Received: 3 September 2022 | Revised: 11 January 2023 | Accepted: 16 February 2023

DOI: 10.1002/ecy.4036

ECOLOGY
ECOLOGICAL SOCIETY OF AMERICA

ARTICLE

Extensive regional variation in the phenology of insects and their response to temperature across North America

Peter O. Dunn[1] | **Insiyaa Ahmed**[2] | **Elise Armstrong**[3] | **Natasha Barlow**[4] | **Malcolm A. Barnard**[5,6] | **Marc Bélisle**[7] | **Thomas J. Benson**[8] | **Lisha L. Berzins**[9] | **Chloe K. Boynton**[10] | **T. Anders Brown**[11] | **Melissa Cady**[12] | **Kyle Cameron**[4] | **Xuan Chen**[13] | **Robert G. Clark**[9,14] | **Ethan D. Clotfelter**[15] | **Kara Cromwell**[16] | **Russell D. Dawson**[17] | **Elsie Denton**[18] | **Andrew Forbes**[19] | **Kendrick Fowler**[20] | **Kevin C. Fraser**[21] | **Kamal J. K. Gandhi**[22] | **Dany Garant**[7] | **Megan Hiebert**[4] | **Claire Houchen**[23] | **Jennifer Houtz**[24] | **Tara L. Imlay**[10,25] | **Brian D. Inouye**[26,27] | **David W. Inouye**[27,28] | **Michelle Jackson**[29] | **Andrew P. Jacobson**[30] | **Kristin Jayd**[31] | **Christy Juteau**[29] | **Andrea Kautz**[32] | **Caroline Killian**[11] | **Elliot Kinnear**[21] | **Kimberly J. Komatsu**[33] | **Kirk Larsen**[34] | **Andrew Laughlin**[35] | **Valerie Levesque-Beaudin**[36] | **Ryan Leys**[4,37] | **Elizabeth Long**[38] | **Stephen C. Lougheed**[39] | **Stuart Mackenzie**[4] | **Jen Marangelo**[40] | **Colleen Miller**[24] | **Brenda Molano-Flores**[8] | **Christy A. Morrissey**[9] | **Emony Nicholls**[41] | **Jessica M. Orlofske**[42] | **Ian S. Pearse**[43] | **Fanie Pelletier**[7] | **Amber L. Pitt**[11] | **Joseph P. Poston**[44] | **Danielle M. Racke**[45] | **Jeannine A. Randall**[17] | **Matthew L. Richardson**[46] | **Olivia Rooney**[24] | **A. Rose Ruegg**[35] | **Scott Rush**[47] | **Sadie J. Ryan**[48] | **Mitchell Sadowski**[49] | **Ivana Schoepf**[39,50] | **Lindsay Schulz**[51] | **Brenna Shea**[40] | **Thomas N. Sheehan**[52] | **Lynn Siefferman**[53] | **Derek Sikes**[54] | **Mark Stanback**[55] | **John D. Styrsky**[56] | **Conor C. Taff**[24] | **Jennifer J. Uehling**[24] | **Kathleen Uvino**[57] | **Thomas Wassmer**[3] | **Kathryn Weglarz**[49] | **Megan Weinberger**[19] | **John Wenzel**[32] | **Linda A. Whittingham**[1]

Correspondence
Peter O. Dunn
Email: pdunn@uwm.edu

Abstract

Climate change models often assume similar responses to temperatures across the range of a species, but local adaptation or phenotypic plasticity can lead

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Ecology. 2023;104:e4036.
https://doi.org/10.1002/ecy.4036

Funding information
Fonds de recherche du Québec—Nature et technologies; National Science Foundation, Grant/Award Number: DEB-2016749; Natural Sciences and Engineering Research Council of Canada; British Columbia Knowledge Development Fund; USDA Agricultural Research Service; USDA-NIFA Agricultural Experiment Station funding; University of Wisconsin-Milwaukee SURF; Canada Foundation for Innovation

Handling Editor: Lee A. Dyer

plants and animals to respond differently to temperature in different parts of their range. To date, there have been few tests of this assumption at the scale of continents, so it is unclear if this is a large-scale problem. Here, we examined the assumption that insect taxa show similar responses to temperature at 96 sites in grassy habitats across North America. We sampled insects with Malaise traps during 2019–2021 ($N = 1041$ samples) and examined the biomass of insects in relation to temperature and time of season. Our samples mostly contained Diptera (33%), Lepidoptera (19%), Hymenoptera (18%), and Coleoptera (10%). We found strong regional differences in the phenology of insects and their response to temperature, even within the same taxonomic group, habitat type, and time of season. For example, the biomass of nematoceran flies increased across the season in the central part of the continent, but it only showed a small increase in the Northeast and a seasonal decline in the Southeast and West. At a smaller scale, insect biomass at different traps operating on the same days was correlated up to ~75 km apart. Large-scale geographic and phenological variation in insect biomass and abundance has not been studied well, and it is a major source of controversy in previous analyses of insect declines that have aggregated studies from different locations and time periods. Our study illustrates that large-scale predictions about changes in insect populations, and their causes, will need to incorporate regional and taxonomic differences in the response to temperature.

KEYWORDS
biomass, climate change, insect diversity, Malaise trap, phenology, population

INTRODUCTION

Many studies have documented (or predicted) large-scale declines in biodiversity and population size due to changes in land use and climate (e.g., Outhwaite et al., 2022; Warren et al., 2018). However, these studies can be confounded if they combine data from different populations and taxonomic groups without controlling for geographic differences in phenology (Duchenne et al., 2022), habitat selection (Bladon et al., 2020), and life history (Belitz et al., 2021; Kingsolver et al., 2011). There is ample evidence that the responses of plant and animal populations to temperature vary geographically (e.g., Louthan et al., 2021; Primack et al., 2009), but climate change predictions based on "ecological niche" or "species distribution" models rarely account for this variation (DeMarche et al., 2019; Hallfors et al., 2016; Zhang & Kubota, 2021). These models usually assume that the effects of climate change (and other factors) on abundance and distribution are consistent across space and time (see Sinclair et al., 2016 for discussion). In the context of climate change studies, this "space-for-time" assumption allows researchers to extrapolate the thermal responses of plants and animals (from different climates) to warmer (or cooler) times in the past or future. The evidence in support of this assumption is mixed, so some researchers have advocated studying spatial variation in population responses to understand better the causes of long-term changes, particularly climate change (Blüthgen et al., 2022).

Ectotherms, such as insects and other arthropods, are particularly sensitive to temperature and, thus, should be good indicators of the effects of climate change (Buckley, 2022). However, the large-scale effects of climate and land use change across insect populations are poorly known and have been widely debated (e.g., Dornelas & Daskalova, 2020; Wagner et al., 2021). Much of the controversy about the extent of declines in insect abundance has involved problems that arise when combining geographically and temporally heterogeneous data from different populations and taxa in meta-analyses (e.g., Desquilbet et al., 2020; Didham et al., 2020; Duchenne et al., 2022). This heterogeneity is illustrated by the Rothamsted Insect Survey in the UK, which is one of the longest-running studies of insect abundance (since 1965). In this survey, there was a decline in the total biomass of all species at one site, but not at three other sites, and the decline was dominated by one species of fly (Shortall et al., 2009). Moths also showed a significant, but fluctuating, decline across the UK, particularly in certain types of habitats (Bell et al., 2020), but aphids showed no temporal decline. These heterogeneous results emphasize that we should not extrapolate population trends across regions and taxa unless we have an understanding of the causes, and, for climate change, the responses of taxa to temperature across their range.

We still know relatively little about how climate change affects the abundance of insects in different populations across entire continents (but see; Crossley et al., 2021, 2022; Outhwaite et al., 2022; Soroye et al., 2020; Warren et al., 2018). To date, most large-scale insect surveys have been designed primarily to examine diversity rather than biomass or abundance (e.g., Steinke et al., 2017), or have focused on specific taxa, such as bumblebees (Kerr et al., 2015; Soroye et al., 2020; Weaver & Mallinger, 2022) or butterflies (e.g., Crone et al., 2019; Forister et al., 2021). Predictions about the influence of climate change on insects depend critically on knowledge of the effect of temperature on biomass or abundance, and, perhaps most importantly, whether those relationships can be extrapolated across large regions and different taxa (e.g., see Hallfors et al., 2016; Zhang & Kubota, 2021). To address this need, we established a network of Malaise traps to test the assumption that insect biomass responds to temperature similarly across North America. A second goal was to use our data to design more efficient sampling schemes and provide a baseline for future long-term studies. There has been relatively little discussion of study design for insect monitoring in terms of the number of samples needed to detect population changes (Lebuhn et al., 2013) or the optimal spacing of traps (Steinke et al., 2021), so we also examined the spatial correlation between samples and estimated sample sizes needed to detect significant evidence of insect declines.

