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Increasing neonicotinoid use and the declining butterfly fauna of lowland California

Short title: Neonicotinoids and butterflies

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The butterfly fauna of lowland Northern California has exhibited a marked decline in recent years that previous studies have attributed in part to altered climatic conditions and changes in land use. Here we ask if a shift in insecticide use towards neonicotinoids is associated with butterfly declines at four sites in the region that have been monitored for four decades. A negative association between butterfly populations and increasing neonicotinoid application is detectable while controlling for land use and other factors, and appears to be more severe for smaller-bodied species. These results suggest that neonicotinoids could influence non-target insect populations occurring in proximity to application locations, and highlight the need for mechanistic work to complement long-term observational data.

Keywords: butterflies, insecticide, neonicotinoids, global change, long-term ecological data

1. INTRODUCTION

Understanding cumulative effects of multiple anthropogenic stressors on wild populations of plants and animals is of prime importance for twenty-first century ecology [1]. With one recent exception [2], the effects of deliberate application of insecticides have not been described for non-target taxa for which effects of other stressors, such as changing climate and land conversion (e.g., urbanization), have also been characterized. Here we address this knowledge gap by examining the use of neonicotinoid insecticides in Northern California, a region with a well-studied butterfly fauna.

Neonicotinoids are a relatively new class of synthetic nicotine-like insecticides that have increased in use during the last 20 years [3], partly because of ease of application: they are water soluble, relatively stable, and can be applied to seeds, soil or growing plants, with systemic uptake as the result [4]. Because they are systemic, effects on insects are not restricted to a particular plant tissue (e.g. leaf surfaces) or to a narrow post-application window. Moreover, runoff containing neonicotinoids from agricultural fields can be incorporated into tissues of plants growing nearby, which might include host plants and nectar resources for non-target insects [5]. Research into neonicotinoid exposure on honey bees and bumblebees has revealed a range of lethal and sublethal effects [6], but little is known regarding effects of neonicotinoids on other non-target insects.

Northern California is home to a rich butterfly fauna that has been monitored biweekly (every other week) for over 40 years [7]. Monitoring has revealed a decline in butterfly populations occurring at low elevations (less than 25 meters) within this region, especially since the late 1990s [8]. Previous analyses have implicated changing patterns of land use and warming fall and summer temperatures [9]. Notably, neither land conversion, nor shifting temperatures show evidence of increased rate of change concomitant with the butterfly declines beginning in the late 1990s. However, neonicotinoid use in the region began to increase dramatically at that time. Here we analyze county neonicotinoid application records in relation to both the total number of butterfly species observed per year, and in relation to occupancy records for individual species at individual sites, while controlling for land use and climatic effects.

2. MATERIALS AND METHODS

(a) *Butterfly and insecticide data*

Butterfly data were generated with biweekly Pollard walks along fixed transects for all species of butterfly (52 spp.) at four sites: Suisun Marsh (studied since 1972), West Sacramento (since 1988), North Sacramento (since 1988), and Rancho Cordova (since 1975); see [7,9] for site descriptions and additional details on data collection. These sites are embedded in a matrix of land use types that includes developed land (urban and suburban) and open spaces (agricultural lands, public recreational areas and others) [9]. For each site, the total number of species observed per year was represented as an effective number of species by taking the exponential of the Shannon diversity index, which combines richness and evenness [10]. Evenness for each species is informed by variation in the number of days observed in a given year out of the total number of visits to a site.

Data describing annual use of insecticides by county were compiled for five common neonicotinoid insecticides, as well as for the four most widely used non-neonicotinoid insecticide classes. These data, originating from the California Department of Pesticide Regulation, were obtained from the US Geological Survey National Pesticide Use database (details in the electronic supplementary material), but do not include all types of use, and thus likely underestimate total application.

(b) *Faunal analyses*

We developed two linear mixed models, one focused on neonicotinoids and a second encompassing other factors of interest, particularly land conversion. Both models included site (N=4) as a random (intercept) effect, the numbers of visits (a control for sampling effort), and the effective number of butterfly species as the dependent variable. The first model also included year, while the second model included average minimum daily summer temperature [9] and "converted land", a county level index (available every other year) of the amount of land that has been converted to urban or suburban spaces. For more information on the index of land conversion, the choice of climatic data, and other details of analyses see the electronic supplementary material. Finally, change through time in the butterfly fauna was visualized with the aid of a spline with a single inflection point as implemented in the R package SiZer [11].

(c) *Species-specific analyses*

In order to investigate species-specific sensitivities to neonicotinoids, we used a hierarchical Bayesian binomial regression that estimates population-level beta coefficients, as described in detail elsewhere [12,13]. The model included annual neonicotinoid totals (kg.) for each county, as well as year, with the response variable being the number of days butterflies were observed (for each species) out of the total number of days that each site was visited. Posterior probability distributions were used to calculate species-specific beta coefficients summarizing associations with neonicotinoid use (further details in electronic supplementary material). Beta coefficients were then examined in simple linear models with the following predictors: wingspan, geographic range, number of broods per year, resident status, overwintering mode, number of host genera, and ruderal status (a composite natural history variable encompassing variation in dispersiveness and association with disturbed habitats [8]). We also considered the relationship between neonicotinoid sensitivities and beta coefficients for year (from the same Bayesian models) to ask if species in more severe decline were estimated to have greater sensitivity to neonicotinoids.

