Butterfly abundance declines over 20 years of systematic monitoring in Ohio, USA

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Abstract

Severe insect declines make headlines, but are rarely based on systematic monitoring outside of Europe. We estimate the rate of change in total butterfly abundance and the population trends for 81 species using 21 years of systematic monitoring in Ohio, USA. Total abundance is declining at 2% per year, resulting in a cumulative 33% reduction in butterfly abundance. Three times as many species have negative population trends compared to positive trends. The rate of total decline and the proportion of species in decline mirror those documented in long-term European monitoring. Multiple environmental changes such as climate change, habitat degradation, and agricultural practices may contribute to these declines in Ohio and shift the makeup of the butterfly community by benefiting some species over others. Our analysis of life-history traits associated with population trends shows an impact of climate change, as species with northern distributions and fewer annual generations declined more rapidly. However, even common and invasive species associated with human-dominated landscapes are declining, suggesting widespread environmental causes for these trends. Declines in common species, although they may not be close to extinction, will have an outsized impact on the ecosystem services provided by insects. These results from the most extensive, systematic insect monitoring programs in North America demonstrate an ongoing defaunation in butterflies that on an annual scale might be imperceptible, but cumulatively has reduced butterfly numbers by a third over 20 years.

Introduction

Defaunation, or the drastic loss of animal species and declines in abundance, threatens to destabilize ecosystem functioning globally (1). In comparison to studies of vertebrate

populations, monitoring of changes in insect diversity is more difficult and far less prevalent (2,3). Despite this, a global analysis of long-term population trends across 452 species estimated that insect abundance had declined 45% over 40 years (1). Recently, more extreme declines in insect biomass have been observed upon resampling after 2-4 decades (4,5). Losses of total biomass or total abundance across all species may be more consequential than local declines in species diversity, as common insect species contribute the most to ecosystem functions, such as pollination (6). However, our knowledge of insect declines is skewed towards European monitoring programs, including in global analyses (1). In this study, we analyze long-term, region-wide trends in abundance across a diversity of species for an entire insect group in North America to examine the scope of insect defaunation.

The best source of data to assess insect defaunation comes from large-scale, systematic monitoring programs of multiple species (3). Through these efforts, trained volunteers or citizen scientists have contributed much of the evidence for biotic responses to anthropogenic climate warming through changes in insect phenology and range distributions (7,8). Unlike citizen science reporting of opportunistic observations or species checklists, many insect monitoring programs use a systematic protocol developed specifically for volunteers to track butterfly abundances through time, both within and between seasons, and over large spatial scales (9). Pollard-based monitoring programs, modeled after the first nationwide Butterfly Monitoring Scheme launched in the United Kingdom in 1977 (UKBMS), use weekly standardized counts on fixed transects (10). Their widespread adoption enables regional comparisons of insect responses to environmental change or defaunation (11,12). We compare our analysis with exemplary long-term monitoring schemes from Europe to test if the rate of insect declines generalizes across continents.

The best source of abundance data for assessment of chronic insect decline, and the most prominent source of data in (1), is within the butterflies. Due to the relative ease and popularity of monitoring butterflies, environmental assessments use them as an indicator taxa for the general trajectory of biodiversity, assuming that they experience comparable pressures from land-use change, climate change, and habitat degradation as other insect taxa (13–15). Intensive long-term monitoring of individual butterfly species has provided rigorous, quantitative estimates of declines. Most prominently, the Eastern North American Monarch has declined by over 85% (16) and the Western North American Monarch by over 95% (17) over the past two decades. Severe declines have also been observed in some of the rarest butterflies (18,19). These data from individual species of conservation concern may not represent a broader trend across butterflies, which is what we aim to document in this study.

Volunteers, organized and trained by The Ohio Lepidopterists, have assembled the most extensive dataset of systematic butterfly counts that stands alone in North America in terms of the spatial extent and sampling frequency of Pollard walks (9). Three other monitoring programs in the United States have documented long-term, multi-species population trends. In Massachusetts, based on species lists from field trips, climate-driven community shifts explain how the relative likelihood of species observations change over 18 years (20). Art Shapiro and colleagues have made biweekly presence/absence observations on 11 fixed transects along an elevational gradient in California over 40 years to document species richness changes in response to climate and land-use, increasing abundance at a high elevation site, and impacts of agricultural practices on abundance at low elevation sites (21,22). Several teams have monitored declines in specialist butterflies restricted to native prairie patches in the Midwestern states with transect or timed survey methods over 26 years (23,24). The number of Pollard-based monitoring

89 programs has increased sharply since 2010 in the United States (9), which could potentially track
90 to test how widespread and consistent butterfly trends are.

91 Here, we used 21 years of weekly butterfly surveys across 104 sites to assess abundance
92 trends for butterflies in Ohio. We estimate population trends for 81 species and test for their
93 association with life-history traits and phylogenetic relatedness. We review findings from
94 European butterfly monitoring schemes for quantitative comparison with the rate of abundance
95 changes in Ohio. This analysis provides evidence of widespread insect defaunation and species'
96 declines from the most extensive, systematic monitoring program in North America.

97

98 **Materials and methods**

99 *Study sites*

100 We studied butterfly population trends across the state of Ohio in the Midwestern USA.
101 Over its 116,100 km^2 land area, Ohio has a mosaic of habitat types due to its partially glaciated
102 history and its place at the confluence of Midwestern prairies, the Appalachian Mountains, and
103 the boreal forest (25). Only remnants of wetland and prairie habitat remain in the state due to
104 human modification of the landscape. Some rare butterflies have declined due to forest
105 succession following suppression of disturbances (26). Agriculture and pastures (50%), forest
106 (30%), and urban development (10%) are the predominant land-use/land cover classes (27).

107 Monitoring sites have a Northeast to Southwest gradient in their mean annual
108 temperatures (mean 18.8°C, range from 14.0°C to 23.6°C) from interpolated daily temperatures
109 from Daymet over 1996-2016 (Thornton et al. 1997). Mean annual temperatures at these sites
110 grew at a linear trend of 0.3°C per decade and growing season length has increased by 60 degree-
111 days (base 5°C) per decade from 1980-2016. Monitoring sites span the state but are concentrated

near cities (Fig 1). On average, within a radius of 2 kilometers, monitoring sites have 24% cropland and pasture, 34% forest, and 30% urban land-use based on the National Land Cover Dataset (29). Although not considered in this study, impervious surfaces from urban development influence temperature-dependent butterfly phenology in Ohio through the urban heat island effect, which may not be captured in these gridded temperature interpolations (30).

Fig 1: Transect locations monitored by volunteers with the Ohio Lepidopterists. Of the 147 sites, this analysis used the 104 sites monitored for three or more years.

Monitoring surveys

Trained volunteers contributed 24,405 butterfly surveys from 1996 to 2016 as part of the Ohio Lepidopterists Long-term Monitoring of Butterflies program. Volunteers survey on fixed paths at approximately weekly intervals during the entire growing season from April through October (median 23 of 30 weeks surveyed per year per site) and count every species within an approximate 5-meter buffer around the observer (10). Surveys are constrained to times of good weather to increase the detectability of butterflies and last a mean 85 minutes in duration. The annual number of monitored sites ranged from 13 in 1996 to a maximum of 80 in 2012. We limited our analysis of abundance trends to the 104 sites with three or more years of monitoring data and 10 or more surveys per year at each site (Fig 1). We included observations of all sites with at least 5 surveys per year in phenology models that we used to interpolate missing counts before estimating abundance (31).

All species (102) with population indices estimated by phenology models contributed to the total abundance analysis. We limited species-specific analysis to 81 with sufficient

population indices for estimating trends (present at five or more sites and for 10 or more years). Species naming conventions in the monitoring program follow those used in Opler and Krizek (1984) and Iftner et al. (1992) except for combining all observations of *Celastrina ladon* (Spring Azure) and *Celastrina neglecta* (Summer Azure) as an unresolved species complex.

Population indices

We estimated population indices for each site x year x species by adapting methods established for the UKBMS that account for missing surveys and butterfly phenology over the season (31,33). We used generalized additive models for each species to estimate variation in counts in order to interpolate missing surveys with model predictions (31,34). To account for seasonal, spatial, and interannual variation in species phenology, we extended the regional generalized additive model approach (12, Supplement 1) by including spatially-explicit site locations and converted calendar dates of observations to degree-days (35), which can improve butterfly phenology predictions. We calculated the population index by integrating over the weekly counts and missing survey interpolations using the trapezoid method (31).

Controlling for confounding factors

We accounted for differences in sampling across sites and years so that our modeled trends would capture changes in abundance rather than changes in detection probability (36). True abundance is confounded with detection probability when using counts from Pollard walks (37). Butterfly monitoring protocols that account for detection probability like distance sampling are commonly used for single-species studies (38), but untenable for scaling up to a statewide program. Most analyses of Pollard walks assume no systematic change in detectability (but see (39)) because counts correlate closely with true abundance estimates from distance sampling (40,41). We used two covariates to account for variation in sampling and its influence on

158 population indices (20,36,42). We tracked the number of species reported in each survey, or list-
159 length, which is a synthetic measure of factors influencing detectability such as weather
160 conditions, site quality, and observer effort (20,43,44). We treated the total duration of surveys in
161 minutes as an offset in the models of population trends, which converts the population indices to
162 a rate of butterflies observed per minute. Because we interpolated missing surveys for the
163 population indices, we projected what the total duration would be if all 30 weeks had been
164 surveyed at the mean duration reported for that site x year.