METHODS

We sampled insect biomass using Malaise traps at 96 sites across Canada and the USA from 2019 to 2021 (Figure 1; Appendix S1: Table S1). Malaise traps primarily sample flying insects that intercept and climb up the mesh walls (Skvarla et al., 2021). We were also interested in studying flying insects because they form the food supply for aerial insectivores, such as swallows, which have shown some

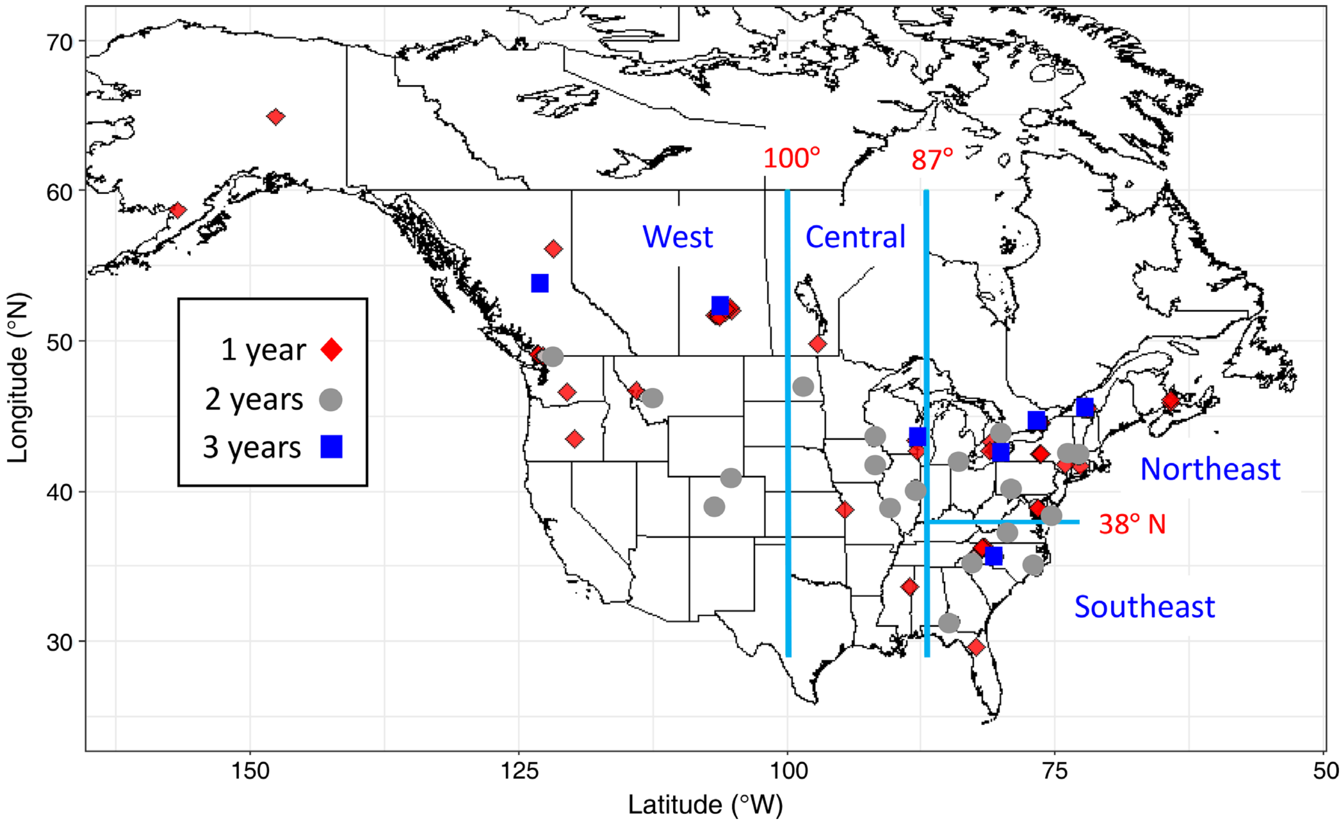

FIGURE 1 Location of Malaise traps used to measure insect biomass in Canada and the USA (2019–2021) by geographic region. West was west of the 100th meridian; Central was 87–100° W; Northeast was east of the 87th meridian and north of 38° N, and Southeast was east of the 87th meridian and south of 38° N. Colors indicate the number of years of sampling. See Appendix S1: Table S1 for more details.

of the greatest declines in population size of North American birds (Spiller & Dettmers, 2019). Traps were located in open areas (>20 m from trees) with mostly grassy vegetation to standardize land cover type (Appendix S1: Figure S1). Collaborators were recruited throughout Canada and the USA in 2018–2020 through email messages to the ECOLOG-L (ECOLOG-L@community.esa.org) and ENTOMO-L (ECOLOG-L@listserv.uoguelph.ca) list-servs, as well as networks of researchers studying aerial insectivores, especially tree swallows (*Tachycineta bicolor*). Aerial insectivores have been declining sharply, especially in eastern North America (Spiller & Dettmers, 2019), and there is concern that this could be caused by a decline in their food supply (flying insects). Thus, one of the reasons for organizing this study was to examine the food supply of tree swallows across their range, although a few of our samples (in Georgia and Florida) came from areas outside their breeding range. We attempted to maximize participation among researchers by using a simple protocol with a small number of sampling periods (three) and minimal sorting and processing of samples. Insect biomass typically increases over the season (mainly May–July) at our sampling locations (see *Results*), so we primarily sampled at three phenologically defined time periods to reduce sampling error from seasonal changes (see below). To simplify post-collection processing, we primarily sorted insects to order, rather than finer taxonomic classifications.

Malaise traps and sampling procedures

To standardize our sampling, we used the same Townes-style model of the Malaise trap (model BT1001 from bugdorm.com, MegaView Science Co. Taiwan) at all sites. The traps were made of black Polyester "no-see-um" fabric (96 × 26 mesh/square inch) with an interception area (center panel) of 1.82 m^2 (1.65 × 1.10 m). Insects were collected in a 500 ml bottle partly filled with 70% ethanol.

Most collaborators on this project operated a single trap each year (28 of 47 individual collaborators or research groups). However, 19 collaborators operated multiple traps in any given year (2–4 per year). The average distance between traps at these multiple-trap locations was 19.6 km (range = 0.1–74.5 km). To account for the geographic clustering of traps, we performed two different types of analyses. First, we used a geographic category, "site," which included all the traps from each collaborator or research group, as a random effect in linear mixed models (see below). Second, we used a continuous measure of distance between nearby traps to examine the spatial correlation between samples from the same region operating during the same time of the season (see below).

At most locations, samples were collected during three sampling periods between April and July. Each sampling period lasted 3 days (72 h), although some collections were conducted over 1 or 2 days (6.5%, 68/1041 samples) due to logistic issues. To account for this variation in sampling duration, we converted all biomass estimates to daily averages. To provide a consistent, phenologically based sampling period across the continent, we chose three main sampling periods based on the breeding biology of tree swallows. Several of the collaborators in this study have been studying tree swallows for >10 years, so we had long-term data on the timing of egg laying, the hatching date of the eggs, and the day nestlings reach 12 days of age, at which point they reach a plateau in growth. We used the average dates of these three events to standardize sampling across the continent. Thus, the starting dates for the three main sampling periods at each site were based on the average: (1) laying date (first egg laid; typically in late May), (2) hatch date (typically 13–14 days after the clutch is completed; early June), and (3) nestling day 12 (12 days post-hatch; late June) of tree swallows. We did not have long-term breeding data on tree swallows at all locations, so we used a regression equation based on laying dates from previous studies across North America (described in Winkler et al., 2014) to define the phenological periods at locations lacking breeding data on swallows (see full data set in Dunn et al., 2023). The equation used latitude, longitude, and elevation as predictors and explained 52% of the variation in laying date (laying date [1 = 1 May] = −23.024 + 1.1118 × decimal_latitude + 0.0912 × decimal_longitude + 0.0111 × elevation [m]; $n = 35$ study populations, $p < 0.001$, $F_{3,31} = 11.45$). In these cases, the hatch date was estimated as 19 days after the laying date, and nestling day 12 was calculated as 12 days after the estimated hatch date (hatch day = 0). References to the "standard" sampling protocol refer to the three periods based on tree swallow nesting phenology (egg laying, hatching, and nestling day 12). In addition to the three phenological periods, some traps were operated continuously throughout the season. These additional samples (647/1041) were included in the overall seasonal analyses, and in these analyses, we controlled for phenology by including sampling (calendar) date in the analysis.

