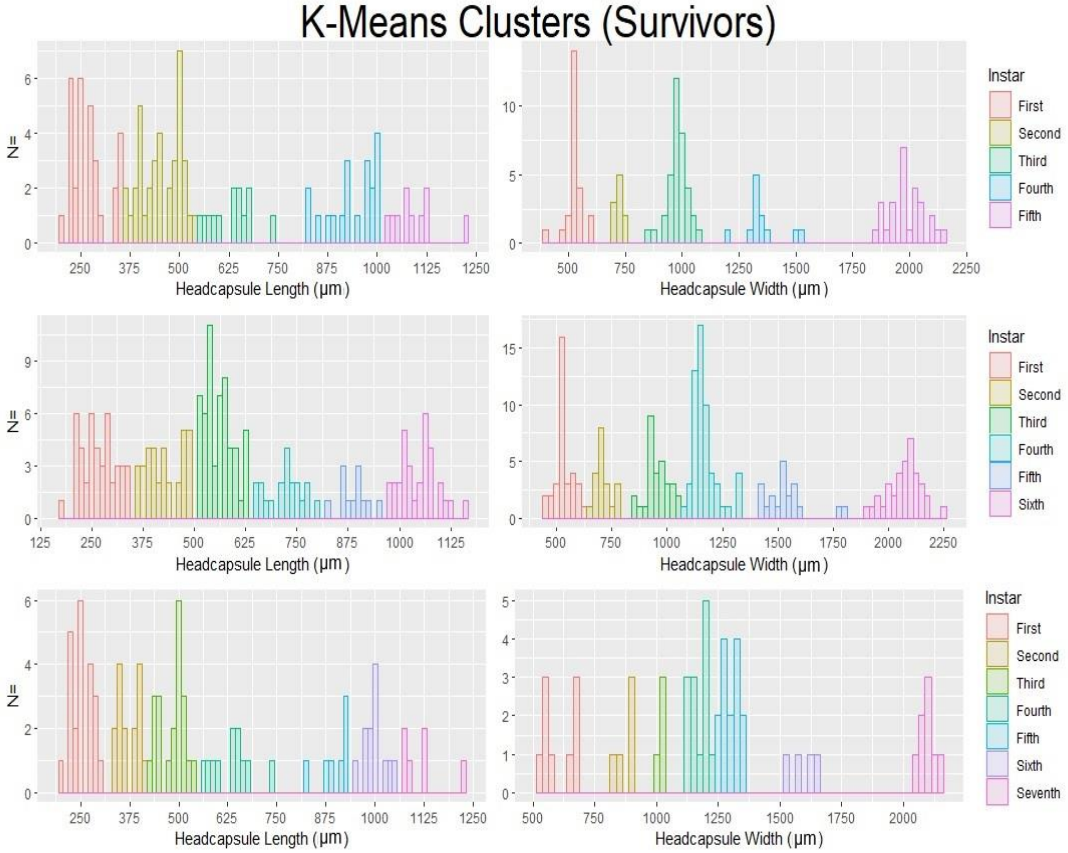


Figure 2.5. Histogram plots of Garita skipperlings' (*O. garita*) head capsule lengths and widths based on k-means clustering. Instars defined by the number of Dyar's values ≥1.1 calculated for each larva's head capsule measurements. All larvae were reared in incubation trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg, and the University of Winnipeg, Manitoba (See Chapter 4). Treatments not considered in instar determination. Widths and lengths given in micrometres.

Larvae with seven total instars had the most consistent size ranges, with more variability in head capsule lengths than widths, excepting the first and sixth instars. The widths for final instars varied by up to 400 micrometres but were similar regardless of the total instar number. The min and max values of first to fourth instars differed by 25 and 30 micrometres for larvae with six instars, by 35 micrometres between the fourth and fifth for larvae with seven instars. All other size ranges for head capsule widths were separated by at least 75 (and up to 400) micrometres, regardless of total instar number. The size ranges for the first to fourth instar were similar between larvae with five, six, or seven instars, while size ranges in the fifth to seventh instars varied with the total number of instars. This was true for size ranges generated for head capsule lengths or widths. However, the differences between the maxima and minima of adjacent clusters were observably lower when head capsule length was used compared to width: from as little as 10 to a maximum of 85 micrometres (Table 2.2)

Table 2.2. Size ranges and average sizes of Garita skipperling head capsule widths and lengths by instar. Table shows ranges and averages for instars determined via k-means clustering of head capsule widths and lengths of Garita skipperling (*O. garita*) larvae reared in static temperature trials from 2019–2020 at Assiniboine Park Zoo, Winnipeg. Larvae grouped by the total number of instars via Dyar's Values. Average head capsule widths and lengths for each instar listed in parentheses. All values listed in micrometres. Number of larvae listed in brackets.

Instar	5 Instars (n = 11)		6 Instars (n = 16)		7 Instars (n = 4)	
	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)
1	395–600 (525)	205–350 (270)	460–620 (535)	170–340 (265)	525–685 (605)	205–300 (255)
2	690–750 (720)	365–535 (455)	650–785 (710)	360–490 (425)	830–905 (875)	330–415 (370)
3	860–1085 (980)	545–735 (630)	840–1055 (955)	505–625 (560)	1005–1030 (1020)	430–535 (480)
4	1200–1525 (1350)	820–1005 (940)	1080–1325 (1170)	650–800 (720)	1125–1215 (1170)	570–735 (635)
5	1855–2150 (1990)	1025–1225 (1090)	1420–1800 (1540)	830–945 (885)	1250–1355 (1300)	820–930 (895)
6	N/A	N/A	1900–2250 (2070)	970–1170 (1045)	1530–1640 (1590)	955–1045 (1000)
7	N/A	N/A	N/A	N/A	2055–2150 (2095)	1070-1225 (1115)

Discussion

DVs ≥ 1.1 had a substantially larger range than values below 1.1 (2.5 to 3 times as large) using larval head capsule widths. In comparison, DVs around 1 calculated using head capsule widths were tightly grouped, with 95% between 0.95 and 1.05. This suggests that the larger range for DVs ≥ 1.1 were not due to errors from measurements, as this would impact range of both Dyar's Values below 1.1 and DVs ≥ 1.1. The wide distribution of Dyar's Values ≥ 1.1 demonstrates that Garita skipperling larvae did not follow Dyar's Rule, regardless of their total instar number. Though larvae grew discontinuously, as indicated by the gap between DVs around 1 and DVs ≥ 1.1, the variation in DVs ≥ 1.1 violated the consistent size ratios between instars assumed under Dyar's Rule (Dyar, 1890; Beck, 1950; Hunt and Chapman, 2001; Grunert *et al.,* 2015; Hawes, 2019). While a variety of caterpillars, and other larvae, follow Dyar's Rule (Dyar, 1890; Hawes, 2019), there are also multiple species which violate one or both of its assumptions (Gains and Campbell, 1935; Beck, 1950; Hunt and Chapman, 2001; Grunert *et al.,* 2015).

Dyar's values calculated using head capsule lengths produced somewhat similar results to DVs generated from head capsule widths. However, DVs centered around 1 or ≥ 1.1 had notably larger ranges with less defined peaks for head capsule length, and numerous datapoints fell outside of these groupings. This is likely attributable to the accuracy of head capsule length measurements versus measurements of head capsule width. The length of head capsules may be a poor indicator of the change in the overall size of larvae's head capsules compared to head capsule widths, and/or is too readily impacted by larvae's orientation. The midline used to estimate head capsule lengths (Fig 2.1) appeared to be impacted by the orientation of larvae's head capsules more than head capsule widths, as it could appear shorter or curved depending on larvae's orientation to the ocular micrometer. Numerous studies on insect larvae have used only widths to determine instars (Dyar, 1890; Capinera, 1984; Gold et al 1999; Brust *et al*., 2005; Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016; Hawes, 2019). Despite prominent midline present in the head capsule, lengths appear to be more error-prone than widths. Head capsule lengths should be omitted from future analyses.

While scree plots indicated that larva which survived to pupation did not undergo a consistent number of instars, the results of scree plots alone did not support the conclusion that larvae underwent between five and seven instars before pupation. Examining the clustering produced at the k-values indicated by scree plots (and at ± 2 k-values) was required to assess the accuracy of grouping larvae into five, six, or seven instars. Only k-means clustering applied at the k-values equal to the number of instars (as indicated by DVs ≥ 1.1) produced clear histograms with distinct size ranges that separated measurements after an apparent moult into separate clusters and did split measurements with DVs <1.1 into separate clusters. This produced comparable results to the Maximum Likelihood Estimation used by Hunt and Chapman (2001) but did not require existing size ranges for each instar. Although individual measurements were not placed into the same instars using head capsule length and width, this was likely due to the aforementioned issues with using head capsule lengths for instar determination.

The results of k-means clustering suggest that Garita skipperlings can undergo between five and seven moults as larvae. This did not appear to be the result of supernumerary instars stemming from static temperatures, as has been observed in Tobacco Hornworm (Grunert *et al.,* 2015), since multiple larvae eclosed after five, six, or seven instars after being reared outdoors. Rather, variability in the total number of instars appears to be a previously unreported trait of Garita skipperling larvae. While it has not been documented for Garita skipperling prior to this study, similar findings have been reported for Western Branded Skipper (*Hesperia colorado*) (MacNeil, 1964; James, 2009). Additional instars can be associated with viral infections, but this is accompanied by a reduction in size with each additional moult (MacNeil, 1964), which was not observed in Garita skipperling.

The variable instar number exhibited by Garita skipperling may stem from compensatory strategies by larvae with different growth rates. Fewer and additional instars have both been attributed to Skipper larvae growing at slower or accelerated rates compared to other larvae in their population (James, 2009). Larvae which have grown slowly enough that they have not reached their final instar by the time that they should pupate (e.g., because of a less nutritional and/or edible food source) can skip the final instar, pupate, and eclose as underweight adults (James, 2009). The opposite can happen to Skipper larvae which reach their final instar prematurely: they can moult an additional time before pupation and eclose as heavier-than-average adults (James, 2009). However, since the weight of Garita skipperling were not assessed at any stage of development, it is unclear from these results if different numbers of instars were associated with differences in adult mass.

