EVALUATING THE RESPONSE OF NATIVE AND INTRODUCED NECTAR PLANTS TO WARMING AND THE ASSOCIATED IMPACTS ON THE BODY COMPOSITION OF THE MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*)

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Abstract

Climate change is altering plant-insect interactions through changes in plant growth, flowering phenology, and nectar resources. These effects may be especially important for migratory insects such as monarch butterflies (*Danaus plexippus*), which rely on late-season floral nectar to accumulate lipid reserves required for migration and overwintering. Introduced plants are frequently used by monarchs as nectar sources, but their quality and response to warming remain poorly understood. I used a field warming experiment in an old-field habitat near Ottawa, Ontario, to examine how passive warming affected the growth and floral production of two native nectar plants, *Solidago canadensis* and *Symphyotrichum novae-angliae*, and two introduced nectar plants, *Lythrum salicaria* and *Trifolium pratense*. I also conducted controlled feeding trails, in which adult monarchs forage on one of the four species in warmed and controlled chambers to determine whether warming has an impact on monarch body composition. The experiment occurred during an unusually dry season, with soil moisture declining to a predicted minimum of 6.74% in mid-August. Warming did not consistently affect plant growth, chlorophyll content, flower abundance, or flowering onset. Instead, warming altered some seasonal plant trajectories, including species-specific stem growth patterns and seasonal flowering distributions, while drought conditions likely constrained floral availability across species. Native species exhibited higher percent stem growth than introduced species overall, but introduced and native species did not differ consistently in their response to warming. Warming also did not significantly affect monarch wet mass, dry fat mass, dry lean mass, or water mass in *S. canadensis* feeding trials. In nectar-source comparisons, monarchs feeding on introduced *L. salicaria* had lower dry lean mass than those feeding on native *S. canadensis*, although wet mass, dry fat mass, and water mass did not differ between species.

These findings suggest that *L. salicaria* did not reduce monarch lipid accumulation relative to *S. canadensis*, though differences in non-fat body components may reflect variation in nectar composition or feeding conditions. Overall, my results suggest that short-term warming alone had limited effects on plant condition and monarch body composition, while drought and seasonal dynamics played a larger role in shaping floral availability and monarch responses. Restoration efforts should therefore prioritize diverse, drought-tolerant, late-season floral communities capable of maintaining nectar availability under increasingly variable climatic conditions.

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1. Introduction

Climate change is intensifying, bringing with it increasingly alarming impacts to biodiversity and plant-insect interactions through changes in species distributions, phenology, and ecosystem functioning (Arneth et al., 2020; Pörtner et al., 2021; Habibullah et al., 2022; Shivanna, 2022). Recent studies have reported long-term declines in insect abundance and biomass that have co-occurred with changes in climate, such as less precipitation and warmer temperatures (Burkle et al., 2013; Dalton et al., 2023; Edwards et al., 2025). As climate warming continues to accelerate, it is vital to evaluate the potential impact on plant-insect interactions.

Despite increasing evidence linking climate change to insect declines (Hallmann et al., 2017; Dalton et al., 2023; de Snoo et al., 2023), the key mechanisms of this decline are poorly understood. Climate change can affect insects directly through physiological responses, and indirectly by altering the plants they rely on for food. Such indirect effects include changes to the timing, availability, and quality of floral resources (Diez et al., 2012; Bartomeus et al., 2013; Byers & Chang, 2017; Inouye, 2022). Warming can alter plant traits that are important to nectar-feeding insects, including flowering phenology, floral abundance, nectar volume, and nectar sucrose concentration (Diez et al., 2012; Takkis et al., 2015; Descamps et al., 2021; McCombs et al., 2022; Montes-Perez et al., 2025; Peel et al., 2026). Although these effects may play an important role in shaping insect responses to climate change, they have been rarely quantified.

Recent work has found that plant responses to climatic warming have been variable, making it difficult to predict how these traits will influence insects (Diez et al., 2012; Takkis et al., 2015; Kopp et al., 2020; Defalque et al., 2025). For example, using herbarium records of 39 native species in North America's Pacific Northwest region from 1901 to 2015, Kopp et al. (2020) found that early-season flowering plants were more sensitive to warming temperature,

advancing their flowering faster than late-flowering species under the same conditions. Diez et al. (2012) noted significant variability in species' phenological responses to temperature, with one community shifting towards earlier phenology with higher temperatures compared to the other community.

The nectar quality and quantity of flowering plants is also differentially sensitive to experimental warming. For example, McCombs et al. (2022) found lower nectar volume and higher sugar concentration in two forb species under warming, whereas another study found that drought and warming decreased nectar sugar concentration in a different forb species (Defalque et al., 2025). A study of two mediterranean plants found the optimal temperature for nectar sucrose concentration was species-specific, and depended on water availability (Takkis et al., 2015). These inconsistent and species-specific responses to warming make it difficult to predict how climate change will alter nectar resources and, consequently, the insects that depend on them.

Understanding these responses is especially important as climate change continues to alter the composition of plant communities through the spread and establishment of introduced species. Introduced plants are well established in modern-day ecosystems, and their introductions are predicted to increase in the coming years due to climate change (Thuiller et al., 2007; Sorte et al., 2013; Guo et al., 2021). Increasingly, there are examples of introduced plants playing key roles for insect communities. For example, introduced plants have been shown to provide nectar to butterflies at critical times in the season (Rivest et al., 2023; Pekos et al., 2025). However, it remains unclear whether these plants are providing good quality nectar and how the plants may respond to climate change. In some contexts, there is evidence that introduced species are demonstrating a stronger response to climate change than native species, shifting their flowering

onset earlier under warmed conditions than native species (Zettlemoyer et al., 2019), whereas other studies have found no difference (Sorte et al., 2013). Additionally, some studies suggest that warming can increase growth of introduced plants. For example, one study found that introduced species grew taller under experimental warming than native species, and several had greater biomass (Ivison et al., 2024). If native and introduced plants differ in their responses to warming, introduced plants may play a different role in plant-insect communities in the future. Without a clear understanding of how introduced nectar plants respond to warming, their potential contribution to pollinator resource availability may be overlooked.

The eastern population of monarch butterflies (*Danaus plexippus*) has faced threats to its persistence for decades and was uplisted to Endangered under the Species at Risk Act in Canada in December, 2023 (Environment and Climate Change Canada, 2023). Climate change is considered one of the most severe of these threats, contributing to both direct and indirect impacts towards population decline (Inamine et al., 2016; Zylstra et al., 2021; Niitepõld et al., 2022; Hobson et al., 2023). A study by Zylstra et al. (2021) found that breeding-season weather was a significant predictor in explaining variation in summer population sizes and subsequent overwintering population sizes of the species. Long-term monitoring of the fall migration period through Cape May New Jersey between 1992 and 2020 found significant delays in the migration timing of the population (Culbertson et al., 2021), while another study conducted in the northeastern breeding ground of Canada found no clear changes in the timing of the fall migration during the same time period (Ethier & Mitchell, 2023). Together, these findings suggest there are likely multiple mechanisms of climate change influencing monarchs.

Another possible contributing factor to the population decline is nectar availability along the fall migratory route (Brower et al., 2006; Agrawal & Inamine, 2018; Inamine et al., 2016; Saunders et al., 2019). Adult monarchs convert nectar sugars into lipid reserves, and lipids are the dominant energy source used to fuel migration and overwintering (Beall, 1948; Brown & Chippendale, 1974; Brower et al., 2006; Hobson et al., 2023). Reduced access to food can also impair monarch body mass, flight physiology, and flight capacity, emphasizing the importance of adequate nectar resources during migration (Niitepõld et al., 2022).

In this study, I use a field warming experiment in an old-field habitat near Ottawa, Ontario, Canada, to test how warming affects monarch butterflies indirectly through changes in our frequently used native and introduced nectar plants: *Solidago canadensis* (Canada goldenrod), *Symphyotrichum novae-angliae* (New-England aster), *Trifolium pratense* (red clover), and *Lythrum salicaria* (purple loosestrife). Large enclosures will be placed around the passive warming chambers, allowing adult monarchs to feed on warmed nectar plants while spending non-feeding periods outside the warming treatment; thus, isolating plant-mediated effects of warming from its direct effects on the butterfly (Figure 1; Peel et al., 2026)

I test four hypotheses about how experimental warming could affect nectar plants and indirectly influence monarch body composition. First, I hypothesize that warming affects plant condition and flowering by altering the balance between thermal stimulation and stress. I define plant stress as an external condition that pushes a plant away from optimal functioning and produces measurable or observable decline in vegetative growth, reproduction, and survival (Lichtenthaler, 1996). Moderate warming can accelerate developmental processes and shift flowering earlier, but more extreme warming can act as a stressor and reduce floral abundance, alter nectar traits, or increase stress when water availability is limited (Diez et al., 2012; Takkis

et al., 2015; McCombs et al., 2022; Inouye, 2022; Defalque et al., 2025). If the degree of warming is above the species' thermal optimum, I predict that plants will show reduced stem growth, lower chlorophyll content, lower open flower abundance, or earlier senescence compared with control plants. If warming is within the species' optimal range, I predict an increase in stem growth, higher leaf chlorophyll content, higher open flower abundance, and an earlier flowering onset in warmed chambers compared to control.

Second, I hypothesize that plant responses to warming are based on local adaptation rather than solely based on origin. Because the introduced nectar plants in this study have been present in North America for hundreds of years, I predict that introduced species will have a similar response to warming to the native species.

Third, I hypothesize that warming will indirectly affect monarch body composition if it changes the condition, abundance, or quality of nectar resources. Given that adult monarchs convert nectar sugars into lipid reserves and reduced access to food has been shown to impair monarch body mass, flight physiology, and flight capacity (Beall, 1948; Brown & Chippendale, 1974; Brower et al., 2006; Niitepõld et al., 2022; Hobson et al., 2023), I predict that warming-driven changes in nectar quality will change fat reserves. If warming reduces floral resources or nectar quality, monarchs will have lower dry fat mass, and vice versa if nectar quality increases. I expected dry lean mass and water mass to be less directly responsive to warming than dry fat mass because lipids are the primary migratory energy reserve in monarchs.

Finally, I hypothesize that monarch body composition will depend more on nectar plant identity than on whether a species is native or introduced. Monarchs are generalist nectar feeders and use both native and introduced floral resources during migration (Brower et al., 2006;

Antonsen et al., 2021; Pekos et al., 2025). Therefore, I predict that monarch body composition will not differ between native and introduced nectar sources. Instead, I predict variation in body composition among individual plant species, reflecting differences in nectar availability, sugar content, amino acid composition, or other nectar traits (Levin et al., 2017; Tigreros et al., 2026).

2. Methods

2.1 Study System

2.1.1 Monarch butterflies

In early fall, the eastern population of the monarch butterfly migrates from breeding grounds in Canada to overwintering habitat in the forests of Michoacán and Estado de México (Rendón-Salinas et al., 2026). Monarchs have experienced long term population declines at overwintering sites since the mid 1990s, prompting debate regarding the most severe threats to the population.

Monarch butterflies complete a nearly 4000km migration from overwintering habitat in Mexico to breeding grounds in southern Ontario. The spring migration begins in March and usually involves multiple generations of monarchs. Adults lay eggs along the migratory pathway of the Gulf Coast and central United States which develop through five larval instars and form a chrysalis during the pupal stage before emerging as an adult. These individuals continue the journey and often repeat the same process, resulting in up to five generations of monarchs being produced before individuals reach the northern part of their breeding range in south-eastern Canada (Flockhart et al., 2013; Miller et al., 2012; Reppert & Roode, 2018). Monarchs generally arrive in Ottawa in early July, where they continue to forage on nectar from flowering plants and lay eggs on milkweed that develop into the final non-breeding generation of adults (beginning

mid-August). These individuals are often referred to as the migratory generation, as they remain in reproductive diapause for energy conservation and live up to nine months to migrate to Mexico (Brower et al., 2006; Reppert & Roode, 2018). Since the journey requires significant energy, the migratory generation spends their time foraging for nectar to build energetic lipid reserves and roosting along the Great Lakes until the trip begins in the fall (Beall, 1948; Brown & Chippendale, 1974; Miller et al., 2012; Hobson et al., 2023).

The adults I used in this study were reared from eggs laid by wild-caught adults to produce individuals of the migratory generation in reproductive diapause. Matching the timing of the controlled rearing to the natural cycle allowed me to more accurately evaluate how climate-warmed nectar resources may impact the population.

2.1.2 Plant species

I used two species of native and two species of introduced nectar sources that were abundant at the site and are known to be frequently used by monarch butterflies in the Ottawa region (Pekos et al., 2025).

Symphyotrichum novae-angliae (New England aster) is a native perennial forb in the family *Asteraceae*. It generally flowers from late August to October, growing in a variety of habitats including thickets, wet meadows, low fields, roadside shoulders, and ditches. The purple petal-like ray flowers grow in rounded clusters at the tops of the stems and have a bright yellow central disk (National Plant Data Team, 2026).

Solidago canadensis (Canada goldenrod) is a native perennial forb in the family *Asteraceae*. It generally flowers from July to October, growing in a variety of moist and dry habitats including meadows, along roadsides, ditches, and upland prairies. Inflorescences of

small yellow ray flowers grow on numerous long, drooping, panicle branches of each stem (National Plant Data Team, 2026).

Lythrum salicaria (purple loosestrife) is an introduced perennial forb in the family *Lythraceae*. It flowers from July until October, preferring moist habitats such as wet meadows and ditches. The small purple flowers grow on numerous spike shaped inflorescences present on each stem (National Plant Data Team, 2026). *Lythrum salicaria* was introduced to North America from Europe in the 1800's and is considered a highly invasive plant by the Ontario Invasive Plant Council due to its negative ecological impacts (National Plant Data Team, 2026; Ontario Invasive Species Council, 2026). Despite invasive status, it was included in this study due to documented use by monarch butterflies and abundance in the Ottawa region (Pekos et al., 2025).

Trifolium pratense (red clover) is an introduced, short-lived perennial forb in the family *Fabaceae*. *Trifolium pratense* originates in Asia Minor and southeastern Europe and was brought to North America in the 1600s (National Plant Data Team, 2026). It is frequently used in agriculture as a cover crop, pasture, and is known to produce high quality forage for livestock. It generally flowers from late spring to mid-summer, tolerating a range of soil types. Pinkish-purple tubular flowers grow in compact clusters at the tips of branches on each stem (National Plant Data Team, 2026). It is not currently considered invasive by the Ontario Invasive Plant Council. *Trifolium pratense* was included in the study to evaluate whether a widely available species with inexpensive seeds could provide high quality nectar for monarchs.