Insect biomass

To estimate insect biomass, we first sorted most samples by order of insect, and then to the suborder Nematocera (including mosquitoes, midges, and blackflies) for dipterans. We were interested in nematocerans because many of them contain high levels of long-chain omega-3 polyunsaturated fatty acids, which are important to the breeding success of tree swallows and other aerial insectivores (Twining et al., 2016). We also sorted and weighed spiders (order Araneae), but they were a relatively small portion of the total biomass, so we do not analyze them here (see Appendix S1: Table S2 for a taxonomic summary). After 1 h of sorting and drying at room temperature, we weighed the samples to 1 mg on electronic balances. This protocol was chosen to allow time to sort samples to order prior to weighing. Biomass was measured separately for each order (suborder for Nematocera) and then summed to calculate total biomass. Some sites did not have separate data for each order, so we only estimated total biomass (13%, 139/1041 samples). The original insect data are available online at figshare (Dunn et al., 2023).

Weather variables

To examine the potential effects of weather on insect biomass, we obtained daily average data for temperature (mean, maximum and minimum), precipitation (rainfall), and wind speed. We used weather stations at each trap site, if available, or from the nearest weather station with complete data in the Global Historical Climatology Network daily (https://www.ncei.noaa.gov/data/global-historical-climatology-network-daily/access/) at the National Center for Environmental Information (Menne et al., 2012). All weather data are provided at figshare (Dunn et al., 2023), and the weather stations used in this study are summarized in Appendix S1: Table S8.

The correlations between average ($r = 0.948$, 95% confidence interval (CI): 0.935–0.958, $N = 317$) and maximum ($r = 0.920$, 95% CI: 0.901–0.936, $N = 311$) temperature, total precipitation ($r = 0.613$, 95% CI: 0.539–0.677, $N = 315$), and average wind speed ($r = 0.642$, 95% CI: 0.510–0.745, $N = 99$) at the trap locations and these GHCN stations were all significant (median distance between traps and stations = 40.3 km). Weather variables were averaged (temperature, wind speed) or summed (precipitation) for the days of each sampling period (usually 3 days). We first examined total biomass in relation to these weather variables to produce a parsimonious set of predictor variables for the inclusion in subsequent analyses (based on corrected Akaike information criterion [$\mathrm{AIC_c}$] values; Appendix S1: Table S3). The initial regression models for total biomass included temperature (mean and maximum), precipitation, wind speed, sampling (calendar) date, latitude, longitude, and elevation as predictors. Mean and maximum temperatures were analyzed separately because they were highly correlated. These initial analyses revealed that maximum temperature ($T_{\max}$) was the best predictor of total biomass (Appendix S1: Table S3; $\beta = 0.031 \pm 0.001$ [SE], $F_{1,1039} = 249.5$, $p < 0.001$), so we focused on it in our main analyses below.

Statistical analyses

We were primarily interested in geographic and seasonal variation in arthropod biomass, especially in response to temperature, so our main analyses included interactions between: (1) location and date, and (2) location and temperature to test for variation in the effects of temperature in different locations and at different times of the season. We first analyzed biomass data from the three main sampling periods (laying date, hatch date, nestling day 12) to control for the inclusion of data from different times of the season, which has been a source of controversy in recent studies (e.g., Welti et al., 2021). Then we examined data from throughout the sampling season, which allowed a larger sample size. Here we used sampling date as a predictor to control for phenological changes across the entire season. Prior to inferential analyses, we examined the distribution of biomass and then applied $\log_{10}$ transformations to improve the normality of residuals.

Mixed models of insect biomass during the three phenological sampling periods included the fixed effects of the phenological period of the sample, the maximum temperature during the sample period, geographic region (see below) and the interactions between region and both phenological period and maximum temperature. Random effects included sampling site and year nested within site (Appendix S1: Tables S3 and S4). In these mixed models we used site to account for the lack of spatial independence of data from nearby traps; however, we also conducted separate analyses of the spatial correlation between traps using the Euclidean distance between traps in km. We included the geographic region in the mixed models (Figure 1), which was coded as four categories: West (west of the 100th meridian), Central (87–100° W), Northeast (east of the 87th meridian and north of 38° N), and Southeast (east of the 87th meridian and south of 38° N). These four regions were chosen because most of our sites were located in a much wider longitudinal (64 to 157° W), than latitudinal (29.6 to 64.9° N) band, except in the east where we had numerous sites in the south, so we divided the eastern region into Northeast and Southeast (Figure 1). We used these regions in analyses of biomass, rather than latitude and longitude, because they explained similar amounts of variation, had lower $\mathrm{AIC_c}$ values, and were simpler to interpret than models with latitude and longitude (Appendix S1: Table S3). We conducted all analyses in JMP v. 16 (SAS Institute, 2021), unless indicated otherwise. We estimated the proportion of variance explained by fixed effects (marginal R^2, R^2_{m}) and by both fixed and random effects (conditional R^2, R^2_{c}) using the r.squaredGLMM command (Nakagawa et al., 2017) in the *MuMIn* package (cran.r-project.org/web/packages/MuMIn) in R v. 4.1 (R Core Team, 2021).

Designing future studies

To help design future studies, we conducted repeatability analyses of total biomass using the *rptR* package (Stoffel et al., 2017), and power analyses using the *simR* package (Green & MacLeod, 2016) in R v. 4.1 (R Core Team, 2021). Previous studies of insect biomass have reported declines of up to 6% annually in Germany (Hallmann et al., 2017), although the average from a meta-analysis was 1% annually for terrestrial insects (van Klink et al., 2020). Therefore, we estimated the minimum necessary sample sizes to achieve a significant effect of year based on annual declines of 1% and 5%. Here we started with the observed biomass values in our phenological data set ($N = 394$ samples) and imposed a constant 1% or 5% annual decline in biomass in future years. On average, we collected 131 samples per year during the three main phenological periods of this study (394 total/3 years = 131 per year), so we assumed 131 samples would be collected each year in the simulated future. Using simR we estimated power in mixed models similar to those above but used the *lme4* package (Bates & Sarkar, 2007) in R.

RESULTS

Over 3 years (2019–2021), we collected 1041 samples from 96 trapping sites during the entire season, which ranged from 1 April to 26 August (mean start date = 5 June; interquartile range [IQR] = 20 May to 19 June). Most sites were sampled 1 year (54%, 52/96 trap sites), while another 35 sites (37%) were sampled 2 years, and nine (9%) were sampled all 3 years (mean = 1.6 years; Figure 1, Appendix S1: Table S1). Total biomass averaged 822.8 mg per day (±864.4 SD, $N = 1041$; median = 532.0 mg/day; IQR = 227.8 to 1124.8 mg/day; maximum = 7365.7 mg/day). Our analyses focused on the insect orders/suborders contributing at least 2% to total biomass: Diptera (33%; suborder Nematocera was 7% of the total), Lepidoptera (19%), Hymenoptera (18%), Coleoptera (10%), Orthoptera (5%), and Hemiptera (2%; see Appendix S1: Table S2 for a complete list).

Trends in biomass while controlling for phenological period

To control for different phenology, we first analyzed data from the three standard sampling periods based on the phenology of tree swallow breeding (laying date, hatching date and nestling day 12), which included 394 trapping sessions from 44 sites (1173 sample days). The starting date for each trapping session averaged 18 May (IQR = 15 to 25 May, $N = 126$) during the laying date period, 6 June (IQR = 2 to 12 June, $N = 138$) during the hatching date period and 19 June (IQR = 14 to 23 June, $N = 130$) during the nestling day 12 period.

Total biomass increased with maximum temperature ($T_{\max}$) and varied across the season (phenological period; Figure 2, Appendix S1: Table S4). However, the changes in biomass with temperature and phenological period (Figure 2) were similar across regions (i.e., the interactions with region were not significant; Figure 2; Appendix S1: Table S4). The results were more variable when we examined each major order separately (orders contributing >2% of total biomass). Again, biomass generally increased with maximum temperature and across phenological periods in most orders (Appendix S1: Table S4). However, there were geographic (regional) differences in the effects of both phenology (Figure 2b) and maximum temperature (Figure 2c) on the biomass of nematocerans, an important food source for aerial insectivores. In particular, during the period when nestling swallows are reaching their maximum weight (day 12 of age), there was greater biomass of Nematocera in the central than the Northeast region, and biomass was the lowest in the Southeast (days 45 to 54 in Figure 2b). Coleoptera also showed a different response to temperature in different regions (Appendix S1: Table S4), while Hemiptera increased across the season at different rates in different regions (Appendix S1: Figure S2f) but exhibited similar responses to temperature (Appendix S1: Table S4).