The sample size for survivors with seven total instars, four individuals, limits the conclusions that can be drawn for these larvae. While their change in head capsule size, as reflected by Dyar's values, and the results of k-means clustering both indicate that they underwent seven larval instars before pupation, more individuals are needed for to finalize the size ranges for larvae with seven instars. Furthermore, the total instar number of deceased larvae was indeterminable, limiting the specificity of derivable mortality statistics, and were not classifiable beyond the fourth instar. This may be achievable following the development of a generalizable growth model (Kingsolver *et al.,* 2009; Grunert *et al.,* 2015), but it was not determinable via remapping the size ranges generated from k-means clustering. Sexual dimorphism can influence the size of head capsule widths in some butterfly species (Capinera 1984), but any differences between male and female Garita skipperlings was indeterminate because sex was not tracked for all survivors due to errors in record-keeping. Any role played by genetics could also not be analyzed, as relatedness has only been tracked with respect to mating trials: the genetics of the *in-situ* founders and *ex-situ* populations are not known.

The inconsistent size ratios (i.e., Dyar's Values ≥ 1.1) between Garita skipperling's different instars reinforce the growing body of research challenging the assumptions of Dyar's Rule (Grunert *et al.,* 2015; Hawes, 2019). Numerous studies have challenged larvae's adherence to Dyar's Rule of fixed, geometric, growth since its inception (Gains and Campbell, 1935; Beck, 1950), but it has nonetheless been applied — often in tandem with other methods — for over a century by some researchers: indicating that some species follow Dyar's Rule (Dyar, 1890; Cazado *et al.,* 2014; Hawes, 2019). Combining k-means clustering with Dyar's Values based on head capsule widths appears to be a promising technique for instar determination. It is seemingly resilient to the errors associated with linear models (Beck, 1950; Hawes, 2019) and scaling errors or confirmation biases of purely visual assessments (Hunt and Chapman, 2001). This appears to be true even when larvae's total instar number is variable, sample size is limited, and non-destructive and minimally invasive methods are used to measure head capsules.

While preliminary, considering head capsule growth relative to previous measurements (i.e., Dyar's values), grouping individuals by their apparent number of moults, and corroboration using k-means clustering — without applying the assumptions of Dyar's Law with respect to fixed, geometric, growth — appears to be an effective method of instar determination. Instar identification via Dyar's values and k-means clustering of head capsule widths additionally appear to be risk-averse enough to use on critically endangered species where destructive sampling is not feasible. More research, including replication on a larger scale over multiple generations (i.e., tracking progeny of Garita skipperling that eclose following a different number of instars), is needed to determine the cause and significance of larvae undergoing a variable number of instars before pupation. The integration of degree-day modelling is also required to finalize analyze instar-specific temperature requirements, and determine which instars are most sensitive to temperature-induced phenological shifts associated with climate change.

References

Beck SD. 1950. Nutrition of the European Corn Borer, *Pyrausta nubilalis* (HBN.). II. Some effects of diet on larval growth characteristics. Physiological Zoology. 23(4):353–361.

Breiter C-J, Burns L. 2018. Grassland butterfly conservation program 2017 annual report. Winnipeg (MB): Assiniboine Park Zoo.

Capinera JL. 1984. Instar number in Range Caterpillar, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae). Journal of the Kansas Entomological Society. 57(2):344–347.

Castañeda-Vildózola Á, González-Hernández H, Equihua-Martínez A, Valdez-Carrasco J, Peña JE, Cazado LE, Franco-Mora O. 2016. Head capsule width is useful for determining larval instar in *Heilipus lauri* (Coleoptera: Curculionidae). The Florida Entomologist. 99(4):822–825.

Catling P. 1977. On the occurrence of *Oarisma garita* (Reakirt) (Lepidoptera: Hesperiidae) in Manitoulin District, Ontario. The Great Lakes Entomologist 10(2): 59–63

Cazado LE, Van Nieuwenhove GA, O'brien CW, Gastaminza GA, Murúa MG. 2014. Determination of number of instars of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) based on head capsule widths. The Florida Entomologist. 97(2): 639–643.

Cordeiro de Amorim R, Hennig C. 2015. Recovering the number of clusters in data sets with noise features using feature rescaling factors. Information Sciences. 324 (1): 126–145.

Dyar HG. 1890. The number of molts of Lepidopterous larvae. Psyche: a Journal of Entomology. 5(1): 175–176.

Everitt BS, Hothorn T.2010. A handbook of statistical analyses using R. 2nd ed. Boca Raton (FL): Chapman & Hall/CRC.

Friberg M, Haugen IMA, Dahlerus J, Gotthard K, Wiklund C. 2011. Asymmetric life-history decision-making in butterfly larvae. Oecologia. 165(2): 301–310.

Gains JC, Campbell FL. 1935. Dyar's Rule as related to the number of instars of the corn ear worm, *Heliothis obsolete* (Fab.), collected in the field. Annals of the Entomological Society of America. 28(1): 445–461.

Gold CS, Neyeme PS, Coe R. 1999. Recognition and duration of larval instars of banana weevil, *Cosmopolites sordidus* Germar, in Uganda. African Entomology 7(1): 49–62.

Grunert LW, Clarke JW, Ahuja C, Eswaran H, Nijhout HF. 2015. A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. PLoS ONE. 10(5): 1–23.

Harris A, Foster R. 2014. Assessment and status report on the Poweshiek skipperling *Oarisma poweshiek* in Canada. Ottawa (ON): Committee on the Status of Endangered Wildlife in Canada (Canada).

Hawes T. 2019. An equation for geometric progression in Brooks-Dyar growth ratios. Invertebrate Reproduction and Development. 64(1): 1–12.

Hunt G, Chapman RE. 2001. Evaluating hypotheses of instar-grouping in arthropods: A Maximum Likelihood Approach. Paleobiology. 27(3): 466–484.

Layberry R, Hall P, Lafontaine D. 1998. Butterflies of Canada- Garita skipperling. Toronto (ON): University of Toronto Press.

MacNeil CD. 1964. The skippers of the genus Hesperia in western North America with special reference to California. University of California Publications in Entomology, University of California Press, Berkeley and Los Angeles. 35: 1–221.

Pogue C, Monfils M, Cuthrell D, Heumann B, Monfils A. 2017. Habitat suitability modeling of the federally endangered Poweshiek skipperling in Michigan. Journal of Fish and Wildlife Management 7(2): 359–368.

Scott J, Epstein M. 1987. Factors affecting phenology in a temperate insect community. The American Midland Naturalist 117(1): p 103–118.

Wilson K. 2023. Comprehensive Report: Species – *Oarisma garita*. Arlington (VI): NatureServe (US).

CHAPTER 4: INCUBATION TRIALS ON GARITA SKIPPERLINGS REARED AT ASSINIBOINE PARK ZOO, MANITOBA

Abstract

I compared the development and survivorship of Garita skipperlings (*Oarisma garita*) reared from 2019–2020 at Assiniboine Park Zoo, Manitoba, at a constant temperature of 21 °C or 28 °C in environmental control chambers to larvae reared outside. These temperatures were chosen to provide the degree-days necessary to induce eclosure by Fall 2019 and April 2020, respectively. Larvae were also added to the 28 °C incubator after several weeks of development outdoors to simulate the effects of high temperatures in the fall. Exposure to a constant 21 °C reduced survivorship by 15% while 28 °C increased it by 6% (and by 57% for late inductees) compared to larvae reared outdoors. However, neonate mortality was not calculable for late inductees to the 28 °C treatment: contributing to their high survivorship. The phenology of all larvae added to the 28 °C treatment, except for one late inductee, was disrupted and all but one adult eclosed before the onset of winter. The two individuals within the 21 °C treatment, and one individual from the 28 °C that overwintered and eclosed, were not accelerated and emerged at similar times and ages as adults reared outdoors. Performance-issues with the 21 °C environmental control chamber likely impacted larval mortality, and possibly ontogeny, in this treatment. While preliminary, it appears that high static temperatures can accelerate Garita skipperlings, but that prolonged exposure to temperatures above 21 °C are needed to force Garita skipperlings to eclose in a single season. This research may provide insight into potential causes of the dramatic declines observed in the closely related and Endangered Poweshiek skipperling.

Introduction

A consistent decline in biomass, abundance, and biodiversity has occurred across insect guilds in recent decades (Vanbergen, 2013; Uchida and Ushimaru, 2014; Hallmann *et al.,* 2017). Biodiversity loss for herbivorous insects has primarily been attributed to increasing land-use intensity reducing the diversity of floral assemblages and abandoned agricultural land remaining less diverse than undisturbed habitats (Pöyry *et al.,* 2006; Uchida and Ushimaru, 2014). Habitat destruction and pesticides are further implicated in widespread pollinator declines (Vanbergen, 2013; Gilburn *et al.,* 2015). Even within protected areas, aerial insects in temperate habitats have exhibited substantial declines in biomass, over 75% in Germany in the last 30 years, which may result in an ecological cascade if it continues (Hallmann *et al.,* 2017).

Anthropogenic threats like habitat loss, land degradation, and resource overexploitation (e.g., groundwater and/or soil depletion) are all considered primary drivers of insect biodiversity loss in the last 50 years, but it is generally accepted that climate change will exacerbate the current biodiversity crisis (Diamond *et al.,* 2014; Diaz *et al*., 2019; Yang *et al.,* 2019). The unprecedented fluctuations in Nearctic temperatures predicted by most climatological models (Blair *et al*., 2019) may cause community-level changes to ecosystems through differential phenological responses. This may result in the desynchronization of plants and pollinators, predators and prey, and many other species interactions: ultimately leading to range declines or the extinction of ecologically significant species (Ovaskainen *et al.,* 2013).