2.2 Experimental design

This study was designed as a 2x2 nested factorial experiment with plant species origin treatment nested within the warming treatment. The design included warmed and control

chambers for two native species (*Solidago canadensis* and *Symphyotrichum novae-angliae*) and two introduced species (*Lythrum salicaria* and *Trifolium pratense*). This design was intended to test whether native and introduced nectar plants differed in their responses to warming, and whether the response of monarch body composition to warming differed based on species origin. However, unexpected complications related to drought conditions limited the ability to evaluate all plant responses, and complete monarch feeding trials across all species and treatment combinations (see results for details). As a result, the full factorial design could only be evaluated for select plant responses.

2.2.1 Location

The field warming experiment took place from June 2 to September 12, 2025, in an old field (approximately 10 acres) on private property in Kinburn, Ontario, Canada.

2.2.2 Warming treatment and controls

I used open-top chambers (OTC) as a passive warming treatment. The OTCs are designed to simulate moderate increases in daytime temperatures that reflect projected climate warming trends in Ottawa. Open-top chambers are widely used as they are generally low cost, easy to move, and can significantly increase the temperature within their environment while minimizing the impact on levels of precipitation (Marion et al., 1997). The effects of OTCs on abiotic factors including air temperature, humidity, and soil moisture are understood to be largely dependent on location and natural conditions, requiring separate measurements to assess impacts (Dabros et al., 2010; Healey et al., 2023).

The OTCs were constructed with an aluminum frame and clear acrylic siding to allow light transmission for plant photosynthesis while maintaining the passive warming design. The OTCs measured 1.2 m^2 at the bottom, tapering to a 0.9 m opening at the top (1.5 m tall). They

were placed in the field on June 2, 2025. The control frames were constructed *in situ* in June with the same dimensions using plastic-coated steel garden stakes connected with PVC corner fittings. There was nothing covering the sides of the control frames during the month of June. On July 9, 2025, the control frames were wrapped with fine mesh tulle to mimic some of the effects of siding on the OTCs (i.e., lower wind impact on plants and to act as a similar physical barrier during the monarch feeding trial). The OTCs were also wrapped then with fine mesh tulle to ensure similar light filtering would occur between control and warming chambers.

In early August, just before the feeding trials began, $2m^2$ enclosures were constructed over the OTCs and control chambers (Figure 1). These enclosures were constructed using plastic-coated steel garden stakes, PVC corner fittings, and a fine white mesh mosquito net which covered the top and sides. The enclosures were designed to keep monarchs in the chamber while allowing enough space for them to spend their non-feeding time outside of the chambers. To ensure the monarch only had access to the targeted experimental flowering plants, all plant material in the space between the chamber and the mesh enclosure (~30 cm) was removed. Effectively, this design allowed us to measure the warming effect from the OTC on the plants while minimizing the direct warming effects on the monarchs.

2.2.3 Location of chambers

On June 2, 2025, I placed 20 pairs of OTCs and control chambers (n=40) over existing single species stands of two native (*S. canadensis* and *S. novae-angliae*) and two introduced (*T. pratense* and *L. salicaria*) flowers, with 5 pairs per species. Efforts were made to place the chambers prior to this date, but identification of all plants was not possible during prior weeks. I selected locations that: i) minimized tree shading; ii) optimized similarity of stem height and density within each pair; and iii) maximized the distance between pairs of the same species. The

maximum distance between warmed and control chambers within the same replicate pair was 2 m. The shortest distance between replicate pairs was ~5 m.

2.2.4 Monarch rearing

Between July 21 and August 1, 2025, I caught six female and four male monarch butterflies from the field site in Kinburn and the Canadensis Garden of Canada in Ottawa, Ontario. Adults were housed outdoors at the University of Ottawa in large mesh enclosures approximately 4 ft tall, with males and females paired to allow mating. Each enclosure was supplied with three to five potted common milkweed plants (*Asclepias syriaca*) for oviposition and a 1:4 sugar-water solution as a nectar substitute. Sugar water was provided in a clean bowl with an unused scouring pad and was replaced every other day. Potted milkweed was used for egg laying, and fresh milkweed leaves used for larval rearing were collected from milkweed plants grown on campus and from the field site as needed.

I checked enclosures for eggs daily. Eggs were collected from milkweed leaves and placed in labelled insect-rearing containers with moistened coffee filters. All eggs were produced by four female and four male parents, with a total of six mating pairs. Eggs and larvae were reared in 240 mL and 360 mL clear insect-rearing containers in a Conviron® Genesis™ GEN100 growth chamber under 16-hour daytime conditions at 27°C and 60% relative humidity, and 8-hour nighttime conditions at 21°C and 60% relative humidity. Larvae were checked daily and provided fresh common milkweed leaves until chrysalis formation.

The adults were brought into the field and introduced into the experiment on the same day they eclosed. Adults were introduced from August 18 to September 7, 2025. Adults were only introduced to enclosures with open flowers. To maximize sample size but minimize wing

injuries, one male or two females were allowed in an enclosure at once. The adults fed *ad libitum* in the enclosures for a period of 5 days before being euthanized.

2.3 Measurements

2.3.1 Temperature, soil moisture, and humidity

Temperature within the chambers were monitored every 30 minutes using 15 HOBO temperature data loggers (Onset HOBO Pendant Temperature/Light 64K Data logger, ±0.53°C) from June 3 to September 5, 2025. A logger was placed in the chambers of two replicate pairs per species aside from *S. novae-angliae* which had loggers in only one replicate pair. The loggers were suspended in the middle of each chamber using zip ties and garden stakes approximately 30 cm off the ground to measure the air temperature around the plants.

I measured the soil moisture weekly from June 16 to September 5, 2025 in all 40 chambers using a portable Field Scout™ TDR 100 Soil Moisture Meter with 8 cm metal probes. The meter uses time-domain reflectometry, in which the travel time of an electromagnetic wave is used to estimate volumetric water content (VWC), defined as the volume of water relative to the total soil volume. Measurements were taken in the middle and close to the four corners of each chamber to determine the average percent VWC per chamber. The meter has an accuracy of ±3.0% volumetric water content with electrical conductivity < 2 dS m –1.

I measured relative air humidity weekly from July 14 to September 5, 2025 in all 40 chambers using a Fisherbrand™ Traceable™ Pocket Hygrometer. The meter uses solid-state sensors to determine relative humidity (RH) with a range between 0.0 to 100.0%, a resolution of 0.1%, and accuracy of ±3% RH. Measurements were taken by placing the meter in the middle of the chamber for 30 seconds prior to recording the reading.

2.3.2 Plants

On June 2, 2025, I flagged 10 random stems within each chamber using flagging tape. The height of each flagged stem was measured from the base of the stem to the apical meristem twice weekly until the plant had completed flowering or died.

To monitor overall plant health, I took chlorophyll concentration measurements bi-weekly from July 14 to August 12, 2025 in all 40 chamber using an Apogee® MC-100 Chlorophyll Meter. The meter determines the chlorophyll concentration present in the leaves through a non-destructive method that measures the transmittance of red and near-infrared light through the leaf. Since chlorophyll strongly absorbs red light but not near-infrared light, the ratio of these wavelengths is used to estimate chlorophyll concentration in absolute units of μmol m^2 of chlorophyll. When possible, I took three readings on a single leaf near the bottom, middle, and top of three stems per chamber and then took the average. Due to insect predation and impact of drought, some leaves on certain areas of the plant were not large enough to cover the sample area of the instrument and could not be recorded.

I recorded the phenophase of flowers on tagged stems twice weekly throughout the duration of the experiment. The number of open and closed buds, and the number of open and senescing flowers were recorded based on the flower phenophase classification protocol from the USA National Phenology Network (Denny et al., 2014).

To determine the floral availability within each chamber during the feeding trials, I counted the total number of open flowers on *T. pratense* and *L. salicaria*. Since individual counts were not feasible for *S. canadensis*, aerial photos of each *S. canadensis* chamber were taken during the monarch feeding trails to estimate the amount of flowers present by multiplying the number of stems present by the average amount of flowers counted on a single stem in the field.

I was not able to include floral counts or flowering period of *S. novae-angliae* in the analysis as the species did not begin to flower until the end of the experiment.

2.3.3 Monarchs

I conducted 5-day feeding trials and weighed adults on days 0, 1, 3, and 5, with day 0 corresponding to the eclosion date. Before the first weighing, I labelled each adult with an identification number on the largest cell of their wing with a fine point Sharpie. Using a Sartorius Research R300S analytical balance with a precision of 0.0001g located at the site, each monarch was gently removed from its container and placed into a separate insect-rearing container that had been tared on the balance. The first stabilized reading displayed on the balance was recorded. Weighing was conducted between mid-morning and noon on each sampling day to maintain a consistent time interval between measurements. Monarchs that could not be introduced to the experiment due to space constraints were released in the local population provided no more than 24 hours had passed from eclosion. By the end of the experiment, 91 (n=56 females, n=35 males) 5-day feeding trails were completed between August 18th to September 12th, 2025.

At the conclusion of the feeding trial, monarchs were humanely euthanized by freezing. Forewing length was measured on euthanized monarchs using a vernier calliper from the base of the thorax to the wing tip. Individuals were stored in a -5°C freezer until they were sent to the lab of Dr. Christopher C. Guglielmo at Western University for body composition analysis including the wet mass, water mass, dry fat mass, and dry lean mass.

To measure wet mass, each butterfly was placed in an individual envelope and weighed to the nearest 0.0001g. Envelope mass was subtracted from the total measurement to obtain the wet mass of the butterfly at the end of the feeding trial. This wet mass measurement was considered more accurate than field mass measurements because monarchs were weighed after

euthanasia, when they were no longer moving. Field measurements were more variable because the balance was highly sensitive to monarch movement. The butterflies were subsequently dried in an oven at 70°C for 3 days until a constant mass was reached. Samples were weighed again to determine the dry mass. The water mass is obtained by subtracting the dry mass from the wet mass of the individuals. Monarchs rely on small puddles, dew and rain on vegetation, and water content of nectar to avoid desiccation, making it an important metric in determining survival during migration (Gibo & McCurdy, 1993; Hobson et al., 2023).

To determine fat mass, the dried samples were subjected to petroleum ether extraction in a Soxhlet extractor, which removes lipids other than membrane phospholipids. Samples were then re-dried for 8 hours and re-weighed. Fat mass was calculated as the difference between dry mass before and after extraction. Lipids are known to be the major source of energy reserves in migrating monarch butterflies, providing more energy per weight than carbohydrates such as glycogen which have a minimal contribution to energy reserves for migration, making fat mass an important metric for migration success (Beall, 1948; Brower et al., 2006; Brown & Chippendale, 1974).

Finally, the dry lean mass was determined as the remaining mass after lipid extraction. This measure represents the dry mass of the butterfly after removing fat and includes structural and tissue components such as muscle, organs, wings, and exoskeleton. While components of dry lean mass, including carbohydrates and proteins, may be used during short-distance flight, they are generally considered to play a limited role in fueling the long-distance migration of monarch butterflies (Brower et al., 2006; Beall, 1948; Brown & Chippendale, 1974). Similarly, in migratory birds, fat is the primary fuel source, with protein traditionally assumed to provide a low amount of energy (Elowe et al., 2023). However, a study by Elowe et al. (2023) found that

lean mass was depleted rapidly early in flight, indicating that protein may play a more significant role in migratory success and could become limiting even before fat reserves are exhausted.

2.4 Statistical Methods

Statistical analyses were performed using R 4.5.1 (R Core Team, 2025). The analysis was split into three parts: I examined the difference in: i) environmental conditions between the warmed and control chambers (‘environmental models); ii) plant responses between warmed and control chambers, and based on origin where possible (‘plant models’); and iii) monarch body composition from feeding trials on *S. canadensis* between warmed and control chambers, and between trials on *S. canadensis* and *L. salicaria* in control chambers.

Models were fitted using the packages mgcv, lme4, lmerTest, emmeans, car, performance, and DHARMa, as appropriate for each analysis. Across all models, model fit and assumptions were evaluated using residual diagnostics. For linear and linear mixed-effects models (LME), I inspected residual-versus-fitted plots, Q-Q plots, and scale-location plots, and assessed heteroscedasticity and influential observations where appropriate. For generalized additive mixed models (GAMMs), I evaluated model diagnostics such as convergence, residual patterns, and basis dimension adequacy using gam.check in mgcv. GAMMs were fitted using restricted maximum likelihood (REML) and model diagnostics such as convergence, residual patterns, and basis dimension adequacy were evaluated using gam.check in the mgcv package in R. DOY was mean-centered in many models to improve interpretation of the model intercept, so that fixed-effect estimates were interpreted at the average sampling date.

The significant drought that occurred during the experiment (see results) resulted in death and suspected phenological delays of some species, restricting the ability to track phenological

changes, measure leaf chlorophyll content, and place monarchs in both treatment chambers for all species. As a result, species without sufficient observations were excluded from some analysis.

2.4.1 Environmental Models

2.4.1.1 Temperature analysis

To evaluate temperature differences between warmed and control chambers over the season, I analyzed temperature data recorded at 30-minute intervals using GAMMs as exploratory analyses indicated that temperature varied non-linearly within a day and over the season. The analysis was restricted to dates between June 3rd and August 12th, where both treatment groups were represented to ensure comparable temporal coverage between treatment chambers. I included time of day (TOD; decimal hours) and day of year (DOY) in the model to account for diel temperature cycles within each 24-h period and seasonal changes in temperature across the field season, respectively. Temperature was modeled using a Gaussian error distribution with identity link. I fitted separate smooth terms for TOD and DOY for each treatment group. Due to the cyclical pattern of TOD, I modeled it using a cyclic cubic spline, while DOY was modeled using a thin plate regression spline to allow for non-linear seasonal change. Chamber was included as a random-intercept smooth to account for repeated measurements within chambers.

To estimate the magnitude of the warming effect both daily and seasonally, I derived population-level predicted differences between warmed and control chambers from the fitted GAM. 95% confidence intervals for these contrasts were estimated from the model linear predictor matrix and coefficient covariance matrix, following standard prediction methods for GAMs in mgcv (Wood, 2017).

2.4.1.2 Soil moisture and relative humidity analysis

To evaluate differences in soil moisture and relative humidity between warmed and control chambers over the season, I analyzed the average proportional soil moisture and relative humidity per chamber across the experiment. Initial data visualization indicated strong non-linear seasonal patterns, so I fitted separate beta GAMMs for both variables. In each model, treatment was included as a fixed effect, treatment-specific smooths of DOY were fitted to account for non-linear seasonal change within each treatment. Chamber identity was included as a random-intercept smooth to account for repeated measurements.