Trends in biomass throughout the season

Next, we compared the results above with those from sites that had additional samples outside the three standard phenological periods (i.e., those based on swallows), including some sites that were sampled continuously throughout most of the season. In this case, we used sample (calendar) date instead of the three main phenological periods to control for differences in phenology. Using this larger data set ($N = 1041$ samples), the patterns were similar to those from the three standard phenological periods (above), but more extreme in some cases, because they included data from earlier and later in the season (Appendix S1: Figure S3). As above, the total biomass and biomass of most insect orders increased as the season progressed (Figure 3), but often at different rates in different regions (Appendix S1: Table S5). In this case, five of the six orders/suborders had significant regional interactions with either sampling date or maximum temperature (Figure 3; Appendix S1: Table S5). For example, the biomass of nematocerans increased across the season in the

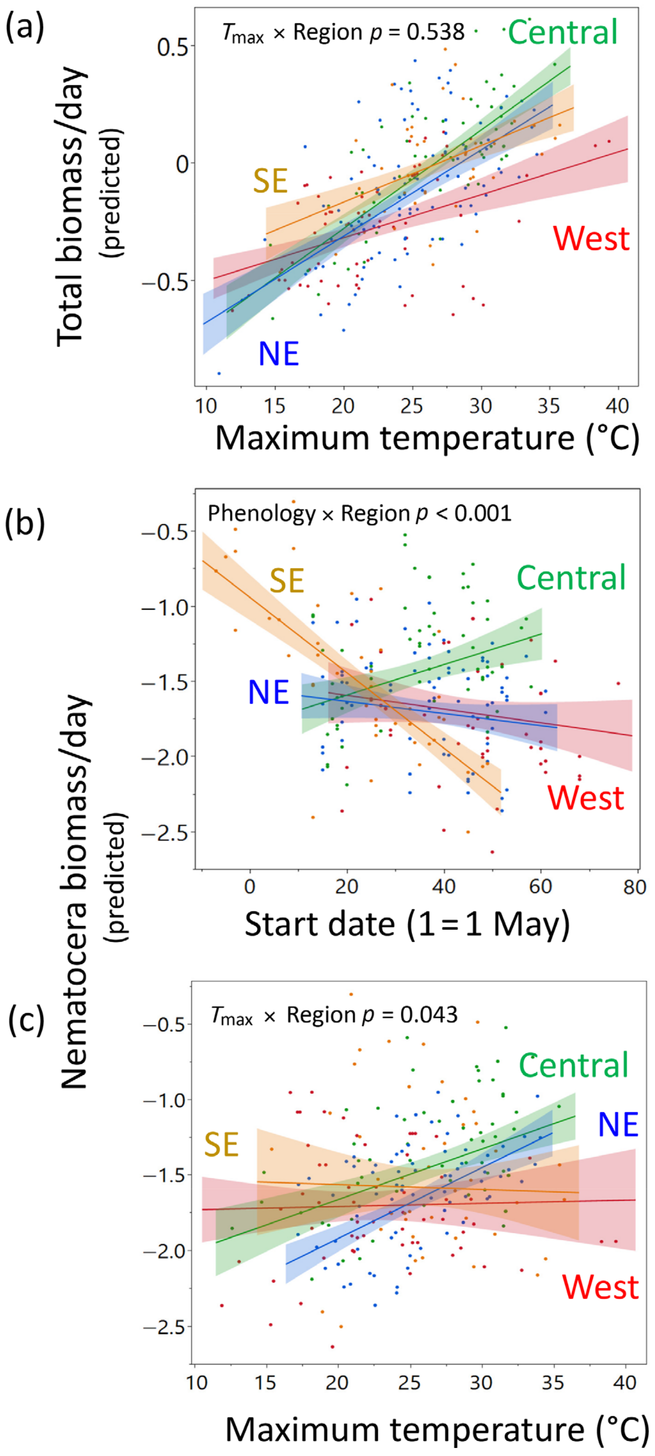

FIGURE 2 Log-transformed biomass (mg) per day (predicted) in relation to region and sampling date for all arthropods (panel a) and Nematocera (panels b), or region and maximum temperature for Nematocera (panel c; $N = 282–394$ samples) from linear mixed models (Appendix S1: Table S4). Lines and 95% CIs (shaded areas) are from the mixed models and points are the conditional predicted values which account for fixed and random effects (see Appendix S1: Table S4 for more details). Detailed statistical results for all taxa are in Appendix S1: Table S4 and Figure S2. NE, Northeast; SE, Southeast.

central region and it was greater than any of the other regions by the end of sampling (Figure 3). In contrast, nematocerans only showed a small increase across the season in the Northeast and they declined across the sampling period in the Southeast and West (Figure 3). One exception to these regional interactions was hemipterans (not shown in Figure 3), which differed in biomass among regions but had nonsignificant interactions between region and date and region and maximum temperature (Appendix S1: Table S5).

Insect biomass also responded differently to maximum temperature in different regions. There were significant interactions between region and temperature for total biomass, other Diptera, hymenopterans, and coleopterans (Figure 4). For total biomass, other Diptera and hymenopterans, biomass increased less steeply with temperature in the Southeast than in other regions, although the Southeast also had a narrower range of temperatures (Figure 4). Nematocerans showed regional differences in biomass, but the rate of change with temperature was similar among regions (the region × T_{max} interaction was not significant; Figure 4).

Geographic differences were also evident in the continuous sampling from sites with at least 10 samples per season. For example, nematocerans showed more peaked (bell-shaped) distributions of biomass across the season at sites in Saskatchewan, Colorado, and Iowa, compared with most of the eastern sites (Figure 5). Other dipterans did not seem to show as much of a peak, except for a few years in Wisconsin. In this sample from seven sites (Figures 5 and 6), the biomass of all five of the orders/suborders showed variation between sites in phenology (significant site × date interaction; Appendix S1: Table S6). Nematocerans, hymenopterans and coleopterans also showed geographic variation in terms of their response to temperature (significant site × T_{max} interaction; Appendix S1: Table S6).

Variation among locations, traps, and years

To understand better the source of geographic and temporal variation in biomass, we examined the proportion of variation explained by year, region, province/state, site (where there were multiple traps) and individual trap locations in a random effects model. This analysis used a reduced data file with only sites that had data from at least two traps with a similar starting day (±2 days; $N = 661$). In this model most of the variation in total biomass was explained by province/state (12%), followed by year (9.4%), the individual trap location (9.2%), region (8.6%), and site (7.6%; i.e., the cluster of traps operated by one research group). The correlation between total