165 Sampling across the state is nonrandom because participants choose transect locations, a
166 common practice in volunteer-based monitoring programs. Since sites generally cluster near
167 human population centers with a greater proportion of developed land-use and a lesser
168 proportion of agriculture, we assumed that population trends at the 104 sites across the state
169 sufficiently capture the broader statewide trends (36). Comparisons between the UKBMS
170 volunteer-placed transects and a broader survey with stratified, random sampling show
171 congruence between species trends estimated from each monitoring strategy (45).

172 *Population Trends*

173 We used generalized linear mixed models to estimate temporal trends in relative
174 abundance for 81 species from their population indices (46). We modeled population indices at
175 each site and year as an over-dispersed Poisson random variable with covariates on the log-link
176 scale.

177 $$\log(PopulationIndex) = \beta_0 + \beta_1 \times year + \beta_2 \times listlength + \log(duration) + siteID + yearID + siteyearID$$

178 We included the year and mean list length for each population index as covariates, which
179 were centered to aid in model fitting and interpretation (47). We used the coefficient for year (β_2)
180 as the annual trend in population indices as our main result. We controlled for changes in

181 sampling by using the total duration of surveys as a model offset, converting the dependent
182 variable to a rate of butterflies counted per minute. Random effects of individual sites and years
183 account for spatial and temporal variation in population counts deviating from the statewide
184 trend. We accounted for over-dispersion in the Poisson-distributed counts with the random effect
185 *siteyearID* for each unique observation (48). We modeled trends in total abundance using the
186 same modeling approach, but summed across 102 species' population indices for each site x year
187 observation. We interpreted trends as an annual rate by taking the geometric mean rate of change
188 between the predicted abundance between two points in time after setting the list-length
189 covariate to its mean and excluding the random effects (46). For comparisons with other
190 monitoring programs, we used a *p*-value threshold of 0.05 to classify trends as positive, negative,
191 or stable.

192 Our approach is similar to that used by the UKBMS and other European monitoring
193 programs which use generalized linear models in TRIM software (49). One key difference is that
194 our site and annual fluctuations from the temporal trend were derived from random effects rather
195 than fixed effects, which reduces spurious detection of trends (42). Another key difference is that
196 TRIM does not allow for continuous covariates, which we used to account for sampling variation
197 instead of assuming no confounding pattern in sampling effort. To validate that our modeling
198 choices did not unreasonably influence the results, we used three alternative approaches: (1) a
199 Poisson-based generalized linear model (equation 1 without the random effect *siteyearID*); (2) a
200 nonlinear generalized additive mixed model with a smoothing spline replacing the linear
201 temporal trend (42); and (3) a TRIM model with over-dispersion and serial temporal correlation
202 but no sampling covariates or offsets (49). We compared similarity in the total abundance trends,

203 the correlation of species' trends between model alternatives, and the classification of species'
204 trends.

205 *Comparison with other studies*

206 We compare our findings to three European long-term, regional butterfly monitoring
207 programs with systematic Pollard walks that publish regular updates on total abundance and
208 species' trends (39,50,51). Although all programs analyzed counts with Poisson regression, we
209 had to standardize them differently depending on the data available and their modeling
210 approaches. The UKBMS reports total abundance indicators as the geometric mean of species
211 trends from two groups: specialist and countryside species (50). We used the reported smoothed
212 annual index values for these indicators because the first year of monitoring is an outlier that
213 exaggerates declines (UK Biodiversity Indicators 2018, http://jncc.defra.gov.uk/page-4236). We
214 used the Dutch Butterfly Monitoring Scheme's reported cumulative annual trend in total
215 butterflies counted across all transects after correction for missing surveys (51). For the Catalan
216 Butterfly Monitoring Scheme, we extracted annual population indices from the 2015-2016
217 annual report (52) with WebPlotDigitizer 4.1 (53) and performed a Poisson regression over time
218 with annual random effects to obtain a comparable abundance trend. We converted total
219 abundance trends into annual percent rates for comparison. We tallied the increases and
220 decreases in species' trends for each region reported by the monitoring program, without
221 accounting for differences in their statistical approaches.

222 *Species' traits*

223 To explore potential mechanisms that might explain species-level variation in abundance
224 trends, we modeled the estimates of species' temporal trends (β_1) as a response to life history
225 traits (20,30). Of the 81 species, 14 were classified as migratory species and 4 (*Colias*

226 *eurytheme*, *Lycaena phlaeus, Pieris rapae*, and *Thymelicus lineola*) were considered naturalized
227 species that were introduced to the state 90 or more years ago (25,32). We analyzed traits models
228 both across all species and after excluding migratory species, which would have population
229 trends driven by factors outside of Ohio. We collected traits that relate to insect responses to
230 climate change and habitat change, as these are two primary drivers of butterfly community
231 changes (7,20,21).

232 We tested if butterflies with traits making them more adaptive to a warming climate have
233 more positive population trends. Voltinism, or the number of generations per year, increases in
234 warmer years and warmer regions within many species in Ohio (54), compared with obligate
235 univoltine species that do not adjust their lifecycle based on changing growing season length. We
236 assigned voltinism observed in Ohio (1-4 generations per year) to an integer scale based on
237 visualization of phenology models and (25). We compared species with different range
238 distributions, assuming that species distributed in warmer, Southern regions would be more
239 3likely to increase in Ohio as the climate warms. We assigned species' ranges as Southern, core,
240 or Northern by range maps and county records (25,32). The life stage in which species
241 overwinter contributes to its ability to respond to warming with shifts in phenology (20,55).

242 We would expect more generalist species, in host plant requirements and habitat
243 preferences, to have more positive population trends in a landscape heavily modified by human
244 use (21,50). For host plant requirements, we gathered two traits from the literature that describe
245 host plant category (forb, graminoid, or woody) and whether the butterfly's host plant
246 requirements span multiple plant families or are limited to one plant family or genus (25). Mean
247 wing size from (32) was used as a surrogate of dispersal ability between habitats, which is
248 expected to increase ability to access resources in a fragmented landscape. Three of the authors

249 assigned species as wetland-dependent or human-disturbance tolerant species, which we
250 aggregated into two binary variables to test if specialist or generalist habitat preferences correlate
251 with abundance trends.

252 We used univariate linear models for each life history trait both for all 81 species and
253 with the 14 migratory species excluded. To account for the phylogenetic relatedness and the non-
254 independence across species, we compared phylogenetic generalized least squares models that
255 estimated branch length transformations with Pagel's lambda by maximum likelihood (56). The
256 phylogenetic models excluded three species without gene sequences available.

257 *Phylogenetic tree*

258 We obtained coding sequences for the most widely used DNA barcoding locus, the
259 mitochondrial cytochrome c oxidase subunit I gene COI-5P, from GenBank (57). For species not
260 found in GenBank, we obtained coding sequences from The Barcode of Life Data System (58).
261 When possible, we obtained sequences from multiple sampling locations in North America.

262 Owing to the relatively small size of our multiple-species alignment—i.e. a single
263 mtDNA locus, 651 base pairs in length—we decided to take both a constrained and
264 unconstrained maximum likelihood approach to estimate the genealogical relationships of our
265 samples. Some of the species from our analysis, though not all, were recently used in a more
266 comprehensive phylogenetic analysis of butterflies (59), thus prompting us to constrain the
267 phylogenetic backbone of our tree using family-level relationships. We report details of our
268 workflow in Supplement 1.

269 *Statistical analysis*

270 We used R 3.5.2 for analysis (60) and share the data and our code on Dryad. We fit
271 generalized additive models with the *mgcv* package (34), generalized linear mixed models with

the *lme4* package (Bates et al. 2015), and phylogenetic generalized least squares models with the *ape* and *caper* packages (62,63). Confidence intervals for the temporal trends were estimated with bootstrapped model fits with the *merTools* package (64). We estimated the goodness of fit with R^2 developed for generalized linear mixed models that give marginal and conditional R^2 values for the fixed effects and the fixed + random effects, respectively (65,66).

Results

The statewide relative abundance across all species declined at an annual rate of 2.0% (β_1 = -0.020, std. err. 0.005, $p < 0.001$), accumulating a 33% decline over 1996-2016 (Table 1, Fig 2). Among population trends, more than three times as many species are declining than increasing in abundance at our threshold of $p < 0.05$ (32 versus 9, respectively) (Table 2, Fig B-D in S1 Appendix). Positive and negative species trends are distributed across the phylogenetic tree (Fig A in S1 Appendix).

Table 1: Generalized linear mixed model of total abundance across all species. The natural logarithm of the total survey duration across the monitoring season was an offset in the model. The model's marginal R^2 was 0.20 for its fixed effects and its conditional R^2 was 0.61 when including variation in sites, years, over-dispersion with random effects parameters.

Fixed effects	*B*	std.error	z statistic	p.value
Intercept	1.33	0.0506	26.4	<0.001
Year (numeric)	-0.0203	0.00496	-4.11	<0.001
List-length	0.104	0.00587	17.7	<0.001
Random effects	std. dev.	# groups		
Site x year	0.278	1005		
Site	0.417	104		
Year (factor)	0.121	21		

Table 2: Species' abundance trends over time. Trends are the coefficient of year in our regression models with the accompanying standard error and *p*-value for the coefficient (equation 1). Included are the number of population indices calculated for each species for use in abundance model (Site x year) and total number of butterflies recorded for all years. Bold font indicates trends classified as increasing or decreasing ($p < 0.05$).