2.4.2 Plant Models

2.4.2.1 Stem growth

I calculated percent stem growth for each sampling date relative to the initial recorded height using the following equation: $(\text{height}_{final} - \text{height}_{initial})/\text{height}_{initial} \times 100$. Percent growth under warming and by origin was separately analyzed using LME models which included linear and quadratic terms for scaled DOY, treatment, species, and their interactions as fixed effects. DOY was centered by subtracting the mean and scaled by dividing by 10 before analysis. Chamber was included as a random intercept to account for differences among chambers, and stem identity was included as a random intercept to account for repeated measurements on the same stems over time. A random slope of DOY for stem identity was included to allow individual stems to vary in their growth trajectories. The statistical significance of fixed effects were assessed using Type III ANOVA with Satterthwaite's approximation for degrees of freedom.

Since the warming model included significant interactions among quadratic DOY, treatment, and species, post hoc trend comparisons were used to determine whether the rate of percent stem growth differed between warmed and control plants within each species at three representative time points across the season. These time points were selected as DOY values at approximately equal intervals across the measurement period: 173 (June 22nd, 2025), 193 (July 12th, 2025), and 212 (July 31st, 2025). The selected DOY values were converted to the centered and scaled DOY variable used in the model. I then used the emtrends function in the emmeans package to compare the estimated slope of percent growth over DOY between warmed and control chambers within each species at each time point. P-values were adjusted using the Holm correction with the base R p.adjust function to account for multiple comparisons.

2.4.2.2 Phenology

To evaluate phenological abundance and timing responses to experimental warming, I analyzed open flower counts using GAMMs, as exploratory plots indicated that flowering responses varied non-linearly over the season. Flower counts were summarized at the chamber level by species, treatment, and DOY. Models were fit using a negative binomial error distribution as it is known to more accurately model floral count data (Reitan & Nielsen, 2016). Chamber was included as a random-effect smooth to account for repeated observations within chambers. *S. novae-angliae* was excluded from the open flower analysis because only one chamber flowered during the experiment.

For open flower abundance, I compared two GAM structures. The first included species-specific seasonal smooths of DOY and assumed that warmed and control chambers shared the same seasonal curve within each species, that is, they did not differ in the timing, duration, or magnitude of flowering across the season. The second included separate seasonal smooths of

DOY for each species × treatment combination, allowing warmed and control chambers to differ in seasonal phenology within species. This comparison tested whether warming altered the shape of the seasonal phenology curve by changing when flowers appear, how long flowering lasts, or how many flowers are open at different points during the season. Models were compared using Akaike information criterion (AIC), with lower AIC values indicating better relative model fit. The model including separate species × treatment seasonal smooths was retained because it had a lower AIC than the simpler flower abundance model (943.16 vs. 957.80), indicating improved fit relative to the additional model complexity. Model diagnostics were evaluated using the gam.check function.

Phenological timing metrics were then derived from the fitted open flower curves rather than the raw data, as phenology metrics were collected based on 10 randomly tagged stems instead of observations of all plants within the chamber. I generated population-level predictions across the observed DOY range for each species × treatment combination while excluding the chamber random-effect smooth. 'Peak flowering' was defined as the DOY with the highest predicted open flower count. The predicted count at this DOY was retained as estimated peak abundance, which was a derived metric from the fitted curve. 'Flowering onset' and 'finish' were defined using a threshold equal to 5% of the predicted peak abundance rather than the predicted day of first flower to avoid onset estimates being driven by isolated early observations. 'Onset' was defined as the first DOY when the fitted curve exceeded this threshold, and 'finish' was defined as the last DOY when the fitted curve remained above this threshold. 'Flowering duration' was calculated as the difference between finish and onset. Uncertainty in the derived flowering timing metrics was estimated by simulating coefficients from the fitted GAM

covariance matrix and recalculating onset, peak timing, finish, duration, and peak abundance across 2,000 simulated curves.

2.4.2.3 Chlorophyll

For three randomly selected stems in each chamber, three chlorophyll meter readings were collected from each plant section: bottom, middle, and top. Readings were averaged within each plant section to calculate one mean chlorophyll value per section per stem. Chlorophyll was analyzed using a linear mixed-effects model in the lme4 and lmerTestpackages. The model included plant section, treatment, species, and centered DOY as fixed effects. Random intercepts were included for chamber and for stem ID nested within chamber to account for the hierarchical structure of the data and repeated measurements within chambers and stems.

Statistical significance of fixed effects was assessed using Type III analysis of variance with Satterthwaite's approximation for denominator degrees of freedom. An additional model including the plant part by treatment interaction was compared with the original model using a likelihood ratio test fit with maximum likelihood. The interaction was not significant, so the simpler model without the interaction was retained. When plant section effects were significant, estimated marginal means were calculated and pairwise comparisons were performed with Tukey adjustment for multiple comparisons.

2.4.3 Monarch models

Prior to evaluating monarch body composition metrics, *S. novae-angliae* and *T. pratense* observations were excluded from the analysis because low flower availability resulted in insufficient feeding trials for these species. No feeding trials were conducted in warmed *L. salicaria* chambers because all flowers had senesced in warmed chambers before trials could be

completed. Monarch mass change and body composition were therefore analyzed separately for two comparisons: i) warmed versus control *S. canadensis* trials, and ii) control *S. canadensis* versus control *L. salicaria* trials. In both analyses, centered eclosion DOY was included to account for seasonal timing, sex was included to account for physiological differences between males and females, and forewing length was included to account for differences in body size. Final inference for linear models was based on Type II ANOVA. Heteroscedasticity-robust HC3 standard errors were used when model diagnostics indicated heteroscedasticity. For non-significant focal effects, I conducted sensitivity analyses to estimate the minimum detectable difference at 80% power and used 90% confidence intervals to assess whether estimated effects were small in magnitude.

To evaluate effects of warming on monarch body composition following feeding trials on *S. canadensis*, I modeled wet mass, dry fat mass, dry lean mass, and water mass as a function of warming treatment using linear models. Quadratic eclosion DOY terms were tested where exploratory plots or model comparisons indicated non-linear seasonal patterns. Final model structure was determined using biological relevance, model diagnostics, and comparison of models using AIC and nested model comparisons where appropriate.

To examine whether monarch body composition differed between nectar plant species in the absence of warming, I compared monarchs from control *S. canadensis* and control *L. salicaria* feeding trials. Wet mass, dry fat mass, dry lean mass, and water mass were modeled separately as a function of plant species. Treatment × eclosion DOY interactions and quadratic eclosion DOY terms were tested for each response and retained only when supported by model comparison.

3. Results

3.1 Environmental conditions during the study

During the experiment, below-average precipitation and above-average temperatures resulted in unusually dry conditions across the study region. In July, the Canadian Drought Monitor (CDM) classified the area as abnormally dry, and by early August conditions had intensified to a D2 severe drought classification (Agriculture and Agri-Food Canada, 2025). According to the CDM, D2 drought conditions historically occur less than once every 10 years (Agriculture and Agri-Food Canada, 2026). Consistent with these regional patterns, the local conservation authority issued a Level II (Moderate) Low Water Advisory on September 5, 2025, reporting that the watershed had received less than 80% of the long-term seasonal average rainfall between June and August (Mississippi Valley Conservation Authority, 2025).

I detected strong seasonal effects of drought on soil moisture throughout the experiment. Soil moisture varied strongly over the season in both control and warmed chambers (control: edf = 7.47, $\chi^2 = 577.3$, $p < 0.001$; warmed: edf = 7.70, $\chi^2 = 525.3$, $p < 0.001$). This indicates that soil moisture did not change at a constant rate through time. Instead, soil moisture followed a seasonal pattern consistent with progressive drying during the drought period (Figure 2). Population-level predictions from the final GAM indicated that soil moisture reached a minimum of 6.74% on approximately August 18, 2025, with the driest predicted period occurring from approximately August 14 to August 22, 2025. These trends aligned closely with the regional drought conditions reported across Ontario during the same period that are described above (Agriculture and Agri-Food Canada, 2025; Ottawa River Regulating Committee, 2025). Conversely, model predicted the highest soil moisture reached a maximum of 31.9% on

approximately July 8, 2025, with the wettest predicted period occurring from approximately July 4 to July 12, 2025.

Plant condition deteriorated substantially following the onset of drought conditions and there were many indications of drought stress. In warmed *L. salicaria* plots, all flowers senesced by July 31, and unopened buds subsequently failed to develop. In *S. novae-angliae* plots, leaves became red, yellow, or brown, and herbivory increased noticeably during July and early August (Figure 3). In *T. pratense* plots, buds failed to open and leaves became limp by July 23 (Figure 3). These effects were sufficiently severe that many species had to be excluded from phenology analyses and monarch feeding trials because too few flowers were available when the experimental monarchs emerged (Figure 4).

3.2 Environmental effects of warming treatment

3.2.1 Temperature

The warming effect of the OTCs changed over both diel and seasonal time scales (Table 1; Figure 4). Based on GAM prediction contrasts, warmed chambers were on average 1.20°C warmer than control chambers across the study period (95% CI: 0.35 to 2.05°C). The magnitude of warming varied through the day: at the median day of the experiment (DOY 189; July 8, 2025), predicted warming effects ranged from 0.30°C at 10:00 (95% CI: -0.68 to 1.28°C) to 1.81°C at 14:00 (95% CI: 0.83 to 2.79°C), with pointwise 95% confidence intervals excluding zero for 13 of 24 hourly predictions. Seasonal contrasts showed a similar pattern when time of day was held constant at approximately midday, with warmed chambers predicted to be 0.97 to 1.90°C warmer than control chambers across the season. Chamber-level variation was also significant (edf = 11.64, $F = 26.41$, $p < 0.001$), indicating that average temperature varied among chambers independent of the warming treatment.

3.2.1 Soil Moisture

There was no overall difference in mean soil moisture between warmed and control chambers (Table 2). Chamber-level differences were substantial (edf = 44.49, $\chi^2 = 1631.4$, $p < 0.001$), indicating that average soil moisture varied considerably among chambers independent of the warming treatment.

3.2.3 Relative Humidity

There was no overall difference in mean relative humidity between warmed and control chambers (Table 3). Relative humidity varied strongly over the season, with significant effects of DOY in both control and warmed chambers (control: edf = 8.43, $\chi^2 = 166.94$, $p < 0.001$; warmed: edf = 8.30, $\chi^2 = 202.43$, $p < 0.001$). The high effective degrees of freedom (edf) indicate that relative humidity fluctuated during the season rather than increasing or decreasing at a constant rate (Figure 5). Chamber-level differences were also significant (edf = 23.35, $\chi^2 = 50.61$, $p < 0.001$), indicating that average relative humidity varied among chambers independent of the warming treatment.

3.3 Plant responses

3.3.1 Stem height

Warming altered the shape of seasonal stem growth trajectories but did not cause an increase in stem growth under warmed conditions as predicted (Figure 6). Although the overall model indicated that the seasonal growth trajectories differed among species and between warmed and control chambers (DOY*warming treatment*species: $F_{6,774.16} = 6.06$, $p < 0.001$; Table 4), the post hoc trend comparisons showed that there was no consistent effect of warming treatment on percent stem growth across species (Table 5). *Trifolium pratense* showed

significantly higher percent growth rates in control chambers mid- (July 12, 2025: mean difference = 7.65, Holm-adjusted p = 0.0012) and late-season (July 31, 2025: estimate = 17.18, Holm-adjusted p < 0.001) time points. No other differences in percent growth rate due to the warming treatment were detected for *S. canadensis*, *L. salicaria*, or *S. novae-angliae* (Table 5). Overall percent growth differed by species ($F_{3,65.03} = 49.46$, $p < 0.001$) and varied over the season (DOY and DOY² : $F_{2,762.26} = 1838.04$, $p < 0.001$) (Table 4).

An origin analysis in the absence of warming showed that percent stem growth differed between native and introduced species (Table 6; Figure 7). Native species had higher percent stem growth than introduced species overall (origin: $F_{1,163.55} = 24.95$, $p < 0.001$), and their seasonal growth trajectories differed in shape across the season (DOY*origin: $F_{2,2855.36} = 9.05$, $p < 0.001$).

3.3.2 Floral availability

A negative binomial GAM showed no effect of warming on open flower abundance (Table 7). Strong seasonal variation was observed in all three species (*S. canadensis*: edf = 1.94, $\chi^2 = 46.4$, $p < 0.001$; *L. salicaria*: edf = 5.04, $\chi^2 = 171.5$, $p < 0.001$; *T. pratense*: edf = 5.01, $\chi^2 = 61.1$, $p < 0.001$). Chamber-level variation was also observed (edf = 23.51, $\chi^2 = 135.4$, $p < 0.001$).

Warming altered the shape of the seasonal distribution of flowering rather than causing a directional shift in individual phenophases of floral abundance (Figure 8). Warming had no impact on the onset, peak, or finish of flowering for any of the species (Figure 9).

3.3.3 Chlorophyll

Average chlorophyll content of leaves did not differ between warmed and control plants (Table 8; Figure 10). Chlorophyll varied significantly among plant parts ($F_{2,446.25} = 45.39$, $p < 0.001$) and declined over the course of the season with increasing day of year ($F_{1,570.75} =$

178.98, $p < 0.001$). Warming treatment did not significantly affect chlorophyll content ($F_{1,36.57} = 0.025$, $p = 0.876$). The treatment by plant part interaction was not retained in the final model, indicating that patterns among plant parts were consistent across warmed and control plants.

3.4 Monarch responses to warming

3.4.1 Warmed vs control

Warming treatment did not significantly affect any monarch body composition metrics (all $p \geq 0.09$; Table 9; Figure 12).

Day of year and forewing length varied with all metrics but in different ways. Wet mass (0.0057 g (0.0015 SE), $t = 3.811$, $p < 0.001$) and water mass (0.0059 g (0.0013 SE), $t = 4.665$, $p < 0.001$) increased with day of year (Table 9). Dry fat mass also increased with day of year, including a quadratic component, indicating that the magnitude of fat mass accumulation was greater in monarch feeding trials that occurred later in the season (DOY: 0.00080 g (0.00033 SE), $t = 2.418$, $p = 0.020$; DOY^2: 0.00010 g (0.00005 SE), $t = 2.070$, $p = 0.044$; Table 9). In contrast, dry lean mass declined with day of year (−0.0011 g (0.00037 SE), $t = -3.025$, $p = 0.004$; Table 9).

Wet mass (0.015 g (0.0039 SE), $t = 3.937$, $p < 0.001$) and dry lean mass (0.0077 g (0.0022 SE), $t = 3.567$, $p < 0.001$) increased with forewing length, whereas dry fat mass decreased with forewing length (−0.013 g (0.0041 SE), $t = -3.166$, $p = 0.003$) and there was no relationship between water mass and forewing length (Table 9).

There was no difference between sexes for any metric (Table 9).

Sensitivity analyses indicated that the study was sufficiently powered (80%) to detect moderate-large treatment effects, corresponding to differences of approximately 0.054 g for wet mass, 0.011 g for dry fat mass, 0.023 g for dry lean mass, and 0.048 g for water mass. Observed

treatment effects were substantially smaller than these thresholds, and 90% confidence intervals for all responses were narrow and centered near zero, suggesting that any true effects of warming were modest in magnitude.