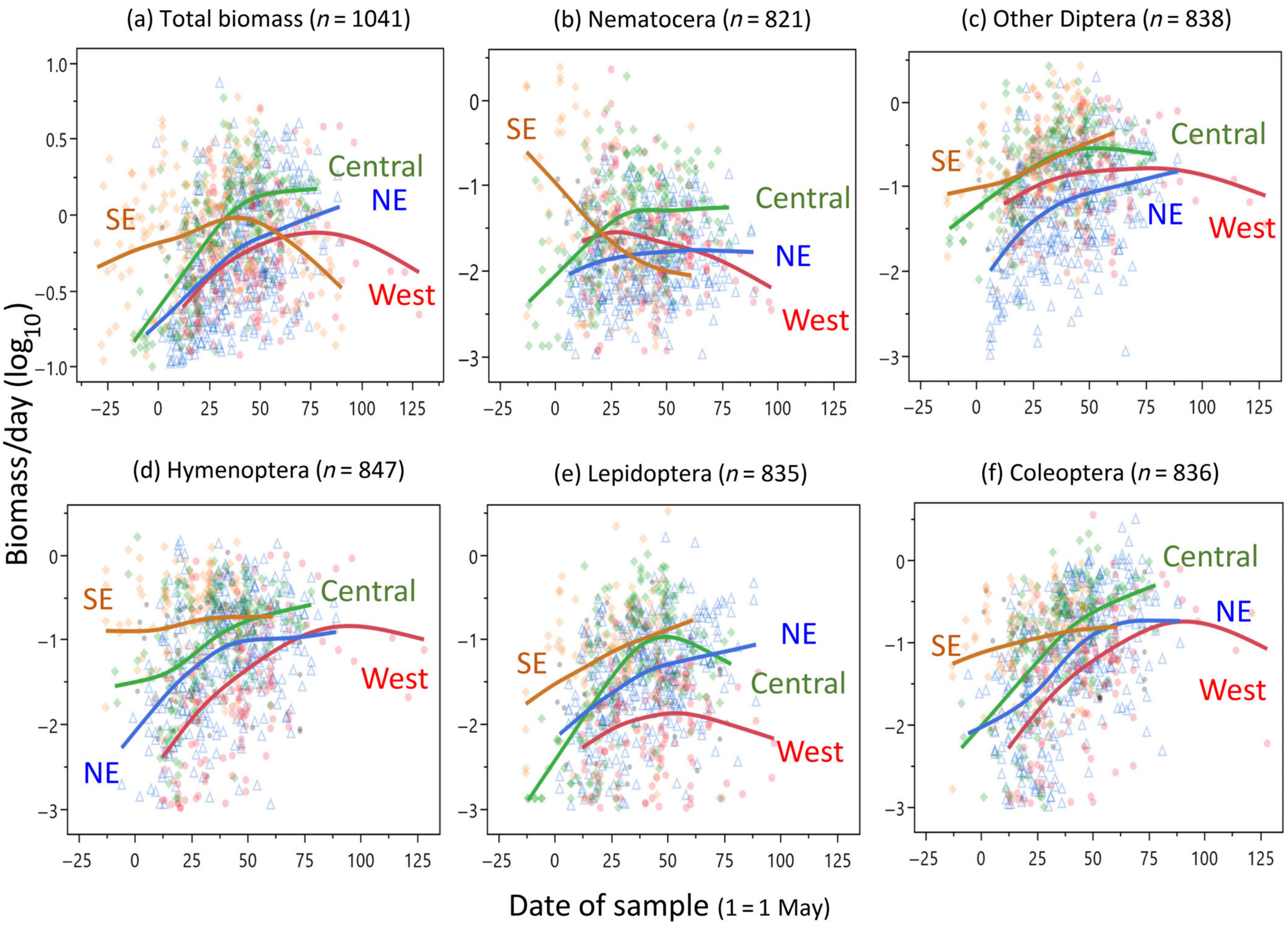

FIGURE 3 Log-transformed biomass (mg) per day of all arthropods (Total, panel a) and major orders of insects (panels b–f) in relation to sample date using data from across the entire season ("all data"; $N = 1041$ samples) for each region (West, Central, Northeast and Southeast) of North America. Biomass generally increased across the season, but at different rates in different regions (see Appendix S1: Table S5 for mixed model results). For illustrative purposes, the figures below show splines through the $\log_{10}$ transformed data ($\log_{10} + 0.1$ for total biomass and $\log_{10} + 0.001$ for other taxa), but statistical analyses are based on generalized linear mixed models (Appendix S1: Table S5). NE, Northeast; SE, Southeast.

biomass at nearby traps (within the same site) operating at the same time (±2 days) was strong ($r = 0.803$, 95% CI: 0.743–0.851; $N = 171$, $p < 0.001$), which likely explains the relatively low proportion of variation (7.6%) explained by traps operating at the same site. This was also evident in the repeatability estimates (R) for total biomass that controlled for the fixed effects of sampling date and maximum temperature (adjusted repeatabilities in rptR; Stoffel et al., 2017). Repeatability was highest for traps operating at the same site (i.e., multiple traps operated by the same researcher) and year (25%, bootstrap $p < 0.001$), followed by traps in the same region (14%, bootstrap $p = 0.128$), the same province/state (1.2%, bootstrap $p = 0.444$), and the same year (<1%, bootstrap $p = 0.999$; See Appendix S1: Figure S4 for more details). Total biomass estimates from the same sampling days were relatively similar up to ~75 km between traps (using data from the same provinces/states); however, at distances >75 km between traps the correlation became nonsignificant (Appendix S1: Figure S5 and Table S7).

How much data are required to detect a decline in biomass?

As a guide for designing future studies, we estimated sample sizes needed to detect significant differences in biomass. For example, to detect a significant difference in total biomass among our four regions (West, Central, Northeast and Southeast) would require ~250 samples with our observed means for each region and assuming power = 0.80 (power calculation in JMP). In this case,

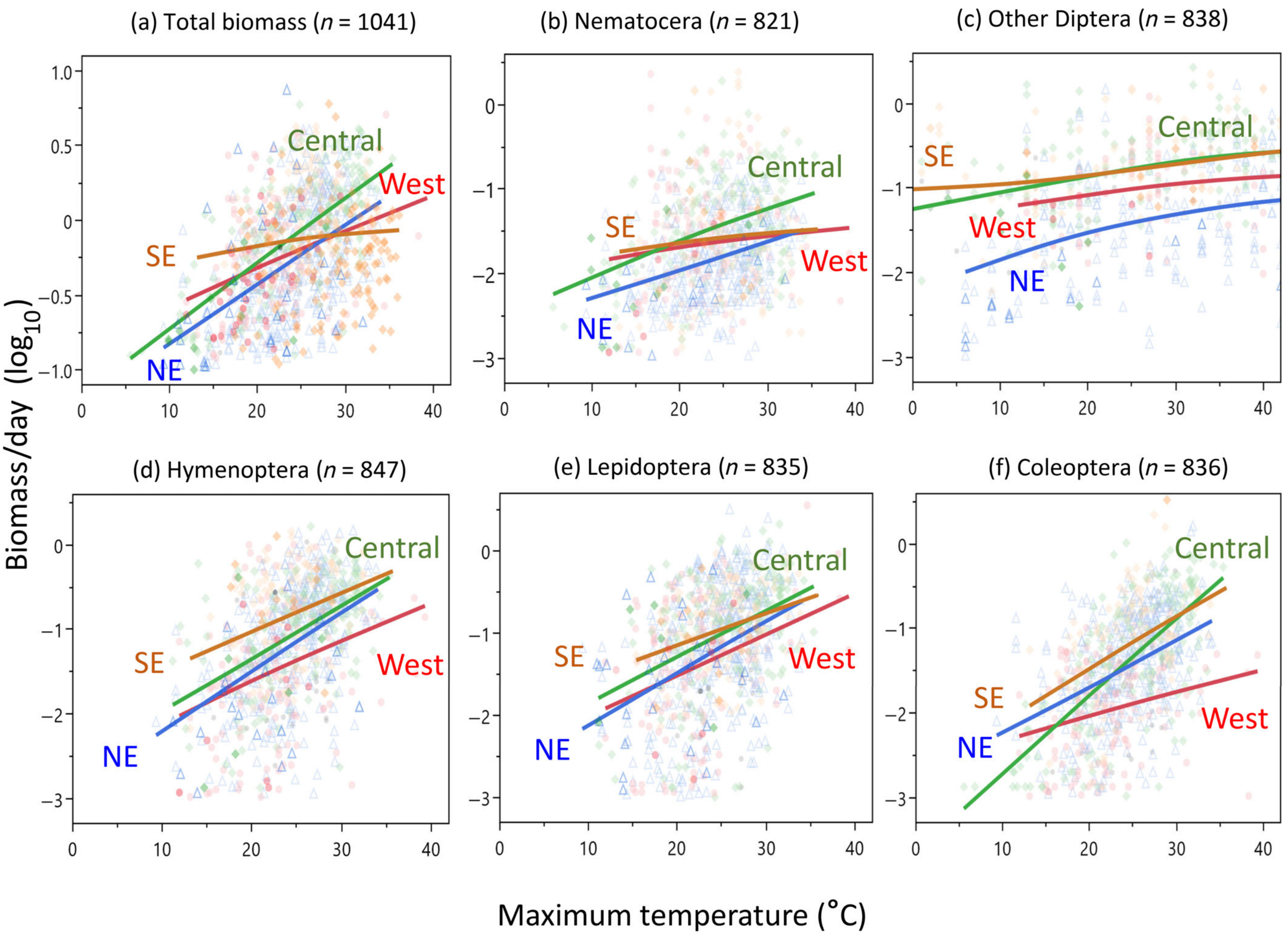

FIGURE 4 Log-transformed biomass (mg) per day of all arthropods (Total, panel a) and major orders of insects (panels b–f) in relation to maximum temperature using data from across the entire season ("all data"; $N = 1041$ samples) for each region (West, Central, Northeast and Southeast) of North America. Biomass increased with temperature for all orders, but at different rates in different regions for total biomass, Other Diptera, Hymenoptera and Coleoptera (i.e., there was a significant region × $T_{\max}$ interaction; see Appendix S1: Table S5 for mixed model results). For illustrative purposes, the figures below show splines through the $\log_{10}$ transformed data ($\log_{10} + 0.1$ for total biomass and $\log_{10} + 0.001$ for other orders), but statistical analyses are based on linear models in Appendix S1: Table S5. NE, Northeast; SE, Southeast.

mean biomass per day varied by ~63% among regions (−0.272 in the west to −0.102 in the central region; SD = 0.377 [$\log_{10} + 0.1$ g/day]).