Species		Sample size				GLMM temporal trend		
Common	Latin	Total # counted	Sites	Years	Site/ year	Trend coef.	Std. error	P
Aphrodite Fritillary	*Speyeria aphrodite*	477	9	16	131	***-0.233***	***0.060***	***<0.001***
Baltimore	*Euphydryas phaeton*	818	7	17	83	***-0.224***	***0.071***	***0.002***
American Copper	*Lycaena phlaeas*	10,255	31	21	359	***-0.193***	***0.024***	***<0.001***
Hoary Edge Skipper	*Achalarus lyciades*	291	7	19	88	***-0.178***	***0.061***	***0.003***
Milbert's Tortoise Shell	*Nymphalis milberti*	140	8	16	101	***-0.174***	***0.065***	***0.008***
European Skipper	*Thymelicus lineola*	46,549	57	21	609	***-0.173***	***0.021***	***<0.001***
Southern Cloudywing	*Thorybes bathyllus*	667	15	20	194	***-0.129***	***0.037***	***<0.001***
Falcate Orangetip	*Anthocharis midea*	756	8	18	103	***-0.123***	***0.040***	***0.002***
Dreamy Duskywing	*Erynnis icelus*	879	18	21	260	***-0.120***	***0.024***	***<0.001***
Swarthy Skipper	*Nastra lherminier*	448	7	17	78	***-0.114***	***0.041***	***0.006***
Tawny Emperor	*Asterocampa clyton*	937	27	19	308	***-0.114***	***0.036***	***0.002***
Leonard's Skipper	*Hesperia leonardus*	1,348	11	20	144	***-0.110***	***0.025***	***<0.001***
White M Hairstreak	*Parrhasius m-album*	95	7	15	110	-0.105	0.081	0.195
Northern Cloudywing	*Thorybes pylades*	547	16	20	210	***-0.095***	***0.033***	***0.004***
Coral Hairstreak	*Satyrium titus*	607	15	21	217	***-0.094***	***0.025***	***<0.001***
Juvenal's Duskywing	*Erynnis juvenalis*	3,838	38	21	487	***-0.083***	***0.020***	***<0.001***
Common Wood Nymph	*Cercyonis pegala*	21,603	77	21	788	***-0.073***	***0.013***	***<0.001***
Common Sooty Wing	*Pholisora catullus*	1,142	34	20	398	***-0.072***	***0.015***	***<0.001***
Sleepy Duskywing	*Erynnis brizo*	811	13	18	156	***-0.071***	***0.032***	***0.027***
Monarch	*Danaus plexippus*	46,070	104	21	1,005	***-0.070***	***0.023***	***0.002***
Red-spotted Purple	*Limenitis arthemis*	6,226	87	21	913	***-0.064***	***0.019***	***<0.001***
Bronze Copper	*Lycaena hyllus*	656	23	21	254	-0.063	0.039	0.103
Northern Broken-Dash	*Wallengrenia egeremet*	5,959	49	21	528	***-0.062***	***0.018***	***<0.001***
Tawny-edged Skipper	*Polites themistocles*	2,322	48	21	541	***-0.058***	***0.016***	***<0.001***
West Virginia White	*Pieris virginiensis*	214	5	16	63	-0.058	0.059	0.329
Fiery Skipper	*Hylephila phyleus*	3,917	57	19	646	-0.057	0.061	0.351
Meadow Fritillary	*Boloria bellona*	5,447	55	21	598	***-0.056***	***0.027***	***0.040***

Orange Sulphur	*Colias eurytheme*	62,160	101	21	996	***-0.055***	***0.021***	***0.008***
Long Dash	*Polites mystic*	1,317	21	21	219	***-0.047***	***0.020***	***0.022***
American Lady	*Vanessa virginiensis*	2,029	54	21	637	-0.045	0.033	0.179
Black Swallowtail	*Papilio polyxenes*	12,410	92	21	941	***-0.044***	***0.015***	***0.004***
Gray Hairstreak	*Strymon melinus*	2,418	49	19	587	-0.044	0.026	0.089
Painted Lady	*Vanessa cardui*	5,564	80	21	873	-0.042	0.054	0.440
Great Spangled Fritillary	*Speyeria cybele*	33,573	90	21	904	***-0.041***	***0.020***	***0.047***
Hobomok Skipper	*Poanes hobomok*	6,863	51	21	576	***-0.040***	***0.014***	***0.005***
Viceroy	*Limenitis archippus*	16,079	85	21	896	***-0.039***	***0.016***	***0.014***
Cabbage White	*Pieris rapae*	304,105	104	21	1,005	***-0.038***	***0.010***	***<0.001***
Hackberry Emperor	*Asterocampa celtis*	9,992	42	20	467	***-0.037***	***0.017***	***0.033***
Striped Hairstreak	*Satyrium liparops*	155	14	18	211	-0.028	0.067	0.682
Variegated Fritillary	*Euptoieta claudia*	956	17	19	204	-0.027	0.052	0.603
Little Wood Satyr	*Megisto cymela*	76,612	87	21	878	***-0.026***	***0.009***	***0.005***
American Snout Butterfly	*Libytheana carinenta*	1,007	36	18	418	-0.025	0.050	0.612
Hickory Hairstreak	*Satyrium caryaevorum*	196	12	20	170	-0.023	0.053	0.656
Mourning Cloak	*Nymphalis antiopa*	3,214	85	21	905	-0.021	0.018	0.256
Clouded Sulphur	*Colias philodice*	49,267	102	21	998	-0.014	0.014	0.286
Spicebush Swallowtail	*Papilio troilus*	25,322	82	21	858	-0.014	0.014	0.324
Dun Skipper	*Euphyes vestris*	1,684	49	21	585	-0.014	0.012	0.224
Question Mark	*Polygonia interrogationis*	6,564	88	21	915	-0.012	0.025	0.640
Delaware Skipper	*Atrytone logan*	1,086	30	21	313	-0.011	0.029	0.697
Horace's Duskywing	*Erynnis horatius*	2,885	31	21	376	-0.011	0.023	0.633
Eastern Tiger Swallowtail	*Papilio glaucus*	29,299	101	21	996	-0.010	0.015	0.483
Pearl Crescent	*Phyciodes tharos*	180,631	104	21	1,005	-0.010	0.014	0.461
Little Yellow	*Eurema lisa*	1,681	24	18	287	-0.008	0.073	0.917
Eastern Comma	*Polygonia comma*	6,222	92	21	944	-0.007	0.011	0.561
Giant Swallowtail	*Papilio cresphontes*	1,109	28	21	322	0.002	0.019	0.912
Banded Hairstreak	*Satyrium calanus*	1,107	36	21	468	0.004	0.031	0.896
Silver-spotted Skipper	*Epargyreus clarus*	54,462	102	21	996	0.005	0.012	0.672
Red Admiral	*Vanessa atalanta*	28,637	97	21	969	0.008	0.044	0.865
Red-banded Hairstreak	*Calycopis cecrops*	795	7	17	91	0.009	0.057	0.879
Crossline Skipper	*Polites origenes*	1,087	27	21	347	0.009	0.020	0.636
Sachem	*Atalopedes campestris*	1,445	19	18	231	0.013	0.058	0.823
Peck's Skipper	*Polites peckius*	23,702	90	21	905	0.014	0.014	0.306
Northern Eyed Brown	*Satyrodes eurydice*	1,342	13	21	174	0.016	0.035	0.651
Eastern Tailed Blue	*Everes comyntas*	56,137	99	21	974	0.016	0.010	0.113
Henry's Elfin	*Callophrys henrici*	330	7	17	76	0.017	0.055	0.752
Little Glassy Wing	*Pompeius verna*	8,658	56	21	632	0.019	0.019	0.307
Silvery Checkerspot	*Chlosyne nycteis*	2,049	20	19	224	0.039	0.022	0.074

Spring/Summer Azure	*Celastrina ladon/neglecta*	63,947	103	21	1,002	***0.047***	***0.021***	***0.022***
Common Buckeye	*Junonia coenia*	15,771	73	19	834	0.050	0.067	0.459
Pipevine Swallowtail	*Battus philenor*	703	23	18	279	0.053	0.033	0.110
Least Skipper	*Ancyloxypha numitor*	27,506	84	21	844	***0.053***	***0.015***	***<0.001***
Appalachian Eyed Brown	*Satyrodes appalachia*	2,118	12	18	118	0.060	0.045	0.181
Zabulon Skipper	*Poanes zabulon*	10,960	71	21	747	***0.061***	***0.022***	***0.004***
Northern Pearly-Eye	*Enodia anthedon*	2,785	37	21	434	***0.071***	***0.020***	***<0.001***
Zebra Swallowtail	*Eurytides marcellus*	1,349	18	18	224	***0.075***	***0.030***	***0.011***
Cloudless Sulphur	*Phoebis sennae*	1,840	27	19	355	0.088	0.057	0.121
Common Checkered-Skipper	*Pyrgus communis*	3,089	33	18	357	***0.092***	***0.046***	***0.046***
Wild Indigo Duskywing	*Erynnis baptisiae*	15,209	51	19	570	***0.106***	***0.020***	***<0.001***
Harvester	*Feniseca tarquinius*	341	11	20	143	***0.122***	***0.061***	***0.046***
Sleepy Orange	*Eurema nicippe*	2,028	6	17	63	0.146	0.134	0.276
Gemmed Satyr	*Cyllopsis gemma*	1,059	6	16	81	***0.228***	***0.052***	***<0.001***

Fig 2: The relative abundance of counted butterflies in Ohio declined by 33% over 1996-2016. Plotted are model predictions for each year based on the fixed effects of year (solid line) and annual random effects (dots) to show annual variation about the trend line. Shading shows 95% confidence interval based on bootstrapped model fits for the temporal trend.