Despite the ecological significance insects in the natural environment, present declines, and potential utility as indicators of ecosystem stressors, their biological responses to changes in temperature remain poorly studied. Research has largely focused on species with direct agricultural or medical significance: pests, domesticated insects, and species that are vectors for viruses, bacteria, and/or parasites (Pyle *et al*., 1981; Saunders *et al*., 2020). While biologists have examined the ecological roles, development, and phenology of various insect species, this has often been biased towards charismatic, less cryptic, taxa and/or those which are easier to rear in an experimental setup (Bossart and Carlton, 2002). For the majority of insect taxa, our ability to predict responses to climate change is severely limited by a lack of foundational research and/or historical data of their past abundances, ranges, etc. (Bossart and Carlton, 2002; Buckley *et al.,* 2010; Ovaskainen *et al.,* 2013)

North America's prairies support a variety of imperiled skippers, family Hesperiidae (Latreille, 1809), that may be threatened by climate change: including the critically endangered Poweshiek skipperling (*O. poweshiek*), its relative (and focus of this study) the Garita skipperling, and the threatened Dakota Skipper (*Hesperia dacotae*). Historically, the prairies formed a wide, heterogenous matrix of flora and fauna across much of central North America (Sampson and Knopf, 1994; Harris and Foster, 2014). Today, this once-expansive assemblage of diverse ecosystems has been all but eradicated following centuries of urbanization, agricultural expansion, and other anthropogenic activities (Selby, 2005; Swengel, 2008; Cuthrell and

Slaughter, 2012; Pogue *et al.,* 2016). In particular, the remaining tallgrass prairies are considered one of the most imperiled habitats globally: less than 1% of North America's native tallgrass prairies remain (Sampson and Knopf, 1994; Swengel, 2008). Despite concerted efforts to protect and study prairie ecosystems, the fate of many of their endemic insects is uncertain due to climate change; complicated by gaps in entomological research (Saunders *et al.*, 2020). This study is focused on the population of Garita skipperling residing in Southern Manitoba.

The severity and frequency of extreme precipitation events (i.e. heavy snow or rainfall) increased in recent decades in southern Manitoba (Blair *et al.,* 2019; Yang *et al.,* 2019). Climate models further indicate that this trend will worsen and impact ecosystems in southern Manitoba (Yang *et al.,* 2019). An average of 19.4 more days above freezing are predicted from 2021–2050 in Southern Manitoba than were averaged between 1976–2005, with a corresponding rise in mean temperature and in the duration and frequency of heat waves (Blair *et al.*, 2019). This has the potential to disrupt insect lifecycles as the likelihood of stochastic weather events and mean temperatures increase with climate change (Ovaskainen *et al.,* 2013; Karlsson, 2014).

Phenological advancements (e.g., early emergence) and extended seasons where individuals are active due to climate change are already well-established for several northern lepidopterans (e.g., butterflies, moths, and skippers) and other insects (Kearney *et al.,* 2010; Ovaskainen *et al.,* 2013; Karlsson, 2014). The predicted outcomes of climate-induced phenological shifts vary, but butterflies with limited ranges, high degrees of host-plant specificity, and/or that overwinter at later developmental stages (e.g. as pupae) typically exhibit greater levels of phenological advancement than other species (Diamond *et al.,* 2011; Diamond *et al.,* 2014). Over 60 species of subartic moths in Finland have flight times that are thermally regulated and are considered at risk of climate change-induced desynchronization with other trophic levels (Valtonen *et al.,* 2011). Numerous insect groups in the Canadian Arctic and Subartic also exhibit accelerated ontogeny to increased body temperature (Danks, 1994); suggesting that climate change may have wide-reaching effects across insect guilds in temperate zones.

Garita skipperling populations in Manitoba are considered stable, so researchers have used them as a surrogate for their endangered close relative, the Poweshiek skipperling, to trial new conservation or experimental methods (Breiter and Burns, 2018). As research on critically endangered species like Poweshiek skipperling must minimize risks, only Garita skipperlings were used in this study. Poweshiek or Garita skipperlings that eclose unseasonably (i.e., outside of the normal flight period) may perish from a lack of nectar sources, produce eggs or larvae which cannot survive hibernation, and/or produce eggs when larval host plants are unavailable. It is essential to investigate the role of temperature in insects' ontogeny in order to understand the potential effects of climate change on their development. The purpose of this study is to test the effects of elevated, temperatures on Garita skipperling's phenology and survivorship compared to larvae reared in diurnal conditions.

Methods

Species selection and egg collection

All larvae in these trials were part of the *ex-situ* population of Garita skipperlings maintained from 2017–2020 at Assiniboine Park Zoo, Manitoba, Canada, sourced from Spruce Woods Provincial Park (Aspen Parkland Ecoregion, Stockton Ecodistrict). All larvae that were used in this study were collected as eggs that were laid in 2019. I collected eggs following mating trials of adults that eclosed at Assiniboine Park Zoo from July 5th to July 29th (APZ Conservation and Research, 2019; unpublished data). After mating, females were left in enclosures that were inspected daily for the presence of eggs, which were transferred onto damp filter papers in petri dishes. The daily output of eggs from each gravid female was defined as an egg batch. Each batch was counted and assigned an identification number to track parentage.

All eggs were collected between July 15th and August 1st, 2019. Eggs were placed inside incubators held at 25 °C and 55% relative humidity to maximize hatch-rates and neonate survivorship (APZ, 2024). However, the development and survivorship of eggs were not considered in this study, which focused on larval ontogeny. Eggs were checked twice daily for signs of development, such as head-capsule formation, and newly hatched larvae (neonates) were removed, imaged, and placed onto Prairie Dropseed (*Sporobolus heterolepis*) host plants. Degree day (DD) calculations for each larva were started on the date they were initially placed onto host plants.

Design of incubation trials using degree-days

I used degree-days to estimate the effects that different temperatures regimes might have on Garita skipperling eclosion by using the DDs calculated for larvae reared under natural conditions (See Chapter 2). Degree days from the 2017 and 2018 generations of Garita skipperlings indicated that their cumulative DDs can vary considerably between generations: averaging 1202 (±115.3) and 1676 (± 87.3) degree-days in 2017 and 2018, respectively. Only the DDs calculated using the standard method were used to estimate the effects of static temperatures, as constant temperatures do not follow the diurnal temperature trends assumed by the double-sine method. The standard method uses the formula:

$$DD = \frac{(T_{max} - T_{min})}{2} - LTT$$

to calculate daily DDs. When static temperatures are used, $(T_{min}+T_{max})/2$ is equal to the the temperature selected (e.g. if the static temperature is 21 °C then (21 °C + 21 °C)/2 is equal to 21 °C), so this equation was simplified to:

$$DD = T_{constant} - LTT$$

The LTT of 6 °C estimated for Poweshiek skipperlings (Dearborn and Westwood, 2014) was used for both species. I then calculated the DDs delivered at various constant temperatures to find static temperatures that would exceed the average DD totaled by larvae from the 2017 and 2018 generations, plus 2 standard deviations (1750 degree-days), by the end of Fall 2019 (hereafter the 28 °C trial) and by the onset of Summer 2020 (hereafter the 21 °C trial). The 28 °C trial provided degree-day values sufficient to eclose before the onset of hibernation and the 21 °C trial provided DDs sufficient to eclose approximately 2–3 months before Garita skipperlings normal eclosure: mid October of 2019 and late April to early May of 2020, respectively. These represented a severe and moderate disruption of Garita skipperlings normal phenology. The eclosure dates predicted for each trial assumed that hibernation was initiated on October 15th and ended on March 15th, and all larvae were added to both treatments by the start of August.

Incubation trials

I reared larvae in Environmental Control (EC) chambers held at constant 21 ℃ or 28 ℃. A control group was raised outdoors, which exposed them to diurnal temperatures, to compare the incubation trials to the natural development of the same cohort. I compared metrics of phenology (i.e., eclosure dates, pupation dates), larval ontogeny, and survival, and examined the differences in survivorship, age, and degree-days accrued at eclosure between treatments using the standard method. Larvae in these treatments were also photographed weekly through an ocular micrometer to measure their head capsules for instar determination. I compared larval development overall using final age, and by instar based on the instars determined from k-means clustering of weekly measurements (See Chapter 3).

I placed larvae into one of two EC chambers. One EC chamber was a Thermo Scientific™ Precision™ Low Temperature BOD Refrigerated Incubator (normally used to overwinter larvae) at Assiniboine Park Zoo, Winnipeg held at 28 ℃. The second was a Forma Scientific™ Model 3740 at the University of Winnipeg held at 21 ℃. Fifty (50) larvae in their 1st instar (neonates) were placed into each treatment upon hatching. As mortality during the neonate stage can be high, dead neonates were replaced until no more newly hatched larvae were available. The EC chambers used fluorescent grow lights connected to timers that were adjusted weekly to match the natural photoperiod throughout the duration of each treatment and were maintained at a relative humidity of 50–70% to prevent desiccation and reduce host-plant withering. Temperature and humidity levels were recorded at 15-minute intervals by Kestrel™ data loggers kept inside the EC chambers and hoop-house throughout the trials to corroborate temperature and humidity readings from the EC chambers, and to determine when to initiate and break hibernation for larvae reared in the hoop-house. A third group of 50 larvae was reared outdoors in hoop-houses at Assiniboine Park Zoo alongside other *ex-situ* larvae as a control group.

Finally, 20 individuals were added to the 28 ℃ EC chamber August 20th, 2019, following approximately 3 weeks of development in the hoop-houses. These were not part of the original experimental design but were added at the as the space available in the 28 ℃ EC chamber allowed me to additionally examine the effects of high static temperatures on Garita skipperlings versus a typical Fall climate. I predicted that they would be accelerated but undergo hibernation (potentially at a different instar than the 21 °C treatment or control), due to the total degree-days that they could accrue before hibernation. I will refer to neonates added to the 28 °C EC chamber before August 2nd as the Original 28 °C treatment and larvae added later (by August 20th) as the Late 28 °C treatment.

The Garita skipperlings inside EC chambers and outdoors were both reared following a methodology already established by Assiniboine Park Zoo Conservancy and Research from rearing previous generations of Poweshiek and Garita skipperling (Breiter and Burns, 2018). I placed larvae individually on Prairie Dropseed plants covered with mesh to contain the larvae and prevent predators from accessing the larvae or the host plant. These individual plants were then placed into the outdoor hoop-house or into an EC chamber. All host plants were inspected weekly, and each larva was located. Any larva which could not be found after three successive weeks of checks was assumed to have died in the first week of its disappearance. Host plant health was assessed during weekly larval checks based on the presence or absence of discoloration, withering, die-back, plant pests, and/or signs of overfeeding by larvae. Watering was provided to plants when needed. Larvae were relocated to new plants if host plants were observed deteriorating over two or more weekly checks.