3.4.2 Control *S. canadensis* vs control *L. salicaria*

Species (nectar source: *S. canadensis* vs. *L. salicaria*) had limited effects on monarch body composition metrics (Table 10; Figure 13). Neither wet mass nor dry fat mass were significantly influenced by species (Table 10). In contrast, individuals feeding in control chambers of *L. salicaria* had lower dry lean mass than those feeding on nectar from *S. canadensis* (−0.0100 g (0.0047 SE), $t = -2.102$, $p = 0.042$; Figure 13).

The only body composition metric that varied with day of year was dry lean mass which declined (−0.00084 g (0.00038 SE), $t = -2.215$, $p = 0.033$; Table 10). Similarly, the only metric that varied with forewing length was dry lean mass which increased (0.0064 g (0.0019 SE), $t = 3.356$, $p = 0.002$; (Table 10).

There was no difference between sexes for any metric (Table 10).

Sensitivity analyses indicated that the study was 80% powered to detect moderate-large species effects, corresponding to differences of approximately 0.0669 g for wet mass, 0.0150 g for dry fat mass, 0.0247 g for dry lean mass, and 0.0576 g for water mass. Observed species effects for wet mass, dry fat mass, and water mass were smaller than these thresholds, and 90% confidence intervals were centered near zero, suggesting that any true differences between nectar sources were modest in magnitude. For dry lean mass, the observed effect (−0.0100) fell within the detectable range and the 90% confidence interval (−0.0181 to −0.0016) supported a small but significant reduction associated with *L. salicaria*.

4. Discussion

Using a field warming experiment, this study evaluated the direct effects of warming on the growth condition and floral production of two native and two introduced plants, and the subsequent effects of both warming and species' origin on monarch body composition. I did not find an effect of warming on plant condition or monarch body composition metrics or a difference in the response of stem growth to warming between native and introduced species. However, I did find an effect of species' origin on average stem growth and monarch dry lean mass. I found that the average stem growth of introduced plant species was found to be significantly lower than native species. I also found that individuals that foraged on flowers of *L. salicaria*, an introduced species, had lower dry lean mass than those that foraged on flowers of native *S. canadensis* chambers, pointing to potential differences in nectar composition between the two species. Together, these findings indicate a need for more research regarding the responses of native and introduced plants to climate warming and associated climatic events (i.e. low precipitation).

4.1 Lack of warming effects on plant growth and floral availability

Contrary to my prediction, there was no consistent positive effect of warming on percent stem growth across species. Instead, *T. pratense* showed significantly higher percent growth rate in control chambers at the middle and late season time points. This may indicate that warming became stressful for *T. pratense* as the season progressed, particularly under increasingly dry conditions. The stronger response in *T. pratense* may therefore reflect greater species-specific sensitivity to warming or drought than the other species. This sensitivity may be related to its earlier flowering phenology, as *T. pratense* flowered substantially earlier than both *L. salicaria*

and *S. canadensis* (first flowers were observed on June 30, 2025 compared with July 9, 2025 and August 1, 2025, respectively; Figure 4).

The absence of an overall warming effect on stem growth is consistent with previous research conducted in the lab in the same system (Peel et al., 2026), and with the lack of warming effect I found on leaf chlorophyll content, which suggests that photosynthetic activity was largely unaffected (Li et al., 2018; Kazemi et al., 2023). At the same time, because these analyses incorporated a robust correction for multiple comparisons, these results are conservative and subtle treatment effects may have gone undetected. Nevertheless, these findings suggest that overall plant condition were not strongly affected by the warming treatment.

Similarly, warming had no consistent effect on flower abundance or phenology. This was contrary to my prediction that warmer temperatures would decrease floral abundance and advance flowering. However, evidence for these effects in the literature is mixed. Some studies have found that warming reduces floral abundance (Takkis et al., 2018; Moss & Evans, 2022; Peel et al., 2026), while others have suggested the effect is more species-specific (McCombs et al., 2022; Defalque et al., 2025). When warming occurs below a species' thermal optimum, it has been shown to advance flowering onset (Kopp et al., 2020; Montes-Perez et al., 2025), but those responses are not universal (Inouye, 2022).

The lack of a response in floral availability is least likely to have resulted from an insufficient magnitude of warming, as previous research conducted in the lab in the same system found that a smaller increase in temperature (0.57°C) than this study (1.20°C) affected floral abundance (Peel et al., 2026). Instead, the absence of an effect may reflect the timing of the warming treatment, which may not have begun early enough to influence floral development. The environmental cues that plants use to regulate flowering timing often occur earlier in the

growing season, and in some cases more than a year before flowering takes place (Diggle & Mulder, 2019; Evers et al., 2021). Future studies should aim to capture the entire developmental process by conducting multi-year warming experiments.

It is also possible that the seasonal drought conditions reduced or masked the effects of warming on plant growth and floral production. The low precipitation conditions may have confounded the effect of warming on several response variables, leading to no consistent observed effect of warming on flowering onset, stem growth, flower abundance, leaf chlorophyll content. Drought and heat stress have both been shown to limit plant growth, and their combined effects may be higher in magnitude than the effect of either stressor alone (Lipiec et al., 2013; Wilschut et al., 2022; Defalque et al., 2025). A study by Diez et al. (2012) found that warm temperatures may have a weaker effect on flowering when soil moisture conditions are not suitable, suggesting there may be an important interaction between temperature and precipitation. Future studies should aim to understand the impact of drought and possible interaction between drought conditions and warming on plant condition, phenological response, and nectar quality.

4.2 Lack of warming on body composition components

I did not find a significant effect of warming on any monarch body composition metric. This was unexpected, given that Peel et al. (2026) reported decreases in monarch fat mass under only 0.57 °C of daytime warming. Considering the greater magnitude of warming present in this study (1.20°C), the absence of an effect on dry fat mass was particularly surprising. One possible mechanism underlying the decrease in monarch fat mass observed by Peel et al. (2026) was the warming-driven decline in nectar sucrose concentration reported in that study. Because I could only evaluate monarch responses to warming in *S. canadensis* chambers, it is possible that nectar

quality in this species was less sensitive to warming than in the other study species. Alternatively, warming may not have affected nectar quality in this study, in which case changes in monarch body composition would not necessarily be expected. Since nectar quality was not measured directly, I was unable to distinguish between these potential mechanisms.

It is also possible that the response of nectar quality to warming depended on water availability. A study by Takkis et al. (2015) found that optimal temperatures for nectar sugar content was dependent on species identity and whether the plants were water stressed. Similarly, Defalque et al. (2025) found that drought and high temperatures reduced nectar volume in a species-specific manner. Since the warming treatment did not affect soil moisture in this study, site-level drought conditions may have moderated warming effects on nectar quality and, consequently, monarch body composition.

Although warming did not significantly affect monarch body composition in this study, comparisons with previous work suggest that broader environmental conditions may have influenced monarch energetic condition. Peel et al. (2026), using the same experimental design during the 2023 field season, found that monarchs feeding on warmed nectar plants had lower mean dry fat mass (0.017 g; Table 11) than monarchs feeding on control plants (0.026 g; Table 11), demonstrating a warming-induced reduction in lipid reserves. In contrast, I found no significant difference in dry fat mass between warmed and control *S. canadensis* chambers. However, the highest mean dry fat mass observed in this study was only 0.018 g (Table 11), meaning that monarchs in both warmed and control treatments had fat reserves that were similar to those of monarchs feeding on warmed plants in Peel et al. (2026) and substantially lower than the control monarchs from that study. While comparisons across years should be interpreted cautiously because of differences in environmental conditions, this pattern suggests that monarch

energetic condition may have been reduced across the entire study system during the 2025 field season. The severe drought experienced during this experiment may have lowered nectar quality or floral resource availability across both warmed and control chambers, thereby reducing monarch fat reserves regardless of treatment and diminishing the ability to detect an additional effect of experimental warming. This interpretation is consistent with the observation that warming did not alter soil moisture within chambers, indicating that all plants experienced similarly dry background conditions. Because fat reserves are critical for migration and overwinter survival in monarch butterflies (Beall, 1948; Brown & Chippendale, 1974; Alonso-Mejía et al., 1997; Brower et al., 2006; Niitepõld et al., 2022), future studies should investigate how interactions between drought and warming influence nectar quality and monarch energetic condition across years.

4.3 Similar growth responses to warming between native and introduced species

As predicted, introduced plants exhibited stem growth responses to warming that were similar to those of native plants. However, because only a small number of species were examined in this study, additional research including a broader range of taxa is needed to determine whether this pattern is generalizable. A study simulating climate change on native and exotic forbs and grasses in British Columbia found that the root and shoot biomass of all species responded differently to warming and water input, with some showing an increase in biomass and some a decrease based on the combination of conditions (Akin-Fajiye et al., 2023). These results suggest a species-specific response to climate warming, rather than an overall response based on origin status.

4.4 Differences in plant growth between native and introduced species

Contrary to my prediction, I found that native species had higher percent stem growth than introduced species under control conditions. One possible explanation is that the native species were better adapted to drought than the introduced species (Frost et al., 2025). Consistent with this idea, a grassland meta-analysis by Frost et al. (2025) found that introduced species were 25.9% more negatively affected by drought than native species. However, the observed differences may also reflect species-specific traits rather than native status itself. In this study, the native species *S. canadensis* and *S. novae-angliae* flowered later than *T. pratense* and *L. salicaria*, which may have contributed to differences in growth responses between native and introduced species. Because I could only include a small number of species, I am unable to distinguish between the two mechanisms. Future studies evaluating the responses of species based on origin should therefore aim to include a higher diversity of species with similar phenological timelines.

4.5 Species-specific differences in monarch body composition

I found that monarchs feeding on *S. canadensis* had higher dry lean mass than those feeding on *L. salicaria*. Although I did not predict differences in body composition based on native status, the inclusion of only one native and one introduced species limits the generality of this comparison. However, species-specific differences in monarch body composition are consistent with recent research conducted by the lab in this system (Veselovsky, in prep). Dry lean mass reflects non-fat body components, including proteins, glycogen, and flight-related tissues, and likely plays a secondary role relative to lipids in fueling migration (Beall, 1948; Brown & Chippendale, 1974; Brower et al., 2006). However, non-fat body components may still

contribute to migratory performance, as protein catabolism has been shown to support flight metabolism in both short- and long-distance migratory warblers (Elowe et al., 2023).

Considering that lipids are the primary energetic fuel for migration, the absence of differences in dry fat mass suggests that *L. salicaria* did not reduce lipid accumulation relative to *S. canadensis*. However, differences in dry lean mass could reflect variation in non-sugar aspects of nectar composition, such as amino acids and other nutrients. Although nectar quality is often evaluated in terms of sugar content, nectar-derived amino acids have also been shown to contribute to flight muscle and tissue maintenance in butterflies (Levin et al., 2017; Tigreros et al., 2026). It is therefore possible that differences in nectar composition between the two flowering species contributed to differences in dry lean mass without affecting dry fat mass. These findings suggest potential species-specific differences in nectar composition and highlight the need for further research examining how nectar plant identity influences monarch condition during migration.

4.6 Seasonal changes in body composition

In the warming analysis, monarchs that eclosed later in the season had higher wet mass, water mass, and dry fat mass than those that eclosed earlier in the season. A positive association between day of year and dry fat mass may reflect seasonal physiological shifts associated with preparation for migration, as lipid reserves are essential for migratory flight and overwintering survival (Brower et al., 2006). Hobson et al. (2023) found that monarchs were able to replenish lipids during migration, suggesting that nectar feeding can rapidly influence lipid accumulation. Since nectar provides both sugar and water, increased nectar intake later in the season could potentially explain the higher fat mass and water mass (and consequently wet mass) observed in

later-eclosing monarchs. The absence of a significant seasonal effect on wet mass, water mass, and dry fat mass in the origin analysis may reflect the greater biological variability introduced by combining observations across two nectar plant species with potentially different nectar traits and flowering dynamics, whereas the warming analysis was based solely on *S. canadensis*.

Conversely, monarchs that eclosed later in the season had lower dry lean mass than those that eclosed earlier in both the warming and origin analyses. This pattern does not necessarily indicate that individual monarchs lost lean mass during the five-day feeding trial, but rather that later-eclosing individuals had lower non-lipid dry mass by the end of the experiment. Because many components of dry lean mass, including flight muscles, structural tissues, wings, and exoskeleton, are largely determined during larval and pupal development, lean mass may be more strongly influenced by developmental conditions than by short-term adult nectar feeding. At the beginning of monarch rearing, larvae were fed exclusively on young milkweed leaves grown outdoors from local seed. As the supply of younger leaves diminished, larvae were fed primarily with milkweed collected from the field site in Kinburn. Since plant traits associated with leaf age can influence herbivore growth and survival (Yang et al., 2020), seasonal changes in milkweed age, source location, or drought exposure may have contributed to the lower lean mass observed in later-eclosing monarchs.

4.7 Limitations

Because the primary focus of this experiment was to evaluate indirect effects of warmed nectar plants on monarch body composition, I did not experimentally separate the direct effects of warming on plants from warming-mediated changes in plant-insect interactions. Plants remained exposed to the surrounding insect community until mesh enclosures were added in

mid-August, meaning that plant responses may have reflected both the abiotic effects of the OTCs and any changes in herbivory or pollinator activity within chambers, as found in other studies (Birkemoe et al., 2016). This could be important because I found evidence that warming altered some plant responses, including species-specific stem growth patterns and changes in the seasonal flowering trajectory. It could be that these responses could be partly attributed to plant-insect interactions in addition to the direct physiological effects of temperature. As a result, my results should be interpreted as the overall response of plants to the warming treatment under semi-natural field conditions. This limitation is common in OTC experiments, which are widely used to estimate plant responses to passive warming but may also alter microclimate and biotic interactions such as herbivory and pollination (Marion et al., 1997).

A second limitation is that since monarch feeding trails under warmed conditions could only be completed for *S. canadensis* and I was not able to directly measure nectar quality or quantity, the results of warming on monarch body composition should be interpreted cautiously. The absence of a detectable warming effect on monarch body composition does not necessarily mean that warming had no effect on nectar resources, but rather that any changes in warmed *S. canadensis* plots did not translate into measurable differences in monarch body composition under the conditions of this experiment. Overall, the plant and monarch responses should therefore be interpreted as responses to the chamber environment under semi-natural field conditions rather than as isolated effects of temperature alone. Future work could more directly separate these pathways by pairing warming treatments with herbivore-exclusion treatments and by directly measuring nectar volume, sugar concentration, and nectar composition.