Both in the total trend in abundance and in the proportion of species with declines, these results are comparable to our review of three European butterfly monitoring schemes (Table 3). Although the longer-running programs show larger cumulative declines, the annual rate of change ranges from -2.0% to -2.6% for Ohio, Catalonia, and the Netherlands, respectively, with the United Kingdom total trends split between generalist species (-0.8%) and specialist species (-2.4%). Across monitoring programs, declining species outnumber increasing species by a factor of two to three (Table 3).

309 Table 3: Comparison of estimated annualized and cumulative rates of change in total abundance and species trends in regional
310 butterfly monitoring programs in Europe compared to this study. Number of sites represents those reported to be analyzed in total, but
311 may no longer be active. Number of butterflies counted per year is based on the most recent years of monitoring described in the
312 references.

					Species' trends			
Region (km^2)	Years	Sites	Counted/year (x 1000)	Annualized trend in total abundance (cumulative)	Positive	Negative	Stable/ not signif.	Reference
United Kingdom (242,500)	41 (1976-2017)	3,164	1,700	-0.8% (-28%) countryside -2.4% (-63%) specialist	11	22	24	(50)
Netherlands (42,508)	25 (1992-2017)	600	250	-2.0% (-40%)	11	23	13	(51)
Catalonia, Spain (32,108)	22 (1994-2016)	116	122	-2.6% (-44%)	15	46	5	(39,52)
Ohio, USA (116,100)	**20 (1996-2016)**	**104**	**80**	**-2.0% (-33%)**	**9**	**32**	**40**	**this study**

313

In general, traits associated with species' responses to climate were more important, based on the predictive ability (adjusted R^2) of univariate models, than traits associated with habitat and host plant restrictions (Fig 3, Tables A and B in S1 Appendix). Phylogenetic signal was included in most traits, so we focus on the phylogenetic generalized least squares results. Migratory species to Ohio had stable population trends on average compared to resident species and the four naturalized species. The Monarch (*Danaus plexippus*) was the only migratory species in decline. Multivoltine species with more annual generations had more positive population trends. Species with more northern geographic ranges were associated with more negative population trends. Species eating forb host plants had negative trends on average, but there was no effect of host plant specialization on population trends.

Fig 3: Species' traits are associated with variation in the statewide trends in abundance. We plot each trend compared to the six most important traits for the 78 species included in the phylogenetic GLS models with full results in Table A in S1 Appendix. Squares represent the regression coefficients with 95% confidence intervals shown in lines. Dots for each species are jittered for visualization.

Our choice of modeling approach did not change the overall evidence of defaunation. Generalized linear mixed models with Poisson-distributed errors and generalized additive mixed models estimated declines in total abundance similar in magnitude at -1.83% and -2.13% annual rates, respectively. The annual trend estimate from TRIM, without sampling covariates, was half the magnitude at -0.96%. Species' trends had high correlations between pairwise comparisons,

but TRIM models had notably more positive trends compared to the other three approaches (Table C in S1 Appendix).

Discussion

We show that the total butterfly abundance has declined by 33% over 20 years in Ohio. This rate is faster than the global abundance trend estimated for Lepidoptera (35% over 40 years) and corresponds more closely to the steeper declines (45% over 40 years) estimated for all insects (1). The Ohio butterfly monitoring program, judged by the weekly frequency, 20-year time period, and statewide spatial extent of its surveys, is the most extensive systematic insect survey in North America and comparable to three exemplary European butterfly monitoring schemes. The annualized 2% rate of decline in this study aligns closely with trends from European butterfly monitoring, confirming the decline of the most closely monitored group of insects in both Europe and North America (Table 3). With less known about other insect taxa, butterflies provide a necessary, if imperfect, surrogate to understand the trajectory and potential mechanisms behind broader insect trends (13). Extensive in both time and space, the decline in butterfly abundance is the current best estimate for the rate of insect defaunation in North America.

The proportion of butterfly species with population declines compared to population increases is similar between Ohio (negative trends three times more numerous) and European studies (negative trends 2-3 times more numerous) (Table 3). In other taxa, moths in the United Kingdom show a similar proportion of species declines (67). Long-term monitoring in protected areas, although less extensive in space, shows more positive species trends for moths in Finland (at 67.7° latitude) and across pollinators in Spain (at 850-1750 m. elevations) (68,69). These

359 counterexamples show how insect communities may shift at high-latitude or high-elevation sites
360 with anthropogenic climate warming (21) or may persist in more remote areas. However,
361 butterfly monitoring in populated areas show a consistency in observed declines (Table 3) that
362 we argue would generalize to other landscapes dominated by human use.

363 We demonstrate declines in species that are generalist, widespread, and not considered
364 vulnerable to extinction (25,70). The four species introduced to Ohio (*Pieris rapae*, *Lycaena*
365 *phlaeus*, and *Thymelicus lineola* from Europe and *Colias eurytheme* from the western USA) are
366 declining more rapidly than native species (Fig 3). Although few may share concern for the most
367 widespread, invasive butterfly in the world's agricultural and urban settings (71), declines in
368 *Pieris rapae* could be indicative of persistent environmental stressors that would affect other
369 species as well. We would expect negative environmental changes to disproportionately affect
370 rare species prone to the demographic dangers of small populations or specialist species that rely
371 on a narrow range of resources or habitat (UKBMS in Table 3, Swengel et al. 2011). This pattern
372 of species declines would lead to biotic homogenization as rarer species are lost and common,
373 disturbance-tolerant species remain (72,73). However, our study adds another example of
374 declines in common butterfly species thought to be well-suited to human-modified habitat
375 (11,21,74).

376 The Eastern North American migratory Monarch (*Danaus plexippus*) abundance in Ohio
377 is declining by 7% per year. The Monarch is the only declining migratory species out of 14 in
378 our analysis. Despite disagreements about whether summer abundance trends have tracked
379 winter colony declines (75,76), our study shows that the long-term trends correspond. However,
380 our study's first two years have very high Monarch population indices which could be outliers
381 (Fig B in S1 Appendix) following the two largest recorded winter population counts (16,77).

382 With these two years removed, the statewide Monarch trend is a 4% decline per year, showing
383 that the magnitude of summer abundance trends are sensitive to the years of inclusion. Our
384 results align with a study using Illinois systematic monitoring data that shows a summer
385 abundance decline for monarchs over two decades, but at different rates across decades (78). A
386 more recent study showed no decline during the summer during 2004-2016 using a population
387 index from NABA counts (77). The trend we document comes from the sum of summer breeding
388 and fall migratory butterflies returning to Mexico; estimates of abundance for these separate
389 generations may be required to model how different stages of the lifecycle contribute to the long-
390 term decline in the winter colonies (77).

391 Even with systematic monitoring, accurate estimates of insect abundance are missing
392 from many species—a fifth of regularly observed species in did not meet our minimum data
393 requirements to for us to estimate trends. None of these species are considered to be of
394 conservation concern, but this also means that we would be limited in our ability to even
395 determine if their populations have reached threatened status. Targeted surveys of selected
396 species, non-adult life stages, or habitats can expand the monitoring to data-deficient species
397 commonly excluded by protocols designed to monitor many species efficiently (50) and can be
398 used to estimate demographic responses to environmental drivers not apparent from adult
399 butterfly counts (79). Additional life-history knowledge about species in our study could inform
400 how worried we should be about extreme population declines, like the Baltimore (*Euphydryas*
401 *phaeton*). We noticed a multiyear population cycle, not captured by log-linear trends or
402 generalized additive models, which reached a nadir at the end of our dataset and exaggerated the
403 Baltimore's decline (Fig B in S1 Appendix). In other cases, density-dependent population

404 regulation may help inform whether species are resilient to temporary declines and guide
405 predictions for future population trajectories (80).

406 Insect declines have multifaceted causes, and the relative impact of these causes is still
407 unknown (81). Although analysis of the causes of site differences in abundance or species trends
408 is beyond the scope of this study, we discuss three environmental drivers commonly associated
409 with global insect declines: climate change, habitat loss and fragmentation, and agricultural
410 intensification (81,82). If species' traits are associated with population trends, then their
411 relationships may suggest environmental changes driving population responses in species sharing
412 these traits (46,81,83). In this study, life-history traits were weakly predictive of population
413 trends, but their associations provide hypotheses that could be tested further (46).

414 *Habitat loss and fragmentation*

415 In Ohio, habitat loss and fragmentation plateaued well before butterfly monitoring
416 started, with human population growth slowing by 1970. In common with other Midwestern
417 states, Ohio had already lost tallgrass prairie species, such as the Regal Fritillary (*Speyeria*
418 *idalia*), due to this habitat conversion to agriculture (25,26). Land-use has changed slowly over
419 the course of the monitoring program; less than 10% of monitoring sites have had more than
420 2.5% change in the surrounding (2.5-km radius) developed, agriculture, or forest land cover from
421 2001-2011. The persistence of butterfly populations in a landscape of habitat fragments are
422 mediated by species' traits that permit them to either move between more isolated resources or
423 persist in smaller, localized populations (82,84). Wing size is one life history trait associated
424 with dispersal ability, but it had no association with species' population trends (Tables A and B
425 in S1 Appendix). However, defining habitat patches by land-use classes overlooks how mobile
426 insect populations are bound by resources, varying across the lifecycle, rather than area (85,86).