Hibernation was induced and broken using methods established previously by researchers at APZ (Breiter and Burns, 2018). I removed larvae from their host plants and placed into paper sheaths within 1.5 mL microcentrifuge tubes held within water saturated Hydrostone™ molds to reduce evaporative losses incurred by overwintering larvae. Larvae were all held in the same EC chamber using a temperature scheme that has incurred minimal mortality in previous years (APZ Conservation and Research, 2018, unpublished data). Hibernation was induced on October 9th for larvae in the Late 28 °C treatment, October 15th for larvae in the control, and October 23rd, 2019, for larvae in the 21 °C treatment. Hibernation was broken for all larvae across treatments on April 30th, 2020. These dates were based on outside temperatures: beginning when overnight lows fell below 0 °C in the fall and ending when they rose above a daily mean of 10 °C in the spring. Larvae in the 21 °C treatment needed to accrue a minimum of 1200 degree-days before hibernation, so the start of hibernation was delayed for this treatment.

To minimize the shock of being introduced to low-temperature overwintering chambers directly from a high temperature treatment, the temperatures of both EC chambers were gradually reduced to below 6 °C from October 9th–15th and from 4 °C to -4 °C over a two-week period starting October 23rd to prepare larvae for hibernation. When reintroduced to incubation trials after overwintering, temperatures were gradually increased from 10 °C back to their original values over 10–14 days, by 2-degree increments with a day to acclimatize in between temperature changes, and rearing was resumed. The end point for all trials and the control was the death or eclosure of all larvae.

Results

Start dates, end dates, and survivorship across treatments

Fifty individuals were added to each treatment upon hatching. Larvae were added to the Original 28 °C treatment over a 20-day period: 50 neonates on July 15–16th and 26 replacements from July 20th– August 2nd. 50 neonates were placed into the control between July 17th–18th, with 12 replacements added by August 7th. 50 neonates were also added to the 21 °C treatment between July 19th and July 20th, with 14 neonates replaced by July 30th. All replacements in the above treatments were neonates and were only added to replace larvae that died without any change in their head capsule length or width (i.e., as neonates). All larvae added to the Original 28 °C treatment either died or eclosed before hibernation. Lastly, 20 additional larvae — which were not neonates — were introduced to the Late 28 °C treatment from August 20th–23rd, 2019 and were not replaced upon death. Fourteen of the larvae in the Late 28 °C treatment also eclosed before hibernation. All other Garita skipperling that survived to adulthood eclosed between June 29th and July 2nd of the following year in both of the treatments and the control (Table 3.1).

Table 3.1. Summary of 2019–2020 incubation trials on Garita skipperlings. Table shows the number of larvae initially added to each treatment (Target), the total number added to each treatment to replace dead neonates (Total), and important dates for each treatment. Larvae that were added to the 28 °C are separated between the original treatment, and a second, late addition of older larvae. Start dates defined by the last larvae added to each treatment.

Treatment	Target (N=)	Total (N=)	Treatment Start Date	Hibernation Start Date	Hibernation End Date	Treatment End Date
Original 28 °C	50	76	August 2nd	NA	NA	September 28th
Late 28 °C	20	20	August 23rd	October 9th	April 30th	June 29th
21 °C	50	64	July 30th	October 15th	April 30th	June 29th
Control	50	62	August 7th	October 23rd	April 30th	July 2nd

Survivorship when hibernation was initiated varied from 85% (n = 17) for larvae added to the Late 28 °C treatment to 13% (n = 8) in the 21 °C treatment, with 37% (n = 23) of control larvae surviving to hibernation. All 18 surviving individuals (24%) added to the Original 28 °C treatment eclosed by October 10th (i.e., before hibernation). 14 larvae (70%) added to the Late 28 °C treatment also eclosed before October 9th and did not undergo hibernation. Of the 3 remaining larvae, 2 individuals survived hibernation compared to 14 of the 23 larvae from the control: 66% and 60% of the larvae which entered hibernation in the Late 28 °C and control, respectively. Survivorship over hibernation was lowest in the 21 °C treatment, where only 2 of 8 individuals which reached hibernation survived (25%). Survivorship at eclosure was highest for larvae introduced to the Late 28 °C treatment with 75% of larvae eclosing, while only 3% (2 of 64 individuals) reared in the 21 °C treatment survived to adulthood. Mortality and survivorship were more similar between the control and Original 28 °C treatment, with 18% (11 out of 62 larvae) and 24% (18 out of 76 larvae) of the Garita skipperlings reaching eclosure, respectively, (Table 3.2).

Table 3.2. Survivorship for treatments and control. Survivorship based on weekly checks of larvae and measured as a proportion of the total larvae added to each treatment and the control (in parentheses). Larvae introduced to the 28 °C EC chamber were separated into 2 treatments, the Original 28 °C and Late 28 °C based on the dates on which larvae were added to the treatment.

Treatment	Initial Sample	Eclosed before Hibernation	Survived to Hibernation	Survived over Hibernation	Eclosed after Hibernation
Original 28C	76	18 (0.24)	NA	N/A	NA
Late 28C	20	14 (0.70)	3 (0.15)	2 (0.10)	1 (0.05)
21C	64	NA	8 (0.13)	2 (0.03)	2 (0.03)
Control	62	NA	23 (0.37)	14 (0.23)	11 (0.18)

Final ages and degree-day accumulation across treatments

Larvae in the Original 28 °C treatment all eclosed prior to the onset of hibernation at an average age of 61.6 days (± 5.4 days; n = 18). Larvae in the Late 28 °C treatment averaged 96.8 days old at eclosure with a notably higher standard deviation (± 67.7 days; n = 15). Individuals that eclosed in the Original 28 °C treatment averaged 1356 (± 118 degree-days) while larvae that reached eclosure in the Late 28 °C treatment averaged 2130 DD (± 1490 degree-days). One larva added to the Late 28 °C treatment eclosed after hibernation at a final age of 349 days (accruing 3190 degree-days), resulting in extreme standard deviations for the final ages and degree-day values averaged by the Late 28 °C treatment. Final ages in the control and 21 °C treatment were very similar, averaging 336.5 (± 2.9) and 340.5 (± 7.8) days respectively, but with more variation among control group larvae. Garita skipperlings in the 21 °C treatment also accrued substantially higher DD accumulation than larvae in the control: 5107 (± 117) versus 1239 (± 46.6) degree-days. The single in the Late 28 °C treatment that eclosed after hibernation accrued 7678 degree-days: the highest value calculated for any larva. Only larvae from the Original 28 °C treatment averaged comparable degree-days at eclosure to the larvae in the control that survived to adulthood (Table 3.3).

Table 3.3. Average ages and DDs of Garita skipperlings that survived to eclosure. Table shows average ages and degree-day (DD) values calculated for Garita skipperlings (*O. garita*) that survived to eclosure in incubation trials and the control. Larvae that were added to the 28 °C are separated between the original treatment, and a second, late addition of older larvae. Number of larvae which survived to eclosure in each treatment also listed. Averages given in days and degree-days, respectively, with standard deviation values (±) in days and degree-days listed in parentheses.

Treatment	Average ages at eclosure	Average DD accumulation at eclosure	Survivors at eclosure (N=)
Original 28 °C	61.6 (± 5.4)	1356 (± 118)	18
Late 28 °C	96.8 (± 67.7)	2130 (± 1490)	15
21 °C	340.5 (± 7.8)	5107 (± 117)	2
Control	336.5 (± 2.9)	1239 (± 46.6)	11

Discussion

As predicted, the larval development of Garita skipperlings was accelerated by prolonged exposure to high static temperatures, resulting in a substantial disruption to their normal phenology. Although neonate mortality was very high in the Original 28 °C treatment, larvae that survived to eclose accumulated similar amounts of DD to the control treatment (Table 3.3). Adults eclosed roughly 15 days before my calculated eclosure date for the Original 28 °C treatment (Oct 15th). Static temperatures can impact the accuracy of predictions made using DD models (Kingsolver *et al.*, 2009), which may account for the difference between their predicted and actual eclosure dates. However, the discrepancy between the predicted eclosure date and the actual eclosure date may relate to the methodology used in this study. The high neonate mortality of the Original 28 °C treatment resulted in many of the initial inductees (n = 26) being replaced by larvae which hatched later than the hatch date used to predict eclosure. Given the low survivorship in the Original 28 °C treatment, the difference between predicted and actual eclosure dates may have been influenced by sample size as Garita skipperlings can have variable eclosure dates (See Chapter 2).

While three of the 20 larva added to the Late 28 °C treatment hibernated, the majority (14 of 20) eclosed in the fall with the survivors of the Original 28 °C treatment, rather than slowing their growth in response to the reduced photoperiod from September–October (Table 3.1, Table 3.2). The 21 °C treatment encountered some technical issues (e.g., grow light failures, poor temperature regulation) so the inferences from this treatment should be evaluated with caution, particularly with respect to survivorship and mortality. However, larva in the 21 °C treatment and Late 28 °C treatment that eclosed after hibernation had similar eclosure dates to larva in the control treatment. These individual accumulated much higher DDs than other treatments (Table 3.3), suggesting that compensatory strategies are available to developing larvae: i.e., that there is some mechanism to control development to ensure emergence for flight and breeding that is timed by a combination of hibernation and/or photoperiod.

Preparing for hibernation appears to slow or halt development in Poweshiek and Garita skipperling between mid to late Fall (Breiter and Burns, 2018). While this may be influenced by temperature, larvae in the 21 °C and Late 28 °C treatments which eclosed after hibernation had comparable eclosure dates to larvae in the control treatment: despite temperatures remaining constant from Summer to Fall. Photoperiod is known to influence the development and phenology of some species of temperate insects (James, 2009; Grevstad and Coop, 2015; Grunert *et al.,* 2015). Since all treatments were exposed to a natural photoperiod, it is possible that photoperiod cues before and after hibernation may be responsible for the similar eclosure dates after hibernation.