Similarly, the species origin comparison could not fully separate plant species effects from species-origin effects. Because drought and flowering phenology limited monarch feeding

trials on several species, the origin analysis was restricted to control *S. canadensis* and control *L. salicaria*. As a result, differences between these groups cannot be interpreted as a general effect of native versus introduced nectar sources, but rather as a comparison between these two focal species under control conditions. Future work should therefore include a broader range of native and introduced nectar plants with overlapping flowering periods to better determine whether introduced species consistently provide nectar resources of similar quality to native species.

5. Conclusion

Overall, I found that the experimental warming treatment had limited effects on plant condition, flowering phenology, floral abundance, and monarch body composition. Warming did not significantly affect stem growth, chlorophyll content, flowering onset, or flower abundance across species, nor did it affect monarch body composition metrics in *S. canadensis* feeding trials. Differences between native and introduced nectar plants were also limited, although monarchs feeding on introduced *L. salicaria* exhibited lower dry lean mass than those feeding on native *S. canadensis*, suggesting potential species-specific differences in nectar composition.

The severe drought conditions that occurred during the experiment likely influenced many of the observed responses and may have moderated the effects of warming. The early senescence of *T. pratense* and *L. salicaria*, together with the near absence of flowering in *S. novae-angliae* during the feeding-trial period, highlights the potential for drought and other climatic extremes to reduce nectar availability for migratory monarchs in Ontario. These findings suggest that interactions between warming and water availability may play an important role in shaping plant-insect interactions under climate change.

This study also has implications for habitat restoration and conservation planning. Future research should further investigate the combined effects of warming and drought on nectar resources and monarch condition across a broader diversity of flowering species. Given the increasing frequency and unpredictability of climatic extremes, restoration efforts should prioritize diverse communities of early- and late-season flowering plants, including drought-tolerant species, to support resilient nectar landscapes capable of sustaining plant-insect interactions under changing environmental conditions.

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Tables

Table 1. Generalized additive model summary for temperature differences between warmed and control chambers across diel and seasonal time scales. The model was fitted with a Gaussian family (identity link) using REML. Sample size n = 18,186; adjusted R^2 = 0.819; deviance explained = 82%; REML score = 54,021; scale estimate = 21.96. Estimated degrees of freedom (edf) indicate the complexity of smooth terms. Bold font indicates statistical significance ($p < 0.05$).

Term	Estimate	SE	t/F	edf	Ref.df	p-value
Parametric						
Intercept	**-13.21**	**3.33**	**-3.97**	—	—	**<0.001**
Smooth terms						
TOD, control plots	—	—	**3967.23**	**9.82**	**9.99**	**<0.001**
TOD, warmed plots	—	—	**3371.95**	**9.76**	**9.99**	**<0.001**
DOY, control plots	—	—	**449.93**	**9.93**	**10**	**<0.001**
DOY, warmed plots	—	—	**379.46**	**8.92**	**9**	**<0.001**
Chamber	—	—	**26.41**	**11.64**	**12**	**<0.001**

Table 2. Generalized additive model summaries and prediction-based treatment contrasts for soil moisture proportion. Model was fitted using a beta regression family (logit link) with REML. Sample size n = 463; adjusted R^2 = 0.875; deviance explained = 86.9%; REML score = −671.08; scale estimate = 1. Estimated degrees of freedom (edf) indicate the complexity of smooth terms. Bold font indicates statistical significance ($p < 0.05$).

Model term	Estimate	SE	z/χ^2	edf	Ref.df	p-value
Parametric terms						
Intercept	**-1.604**	**0.115**	**-13.978**	—	—	**<0.001**
Warming treatment	-0.166	0.172	-0.963	—	—	0.335
Smooth terms						
DOY, control plots	—	—	**577.3**	**7.471**	**8.38**	**<0.001**
DOY, warmed plots	—	—	**525.3**	**7.701**	**8.55**	**<0.001**
Site	—	—	**1631.4**	**44.486**	**49**	**<0.001**

Table 3. Generalized additive model summaries and prediction-based treatment contrasts for hygrometer-derived humidity proportion. Model was fitted using a beta regression family (logit link) with REML. Sample size n = 303; adjusted R^2 = 0.621; deviance explained = 67.2%; REML score = −290.65; scale estimate = 1. Estimated degrees of freedom (edf) indicate the complexity of smooth terms. Bold font indicates statistical significance ($p < 0.05$).

Term	Estimate	SE	z/χ^2	edf	Ref.df	p-value
Parametric						
Intercept	**-0.258**	**0.038**	**-6.879**	**—**	**—**	**<0.001**
Warming treatment	0.017	0.055	0.314	—	—	0.754
Smooth terms						
DOY, control plots	**—**	**—**	**166.94**	**8.425**	**8.842**	**<0.001**
DOY, warmed plots	**—**	**—**	**202.43**	**8.297**	**8.785**	**<0.001**
Chamber	**—**	**—**	**50.61**	**23.35**	**49**	**<0.001**

Table 4. Quadratic mixed-effects model results for percent stem growth. The model was fitted using REML with Satterthwaite degrees of freedom; n = 5,860. Percent stem growth was modelled using species, warming treatment, scaled day of year, and their interactions, with day of year included as a quadratic term. Marginal R^2 represents variance explained by fixed effects only, while conditional R^2 represents variance explained by both fixed and random effects. Bold font indicates statistical significance ($p < 0.05$).

Response	Fixed effect	Num df	Den df	F statistic	p	Marginal R^2	Conditional R^2
Percent stem growth	**Species**	**3**	**65.03**	**49.46**	**<0.001**	0.571	0.904
	Warming treatment	1	41.97	0.56	0.46		
	Warming treatment:speceis	3	65.03	1.02	0.388		
	DOY + DOY²	**2**	**762.26**	**1838.04**	**<0.001**		
	DOY:warming treatment	**2**	**762.26**	**30.48**	**<0.001**		
	DOY:species	**6**	**774.16**	**296.4**	**<0.001**		
	DOY:warming treatment:species	**6**	**774.16**	**6.06**	**<0.001**		

Table 5. Holm-corrected post hoc contrasts for percent stem growth. Contrasts compare predicted percent stem growth between control and warmed treatments within each species at representative dates across the growing season. Estimates are expressed as control minus warmed (C − W), so positive values indicate higher predicted percent growth in control chambers, while negative values indicate higher predicted percent growth in warmed chambers. P-values were adjusted for multiple comparisons using the Holm method. Bold font indicates statistical significance ($p < 0.05$).

Species	Date (2025)	Estimate (C − W)	SE	z	Raw p	Holm-adjusted p	Significant after Holm?	Interpretation
S. canadensis	22-Jun	-2.372	1.755	-1.351	0.177	1	No	No significant difference
L. salicaria	22-Jun	-4.501	1.76	-2.557	0.0106	0.1056	No	No significant difference
S. novae-angliae	22-Jun	-4.143	1.766	-2.346	0.019	0.171	No	No significant difference
T. pratense	22-Jun	-2.379	1.8	-1.321	0.186	1	No	No significant difference
S. canadensis	12-Jul	-1.663	1.679	-0.991	0.322	1	No	No significant difference
L. salicaria	12-Jul	-0.442	1.809	-0.245	0.807	1	No	No significant difference
S. novae-angliae	12-Jul	-1.845	1.649	-1.119	0.263	1	No	No significant difference
T. pratense	12-Jul	**7.652**	**1.974**	**3.877**	**0.000106**	**0.00116**	**Yes**	**Control higher percent growth**
S. canadensis	31-Jul	-0.99	2.025	-0.489	0.625	1	No	No significant difference
L. salicaria	31-Jul	3.413	2.563	1.332	0.183	1	No	No significant difference
S. novae-angliae	31-Jul	0.339	1.721	0.197	0.844	1	No	No significant difference
T. pratense	31-Jul	**17.182**	**2.909**	**5.907**	**3.49E-09**	**4.19E-08**	**Yes**	**Control higher percent growth**

Table 6. Quadratic mixed-effects model results for percent stem growth by origin in control chambers. The model was fit using maximum likelihood with a Satterthwaite degrees of freedom approximation. Percent stem growth was modelled using plant origin, scaled day of year, and their interaction, with day of year included as a quadratic term. Chamber was included as a random intercept, and stem identity was included as a random intercept with a random slope of DOY. Marginal R^2 represents variance explained by fixed effects only, while conditional R^2 represents variance explained by both fixed and random effects. Bold font indicates statistical significance ($p < 0.05$).

Response	Fixed effect	Num df	Den df	F statistic	p	Marginal R^2	Conditional R^2
Percent stem growth	**DOY + DOY²**	**2**	**24.87**	**423.76**	**<0.001**	0.345	0.73
	Origin	**1**	**163.55**	**24.95**	**<0.001**		
	DOY × Origin	**2**	**2855.36**	**9.05**	**<0.001**		

Table 7. Generalized additive model summaries and prediction-based treatment contrasts for open flower abundance. Model was fitted using a negative binomial family (log link) with REML. Sample size n = 470; adjusted R^2 = 0.174; deviance explained = 95.9%; REML score = 491.17; scale estimate = 1. The best-supported model included separate day-of-year smooths for each species × treatment combination, which improved fit relative to the reduced species-only seasonal model, χ^2 = 37.432, $p < 0.001$, and had lower AIC (943.16 vs. 957.80). Estimated degrees of freedom (edf) indicate the complexity of smooth terms. Bold font indicates statistical significance ($p < 0.05$).

Model term	Estimate	SE	z/χ^2	edf	Ref.df	p-value
Parametric terms						
Intercept	-77.274	419.442	-0.184	—	—	0.854
L. salicaria	71.907	419.451	0.171	—	—	0.864
T. pratense	75.218	419.442	0.179	—	—	0.858
Warming treatment	-0.455	1.445	-0.315	—	—	0.753
Smooth terms						
DOY, *S. canadensis* control	—	—	**20.604**	**2.073**	**2.143**	**<0.001**
DOY, *L. salicaria* control	—	—	**128.258**	**4.759**	**5.326**	**<0.001**
DOY, *T. pratense* control	—	—	**37.004**	**4.291**	**5.26**	**<0.001**
DOY, *S. canadensis* warmed	—	—	0.717	2.758	2.843	0.85
DOY, *L. salicaria* warmed	—	—	**90.706**	**4.972**	**5.608**	**<0.001**
DOY, *T. pratense* warmed	—	—	**43.04**	**4.573**	**5.498**	**<0.001**
Chamber	—	—	**171.156**	**22.726**	**34**	**<0.001**

Table 8. Linear mixed-effects model results for average leaf chlorophyll content. The model was fitted using REML with Satterthwaite degrees of freedom; n = 646, with 240 stems nested within 42 chambers. Sample sizes by plant part were: bottom, n = 227; middle, n = 200; top, n = 219. Day of year was mean-centred before analysis. Random intercepts were included for stems nested within chambers. Bold font indicates statistical significance ($p < 0.05$).

Response	Fixed effect	Estimate	SE	Num df	Den df	t/F statistic	p-value
Average chlorophyll	**Plant part**	—	—	**2**	**446.25**	**45.39**	**<0.001**
	Middle vs. bottom	**52.162**	**7.295**	—	**467.13**	**7.151**	**<0.001**
	Top vs. bottom	-10.567	6.926	—	445.24	-1.526	0.128
	Warming treatment	—	—	1	36.57	0.025	0.876
	Warmed vs. control	1.852	11.793	—	36.57	0.157	0.876
	DOY	**-4.189**	**0.313**	**1**	**570.75**	**178.98**	**<0.001**

Table 9. The response of five monarch body composition metrics to the indirect effects of warming from the temperature treatment in *Solidago canadensis* plots. Responses included change in wet mass, dry fat mass, dry lean mass, and water mass. Final analyses were conducted using linear models. Reported values include the effect estimate (β), standard error (SE), test statistic (t), p-value (p), and model R^2. Reported fixed effects and covariates varied among final models and included treatment or species, centered day of year, quadratic DOY terms where retained, sex where retained, and forewing length where retained. Where model assumptions indicated mild heteroscedasticity or non-normal residuals, inference was based on HC3 heteroscedasticity-robust standard errors. Bold font indicates statistical significance ($p < 0.05$).

Response	Model	Fixed effect	Effect size	SE	Statistic	p	R^2	Inference used
Wet mass (g)	Linear model	Treatment	-0.01041	0.01612	t = -0.646	0.522	0.32	Standard
		DOY	**0.00565**	**0.00148**	**t = 3.811**	**<0.001**		
		Forewing length	**0.01535**	**0.0039**	**t = 3.937**	**<0.001**		
		Sex	-0.0018	0.01799	t = -0.100	0.921		
Dry fat mass (g)	Quadratic linear model	Treatment	0.000307	0.00359	t = 0.086	0.932	0.24	Standard
		DOY	**0.000798**	**0.00033**	**t = 2.418**	**0.02**		
		DOY²	**0.000104**	**0.00005**	**t = 2.070**	**0.044**		
		Forewing length	**-0.01308**	**0.00413**	**t = -3.166**	**0.003**		
		Sex	0.000618	0.000868	t = 0.712	0.48		
Dry lean mass (g)	Linear model	Treatment	-0.00844	0.00488	t = -1.730	0.09	0.66	Robust HC3
		DOY	**-0.0011**	**0.000365**	**t = -3.025**	**0.004**		
		Forewing length	**0.00774**	**0.00217**	**t = 3.567**	**<0.001**		
		Sex	0.00142	0.00575	t = 0.247	0.806		
Water mass (g)	Linear model	Treatment	-0.00225	0.01473	t = -0.152	0.88	0.38	Robust HC3
		DOY	**0.00594**	**0.00127**	**t = 4.665**	**<0.001**		
		Forewing length	0.00692	0.00545	t = 1.270	0.211		
		Sex	0.00776	0.01495	t = 0.519	0.606		

Table 10. The responses of four monarch body composition metrics to nectar from *Solidago canadensis* (native) or *Lythrum salicaria* (introduced) flowers. Responses included wet mass, dry fat mass, dry lean mass, and water mass for monarchs from control S. canadensis and control L. Salicaria plots. Final analyses were conducted using linear models. Reported values include the effect estimate (β), standard error (SE), test statistic (t), p-value (p), and model R^2. Reported fixed effects and covariates varied among final models and included species, centered day of year, and forewing length where retained. Where model assumptions indicated mild heteroscedasticity or non-normal residuals, inference was based on HC3 heteroscedasticity-robust standard errors. Bold font indicates statistical significance ($p < 0.05$).