427 Although there has been little wholesale habitat conversion around our study transects,
428 degradation of the remaining habitat could be a cause of the general decline in butterfly
429 abundance.

430 *Climate change*

431 Species trends are associated with two life-history traits, voltinism and range distribution,
432 which suggest that the butterfly community is changes with the warming climate. Species that
433 complete more annual generations, or multivoltine species, had more positive abundance trends.
434 This aligns with obligate univoltine species becoming less common in Massachusetts (20), but is
435 the opposite of the findings in Spain where multivoltine species are in steeper declines with
436 exposure to increasingly dry summers (39). Multivoltine species may be more adaptive to annual
437 and spatial variation in seasonal temperatures as many have plasticity in the voltinism observed
438 within Ohio (25). For most of the species with flexible voltinism in Ohio, adding an extra
439 generation in warmer summers increases their annual population growth rates (54). Northern-
440 distributed species have more negative population trends compared to widely-distributed or
441 southern species. This corresponds with findings from Massachusetts and Europe that warm-
442 adapted species are replacing cool-adapted species as range distributions shift (20,87). Even
443 though these two traits should be increasing butterfly abundance for some species as the climate
444 warms, it has not been enough to prevent the overall decline in butterfly abundance.

445 *Agricultural intensification*

446 Cropland and pasture make up half of Ohio's land area, so we would expect agricultural
447 practices to affect statewide insect abundance. One assessment of pollinator habitat suitability
448 based on land-use, acres in conservation reserve programs, and crop type estimated an increase
449 in resources in Ohio from 1982 through 2002, followed by a stable trend (88). However,

450 agricultural practices can decrease insect abundance with systemic insecticides, herbicide use on
451 host plants or nectar resources, and nitrogen fertilization that alters the composition of
452 surrounding plant communities.

453 In Ohio, the use of neonicotinoids rapidly increased after 2004 when they became widely
454 used on corn and soybeans (89,90). The mechanistic link between neonicotinoid insecticides and
455 insect declines is established and observational studies have shown widespread impacts of their
456 use (91–93). Even though seed-coatings with neonicotinoids reduce broadcast spraying, the
457 mechanical planting of these seeds exposes widespread areas around farms to contaminated dust
458 that is incorporated into non-target plants and insects (94,95). In the United Kingdom and
459 California, neonicotinoids are associated with butterfly declines (22,96) and hinder butterfly
460 larval development on host plants (97). We did not design this study to test whether
461 neonicotinoids affect butterfly abundance in Ohio. However, the observed declines across
462 common, invasive species, which would typically be predicted to exploit an agricultural or
463 human-altered landscape, would be consistent with widespread exposure to insecticides.

464 Species that eat forbs as larvae have negative population declines (Fig 3). Both herbicide
465 use and nitrogen deposition may alter plant communities to favor grasses over forbs (98).
466 Milkweed losses contribute to declines in Monarch butterfly abundance, as they lose host333
467 plants as a result of herbicide use (78,79). In Ohio, glyphosate use has increased linearly, and is
468 now applied at 6 times the rate it was in 1996 (89,90). Nitrogen increases have been linked to
469 declines in grassland species adapted to low-nitrogen environments (99–101) and to higher
470 mortality during larval development on enriched host plants (102).

471

472 **Conclusions**

Systematic, long-term surveys of butterflies provide the most rigorous evidence for the rate of insect declines. This study demonstrates that defaunation is happening in North America similarly to Europe. In landscapes comprising natural areas amid heavy human land-use, butterfly total abundance is declining at 2% per year and 2-3 times more species have population trends declining rather than increasing. Additional Pollard-based monitoring programs in North America, listed in (9), will enable tracking insect trends over larger spatial extents as with efforts to integrate data across European monitoring schemes (11). The rates for other insect groups may deviate from this baseline and were previously estimated to be declining more rapidly than Lepidoptera (1). Expanded monitoring and support for taxonomists are imperative for other taxa and under sampled regions, like the Tropics where most insect diversity resides. Besides the evaluation if butterfly trends generalize to other insects, the most urgent need for science and conservation is understanding the causes of decline and testing mitigation actions. As butterflies are the best-monitored insect taxa, they are the best indicator of the baseline threat to the 5.5 million insects, the most diverse group of animals on earth.

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497 **References**

498 1. Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. Defaunation in the
499 Anthropocene. Science. 2014 Jul 25;345(6195):401–6.

500 2. May RM. How many species are there on earth? Science. 1988;241(4872):1441–1449.

501 3. Conrad KF, Fox R, Woiwod IP. Monitoring biodiversity: measuring long-term changes in
502 insect abundance. Insect Conserv Biol. 2007;203–225.

503 4. Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. More than 75
504 percent decline over 27 years in total flying insect biomass in protected areas. Lamb EG,
505 editor. PLOS ONE. 2017 Oct 18;12(10):e0185809.

506 5. Lister BC, Garcia A. Climate-driven declines in arthropod abundance restructure a
507 rainforest food web. Proc Natl Acad Sci. 2018 Oct 30;115(44):E10397–406.

508 6. Winfree R, W. Fox J, Williams NM, Reilly JR, Cariveau DP. Abundance of common
509 species, not species richness, drives delivery of a real-world ecosystem service. Shea K,
510 editor. Ecol Lett. 2015 Jul;18(7):626–35.

511 7. Warren MS, Hill JK, Thomas JA, Asher J, Fox R, Huntley B, et al. Rapid responses of
512 British butterflies to opposing forces of climate and habitat change. 2001;414:5.

513 8. Parmesan C. Ecological and Evolutionary Responses to Recent Climate Change. Annu
514 Rev Ecol Evol Syst. 2006 Dec;37(1):637–69.

515 9. Taron D, Ries L. Butterfly Monitoring for Conservation. In: Daniels JC, editor. Butterfly
516 Conservation in North America [Internet]. Dordrecht: Springer Netherlands; 2015 [cited
517 2018 Dec 7]. p. 35–57. Available from: http://link.springer.com/10.1007/978-94-017-
518 9852-5_3

519 10. Pollard E, Yates TJ. Monitoring butterflies for ecology and conservation: the British
520 butterfly monitoring scheme. Springer Science & Business Media; 1994.
521 11. Van Swaay C, Van Strien A, Aghababyan K, Astrom S, Botham M, Brereton T, et al. The
522 European Butterfly Indicator for Grassland species: 1990-2013. 2015;
523 12. Schmucki R, Pe'er G, Roy DB, Stefanescu C, Van Swaay CAM, Oliver TH, et al. A
524 regionally informed abundance index for supporting integrative analyses across butterfly
525 monitoring schemes. Müller J, editor. J Appl Ecol. 2016 Apr;53(2):501–10.
526 13. Thomas JA. Monitoring change in the abundance and distribution of insects using
527 butterflies and other indicator groups. Philos Trans R Soc B Biol Sci. 2005 Feb
528 28;360(1454):339–57.
529 14. Brereton T, Roy DB, Middlebrook I, Botham M, Warren M. The development of butterfly
530 indicators in the United Kingdom and assessments in 2010. J Insect Conserv. 2011
531 Apr;15(1–2):139–51.
532 15. Dennis EB, Morgan BJT, Roy DB, Brereton TM. Urban indicators for UK butterflies.
533 Ecol Indic. 2017 May;76:184–93.
534 16. Agrawal AA, Inamine H. Mechanisms behind the monarch's decline. Science.
535 2018;360(6395):1294–1296.
536 17. Schultz CB, Brown LM, Pelton E, Crone EE. Citizen science monitoring demonstrates
537 dramatic declines of monarch butterflies in western North America. Biol Conserv.
538 2017;214:343–346.
539 18. Belitz MW, Hendrick LK, Monfils MJ, Cuthrell DL, Marshall CJ, Kawahara AY, et al.
540 Aggregated occurrence records of the federally endangered Poweshiek skipperling
541 (Oarisma poweshiek). Biodivers Data J. 2018;(6).

19. Haddad NM. Resurrection and resilience of the rarest butterflies. PLOS Biol. 2018 Feb 6;16(2):e2003488.

20. Breed GA, Stichter S, Crone EE. Climate-driven changes in northeastern US butterfly communities. Nat Clim Change. 2013 Feb;3(2):142–5.

21. Forister ML, McCall AC, Sanders NJ, Fordyce JA, Thorne JH, O'Brien J, et al. Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity. Proc Natl Acad Sci. 2010 Feb 2;107(5):2088–92.

22. Forister ML, Cousens B, Harrison JG, Anderson K, Thorne JH, Waetjen D, et al. Increasing neonicotinoid use and the declining butterfly fauna of lowland California. Biol Lett. 2016 Aug;12(8):20160475.

23. Schlicht D, Swengel A, Swengel S. Meta-analysis of survey data to assess trends of prairie butterflies in Minnesota, USA during 1979–2005. J Insect Conserv. 2009 Aug;13(4):429–47.

24. Swengel SR, Schlicht D, Olsen F, Swengel AB. Declines of prairie butterflies in the midwestern USA. J Insect Conserv. 2011 Apr;15(1–2):327–39.