Next, we estimated the required sample size to detect a 1% or 5% annual decline in biomass in future years, using samples from the three phenological sampling periods and a mixed model with year, maximum temperature, and phenology as fixed factors, and site and year nested within site as random effects (see *Methods*). For a 1% annual decline, the power to detect a decline reached 77% with 5 additional years of data; this would lead to a total sample size of 915 (Appendix S1: Figure S6). For a 5% decline, the power to detect a decline reached 100% with just one additional year of sampling (Appendix S1: Figure S6), which would lead to a total sample size of 535. In our analysis of the entire season there was no detectable change in total biomass over the 3 years of the study (2019–2021, $N = 1041$; Year: $F_{1,50} = 0.227$, $p = 0.636$), using a mixed model similar to the one above, but including sampling date instead of phenological period. Consequently, our power to detect a decline in total biomass over the 3 years of our study was low (0.124; 95% CI: 0.050–0.786) because the change in biomass was small (year slope = −0.016, 95% CI: −0.086 to 0.053 from the mixed model).

DISCUSSION

Geographic and temporal variation in insect biomass

Across North America, we found strong regional differences in the biomass of insects and their response to

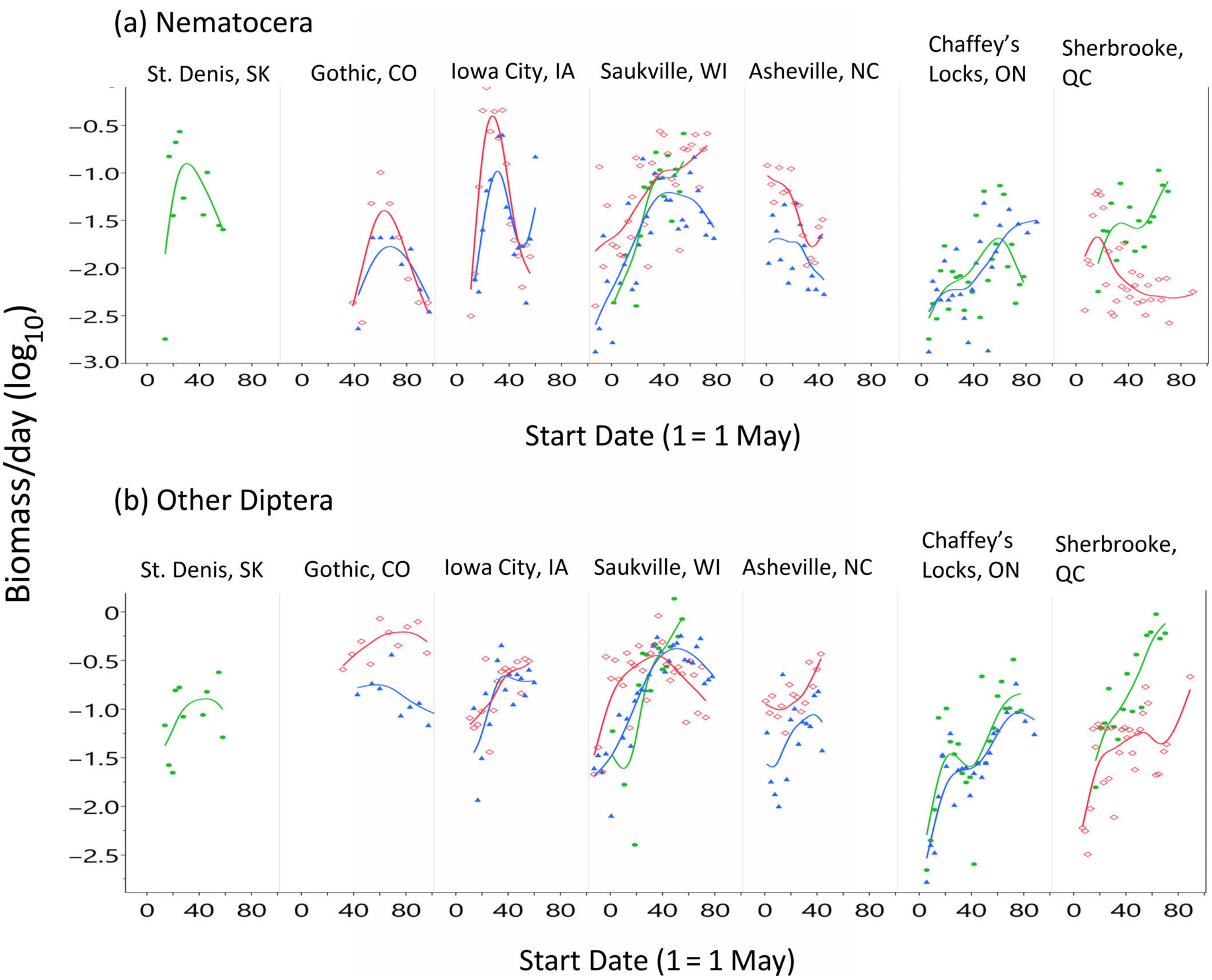

FIGURE 5 Seasonal patterns of (a) Diptera: Nematocera, and (b) Other Diptera biomass (mg/day $\log_{10}$ transformed) at seven representative sites in North America with at least 10 samples from a single trap in a season (not all sites are shown). Different colors indicate different years (green—2019, blue—2020, red—2021). Lines are splines (lambda = 1000). Start dates for sampling range from 19 April (−12 in Saukville) to 26 August (128 in Gothic). Interquartile range of sample dates = 22 May to 22 June.

temperature, even within the same taxonomic group and in similar habitats at the same stage of phenology (e.g., Figures 3 and 4). While total biomass fluctuated in a similar manner throughout the season, this pattern was not always consistent for each order, which emphasizes the need for studies that examine finer taxonomic levels. Different taxonomic responses were especially obvious with the biomass of Nematocera (e.g., midges, mosquitoes), which declined or remained stable throughout the season in all regions, except in the central region where it continued to increase and then plateaued later in the season (Figures 2 and 3). Although regional differences in mean biomass might be expected, it is noteworthy that most orders of insects (except Hemiptera) showed differences in the rate of seasonal change among regions (i.e., the region × date interaction was significant) even while controlling for temperature (Figure 4). These interactions were not always significant in the analysis of the data from the three main phenological periods (Appendix S1: Table S4), which may be a consequence of smaller sample sizes and a more restricted set of sampling dates.

Geographic and temporal variations in the response of insects to temperature can have an important effect on interpreting insect trends. This issue is illustrated by the study of Hallmann et al. (2017) which has been widely cited because it reported a 76% decline in total biomass over 27 years (1989–2016) in Germany. Hallmann et al. (2017) concluded that the decline was unlikely to be due to climate change because the temperature change in their study was small and not significant, and, in any case, it should have led to an increase in biomass. Interestingly, we reanalyzed their data and found that the relationship between biomass