However, elevated temperatures had mixed effects on larvae's development, depending on the temperature and when larvae were added to treatments. Only larvae reared at 28 °C eclosed before hibernation, while all larvae that placed into hibernation had comparable eclosure dates to larvae reared outdoors (i.e., in the control), regardless of treatment. It appears that Garita skipperling's phenology can be disrupted by increased temperatures, but it requires exposure to high temperatures starting early in development to accelerate them to the point of eclosing in the same year as they hatched. In contrast, larval phenology after hibernation does not appear to be strongly influenced by temperature, with similar eclosure dates for adults that overwintered in the treatments and control. Many larvae in temperate environments have been observed slowing their development in response to shortening daylength (Friberg *et al.,* 2011). Similarly, Garita skipperling's resilience to disruptions of their phenology may be the result of the chilling during hibernation and/or larvae responding to photoperiod cues, which were matched across treatments throughout the duration of the experiment.

Larvae held at 21 °C exhibited a 97% mortality rate versus the 82% observed in control or 76% and 25% rate of the Original 28 °C and Late 28 °C treatments, respectively (Table 3.2). While EC chambers were setup with the same host plants, lighting, and watering regimens, dataloggers indicated that the 21 °C treatment were subject to less constant temperatures: likely due older equipment. Similarly, more grow light failures were noted in this chamber, possibly adding further stressors to host plants and/or larva in addition to any effects of the lower temperature: likely impacting survivorship in the 21 °C treatment. Kingsolver *et al.* (2009) proposed that mean temperatures and thermal extremes exert strong selective pressures on larvae's thermal reaction norms. Since larvae were reared at one of two constant temperatures or diurnal temperatures, the differences between mean temperatures and lack of thermal extremes in the experimental groups may have had different impacts on survivorship in each treatment and the control. This may have also contributed to the large number of neonates which died in the Original 28 °C treatment, as instars can be sensitive to different temperatures (Etilé and Despland, 2008; Kingsolver *et al.,* 2009).

Different mortality rates may also be attributable the effects of static temperatures and/or other abiotic conditions (e.g. relative humidity) on the nutritional value of the host plants in this study, which can impact growth and survivorship (Beck, 1950; Grunert *et al.,* 2015). While host plants' health was assessed weekly, the effects of incubation trials on their nutritional value and palatability were not estimable. Furthermore, larvae added to the Late 28 °C treatment were several weeks old and not neonates. Since the mortality rate in the neonate stage is high (Table 3.1), this inflated the survivorship value calculated for the Late 28 °C treatment. In contrast, the Original 28 °C treatment had a similar (though somewhat higher) survivorship rate to the control, despite the high number of neonates that died in the Original 28 °C treatment. High temperatures may reduce survivorship in the neonate stage more than the survival of later instars, which is commonly associated with instar-specific responses to temperature (Etilé and Despland, 2008; Kingsolver *et al.,* 2009).

The degree-day values of larvae that eclosed in the Original 28 °C treatment (before hibernation) were similar to the DDs accrued by larvae in the control (after hibernation): averaging 1356 degree-days and 1239 degree-days at eclosure, respectively (Table 3.3). This may relate to the minimum DDs needed to develop to eclosure. Supporting this observation is the similarity of these DDs to the lowest DDs averaged by any generation of Garita skipperlings reared at APZ from 2017–2020, which also had similar standard deviations (See Chapter 2). However, further research is needed to determine whether these values represent the minima required by Garita skipperling.

It is when temperature conditions deviate that larvae have to adjust development to compensate. For example, the few larvae in the Late 28 °C and 21 °C treatment that overwintered and survived to eclosure had notably higher DDs, suggesting that they were able to compensate by slowing development. The extreme example of this was a single larvae added to the 28 °C treatment in Late August that eclosed following hibernation after accruing a staggering 7678 degree-days. While constant temperatures can affect the reliability of DD-based predictions (Kingsolver, *et al.,* 2009), this only appeared to be true of Garita skipperling larvae reared at constant temperatures that underwent hibernation. Larvae from both the experimental treatments which eclosed after diapause accrued more than 3× the highest DD accrued larvae in the control, or by any larva reared outdoors from 2017 to 2020 (Table 3.3, See Chapter 2). Although sample sizes of incubation trials were low, similar responses have been observed in larger-scale studies on Cabbage White butterflies, *Pieris rapae* (Linnaeus, 1758). Researchers have observed different responses from Cabbage Whites reared at constant temperatures depending on whether or not their development was stalled by hibernation (Kivelä *et al.,* 2015), suggesting that hibernation may have altered the response of Garita skipperlings to elevated temperatures. Further trials are likely needed to fully assess the affects of temperature on the DD accumulation of Garita skipperlings, and the role of hibernation in stalling development.

Larvae that accrued extremely high DDs after overwintering had more similar final ages to larvae in the control than larvae reared at 28 °C that eclosed before hibernation: averaging 340.5 days in the 21 °C treatment and 349 days for the single larvae in the Late 28 °C treatment that eclosed post-hibernation. These were comparable to the 336.5 days averaged by larvae in the control. In contrast, the Original 28 °C treatment averaged 61.6 days old at eclosure and the Late 28 °C treatment averaged and 96.8 days old (64.9 days for larvae which eclosed before hibernation). Larvae in treatments that overwintered also had more similar eclosure dates to larvae in the control than larvae that eclosed before hibernation (Table 3.3), indicating that their phenology had not been disrupted despite being reared at constant temperatures.

While sample size limits the conclusions that can be made, larvae reared in incubation trials had either similar ages and eclosure dates or DDs compared to larvae in the control group (Table 3.3). Hibernation can induce adaptive thermal responses in other species reared at constant temperatures which differ from the responses of larvae that develop without hibernating (Kivelä *et al.,* 2015). This can be complicated by photoperiod cues to enter hibernation, which can mitigate the effects of high temperatures (Kivelä *et al.,* 2015). Reduced daylength has been observed to stall the development of a variety of temperate butterfly species (Kingsolver *et al.,* 2009; Friberg *et al.,* 2011; Karlsson, 2014; Kivelä *et al.,* 2015; Saunders *et al.,* 2020). It is possible that photoperiod cues in the Fall similarly caused larvae reared at higher temperatures to either pause their development or pupate and eclose before hibernation, depending on whether larvae were in a photoperiod-sensitive stage of development in the Fall, or had developed past them. Future research could focus on the role that photoperiod plays in the phenology and ontogeny of Garita skipperlings.

While Garita skipperlings appear to retain the ability to enter hibernation late into development, prolonged exposure to a constant temperature of 28 °C is sufficient to induce larvae to pupate before hibernation; despite any cues from the shortening photoperiod to enter hibernation (Table 3.2). However, once committed to overwintering, other seasonal cues like daylength and/or the effects of entering and breaking hibernation may have countered the effects of elevated temperatures on eclosure dates for larvae that hibernated. Garita skipperling, and their endangered sister-species, the Poweshiek skipperling, have been observed to vary their eclosure by up to a month across generations (Dearborn and Westwood, 2014; Breiter and Burns, 2018): suggesting that temperature in the wild influences the development of larvae after hibernation despite any role photoperiod may play in their phenology post-hibernation. Replicating the methods of Friberg *et al.* (2011), where Tobacco Hornworm larvae were reared in long or short photoperiods and a portion of each sample was swapped between treatments, with Garita and Poweshiek skipperling larvae could further our understanding of the role of photoperiod in both species' development and phenology. Combining the manipulation of DDs with instar determination could also further our understanding of the specific roles played by temperature and photoperiod in regulating the onset or end of hibernation for larvae in the wild.

Research in the Palearctic has documented phenological shifts in response to climate change (Valtonen *et al.,* 2011; Ovaskainen *et al.,* 2013), and similar responses to increased temperature have been documented in insects from the Nearctic (Danks, 1994). Given the role that temperature appears to play in governing Garita's phenology, it is likely vulnerable to the same threats associated with climate change as other temperate and subarctic insects. However, the potentially mitigating effects of photoperiod cues must be investigated to further assess the risk that climate change poses. Climate change may cause Garita skipperlings to accelerate their development before hibernation, despite any cues from photoperiod. This could result from larvae developing past the photoperiod-sensitive life stages (e.g., instars that typically overwinter) before the onset of Fall, thus exposing later instars to abnormal photoperiods (Grevstad and Coop, 2015). Conversely, it is possible that photoperiod cues may offset any climate change-induced phenological shifts that have been documented in other insects (Ovaskainen *et al.,* 2013; Diamond *et al.,* 2014). However, the observation of less variation in the lifecycle of Poweshiek skipperling (See Chapter 2) may suggest they are less resilient to the climate changes. It is therefore essential to understand the roles of temperature and photoperiod in both species' phenology and ontogeny. Future research should examine the roles that temperature and photoperiod plays in the preparation for hibernation and timing of Poweshiek and Garita skipperlings eclosure to further our understanding of the threat that climate change poses to both species.

References

APZ. 2024. Grassland Butterfly Conservation Program: Animal Care and Research Protocols for Poweshiek skipperling and Dakota skipper (2024 update). Assiniboine Park Conservancy, Winnipeg, MB. 61pp.

Blair D, Casson N, Allen C, Kreutzer M, Loxley M, Huson B, MacPherson E, Robak I, Ebhuoma E, Mauro I. 2019. The climate atlas of Canada. Winnipeg (MB): Prairie Climate Centre (Canada).

Bossart JL, Carlton CE. 2002. Insect conservation in America: status and perspectives. American Entomologist. 48(2):82–92.

Breiter C-J, Burns L. 2018. Grassland butterfly conservation program 2017 annual report. Winnipeg (MB): Assiniboine Park Zoo.

Buckley LB, Urban M, Angiletta M, Crozier L, Rissler L, Sears M. 2010. Can mechanism inform species' distribution models? Ecology Letters 13(8): 1041–1054.

Burns L, Grottoli A, Papineau A, Petersen SD. 2022. Grassland Butterfly Conservation Program: 2021 Annual Report. Assiniboine Park Conservancy, Winnipeg (MB): 1–45.

Cuthrell DL, Slaughter SL. 2012. Special animal abstract for *Oarisma poweshiek* (Poweshiek skipperling). Lansing (MI): Michigan Natural Features Inventory (US).