25. Iftner DC, Shuey JA, Calhoun JV. Butterflies and skippers of Ohio. College of Biological Sciences, Ohio State University; 1992.

26. Shuey JA, Calhoun JV, Iftner DC. Butterflies that are endangered, threatened, and of special concern in Ohio. 1987;

27. Bigelow D, Borchers A. Major uses of land in the United States, 2012. 2017.

28. Thornton PE, Running SW, White MA. Generating surfaces of daily meteorological variables over large regions of complex terrain. J Hydrol. 1997 Mar;190(3–4):214–51.

29. Homer C, Dewitz J, Yang L, Jin S, Danielson P, Xian G, et al. Completion of the 2011 National Land Cover Database for the conterminous United States–representing a decade of land cover change information. Photogramm Eng Remote Sens. 2015;81(5):345–354.

30. Diamond SE, Cayton H, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, et al. Unexpected phenological responses of butterflies to the interaction of urbanization and geographic temperature. Ecology. 2014 Sep;95(9):2613–21.

31. Dennis EB, Freeman SN, Brereton T, Roy DB. Indexing butterfly abundance whilst accounting for missing counts and variability in seasonal pattern. O'Hara RB, editor. Methods Ecol Evol. 2013 Jul;4(7):637–45.

32. Opler PA, Krizek GO. Butterflies east of the Great Plains: an illustrated natural history. Johns Hopkins Univ Pr; 1984.

33. Dennis EB, Morgan BJT, Freeman SN, Brereton TM, Roy DB. A generalized abundance index for seasonal invertebrates: A Generalized Abundance Index for Seasonal Invertebrates. Biometrics. 2016 Dec;72(4):1305–14.

34. Wood SN. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC; 2017.

35. Cordano EE& E. Interpol.T: Hourly interpolation of multiple temperature daily series [Internet]. 2013. Available from: https://CRAN.R-project.org/package=Interpol.T

36. Link WA, Sauer JR. Estimating Population Change from Count Data: Application to the North American Breeding Bird Survey. Ecol Appl. 1998 May;8(2):258.

37. Pellet J, Bried JT, Parietti D, Gander A, Heer PO, Cherix D, et al. Monitoring Butterfly Abundance: Beyond Pollard Walks. Schweiger O, editor. PLoS ONE. 2012 Jul 30;7(7):e41396.

587 38. Henry EH, Anderson CT. Abundance estimates to inform butterfly management: double-
588 observer versus distance sampling. J Insect Conserv. 2016 Jun;20(3):505–14.
589 39. Melero Y, Stefanescu C, Pino J. General declines in Mediterranean butterflies over the last
590 two decades are modulated by species traits. Biol Conserv. 2016 Sep;201:336–42.
591 40. Haddad NM, Hudgens B, Damiani C, Gross K, Kuefler D, Pollock K. Determining
592 Optimal Population Monitoring for Rare Butterflies: *Monitoring Rare Butterflies*. Conserv
593 Biol. 2008 Aug;22(4):929–40.
594 41. Isaac NJB, Cruickshanks KL, Weddle AM, Marcus Rowcliffe J, Brereton TM, Dennis
595 RLH, et al. Distance sampling and the challenge of monitoring butterfly populations:
596 Distance sampling and monitoring butterflies. Methods Ecol Evol. 2011 Dec;2(6):585–94.
597 42. Knape J. Decomposing trends in Swedish bird populations using generalized additive
598 mixed models. Siriwardena G, editor. J Appl Ecol. 2016 Dec;53(6):1852–61.
599 43. Szabo JK, Vesk PA, Baxter PWJ, Possingham HP. Regional avian species declines
600 estimated from volunteer-collected long-term data using List Length Analysis. Ecol Appl.
601 2010;20(8):2157–69.
602 44. van Strien AJ, van Swaay CAM, Termaat T. Opportunistic citizen science data of animal
603 species produce reliable estimates of distribution trends if analysed with occupancy
604 models. Devictor V, editor. J Appl Ecol. 2013 Dec;50(6):1450–8.
605 45. Roy DB, Ploquin EF, Randle Z, Risely K, Botham MS, Middlebrook I, et al. Comparison
606 of trends in butterfly populations between monitoring schemes. J Insect Conserv. 2015
607 Apr;19(2):313–24.

46. Soykan CU, Sauer J, Schuetz JG, LeBaron GS, Dale K, Langham GM. Population trends for North American winter birds based on hierarchical models. Ecosphere. 2016;7(5):e01351.

47. Kraemer HC, Blasey CM. Centring in regression analyses: a strategy to prevent errors in statistical inference. Int J Methods Psychiatr Res. 2004;13(3):141–51.

48. Harrison XA. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ. 2014 Oct 9;2:e616.

49. Pannekoek J, Van Strien A. Trim 3 Manual (TRends & Indices for Monitoring data)– Statistics Netherlands. Voorburg; 2001.

50. Brereton T, Botham M, Middlebrook I, Randle Z, Noble D, Harris S, et al. United Kingdom Butterfly Monitoring Scheme report for 2017. Centre for Ecology & Hydrology & Butterfly Conservation; 2018.

51. Van Swaay CAM, Bos G, Van Grunsven RHA, Kok J, Huskens K, Van Deijk JR, et al. Vlinders en libellen geteld: Jaarverslag 2017 [Internet]. De Vlinderstichting, Wageningen; 2018 [cited 2019 Feb 14]. Report No.: Rapport VS2018.006. Available from: https://assets.vlinderstichting.nl/docs/6d51f174-b497-4777-b84a-362e344c3528.pdf

52. Stefanescu C. Resum de les temporades 2015 i 2016. Cynthia Butlletí Butterfly Monit Scheme Catalunya [Internet]. 2018 [cited 2019 Feb 14];14. Available from: http://www.catalanbms.org/es/cynthia/

53. Rohatgi A. WebPlotDigitizer. 2011.

54. Wepprich TM. Effects of Climatic Variability on a Statewide Butterfly Community. North Carolina State University; 2017.

630 55. Diamond SE, Frame AM, Martin RA, Buckley LB. Species' traits predict phenological
631 responses to climate change in butterflies. 2011;92(5):8.
632 56. Pagel M. Inferring the historical patterns of biological evolution. Nature. 1999
633 Oct;401(6756):877–84.
634 57. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids
635 Res. 2016 Jan 4;44(Database issue):D67–72.
636 58. Ratnasingham S, Hebert PD. BOLD: The Barcode of Life Data System (http://www.
637 barcodinglife. org). Mol Ecol Notes. 2007;7(3):355–364.
638 59. Espeland M, Breinholt J, Willmott KR, Warren AD, Vila R, Toussaint EFA, et al. A
639 Comprehensive and Dated Phylogenomic Analysis of Butterflies. Curr Biol. 2018
640 Mar;28(5):770-778.e5.
641 60. R Core Team. R: A Language and Environment for Statistical Computing [Internet].
642 Vienna, Austria: R Foundation for Statistical Computing; 2018. Available from:
643 https://www.R-project.org/
644 61. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using
645 **lme4**. J Stat Softw [Internet]. 2015 [cited 2018 Dec 4];67(1). Available from:
646 http://www.jstatsoft.org/v67/i01/
647 62. Orme D, Freckleton R, Thomas G, Petzoldt T. The caper package: comparative analysis of
648 phylogenetics and evolution in R. R Package Version. 2013;5(2).
649 63. Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary
650 analyses in R. Bioinformatics. 2018;xx:xxx–xxx.
651 64. Knowles JE, Frederick C. merTools: Tools for Analyzing Mixed Effect Regression
652 Models [Internet]. 2018. Available from: https://CRAN.R-project.org/package=merTools

653 65. Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from
654 generalized linear mixed-effects models. O'Hara RB, editor. Methods Ecol Evol. 2013
655 Feb;4(2):133–42.
656 66. Barton K. MuMIn: Multi-Model Inference [Internet]. 2018. Available from:
657 https://CRAN.R-project.org/package=MuMIn
658 67. Conrad KF, Woiwod IP, Parsons M, Fox R, Warren MS. Long-term population trends in
659 widespread British moths. J Insect Conserv. 2004;8(2–3):119–136.
660 68. Hunter MD, Kozlov MV, Itämies J, Pulliainen E, Bäck J, Kyrö E-M, et al. Current
661 temporal trends in moth abundance are counter to predicted effects of climate change in an
662 assemblage of subarctic forest moths. Glob Change Biol. 2014 Jun;20(6):1723–37.
663 69. Herrera CM. Complex long-term dynamics of pollinator abundance in undisturbed
664 Mediterranean montane habitats over two decades. Ecol Monogr. 2019 Feb
665 1;89(1):e01338.
666 70. Cech R, Tudor G, others. Butterflies of the east coast. Princeton University Press; 2005.
667 71. Ryan SF, Lombaert E, Espeset A, Vila R, Talavera G, Dincă V, et al. Global invasion
668 history of the world's most abundant pest butterfly: a citizen science population genomics
669 study. bioRxiv. 2018 Dec 26;506162.
670 72. McKinney ML, Lockwood JL. Biotic homogenization: a few winners replacing many
671 losers in the next mass extinction. Trends Ecol Evol. 1999;14(11):450–453.
672 73. Clavel J, Julliard R, Devictor V. Worldwide decline of specialist species: toward a global
673 functional homogenization? Front Ecol Environ. 2011;9(4):222–8.