Response	Model	Fixed effect	Estimate	SE	Statistic	p	R^2	Inference used
Wet mass (g)	Linear model	Species	-0.0361	0.0234	t = -1.547	0.131	0.24	Robust HC3
		DOY	0.00327	0.00196	t = 1.672	0.103		
		Forewing length	0.01136	0.0087	t = 1.307	0.199		
		Sex	0.02292	0.02455	t = 0.933	0.357		
Dry fat mass (g)	Linear model	Species	0.00107	0.00484	t = 0.222	0.826	0.1	Standard
		DOY	0.000803	0.000454	t = 1.767	0.086		
		Forewing length	0.00144	0.00112	t = 1.286	0.206		
		Sex	-0.00477	0.00488	t = -0.977	0.335		
Dry lean mass (g)	Linear model	**Species**	**-0.00995**	**0.00474**	**t = -2.102**	**0.042**	0.65	Robust HC3
		DOY	**-0.000837**	**0.000378**	**t = -2.215**	**0.033**		
		Forewing length	**0.0064**	**0.00191**	**t = 3.356**	**0.002**		
		Sex	0.00116	0.0055	t = 0.211	0.834		
Water mass (g)	Linear model	Species	-0.0273	0.0198	t = -1.377	0.177	0.24	Robust HC3
		DOY	0.0033	0.00176	t = 1.874	0.069		
		Forewing length	0.00353	0.00585	t = 0.603	0.55		
		Sex	0.02652	0.01977	t = 1.342	0.188		

Table 11. Comparison of mean dry fat mass of adult monarch butterflies between the 2025 field season (this study) and the 2023 field season conducted by Peel et al. (2026). The 2025 values are presented for all monarchs combined and for butterflies associated with control and warmed *S. canadensis* plots and control *L. salicaria* plots. The Peel et al. (2026) values are shown for monarchs associated with control and warmed nectar treatments during the 2023 field season. Sample sizes (*n*) and mean dry fat mass (g) are presented for each treatment group.

Study / season	Nectar source or treatment	n	Mean dry fat mass (g)
2025 field season	All monarchs measured	69	0.017
2025 field season	Control *S. canadensis*	25	0.017
2025 field season	Warmed *S. canadensis*	27	0.018
2025 field season	Control *L. salicaria*	17	0.017
Peel et al. 2026 2023 field season	Control nectar, 2023 field season	29	0.026
Peel et al. 2026 2023 field season	Warmed nectar, 2023 field season	30	0.017

Figures

Figure 1. Experimental design of warming chambers and enclosures. **a)** control chamber made of plastic-coated steel garden stakes and PVC corner fittings (left) and open-top chamber (right) on stems of *Solidago canadensis* at the field site in Kinburn, ON, Canada on August 6, 2025. The open-top chambers and plastic-coated steel garden stakes are approximately 1.5 meters tall and 1.2 maters wide at the base. Both chambers are wrapped with mesh tulle. Large monarch enclosure frames made of plastic-coated steel garden stakes and PVC corner fittings are constructed on top of each chamber. **b)** large monarch enclosures covered with mosquito netting over top of control chamber (left) and open-top chamber (right) on stems of *Solidago canadensis* on August 26, 2025. The large enclosures have 40+ cm of clearance around all sites to allow monarchs to fly and rest outside of each chamber. Camera set up for a separate observational study is constructed between the two enclosures.

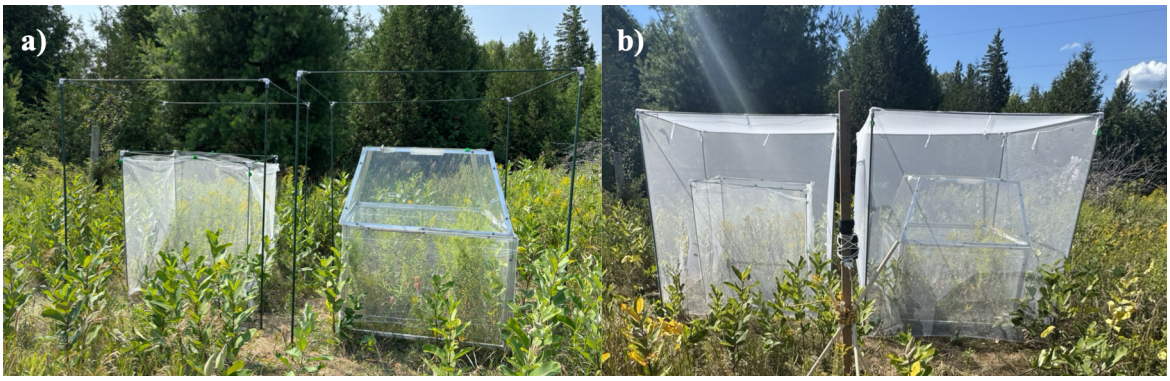

Figure 2. Average proportional soil moisture responses to temperature treatment. Points show observed average soil moisture values for individual chamber measurements (control = blue, warmed = red). Lines represent fitted GAMM smooths for each treatment (control = blue, warmed = red), with shaded bands representing the calculated 95% confidence intervals for each treatment curve.

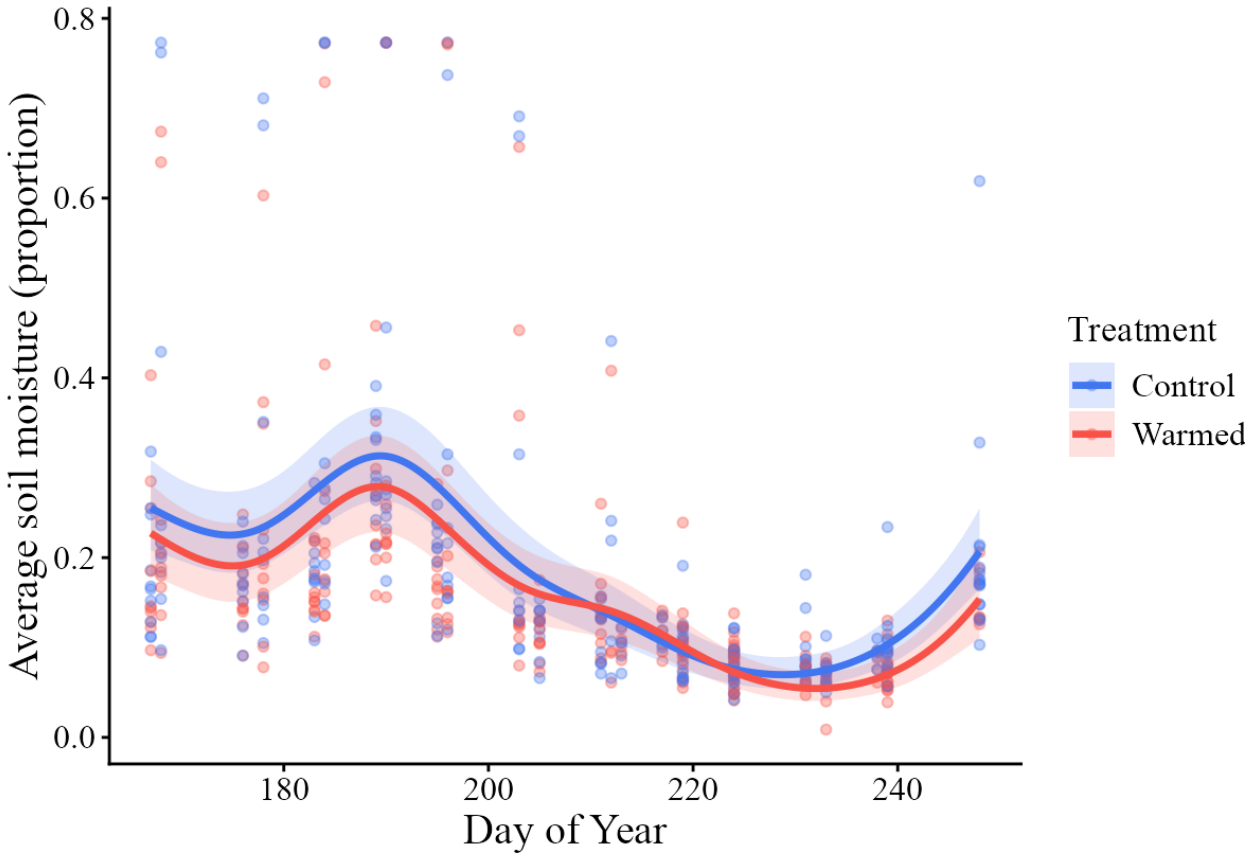

Figure 3. a) Senesced flowers of *Trifolium pratense* in a control chamber on August 11, 2025. **b)** stems of *Symphyotrichum novae-angliae* in a warmed chamber on August 5, 2025. Most leaves are curled or limp, some middle leaves turning red or yellowing, and bottom leaves dead. Evidence of leaf herbivory is present on most of the plants. **c)** senesced flowers of *Lythrum salicaria* in a warmed chamber on July 31, 2025. Many leaves turning yellow and curling with evidence of leaf herbivory. **d)** open-top (left) and control (right) chamber pair of *L. salicaria* on July 31, 2025. All flowers in the open-top chamber have senesced while numerous flowers in the control chamber are open.

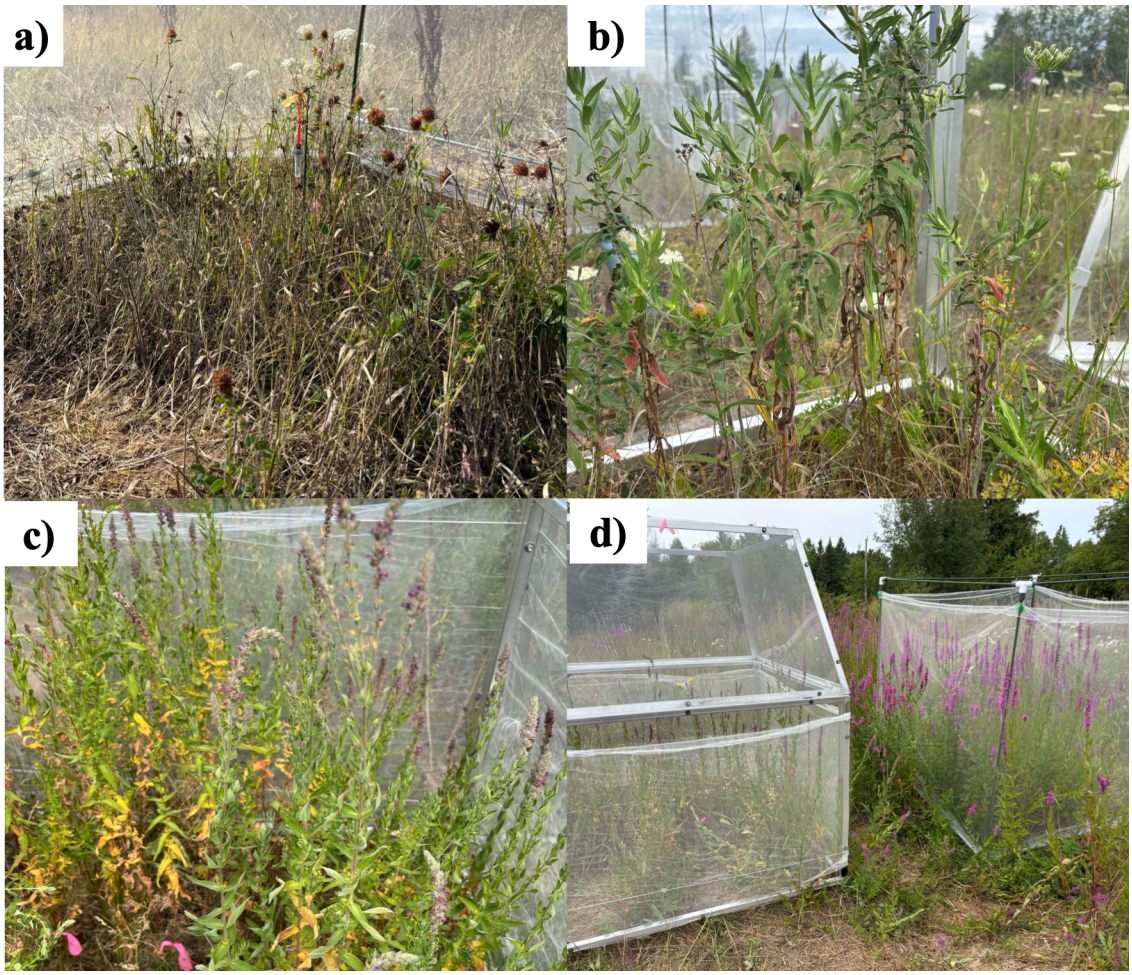

Figure 4. Timeline of the experiment from June 15 to September 15. The red line indicates the flowering period of *Trifolium pratense*, the pink like indicates the flowering period of *Lythrum salicaria*, the yellow line indicates the flowering period of *Solidago canadensis*, and the purple like indicates the flowering period of *Symphyotrichum novae-angliae*. The asterisk indicates the period in which warmed *L. salicaria* flowers completely senesced, while the continuation of the line represents the continued flowering period of control chambers. The arrows represent continued flowering not captured past the conclusion of the experiment. *S. novae-angliae* did not fully flower until the end of the feeding trails. The orange line and shading represent the total duration of the monarch feeding trials.

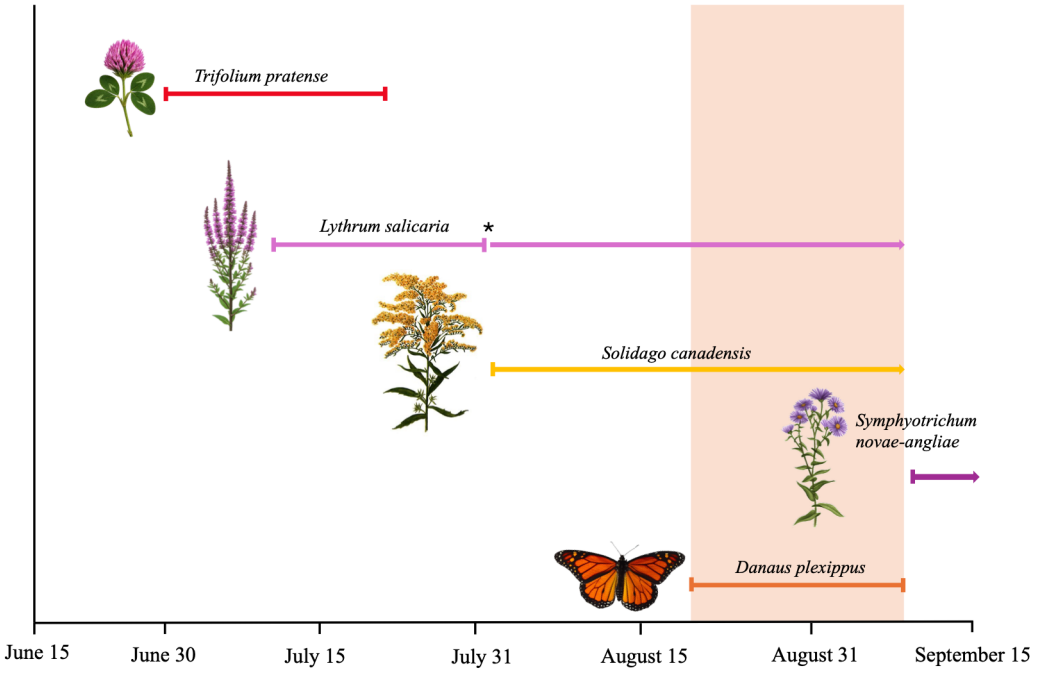

Figure 5. Predicted chamber temperature patterns from the generalized additive model by treatment condition, showing **a)** the seasonal pattern in predicted temperature across day of year, estimated at the median time of day, and **b)** the diel pattern in predicted temperature across time of day, estimated at the median day of year. Lines represent model-predicted mean temperature, with blue representing control chambers and red representing warmed chambers. Shaded ribbons represent 95% confidence intervals.