Danks H, Kukal O, Ring R. 1994. Insect cold-hardiness: insights from the arctic. Arctic 47(4): 391–404.

Diamond SE, Cayton HL, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, Ries L. 2014. Unexpected phenological responses of butterflies to the interactions of urbanization and geographic temperature. Ecology. 95(1): 2613–2621.

Diamond SE, Frame AM, Martin RA, Buckley LB. 2011. Species' traits predict phenological responses to climate change in butterflies. Ecology. 92(5): 1005–1012.

Díaz S, Settele L, Brondízio ES, Ngo HT, Guèze M, Agard J, Arneth A, Balvanera P, Brauman KA, Butchart SHM, Chan KMA, *et al.* 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Bonn (DE): IPBES (International); 2019 Report.

Etilé E, Despland E. 2008. Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. Oikos. 117(1): 135–143.

Gilburn A, Bunnefeld N, Wilson J, Botham M, Brereton T, Fox R, Goulson D. 2015. Are neonicotinoid insecticides driving declines of widespread butterflies? PeerJ. 1402(3): 1–13.

Hallmann C, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Ller A, Sumser H, Hörren T, *et al.* 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE. 12(1): 1–21.

Harris A, Foster R. 2014. Assessment and status report on the Poweshiek skipperling *Oarisma poweshiek* in Canada. Ottawa (ON): Committee on the Status of Endangered Wildlife in Canada (Canada).

Karlsson B. 2014. Extended season for northern butterflies. International Journal of Biometeorology. 58(5): 691–701.

Kearney M, Briscoe N, Karoly D, Porter W, Norgate M, Sunnucks P. 2010. Early emergence in butterfly causally link to anthropogenic warming. Biology Letters. 6 (1): 674–677.

Kingsolver J, Ragland G, Diamond S. 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. Evolution 63 (2): 537–541.

Kivelä S, Svennson B, Tiwe A, Gotthard K. 2015. Thermal plasticity of growth and development varies among alternative developmental pathways. Evolution 69(9): 2399–2413.

Ovaskainen O, Skorokhodova S, Yakovleva M, Sukhov A, Kutenkov A, Kutenkova N, Shcherbakov A, Meyke E, del Mar Delgado M. 2013. Community-level phenological response to climate change. Proceedings of the National Academy of Sciences (USA). 110(33): 13434–13439.

Pöyry J, Luoto M, Paukkunen J, Pykälä J, Raatikainen K, Kuussaari M. 2006. Different responses of plants and herbivore insects to a gradient of vegetation height: an indicator of the vertebrate grazing intensity and successional age. Oikos. 115(3): 401–412.

Pyle R, Bentzien M, Opler P. 1981. Insect conservation. Annual Review of Entomology. 26(1):233–258.

Sampson F, Knopf F. 1994. Prairie conservation in North America. Other Publications in Wildlife Management 6(1): 418–421.

Saunders ME, Janes JK, O’Hanlon JC. 2020. Moving on from the insect apocalypse narrative: engaging with evidence-based insect conservation. BioScience. 70(1): 80–89.

Selby G. 2005. Status assessment and conservation guidelines: Poweshiek skipperling (*Oarisma poweshiek* (Parker) (Lepidoptera: Hesperiidae). Bloomington (MN): U.S. Fish and Wildlife Service (US).

Swengel A. 2008. Poweshiek paradise lost. American Butterflies 16(4): 16–33.

Uchida K, Ushimaru A. 2014. Biodiversity declines due to abandonment and intensification of agricultural lands: patterns and mechanisms. Ecological Monographs. 84(4): 637–658.

Valtonen A, Ayres M, Roininen H, Pöyry J, Leinonen R. 2011. Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. Oecologia 165(1): 237–248.

Vanbergen AJ, Initiative IP. 2013. Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment. 11(5): 251–259.

Yang Y, Gan TY, Tan X. 2019. Spatiotemporal changes in precipitation extremes over Canada and their teleconnections to large-scale climate patterns. Journal of Hydrometeorology. 20(2): 275–296.

CONCLUSION

Despite international conservation efforts, many aspects of Poweshiek skipperling's biology and Genus *Oarisma* remain understudied. To advance our knowledge of the species and genus, I focused on the Poweshiek and Garita skipperling reared at Assiniboine Park Zoo from 2017–2020 thermal requirements as larvae and pupae using degree-days (DD) and compared their variation between the species. I compared the DD accumulation before and after hibernation, during pupation, and from hatching to eclosure for all Poweshiek and Garita skipperling larvae which survived to eclosure.

Although similar hatch, pupation, eclosure dates, and final ages at eclosure were observed from 2017–2020 across the generations of both species, Garita skipperlings hatched and eclosed slightly earlier, at a higher age, and were more variable between individuals and generations than Poweshiek skipperlings outside of pupation (Tables 1.1, 1.4). Degree day accumulation followed a similar trend, excepting pupation where Poweshiek DDs were notably more variable than Garita skipperlings (Tables 1.3, 1.4). In Manitoba, Poweshiek skipperlings pupate and eclose within a span of one to two weeks annually (Dearborn and Westwood, 2014; Breiter and Burns, 2018), while Garita skipperlings emerge as adults over the course of three or more weeks (Harris and Foster, 2014; Breiter and Burns, 2018). Their higher synchrony may relate to Poweshiek skipperling pupae being sensitive to photoperiod cues that help synchronize their eclosure, and Garita skipperling being less sensitive (or insensitive). This may also explain the higher variability in DDs and ages that Poweshiek skipperlings averaged during pupation, as photoperiod cues can affect DD accumulation (Forrest and Thomson, 2011; Friberg *et al.,* 2011). Future studies should, in part, examine the role of photoperiod in Poweshiek and Garita skipperling phenology.

The greater constraint on age and DDs across their lifespan may also play a role in Poweshiek skipperling's synchronous eclosure and the greater variability of Garita skipperling may influence their longer, less synchronized, eclosure. While their hatch and eclosure dates indicated that the phenology of both species are similar in Manitoba, Garita skipperling's higher variability indicate that it is more flexible with respect to the DD accumulation and ages necessary to overwinter and/or survive to eclosion than Poweshiek skipperling. Given their low variability outside of pupation, Poweshiek skipperlings appear to be at a higher risk of climate change-induced phenological shifts than Garita skipperlings.

From 2019–2020, I also compared the effects of a high and moderate-temperature constant temperatures to natural, diurnal, temperatures on the development and survivorship of Garita skipperling by placing them on host plants in either a 21 °C or 28 °C EC chamber and comparing them to larvae reared outdoors. All treatments had the same photoperiod throughout, and larvae that had not pupated and eclosed were placed into hibernation from October 2019 to April 2020, with all remaining larvae eclosing in June 2020. While Garita skipperling's phenology was disrupted by temperature, it required exposure to high temperatures starting early in development. Phenology could only be disrupted such that larvae pupated and eclosed before hibernation: development post-hibernation was comparable across treatments and the control.

Preparing to hibernate appears to stall the development of Poweshiek and Garita skipperling larvae starting in mid to late Fall (Breiter and Burns, 2018). Photoperiod can influence the development and phenology of some species of temperate insects before and after hibernation (James, 2009; Grevstad and Coop, 2015; Grunert *et al.,* 2015). Responses to constant temperatures can depend on whether or not larvae hibernated (Kivelä *et al.,* 2015). This could explain the difference in DDs accrued by Garita skipperling that did and did not hibernate in my incubation trials: hibernation induced an adaptive thermal responses that differed from the response of larvae that did not hibernate (Kivelä *et al.,* 2015).

Reduced daylength can also stall development in numerous species of temperate butterflies (Kingsolver *et al.,* 2009; Friberg *et al.,* 2011; Karlsson, 2014; Kivelä *et al.,* 2015; Saunders *et al.,* 2020). Since all treatments were exposed to a natural photoperiod, it is possible that the Garita skipperling which overwintered were at developmental stages that were sensitive to photoperiod cues before hibernation. Conversely, larvae which eclosed before hibernation may have developed past the stage that is sensitive to photoperiod cues for hibernation: causing larvae to stop growing and prepare for hibernation or to continue to develop and eclose before hibernation depending on their stage of development a may be responsible for the similar eclosure dates after hibernation.

However, both Poweshiek and Garita skipperling can vary their eclosure by up to a month across generations (Dearborn and Westwood, 2014; Breiter and Burns, 2018), which suggests that temperature also influences the development and phenology of larvae after hibernation. The results of my incubation trials further indicate that the role of photoperiod in the development of Poweshiek and Garita skipperlings should be examined. Replicating the methods used in Friberg *et al.* (2011) by rearing larvae either in long or short photoperiods, then swapping a portion of each sample between treatments part way through development, would elucidate the role of photoperiod in both species' development.

I also measured the head capsule widths and lengths of Garita skipperlings in my EC chambers and control weekly with an ocular micrometer until pupation to track their growth and determined the instars of larvae imaged from hatching to eclosure. I classified instars by dividing successive measurements by larvae's preceding measurement (dubbed Dyar's Values), determined when moults occurred using Dyar's Values ≥ 1.1, and used k-means clustering and histograms to evaluate these grouping' fitness. Garita skipperling larvae from my incubation trials did not maintain a consistent size ratio between instars, in violation of Dyar's Rule (Dyar, 1890; Hawes, 2019), as has been observed in multiple other species (Beck, 1950; Hunt and Chapman, 2001; Grunert *et al.,* 2015). This casts further doubt on the generalizability of Dyar's Rule when determining instars.

Using head capsule width, Dyar's Values, and k-means clustering appears to be effective at determining instars without assuming Dyar's Rule and/or using destructive sampling or pre-existing size ranges for each instar (Hunt and Chapman, 2001; Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016). While head capsule lengths produced similar groupings as head capsule widths, the prominent midline of Garita skipperling head capsules does not appear to be a reliable metric for larval growth as it can be readily impacted by the orientation of larvae to the camera (Fig 2.1, Fig 2.5). Future analyses should omit head capsule lengths in favor of head capsule widths, as done by other researchers (Dyar, 1890; Capinera, 1984; Gold *et al.,* 1999; Brust *et al.,* 2005; Cazado *et al.,* 2014; Castañeda-Vildózola *et al.,* 2016; Hawes, 2019).