74. Van Dyck H, Van Strien AJ, Maes D, Van Swaay CAM. Declines in Common, Widespread Butterflies in a Landscape under Intense Human Use. Conserv Biol. 2009 Aug;23(4):957–65.

75. Ries L, Oberhauser K, Taron D, Battin J, Rendon-Salinas E, Altizer S, et al. Connecting eastern monarch population dynamics across their migratory cycle. Monarchs Chang World Biol Conserv Iconic Insect Cornell Univ Press Ithaca NY. 2015;268–281.

76. Pleasants JM, Williams EH, Brower LP, Oberhauser KS, Taylor OR. Conclusion of No Decline in Summer Monarch Population Not Supported. Ann Entomol Soc Am. 2016 Mar 1;109(2):169–71.

77. Saunders SP, Ries L, Neupane N, Ramírez MI, García-Serrano E, Rendón-Salinas E, et al. Multiscale seasonal factors drive the size of winter monarch colonies. Proc Natl Acad Sci. 2019 Mar 18;201805114.

78. Saunders SP, Ries L, Oberhauser KS, Thogmartin WE, Zipkin EF. Local and cross-seasonal associations of climate and land use with abundance of monarch butterflies *Danaus plexippus*. Ecography. 2018 Feb;41(2):278–90.

79. Pleasants JM, Oberhauser KS. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population: *Herbicide use and monarch butterflies*. Insect Conserv Divers. 2013 Mar;6(2):135–44.

80. Oliver TH, Roy DB. The pitfalls of ecological forecasting. Biol J Linn Soc. 2015 Jul;115(3):767–78.

81. Fox R. The decline of moths in Great Britain: a review of possible causes: *The decline of moths in Great Britain*. Insect Conserv Divers. 2013 Jan;6(1):5–19.

82. Thomas JA. Butterfly communities under threat. Science. 2016 Jul 15;353(6296):216–8.

697 83. Wong MKL, Guénard B, Lewis OT. Trait-based ecology of terrestrial arthropods. Biol
698 Rev [Internet]. 2018 [cited 2018 Dec 14];0(0). Available from:
699 https://onlinelibrary.wiley.com/doi/abs/10.1111/brv.12488
700 84. Habel JC, Schmitt T. Vanishing of the common species: Empty habitats and the role of
701 genetic diversity. Biol Conserv. 2018 Feb;218:211–6.
702 85. Dennis RLH, Shreeve TG, Dyck HV. Towards a Functional Resource-Based Concept for
703 Habitat: A Butterfly Biology Viewpoint. Oikos. 2003;102(2):417–26.
704 86. Curtis RJ, Brereton TM, Dennis RLH, Carbone C, Isaac NJB. Butterfly abundance is
705 determined by food availability and is mediated by species traits. Diamond S, editor. J
706 Appl Ecol. 2015 Dec;52(6):1676–84.
707 87. Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, et al. Poleward
708 shifts in geographical ranges of butterfly species associated with regional warming.
709 Nature. 1999 Jun;399(6736):579–83.
710 88. Hellerstein D. Land Use, Land Cover, and Pollinator Health: A Review and Trend
711 Analysis. :47.
712 89. Baker NT, Stone WW. Estimated annual agricultural pesticide use for counties of the
713 conterminous United States, 2008–12: U.S. Geological Survey Data Series 907 [Internet].
714 US Geological Survey; p. 9. (U.S. Geological Survey Data Series 907). Available from:
715 https://dx.doi.org/10.3133/ds907.
716 90. Stone WW. Estimated annual agricultural pesticide use for counties of the conterminous
717 United States, 1992–2009. US Geological Survey; 2013. (U.S. Geological Survey Data
718 Series 752).

719 91. Goulson D. REVIEW: An overview of the environmental risks posed by neonicotinoid
720 insecticides. Kleijn D, editor. J Appl Ecol. 2013 Aug;50(4):977–87.
721 92. Hallmann CA, Foppen RPB, van Turnhout CAM, de Kroon H, Jongejans E. Declines in
722 insectivorous birds are associated with high neonicotinoid concentrations. Nature. 2014
723 Jul;511(7509):341–3.
724 93. Pisa L, Goulson D, Yang E-C, Gibbons D, Sánchez-Bayo F, Mitchell E, et al. An update
725 of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts
726 on organisms and ecosystems. Environ Sci Pollut Res [Internet]. 2017 Nov 9 [cited 2019
727 Feb 1]; Available from: https://doi.org/10.1007/s11356-017-0341-3
728 94. Douglas MR, Tooker JF. Large-Scale Deployment of Seed Treatments Has Driven Rapid
729 Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S.
730 Field Crops. Environ Sci Technol. 2015 Apr 21;49(8):5088–97.
731 95. Krupke CH, Holland JD, Long EY, Eitzer BD. Planting of neonicotinoid-treated maize
732 poses risks for honey bees and other non-target organisms over a wide area without
733 consistent crop yield benefit. Diamond S, editor. J Appl Ecol. 2017 Oct;54(5):1449–58.
734 96. Gilburn AS, Bunnefeld N, Wilson JM, Botham MS, Brereton TM, Fox R, et al. Are
735 neonicotinoid insecticides driving declines of widespread butterflies? PeerJ. 2015 Nov
736 24;3:e1402.
737 97. Basley K, Goulson D. Effects of Field-Relevant Concentrations of Clothianidin on Larval
738 Development of the Butterfly Polyommatus icarus (Lepidoptera, Lycaenidae). Environ Sci
739 Technol. 2018 Apr 3;52(7):3990–6.

98. Kleijn D, Snoeijing GIJ. Field Boundary Vegetation and the Effects of Agrochemical Drift: Botanical Change Caused by Low Levels of Herbicide and Fertilizer. J Appl Ecol. 1997;34(6):1413–25.

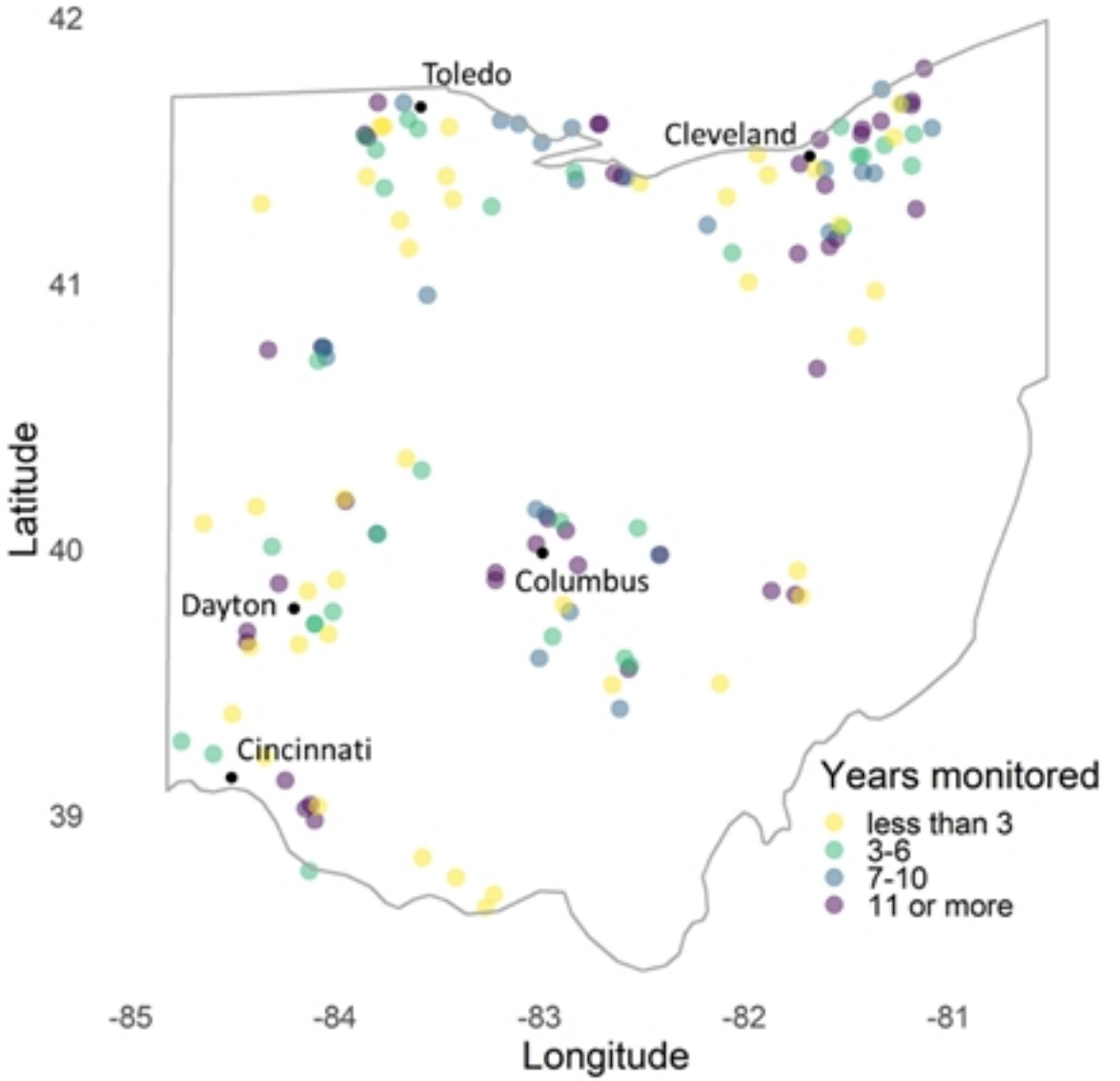
99. Weiss SB. Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. Conserv Biol. 1999;13(6):1476–1486.