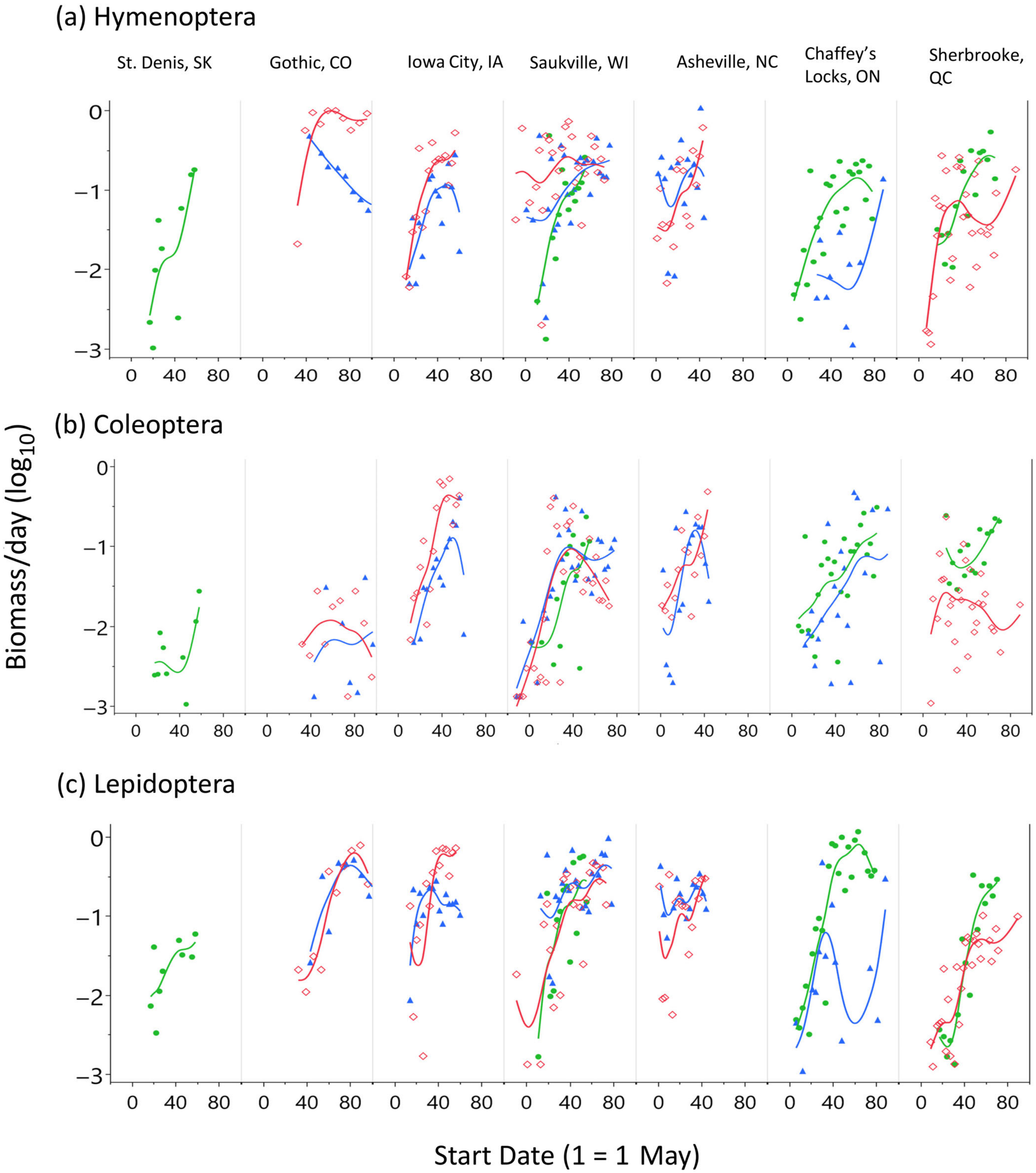

FIGURE 6 Seasonal patterns of (a) Hymenoptera, (b) Coleoptera, and (c) Lepidoptera biomass (mg/day $\log_{10} + 0.001$ transformed) at the same seven representative sites in North America as in Figure 5. Only sites with at least 10 samples from a single trap in a season are shown (not all sites are shown). Different colors indicate different years (2019–2021). Lines are splines (lambda = 1000). Start dates for sampling range from 19 April (−12 in Saukville) to 26 August (128 in Gothic). Interquartile range of sample dates = 22 May to 22 June.

and temperature has changed over time (Appendix S1: Figure S7). In particular, there was lower biomass of insects in more recent years, even at the same temperature (i.e., we found a significant temperature × year interaction). Apparently, Hallmann et al. (2017) did not test for this interaction, but it reinforces their conclusion that temperature was not the main driver of the insect decline. Thus, other factors, most likely agricultural practices, were responsible (Hallmann et al., 2017). It also serves as a caution that the relationship between insects and temperature is likely to vary geographically and temporally due to many other factors (e.g., land use and pesticides) that could confound analyses. In our study we restricted sampling to open, mostly grassy areas to avoid differences in habitat, but we still found regional differences in response to temperature within the same orders of insects (e.g., Figures 4 and 5). Thus, there are likely to be other ecological and anthropogenic differences among regions that produced the differences in biomass we observed. In contrast with the long-term Hallmann et al. study, our sampling only spanned 3 years, so it suggests that there are important regional differences in ecology (natural or anthropogenic) that are currently influencing insect biomass across North America. One possible explanation for the different responses is that there were different species in the regions we studied (i.e., community composition varies), but studies have also found that even within the same species there can be a local adaptation to temperature that influences population growth rates (e.g., Kingsolver et al., 2011; Porcelli et al., 2017), and therefore abundance and biomass (see also González-Tokman et al., 2020; Walter et al., 2022).

The regional variation observed in our study is relevant for the design of large-scale monitoring schemes. In our study, nearby traps had similar (correlated) biomass estimates up to 75 km apart (Appendix S1: Figure S5). Aphid abundance from suction traps was correlated up to 350 km apart in the midwestern United States (Schmidt et al., 2012), but these traps tend to catch higher altitude, wind-dispersed species, which likely disperse longer distances. Thus, a more powerful approach to detecting large-scale changes in insect biomass might be to spread out multiple traps so they are at least 75 km apart. Nonetheless, with our current sampling design, we estimated that just 1 or 5 years of additional sampling would be sufficient to detect declines in insect biomass of 5 or 1% ($N = 597$ or 1014 total samples, respectively). Differences among regions, which were larger (up to 63% on a log scale), could potentially be detected with ~250 samples, assuming the samples were collected from open areas; other types of habitat might show different levels of variation. These sample sizes are similar to some previous estimates for monitoring bees. For example, Lebuhn et al. (2013) estimated 200–250 sampling locations each sampled twice over a span of 5 years would provide sufficient power to detect 2%–5% annual declines in total abundance. They estimated that such a survey would cost US$2 million, but they were assuming that bees were identified as morphospecies, which increases processing time and labor costs (>US$650,000 per year). Our study was primarily self-funded by researchers as an incidental part of other projects, so the total cost was likely <US$100,000 per year.

Large-scale versus long-term sampling

It has recently been argued that large-scale studies can substitute for long-term studies when searching for the causes of change in insect populations, that is, a "space-for-time" substitution (Blüthgen et al., 2022). For example, given the same sampling effort, short-term studies could devote relatively more resources to sampling in different habitats or treatments (e.g., agricultural practices) that might be the cause of long-term insect declines. Long-term sampling at a few sites seems less likely to reveal causes of declines, and the results may not be representative of larger regions, as our study and others suggest. For example, aphid abundance throughout the midwestern USA also shows strong regional (and temporal) variation (Schmidt et al., 2012). The strong taxonomic, geographic, and phenological variations that we observed in this study also illustrate the problems that arise when combining data in meta-analyses, and it is these statistical issues that have generated much of the controversy about insect declines (e.g., Desquilbet et al., 2020; Didham et al., 2020; Duchenne et al., 2022). Thus, to understand the causes of changes in insect populations, the sampling design might benefit from the standardized sampling of locations with targeted differences in land use and ecology. For example, Garrett et al. (2022) monitored Diptera on 40 farms in Québec, Canada along a gradient of agricultural intensification and found a strong effect of agriculture on the biomass of Diptera. Similar types of focused studies on a larger geographic scale will help us better understand the scale and magnitude of insect declines, and, most importantly, their causes (see e.g., Hemberger et al., 2021; Meehan et al., 2011; Paredes et al., 2021).

Implications for the design of monitoring networks

Our study has important implications for the design of insect monitoring networks and their potential for detecting declines or predicting future effects of climate change. First, there were significant regional and taxonomic differences in the phenology of insects and their response to temperature. In the UK the phenology of aphids, butterflies, and moths has shifted earlier, but the shifts also appear to differ depending on location and habitat (Bell et al., 2019). Thus, the potential for geographic variation in insect responses to temperature needs to be incorporated into large-scale and long-term monitoring studies that forecast changes in insect populations. Second, although there were regional differences, insect biomass at different traps operating on the same days was correlated up to ~75 km apart. This suggests that for large-scale studies replication of traps within 75 km will not be as informative as additional traps farther apart. Conversely, studies that focus on the effects of differences in habitat (natural or anthropogenic) or specific treatments (e.g., pesticides) on insect populations will likely benefit from the lower measurement error provided by replicating nearby traps. Our study also illustrates that collaborative networks can be formed relatively quickly and at low cost, given sufficient interest and a simple protocol. Although our Malaise traps are moderately expensive (~US$200 each), they are simple to operate, and in our study we limited the level of sorting to order (or suborder for Nematocera) which likely increased the number and diversity (e.g., universities, government agencies, nature centers) of collaborators. In surveys such as ours, there is a trade-off between maximizing the number of participants (and samples) and the level of taxonomic identification, since time and expertise for sorting samples is often the limiting factor. Thus, we do not have information at the species level, which would be valuable for analyses of changes in population size and species composition, as well as ecosystem services, such as pollination. One compromise might be to store samples for more detailed taxonomic analysis in the future, perhaps using DNA barcoding. In any case, our protocol illustrates a balance that might be suitable for designing future large-scale studies, which will be key for understanding broad trends in insect populations.