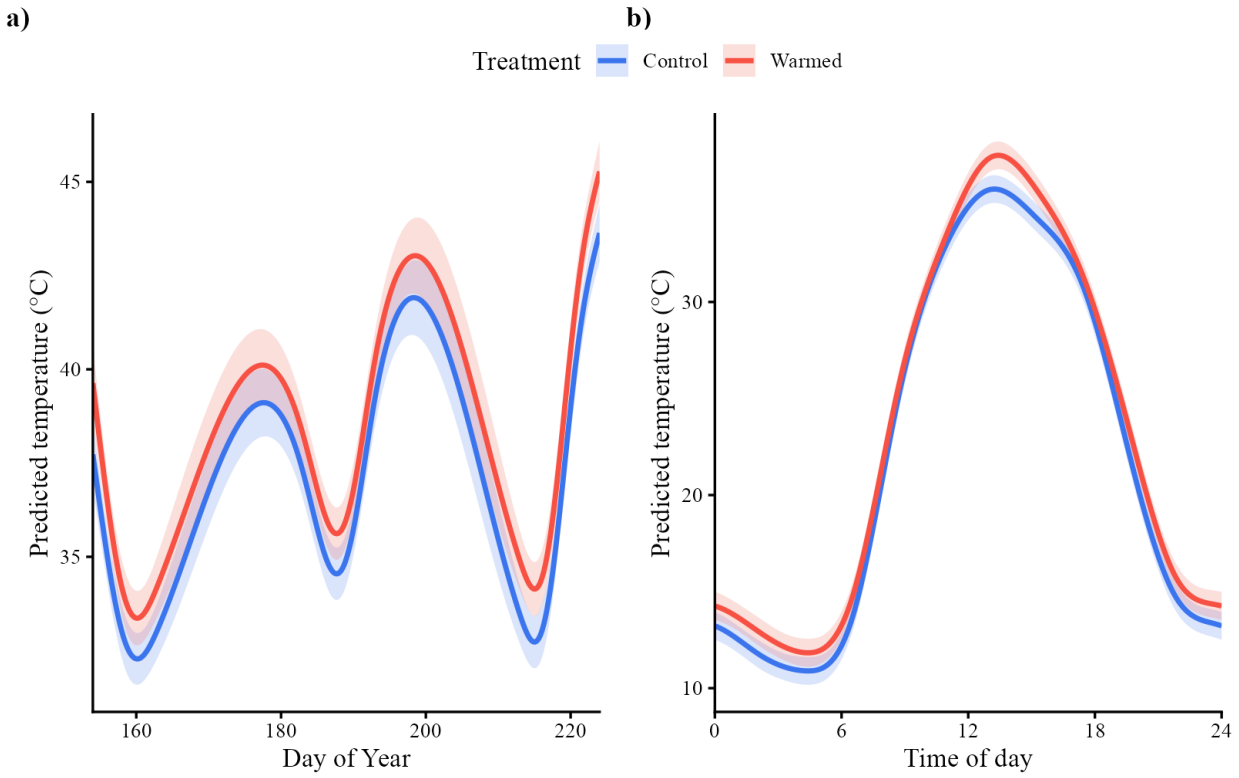

Figure 6. Proportional relative humidity responses to temperature treatment. Points show observed average relative humidity values for individual chamber measurements (control = blue, warmed = red). Lines represent fitted GAM smooths for each treatment (control = blue, warmed = red), with shaded bands representing the calculated 95% confidence intervals for each treatment curve.

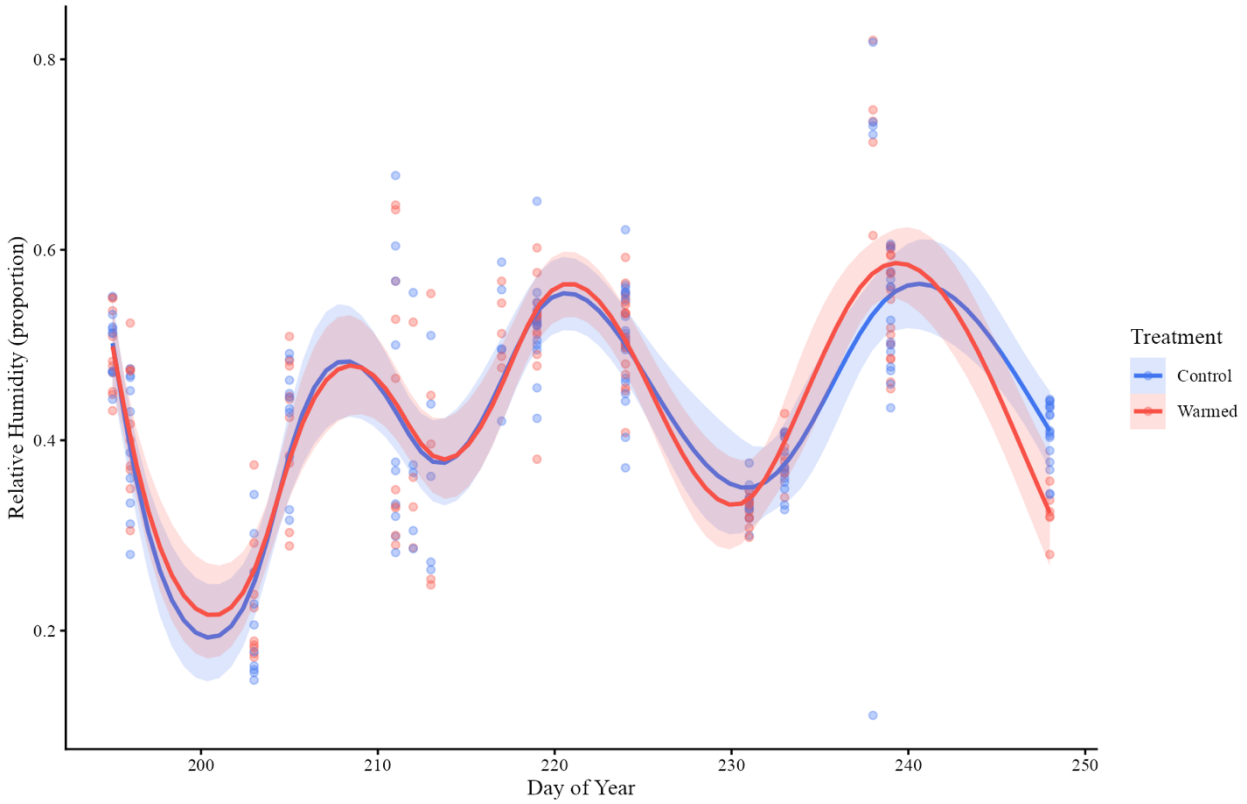

Figure 7. Percent stem growth from first measurement across day of year for each plant species under control and warmed treatments. Points represent raw observations, and lines represent fixed-effect predictions from the quadratic mixed-effects model. Control plants are shown in blue and warmed plants are shown in red.

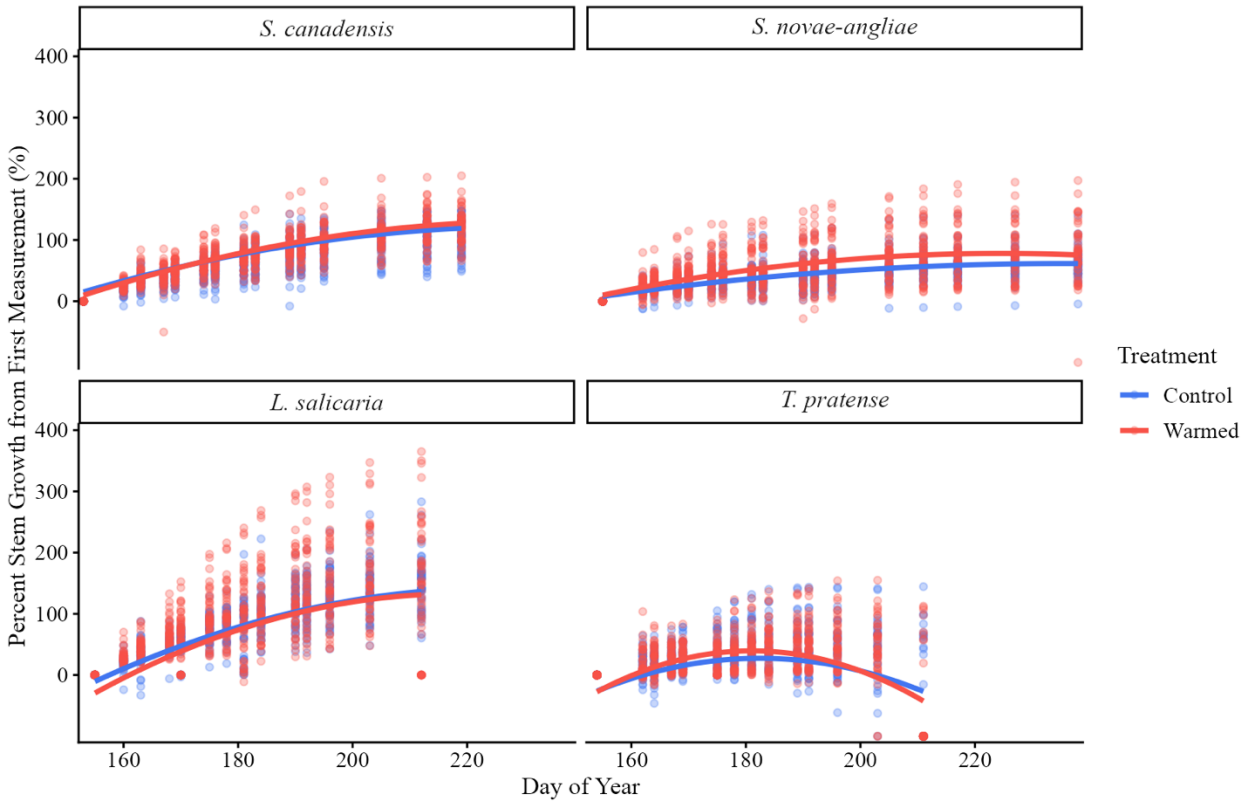

Figure 8. Model-predicted percent stem growth across day of year for native (*Solidago canadensis* and *Symphyotrichum novae-angliae*) and introduced (*Lythrum salicaria* and *Trifolium pratense*) plant species in the absence of warming. Lines show fixed-effect predictions from the quadratic mixed-effects model, points show individual stem observations, and shaded bands represent approximate 95% confidence intervals. Native species are shown in green, and introduced species are shown in brown.

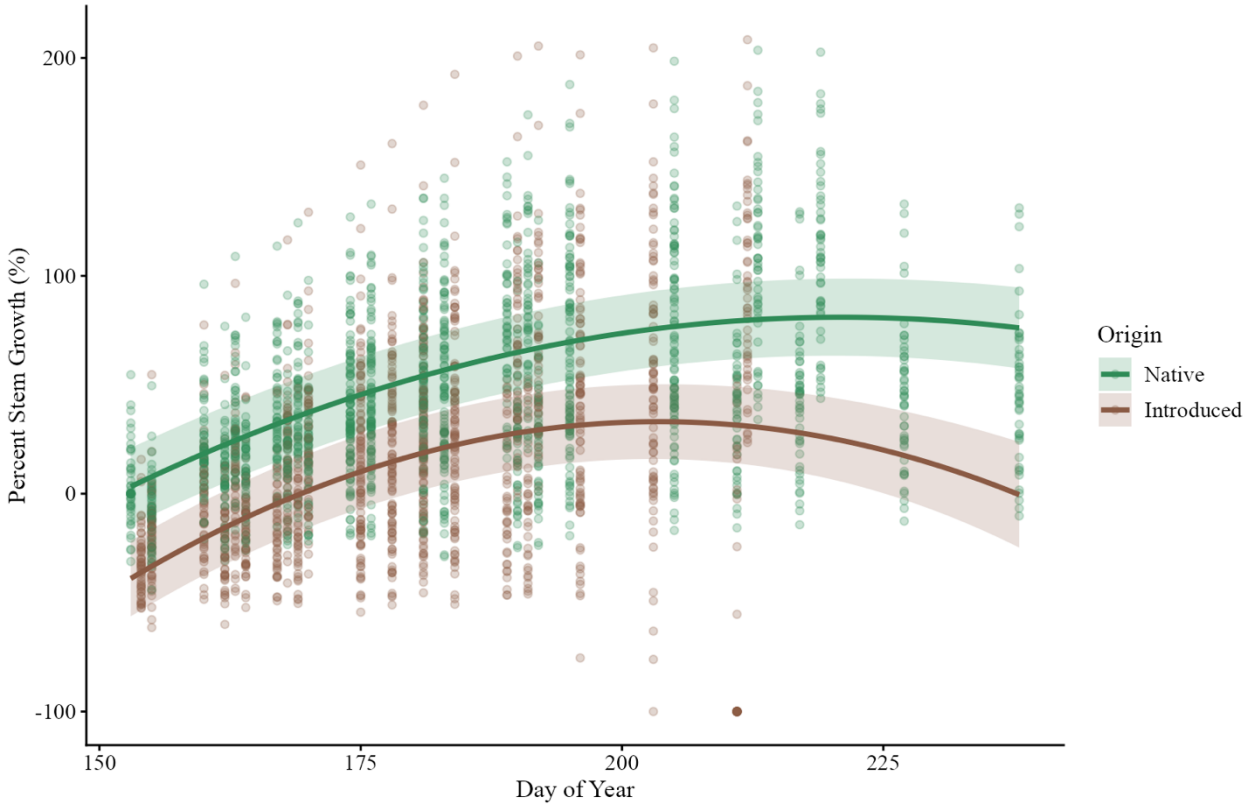

Figure 9. Model-predicted seasonal patterns in open flower abundance across day of year for each plant species and warming treatment. Curves show predicted open flower counts from the fitted GAM, with chamber random effects excluded from predictions. Species are represented by colour: *S. canadensis* in goldenrod, *L. salicaria* in purple, *T. pratense* in red, and *S. novae-angliae* in dark purple. Line type represents treatment, with dashed lines indicating control chambers and solid lines indicating warmed chambers. Predicted flower abundance is shown on a square-root transformed y-axis to improve visualization of differences among species.