100. Öckinger E, Hammarstedt O, Nilsson SG, Smith HG. The relationship between local extinctions of grassland butterflies and increased soil nitrogen levels. Biol Conserv. 2006 Apr 1;128(4):564–73.

101. WallisDeVries MF, van Swaay CAM. A nitrogen index to track changes in butterfly species assemblages under nitrogen deposition. Biol Conserv. 2017 Aug;212:448–53.

102. Kurze S, Heinken T, Fartmann T. Nitrogen enrichment in host plants increases the mortality of common Lepidoptera species. Oecologia. 2018 Dec;188(4):1227–37.

S1 Appendix. Supplementary methods and results. Includes detailed methods for phenology models and phylogenetic trees, one figure of species trends plotting on a cladogram, three figures showing population trends and annual variation for 81 species, two tables of model results from the trait analysis, and a table comparing our trend estimates with three other approaches.


42
41
40
39
Latitude
-85
-84
-83
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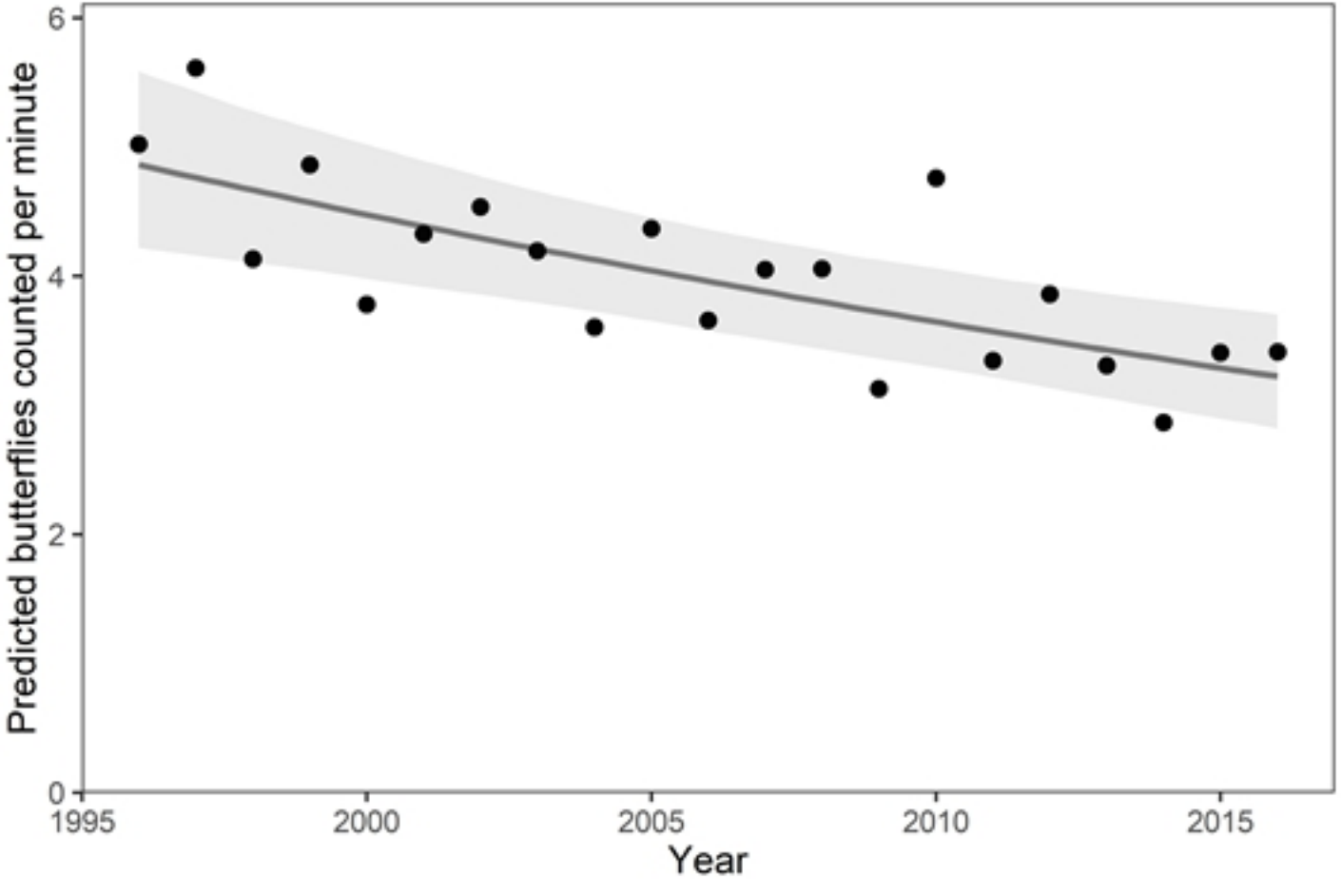
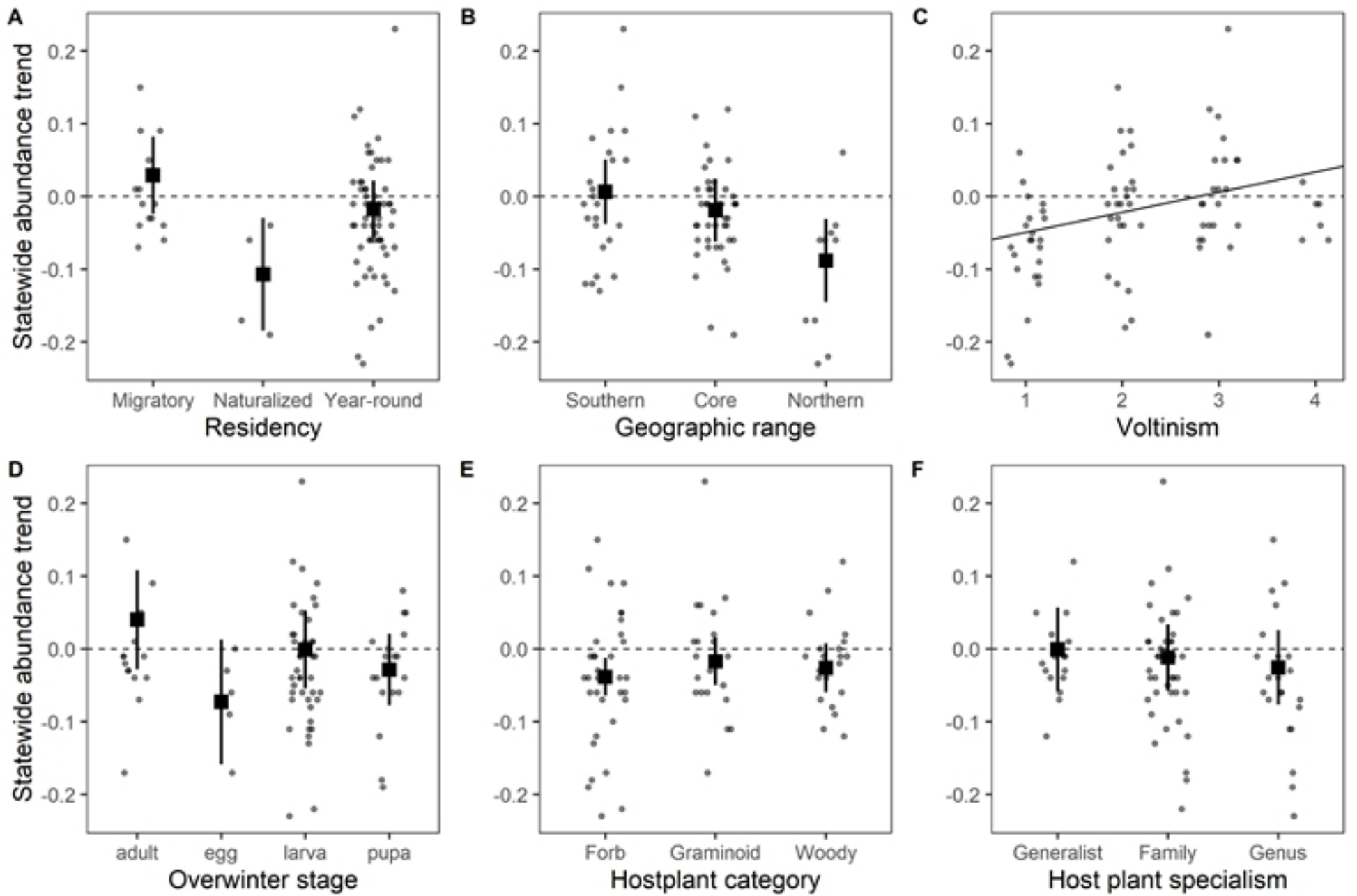
-81
Longitude
Toledo
Cleveland
Columbus
Dayton
Cincinnati
Years monitored
less than 3
3-6
7-10
11 or more

Figure


Predicted butterflies counted per minute
0
2
4
6
1995
2000
2005
2010
2015
Year

Figure


A
B
C
D
E
F
Statewide abundance trend
0.2
0.1
0.0
-0.1
-0.2
Migratory
Naturalized
Year-round
Residency
Southern
Core
Northern
Geographic range
1
2
3
4
Voltinism
adult
egg
larva
pupa
Overwinter stage
Forb
Graminoid
Woody
Hostplant category
Generalist
Family
Genus
Host plant specialism

Figure