AFFILIATIONS

[1]Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA
[2]U.S. Army Corps of Engineers, Rivers Project Office, West Alton, Missouri, USA
[3]Department of Biology, Siena Heights University, Adrian, Michigan, USA
[4]Birds Canada, Port Rowan, ON, Canada
[5]Institute of Marine Science, University of North Carolina at Chapel Hill, Morehead City, North Carolina, USA
[6]Department of Biology, Baylor University, Waco, Texas, USA
[7]Département de Biologie, Université de Sherbrooke, Sherbrooke, QC, Canada
[8]Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA
[9]Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada
[10]Pacific Wildlife Research Centre, Environment and Climate Change Canada, Delta, BC, Canada
[11]Environmental Science Program and Biology Department, Trinity College, Hartford, Connecticut, USA
[12]Alaska Peninsula and Becharof National Wildlife Refuges, U.S. Fish and Wildlife Service, King Salmon, Alaska, USA
[13]Department of Biology, Salisbury University, Salisbury, Maryland, USA
[14]Wildlife Research Division, Environment and Climate Change Canada, Saskatoon, SK, Canada
[15]Department of Biology and Environmental Studies, Amherst College, Amherst, Massachusetts, USA
[16]Division of Biological Sciences, University of Montana, Missoula, Montana, USA
[17]Department of Ecosystem Science and Management, University of Northern British Columbia, Prince George, BC, Canada
[18]US Department of Agriculture, Agricultural Research Service, Rangeland and Meadow Forage Unit, Burns, Oregon, USA
[19]Department of Biology, University of Iowa, Iowa City, Iowa, USA
[20]Hawthorne Valley Farmscape Ecology Program, Ghent, New York, USA
[21]Department of Biological Sciences, University of Manitoba, Manitoba, MB, Canada
[22]D.B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, USA
[23]Department of Biological Sciences, George Washington University, Washington, DC, USA
[24]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York, USA
[25]Department of Biology, Dalhousie University, Halifax, NS, Canada
[26]Department of Biological Science, Florida State University, Tallahassee, Florida, USA
[27]Rocky Mountain Biological Laboratory, Crested Butte, Colorado, USA
[28]Department of Biology, University of Maryland, College Park, Maryland, USA
[29]A Rocha Canada, Surrey, BC, Canada
[30]Department of Environment and Sustainability, Catawba College, Salisbury, North Carolina, USA
[31]Department of Entomology, University of Maryland, College Park, Maryland, USA
[32]Carnegie Museum of Natural History, Powdermill Nature Reserve, Rector, Pennsylvania, USA
[33]Smithsonian Environmental Research Center, Edgewater, Maryland, USA
[34]Department of Biology, Luther College, Decorah, Iowa, USA

[35]Department of Environmental Studies, University of North Carolina – Asheville, Asheville, North Carolina, USA
[36]Centre for Biodiversity Genomics, University of Guelph, Guelph, ON, Canada
[37]Department of Biology, University of Waterloo, Waterloo, ON, Canada
[38]Mohonk Preserve, New Paltz, New York, USA
[39]Department of Biology, Queen's University, Kingston, ON, Canada
[40]Missoula Butterfly House and Insectarium, Missoula, Montana, USA
[41]Ministry of Forests, Lands and Natural Resource Operations, Fort St. John, BC, Canada
[42]Biological Sciences Department, University of Wisconsin-Parkside, Kenosha, Wisconsin, USA
[43]U.S. Geological Survey, Fort Collins Science Center, Ft. Collins, Colorado, USA
[44]Department of Biology, Catawba College, Salisbury, North Carolina, USA
[45]Claytor Nature Center, University of Lynchburg, Bedford, Virginia, USA
[46]Center for Urban Research, Engagement and Scholarship, University of the District of Columbia, Washington, DC, USA
[47]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University, Mississippi State, Mississippi, USA
[48]Department of Geography and Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA
[49]Biology Department, Westfield State University, Westfield, Massachusetts, USA
[50]University of Alberta, Augustana Campus, Camrose, AB, Canada
[51]Cultural and Environmental Resource Management, Central Washington University, Ellensburg, Washington, USA
[52]The Jones Center at Ichauway, Newton, Georgia, USA
[53]Department of Biology, Appalachian State University, Boone, North Carolina, USA
[54]University of Alaska Museum, University of Alaska Fairbanks, Fairbanks, Alaska, USA
[55]Department of Biology, Davidson College, Davidson, North Carolina, USA
[56]Department of Biology, University of Lynchburg, Lynchburg, Virginia, USA
[57]Environmental Science, University of Jamestown, Jamestown, North Dakota, USA

ACKNOWLEDGMENTS

We wish to thank the landowners who allowed us to collect using our Malaise traps, including Bruce Bolin (ON), and Scott Innes of Blythe Dale Sand and Gravel (ON), B. Bryant Farms (MS), Campbell Valley Park (BC), Carpenter and Pettit Farms (MI), Champaign County Forest Preserve District and the University of Illinois Natural Areas (IL), Cornell University Botanic Gardens (NY), Lake View Park Board of Commissioners (NC), Lonesome Fir Ranch and City of Prince George (BC), Martin Van Buren National Historic Site (NY), Northern Great Basin Experimental Range and the Eastern Oregon Agricultural Research Center (OR), Powdermill Nature Reserve (PA), Queen's University Biological Station (ON), Rocky Mountain Biological Laboratory (CO), University of Guelph Arboretum (ON), University of Iowa Ashton Prairie Living Laboratory (IA), University of Lynchburg Claytor Nature Center (VA), University of Wisconsin-Milwaukee Field Station (WI), and Wilkus Farm Preserved Open Space, Town of Wethersfield (CT). We also thank the assistants and volunteers who helped collect and process insect samples, including Caitlin Arlotta, Shelley Bennett, Emily Bornestig, Christian Buchanan-Fraser, Katie Collins, Isabel Davis, Kira Dayton, Claire Ducharme, Chelsea Eby, Steven Esau, Emily Freilich, Paul Gehl, Brian Hidde, Callie Hundley, Benjamin Kuznicki, Andie Mazer, Gretchen Meyer, Michael Minard, Andrew Mitchell, Caitlin Mitchell, Ryan Nicol, Jacob Olichney, Rebecca Prather, Emily Ruder, Brielle Shortreed, Cheyenne Smith, Benjamin Sterrett, Jennifer Styrsky, Ron Tagye, Kailey Tobin, and Aija White. Birds Canada wishes to thank Yolanda Morbey and Greg Mitchell for project design, implementation, and ongoing support, and Michelle Jackson thanks Christine Bishop for the purchase of Malaise traps. Financial support was provided by a team grant from Fonds de recherche du Québec—Nature et Technologies to Marc Bélisle, Dany Garant, and Fanie Pelletier, National Science Foundation (grant DEB-2016749) to Brian D. Inouye and David W. Inouye, Natural Science and Engineering Research Council (NSERC, Discovery grant and Steacie Memorial Fellowship to Fanie Pelletier), Canada Foundation for Innovation and British Columbia Knowledge Development Fund grants to Russell D. Dawson, Province of Ontario to Birds Canada, Trinity College to Amber L. Pitt, USDA Agricultural Research Service to Elsie Denton, USDA-NIFA Agricultural Experiment Station funding to Matthew L. Richardson, and the University of Wisconsin-Milwaukee SURF program to Peter O. Dunn. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the US Government or any funders. Some weather data were provided by NOAA (through Howard University): http://ncas-m.org/research/data-management/. We thank Eliza Grames and David Wagner for their helpful comments on the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Dunn et al., 2023) are available in Figshare at https://doi.org/10.6084/m9.figshare.20415819.

ORCID

Peter O. Dunn https://orcid.org/0000-0002-7450-4194
Malcolm A. Barnard https://orcid.org/0000-0003-4192-574X
Tara L. Imlay https://orcid.org/0000-0001-9011-3217
Brian D. Inouye https://orcid.org/0000-0003-3994-2460
Kimberly J. Komatsu https://orcid.org/0000-0001-7056-4547
Colleen Miller https://orcid.org/0000-0002-2534-2109
Ian S. Pearse https://orcid.org/0000-0001-7098-0495
Amber L. Pitt https://orcid.org/0000-0002-6748-4164
Sadie J. Ryan https://orcid.org/0000-0002-4308-6321
Ivana Schoepf https://orcid.org/0000-0001-5437-337X
Conor C. Taff https://orcid.org/0000-0003-1497-7990
Thomas Wassmer https://orcid.org/0000-0002-1456-3475
Kathryn Weglarz https://orcid.org/0000-0003-1095-0934

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dunn, Peter O., Insiyaa Ahmed, Elise Armstrong, Natasha Barlow, Malcolm A. Barnard, Marc Bélisle, Thomas J. Benson, et al. 2023. "Extensive Regional Variation in the Phenology of Insects and Their Response to Temperature across North America." *Ecology* 104(5): e4036. https://doi.org/10.1002/ecy.4036