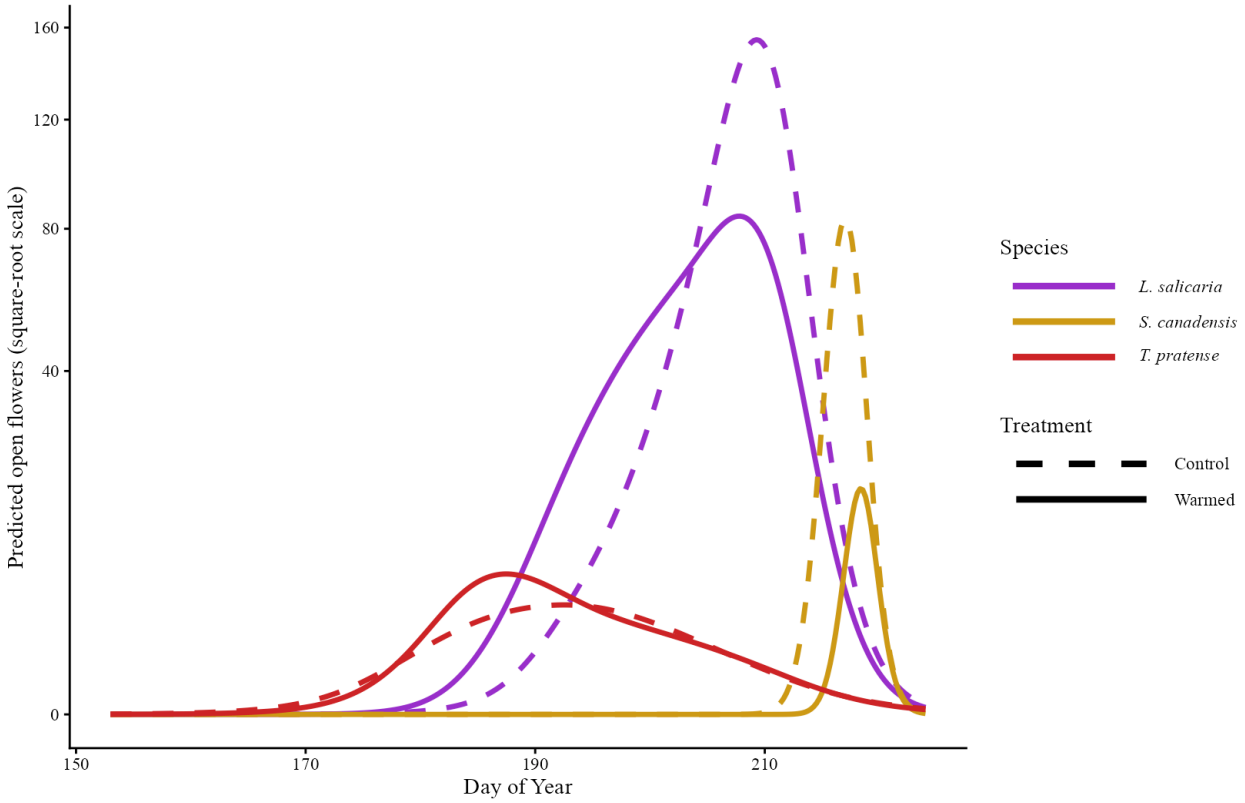

Figure 10. Model-estimated differences in flowering timing between warmed and control chambers for each plant species. Points represent estimated warmed minus control differences, and horizontal bars represent 95% confidence intervals. The dashed vertical line at zero indicates no difference between treatments; values to the right of zero indicate later flowering in warmed chambers, while values to the left indicate earlier flowering in warmed chambers. Panels show differences in flowering onset, peak flowering date, flowering finish, and flowering duration for *Solidago canadensis*, *Lythrum salicaria*, *Trifolium pratense*, and *Symphyotrichum novae-angliae*.

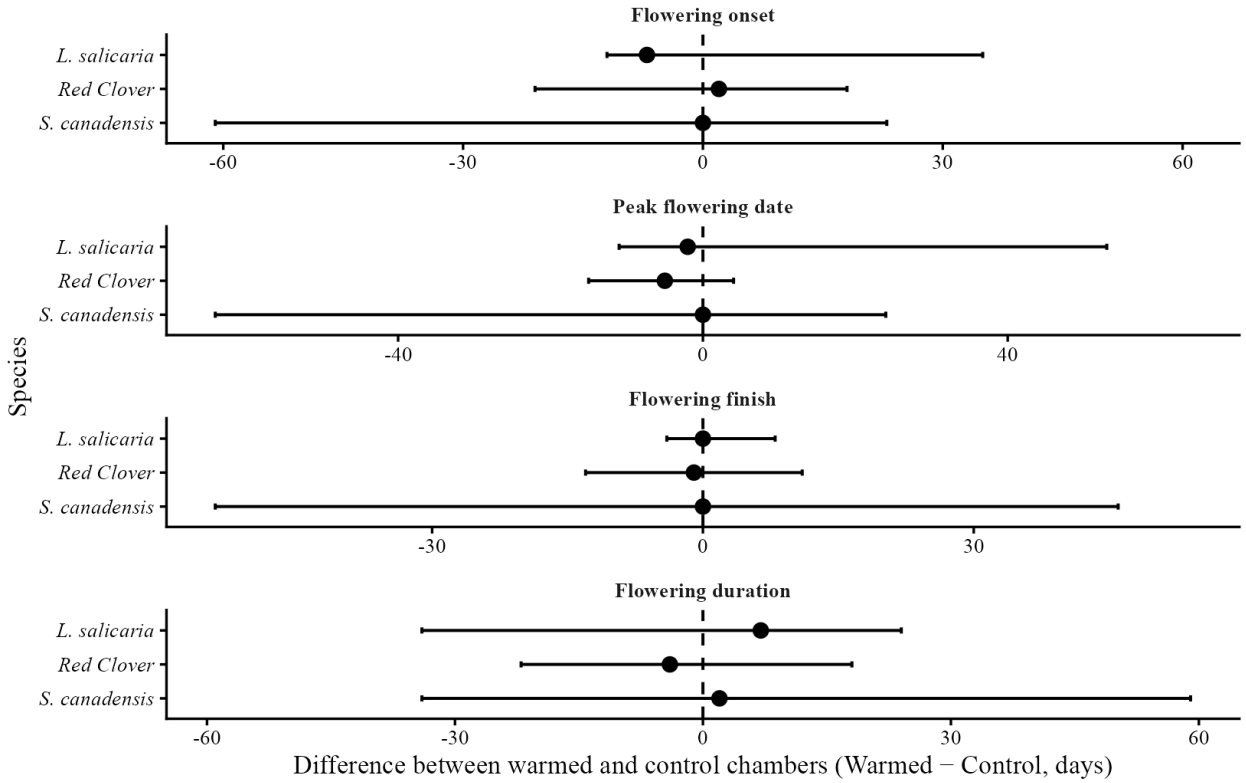

Figure 11. Model-predicted seasonal change in average leaf chlorophyll for plants in control and warmed chambers. Lines show fixed-effect predictions from a linear mixed-effects model of average chlorophyll as a function of treatment, day of year, and plant part, with chamber/stem included as a nested random effect. Shaded ribbons represent 95% confidence intervals. Blue represents control chambers and red represents warmed chambers.

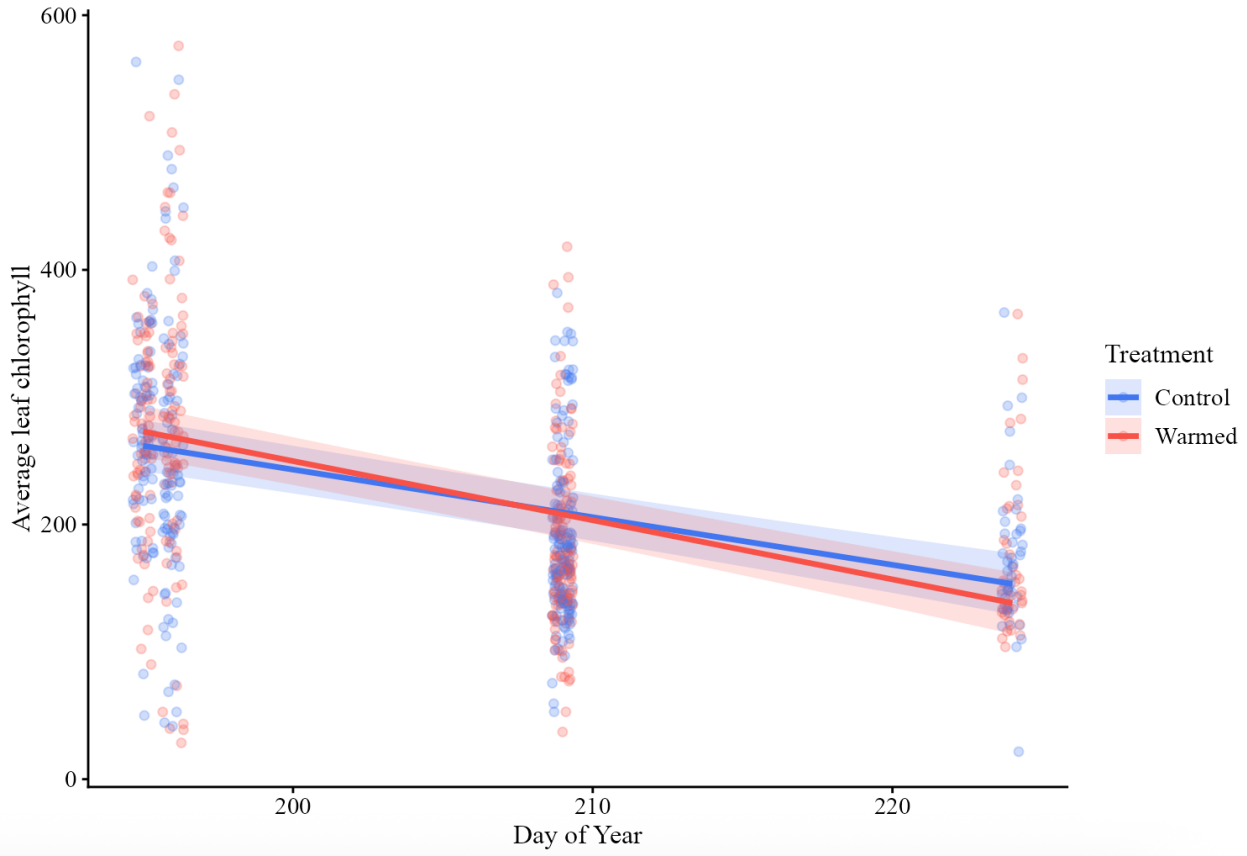

Figure 12. Model-predicted relationships between monarch body composition and eclosion day of year in control (blue) and warmed (red) *Solidago canadensis* plots. Panels show wet mass, dry fat mass, dry lean mass, and water mass. Points represent observed values for individual monarchs, lines show predicted values from the final linear models, and shaded bands represent 95% confidence intervals.

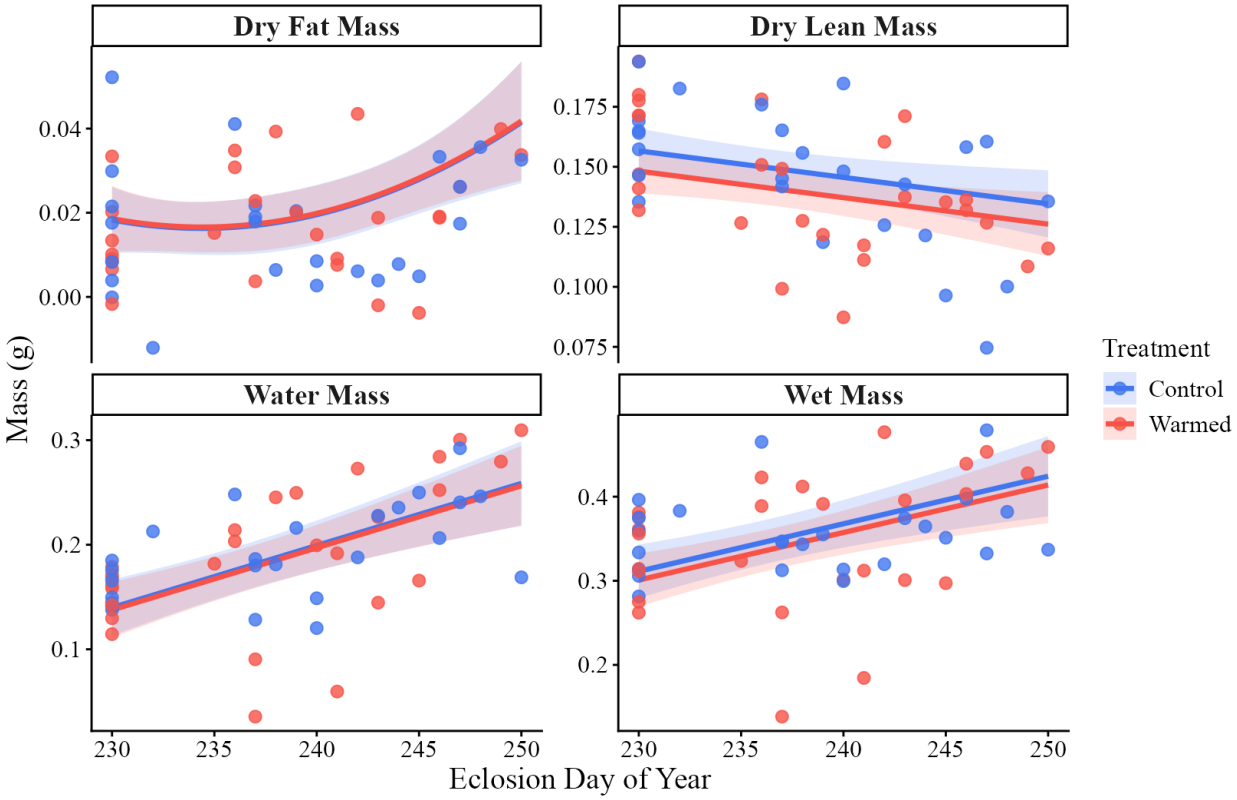

Figure 13. Model-predicted relationships between monarch body composition and eclosion day of year in control *Solidago canadensis* (yellow) and *Lythrum salicaria* (purple) plots. Panels show wet mass, dry fat mass, dry lean mass, and water mass. Points represent observed values for individual monarchs, lines show predicted values from the final linear models, and shaded bands represent 95% confidence intervals.