Garita skipperling larvae had a variable number of instars (Fig 2.5). This occurred for larvae in my incubation trials and control, indicating that the variable instar number was not the result of elevated (or constant) temperatures. Variation in the total number of instars may be the result of Garita skipperling larvae growing at a reduced or accelerated rate relative to other larvae, as had been observed in other species of Skipper (James, 2009). Larvae that developed more slowly may have failed to reach their final instar before pupation: causing them to skip their final instar and thus pupate after only 5 instars. Similarly, Garita skipperlings that grew faster than other larvae in their treatment may have outgrown their final instar before it was time for them to pupate: allowing them to moult an additional time and pupate after 7 instars. Fewer and additional instars can cause underweight and heavier adults, respectively, in Skippers (James, 2009). Future research should compare the weights of adults which eclose after a different number of instars to test whether instar number affects the size of Garita skipperling adults.

Garita skipperling are likely vulnerable to climate change, which appears to also be true for the Poweshiek skipperling. Climate change could cause Poweshiek and Garita skipperlings to develop past any photoperiod-sensitive stages before receiving the photoperiod cues to slow their development. However, photoperiod cues have the potential to mitigate the effects of climate change, which should be investigated to determine the risk that climate change poses to both species in the short versus long-term. Photoperiod cues could offset the affect of climate change on both species' phenology, as documented in other insects (Ovaskainen *et al.,* 2013; Diamond *et al.,* 2014), though the reduced variation observed in the growth and phenology of Poweshiek skipperling may make them more susceptible to climate-change induced phenological shifts.

Combining the manipulation of DDs with instar determination could further our understanding of the specific roles played by temperature in regulating the onset or end of hibernation. By combining DD data with larval instars (possibly across multiple generations) of Poweshiek and Garita skipperlings, these techniques could be used to develop a model of Garita and Poweshiek larval development and identify the thermal requirements and tolerances of each instar. This would deepen our understanding of the thermal requirements of Poweshiek and Garita skipperling's individual instars, elucidate the similarities and differences in the species' development, and help researchers determine the threat posed by climate change to each species at each stage of larval development.

REFERENCES

APZ. 2024. Grassland Butterfly Conservation Program: Animal Care and Research Protocols for Poweshiek skipperling and Dakota skipper (2024 update). Assiniboine Park Conservancy, Winnipeg, MB. 61pp.

Beck SD. 1950. Nutrition of the European Corn Borer, *Pyrausta nubilalis* (HBN.). II. Some effects of diet on larval growth characteristics. Physiological Zoology. 23(4):353–361.

Belitz M, Hendrick L, Monfils M, Cuthrell D, Marshall C, Kawahara A, Cobb N, Zaspel J, Horton A, Huber S, Warren A, Forthaus G, Monfils A. Aggregated occurrence records of the federally endangered Poweshiek skipperling (*Oarisma poweshiek*). Biodiversity Data Journal 6(1): 1-15.

Blair D, Casson N, Allen C, Kreutzer M, Loxley M, Huson B, MacPherson E, Robak I, Ebhuoma E, Mauro I. 2019. The climate atlas of Canada. Winnipeg (MB): Prairie Climate Centre (Canada).

Borkin, S. 1994. Ecological studies of the Poweshiek skipper (*Oarisma poweshiek*) in Wisconsin, 1993. Madison (WI): Wisconsin Department of Natural Resources (US).

Bossart JL, Carlton CE. 2002. Insect conservation in America: status and perspectives. American Entomologist. 48(2):82–92.

Breiter C-J, Burns L. 2018. Grassland butterfly conservation program 2017 annual report. Winnipeg (MB): Assiniboine Park Zoo.

Buckley LB, Urban M, Angiletta M, Crozier L, Rissler L, Sears M. 2010. Can mechanism inform species' distribution models? Ecology Letters 13(8): 1041-1054.

Buckley LB, Kingsolver JG. 2012. Functional and phylogenetic approaches to forecasting species' responses to climate change. Annual Review of Ecology, Evolution, and Systematics. 43:205–226.

Burke R, Fitzsimmons J, Kerr J. 2011. A mobility index for Canadian butterfly species based on naturalists' knowledge. Biodiversity and Conservation. 20 (1): 22773-2295.

Burns L, Grottoli A, Papineau A, Petersen SD. 2022. Grassland Butterfly Conservation Program: 2021 Annual Report. Assiniboine Park Conservancy, Winnipeg (MB): 1-45.

Capinera JL. 1984. Instar number in Range Caterpillar, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae). Journal of the Kansas Entomological Society. 57(2):344–347.

Castañeda-Vildózola Á, González-Hernández H, Equihua-Martínez A, Valdez-Carrasco J, Peña JE, Cazado LE, Franco-Mora O. 2016. Head capsule width is useful for determining larval instar in *Heilipus lauri* (Coleoptera: Curculionidae). The Florida Entomologist. 99(4):822–825.

Catling P. 1977. On the occurrence of *Oarisma garita* (Reakirt) (Lepidoptera: Hesperiidae) in Manitoulin District, Ontario. The Great Lakes Entomologist 10(2): 59-63

Catling P, and Lafontaine J. 1986. First documented record of *Oarisma poweshiek* (Lepidoptera: Hesperiidae) in Canada. The Great Lakes Entomologist 19(2): 63-66

Cayton HL, Haddad NM, Gross K, Diamond SE, Ries L. 2015. Do growing degree days predict phenology across butterfly species? Ecology. 96(6): 1473–1479.

Cazado LE, Van Nieuwenhove GA, O'brien CW, Gastaminza GA, Murúa MG. 2014. Determination of number of instars of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) based on head capsule widths. The Florida Entomologist. 97(2): 639–643.

Clench HK. 1966. Behavioral thermoregulation in butterflies. Ecology. 47(6): 1021–1034.

Cordeiro de Amorim R, Hennig C. 2015. Recovering the number of clusters in data sets with noise features using feature rescaling factors. Information Sciences. 324 (1): 126-145.

Crozier L. 2003. Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*. Oecologica. 135(4): 648-656.

Cuthrell DL, Slaughter SL. 2012. Special animal abstract for *Oarisma poweshiek* (Poweshiek skipperling). Lansing (MI): Michigan Natural Features Inventory (US).

Dana, R.P. 1991. Conservation management of the prairie skippers *Hesperia dacotae* and *Hesperia ottoe*: basic biology and threat of mortality during prescribed burning in spring. St. Paul (MN): University of Minnesota.

Danks H, Kukal O, Ring R. 1994. Insect cold-hardiness: insights from the arctic. Arctic 47(4): 391-404.

Dearborn S, Westwood R. 2014. Predicting adult emergence of Dakota Skipper and Poweshiek skipperling (Lepidoptera: Hesperiidae) in Canada. Insect Conservation 18(1): 875-884.

Diamond SE, Cayton HL, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, Ries L. 2014. Unexpected phenological responses of butterflies to the interactions of urbanization and geographic temperature. Ecology. 95(1): 2613–2621.

Diamond SE, Frame AM, Martin RA, Buckley LB. 2011. Species' traits predict phenological responses to climate change in butterflies. Ecology. 92(5): 1005–1012.

Díaz S, Settele L, Brondízio ES, Ngo HT, Guèze M, Agard J, Arneth A, Balvanera P, Brauman KA, Butchart SHM, Chan KMA, *et al.* 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Bonn (DE): IPBES (International); 2019 Report.

Dupont-Morozoff J, Westwood R, Henault J. 2022. An assessment of prairie management practices for maintaining habitat quality for the endangered Poweshiek skipperling butterfly in Canada. The American Midland Naturalist 188(1): 74-100

Dyar HG. 1890. The number of molts of Lepidopterous larvae. Psyche: a Journal of Entomology. 5(1): 175–176.

Etilé E, Despland E. 2008. Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. Oikos. 117(1): 135–143.

Everitt BS, Hothorn T. 2010. A handbook of statistical analyses using R. 2nd ed. Boca Raton (FL): Chapman & Hall/CRC.

Forrest JRK, Thomson JD. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecological Monographs. 81(3): 469–491.

Friberg M, Haugen IMA, Dahlerus J, Gotthard K, Wiklund C. 2011. Asymmetric life-history decision-making in butterfly larvae. Oecologia. 165(2): 301–310.

Gains JC, Campbell FL. 1935. Dyar's Rule as related to the number of instars of the corn ear worm, *Heliothis obsolete* (Fab.), collected in the field. Annals of the Entomological Society of America. 28(1): 445-461.

Gilburn A, Bunnefeld N, Wilson J, Botham M, Brereton T, Fox R, Goulson D. 2015. Are neonicotinoid insecticides driving declines of widespread butterflies? PeerJ. 1402(3): 1-13.

Gold CS, Neyeme PS, Coe R. 1999. Recognition and duration of larval instars of banana weevil, *Cosmopolites sordidus* Germar, in Uganda. African Entomology 7(1): 49-62.

Grevstad FS, Coop LB. 2015. The consequences of photoperiodism for organisms in new climates. Ecological Applications. 25(6): 1506–1517.

Grunert LW, Clarke JW, Ahuja C, Eswaran H, Nijhout HF. 2015. A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. PLoS ONE. 10(5): 1-23.

Hallmann C, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Ller A, Sumser H, Hörren T, *et al.* 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE. 12(1): 1–21.

Harris A, Foster R. 2014. Assessment and status report on the Poweshiek skipperling *Oarisma poweshiek* in Canada. Ottawa (ON): Committee on the Status of Endangered Wildlife in Canada (Canada).

Hawes T. 2019. An equation for geometric progression in Brooks-Dyar growth ratios. Invertebrate Reproduction and Development. 64(1): 1-12.

Hellmann J, Pelini S, Prior K, Dzurisin J. 2008. The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. Oecologica. 157(4): 583-592.

Henault J, Westwood R. 2022. Endangered *Oarisma poweshiek* larvae vary their graminoid forage in Manitoba, Canada. The Canadian Entomologist 154 (e49): 1-27.

Henault J, Westwood R. 2023. Adult activities of endangered *Oarisma poweshiek* butterflies are associated with a soil moisture gradient in tall grass prairie in Manitoba, Canada. Journal of Insect Conservation 27 (1): 825-839.

Hodkinson ID, Bird J. 1998. Host-specific insect herbivores as sensors of climate change in arctic and alpine environments. Arctic and Alpine Research. 30(1): 78–83.

Holzman R. 1972. Eastern range extension for *Oarisma poweshiek* Parker (Lepidoptera: Hesperiidae). The Great Lakes Entomologist 5(4): 111-114

Hooshmandi M. 2016. A critical habitat suitability model for Poweshiek skipperling (*Oarisma poweshiek*): with a focus on micro-habitat characteristics [thesis]. [Winnipeg (MB)]: University of Winnipeg.

Hunt G, Chapman RE. 2001. Evaluating hypotheses of instar-grouping in arthropods: A Maximum Likelihood Approach. Paleobiology. 27(3): 466–484.

James, D. 2009. Comparative studies on the immature stages and biology of *Hesperia colorado idaho* and *Hesperia juba* (Hesperiidae). Journal of the Lepidopterists' Society 63(3): 129-136.

Karlsson B. 2014. Extended season for northern butterflies. International Journal of Biometeorology. 58(5): 691–701.

Kearney M, Briscoe N, Karoly D, Porter W, Norgate M, Sunnucks P. 2010. Early emergence in butterfly causally link to anthropogenic warming. Biology Letters. 6 (1): 674–677.

Kingsolver J. 2000. Feeding, growth, and the thermal environment of cabbage white caterpillars, *Pieris rapae* L. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches 73 (5): 621-628.

Kingsolver J, Ragland G, Diamond S. 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. Evolution 63 (2): 537-541.

Kivelä S, Svennson B, Tiwe A, Gotthard K. 2015. Thermal plasticity of growth and development varies among alternative developmental pathways. Evolution 69(9): 2399-2413.

Klassen P, Westwood AR, Preston WB, Mcillop WB. 1989. The butterflies of Manitoba. Winnipeg (MB): Manitoba Museum of Man and Nature.

Layberry R, Hall P, Lafontaine D. 1998. Butterflies of Canada- Garita skipperling. Toronto (ON): University of Toronto Press.

Lazerte S, Albers S. 2018. Weathercan: download and format weather data from environment and climate change Canada. The Journal of Open Source Software 3(22): 571.

MacNeil CD. 1964. The skippers of the genus Hesperia in western North America with special reference to California. University of California Publications in Entomology, University of California Press, Berkeley and Los Angeles. 35: 1–221.

Moore JL, Liang S, Akullian A, Remais JV. 2012. Cautioning the use of degree-day models for climate change projections in the presence of parametric uncertainty. Ecological Applications. 22(8): 2237–2247.

Navarro-Cano J, Karlsson B, Posledovich D, Toftegaard T, Wiklund C, Ehrlén J, Gotthard K. Climate change, phenology, and butterfly host plant utilization. Ambio. 44(1): 78-88.

Nordmeyer C, Runquist E, Stapleton, S. 2021. Invasive grass negatively affects growth and survival of an imperiled butterfly. Endangered Species Research. 45: 301–314.

Oliphant A, Hauton C, Thatje S, Dam H. 2013. The implications of temperature-mediated plasticity in larval instar number for development within a marine invertebrate, the shrimp *Palaemonetes varians*. PLoS ONE 8(9): 1-9.

Ovaskainen O, Skorokhodova S, Yakovleva M, Sukhov A, Kutenkov A, Kutenkova N, Shcherbakov A, Meyke E, del Mar Delgado M. 2013. Community-level phenological response to climate change. Proceedings of the National Academy of Sciences (USA). 110(33): 13434–13439.

Perkins O. 2007. Using degree-day modelling to ascertain early flight periods of Michigan butterflies and Skippers. The Great Lakes Entomologist 40(3,4): 103-119.

Pivnick KA, McNeil JN. 1986. Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). Ecology. 67(4): 1024–1035.

Pogue C, Monfils M, Cuthrell D, Heumann B, Monfils A. 2017. Habitat suitability modeling of the federally endangered Poweshiek skipperling in Michigan. Journal of Fish and Wildlife Management 7(2): 359-368.

Poweshiek Working Group. 2011. Minutes from the Poweshiek skipperling workshop, March 24 and 25. Winnipeg (MB): International Poweshiek Working Group (Canada).

Poweshiek Recovery Group. 2018. Minutes from the Poweshiek skipperling Recovery Group annual meeting, November 28, Winnipeg, Manitoba, Canada.

Poweshiek International Workshop. 2019. Poweshiek skipperling conservation partnership: proceedings and priorities. Apple Valley (MN): International Poweshiek Working Group (US).

Pöyry J, Luoto M, Paukkunen J, Pykälä J, Raatikainen K, Kuussaari M. 2006. Different responses of plants and herbivore insects to a gradient of vegetation height: an indicator of the vertebrate grazing intensity and successional age. Oikos. 115(3): 401–412.

Pyle R, Bentzien M, Opler P. 1981. Insect conservation. Annual Review of Entomology. 26(1):233–258.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Runquist E, Nordmeyer C. 2018. Minnesota Zoo prairie butterfly conservation program 2018 report. Apple Valley (MN): Minnesota Zoo (US).

Safranek L, Williams CM. 1984. Determinants of larval molt initiation in the Tobacco Hornworm, *Manduca sexta*. Biological Bulletin. 167(3): 568–578.

Sampson F, Knopf F. 1994. Prairie conservation in North America. Other Publications in Wildlife Management 6(1): 418-421.

Saunders ME, Janes JK, O’Hanlon JC. 2020. Moving on from the insect apocalypse narrative: engaging with evidence-based insect conservation. BioScience. 70(1): 80–89.

Schweitzer DF, Opler PA. 2023. Comprehensive report: species – *Oarisma poweshiek*. Arlington (VI): NatureServe (US).

Scott J, Epstein M. 1987. Factors affecting phenology in a temperate insect community. The American Midland Naturalist 117(1): p 103-118.

Selby G. 2005. Status assessment and conservation guidelines: Poweshiek skipperling (*Oarisma poweshiek* (Parker) (Lepidoptera: Hesperiidae). Bloomington (MN): U.S. Fish and Wildlife Service (US).

Spieles JB, Comer PJ, Albert DA, Kost MA. 1999. Natural community abstract for prairie fen. Lansing (MI): Michigan Natural Features Inventory (US).

Summerville K, Clampitt C. 1999. Habitat characterization of five rare insects in Michigan (Lepidoptera: Hesperiidae, Riodinidae, Satyridae; Homoptera: Cercopidae). The Great Lakes Entomologist 32(3): 225-238.

Swengel A. 2008. Poweshiek paradise lost. American Butterflies 16(4): 16-33.

Swengel A, Swengel S. 1999. Observations of prairie Skippers (*Oarisma poweshiek, Hesperia dacotae, H. ottoe, H. leonardus pawnee*, and *Atrytone argos iowa* [Lepidoptera: Hesperiidae]) in Iowa, Minnesota, and North Dakota During 1988-1997. The Great Lakes Entomologist 32(4): 267-292.

Uchida K, Ushimaru A. 2014. Biodiversity declines due to abandonment and intensification of agricultural lands: patterns and mechanisms. Ecological Monographs. 84(4): 637–658.

USFWS. 2011. US Fish and Wildlife Service species assessment and listing priority assignment form. Washington (DC): Department of the Interior (US).

Valtonen A, Ayres M, Roininen H, Pöyry J, Leinonen R. 2011. Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. Oecologia 165(1): 237-248.

Vanbergen AJ, Initiative IP. 2013. Threats to an ecosystem service: pressures on pollinators. Frontiers in Ecology and the Environment. 11(5): 251–259.

Wilson K. 2023. Comprehensive Report: Species – *Oarisma garita*. Arlington (VI): NatureServe (US).

Yang Y, Gan TY, Tan X. 2019. Spatiotemporal changes in precipitation extremes over Canada and their teleconnections to large-scale climate patterns. Journal of Hydrometeorology. 20(2): 275–296.

Taxonomic References

Integrated Taxonomic Information System. 2023. 1st edition. International: United States Federal Government. Available from: https://www.itis.gov/

- Ecdysozoa (Ruggiero *et al.,* 2015)
- Hesperiidae (Latreille, 1809)
- *Hesperia juba* (Scudder, 1872)
- *Helicoverpa zea* (Boddie 1850)
- *Manduca sexta* (Linnaeus, 1753)
- *Oarisma sp.* (Scudder, 1872)
- *Oarisma garita* (Parker, 1970)
- *Oarisma poweshiek* (Parker 1870)
- *Pieris rapae* (Linnaeus, 1758)
- *Thymelicus lineola* (Ochsenheimer, 1808)

World Flora Online. 2024. 1st edition. International: WFO Consortium. Available from: https://wfoplantlist.org/

- *Ancyloxypha numitor* (Fabricus, 1793)
- *Andropogon sp* (Linnaeus 1753)
- *Andropogon gerardi* (Vitman, 1792)
- *Archips fumiferana* (Clemens, 1865)
- *Bouteloua curtipendula* (Torr., 1912)
- *Carex sp.* (Linnaeus, 1753)
- *Dasiphora fruticosa* (Linnaeus, 1753)
- *Echinacea angustifolia* (DC, 1813)
- *Eleocharis elliptica* (Kunth, 1837)
- *Epargyreus clarus* (Cramer, 1775)
- *Galium boreale* (Linnaeus, 1753)
- *Glycyrrhiza lepidota* (Pursh, 1813)
- *Heliopsis helianthoides* (Linnaeus 1753)
- *Muhlenbergia richardsonis* (Trin., 1996)
- *Packera* sp. (Á. Löve and D. Löve**,** 1976)
- *Phragmites australis* (Trin., 1996)
- *Rudbeckia hirta* (Linnaeus, 1753)
- *Schizachyrium scoparium* (Michx., 1903)
- *Sporobolus heterolepis* (Gray, 1857)
- *Triantha glutinosa* (Michx.)
- *Typha angustifolia* (Linnaeus, 1753)