3. RESULTS

Our four study sites exhibited a dramatic decline in the numbers of butterfly species observed annually starting in the late 1990s: the breakpoint estimated by spline inflection was 1997 (figure 1a). Neonicotinoid use began in the region in 1995 and has been increasing dramatically (figure 1b) in comparison with other insecticide classes showing largely static or declining usage (with the exception of a recent increase in pyrethroids; figure 1b). A negative relationship between neonicotinoid use and annual variation in butterfly species observations was readily detectable (likelihood ratio 7.16, $P = 0.0075$; table 1, figure 1c), which was true while controlling for year as an independent variable. Although a less powerful approach, we also considered a simple correlation between detrended variables: with the annual trend in both neonicotinoids and butterfly richness removed prior to analysis, the negative relationship is still detected (Pearson correlation coefficient -0.25, $P = 0.066$).

A relationship between neonicotinoid application and the number of butterfly species was also successfully modeled while accounting for effects of summer temperature and land conversion, with the effect of the latter roughly equal to the effect of neonicotinoids (table 1b). At the level of individual species, those with the strongest negative association with neonicotinoid use also experienced more severe declines (see the year effect in table 2). They also tended to be smaller-bodied species (figure 1d) with fewer generations per year (table 2): the mean (± s.e) neonicotinoid effect for single brooded species was negative (-0.05 ± 0.078), and positive for multiple-brooded species (0.013 ± 0.072).

4. DISCUSSION

California is a hotspot of biological diversity, as well as an area of rapid human population growth and land development [14]. The Central Valley of California has also seen some of the most intense use of neonicotinoids in the country [3]. Here, we find that neonicotinoid application is negatively associated with butterfly populations in the region. Furthermore, the effect of neonicotinoids is detectable while accounting for land conversion, and effects of the two factors are roughly equal in magnitude. The species most negatively associated with neonicotinoids are smaller bodied and have fewer generations per year, traits that may confer a reduced capacity for response to stressors.

Our results derive from observations aggregated at a broad spatial scale, specifically at the county level (for insecticide and land use data), which should limit our ability to detect associations between stressors and butterfly declines. However, detection of associations even at this crude spatial scale raises the possibility that neonicotinoid insecticides are having a negative effect on butterfly populations occurring in areas undergoing insecticide application. Experimental work documenting non-target effects of neonicotinoids on honey bees and bumblebees has been extensive [15,16], and while only one experimental study on butterflies has been reported [5], many studies have documented negative effects of neonicotinoids on pest moths [e.g., 17]. The findings reported here should encourage researchers to broaden the scope of investigations beyond narrow temporal and spatial windows of application to understand spillover effects on non-target species and possible indirect effects on other species, including bats and insectivorous birds.

Data accessibility. Butterfly data are available at AMS's site (http://butterfly.ucdavis.edu/), and insecticide data are publically available, as explained in text.

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Competing interests. We have no competing interests. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Figure legend

Figure 1. *(a)* The number of observed butterfly species at four sites (SM: Suisun Marsh; WS: West Sacramento; NS: North Sacramento; RC: Rancho Cordova). The response variable (in *a* and *c*) is the exponential of Shannon diversity, i.e., the effective number of species; the spline knot in *a* is 1997 (95% confidence interval: 1990-2001). *(b)* Insecticide application for neonicotinoids in focal counties (colored lines), and for the four most commonly-applied non-neonicotinoid classes (gray lines). The non-neonicotinoids are, in decreasing order of line elevation in 1995, organophosphates, carbamates, pyrethroids, and organochlorines (lines are county averages). Note the different range of years in the first two panels, as *(b)* starts in the year in which neonicotinoids are first reported. *(c)* Relationship between number of butterfly species and neonicotinoids (values of the latter at zero jittered for visualization). *(d)* Response of individual species to neonicotinoids as predicted by wingspan; more negative values on the y-axis indicate species with more negative associations with neonicotinoids. Gray polygons in panels *(a)*, *(c)*, and *(d)* are 95% confidence intervals. *Pyrgus scriptura* (in *d)*, is one of the smallest species in the fauna; drawing by MLF.

Table 1. Results from linear mixed models, showing standardized beta coefficients and likelihood ratio tests for fixed effects. Model in *(a)* includes only neonicotinoid application, year, and visits (for sampling effort), while *(b)* includes the effect of land use ("converted land") as well as the previous summer's average daily minimum temperature ("summer temp."). Both models included site as a random effect, and the response variable in both cases was the effective number of butterfly species.

Factor	Estimate (± SE)	Lik. ratio	P
(a)			
Neonicotinoids	-0.32 (0.12)	7.16	0.0075
Year	-0.49 (0.11)	17.81	<0.0001
Visits	-0.075 (0.07)	1.39	0.24
(b)			
Neonicotinoids	-0.43 (0.15)	8.24	0.0041
Converted land	-0.48 (0.17)	6.91	0.0086
Summer temp.	-0.074 (0.084)	0.96	0.33
Visits	-0.025 (0.13)	0.068	0.79

Table 2. Results from analyses of species-specific properties and sensitivity to neonicotinoids. Each row is a separate model (linear regressions in *(a)* and analyses of variance in *(b)*) with different independent variables and the response variable in all cases being the standardized beta coefficients from hierarchical Bayesian models estimating the association between neonicotinoid usage and interannual variation in butterfly observations. Estimates of standardized beta coefficients are shown for regressions.

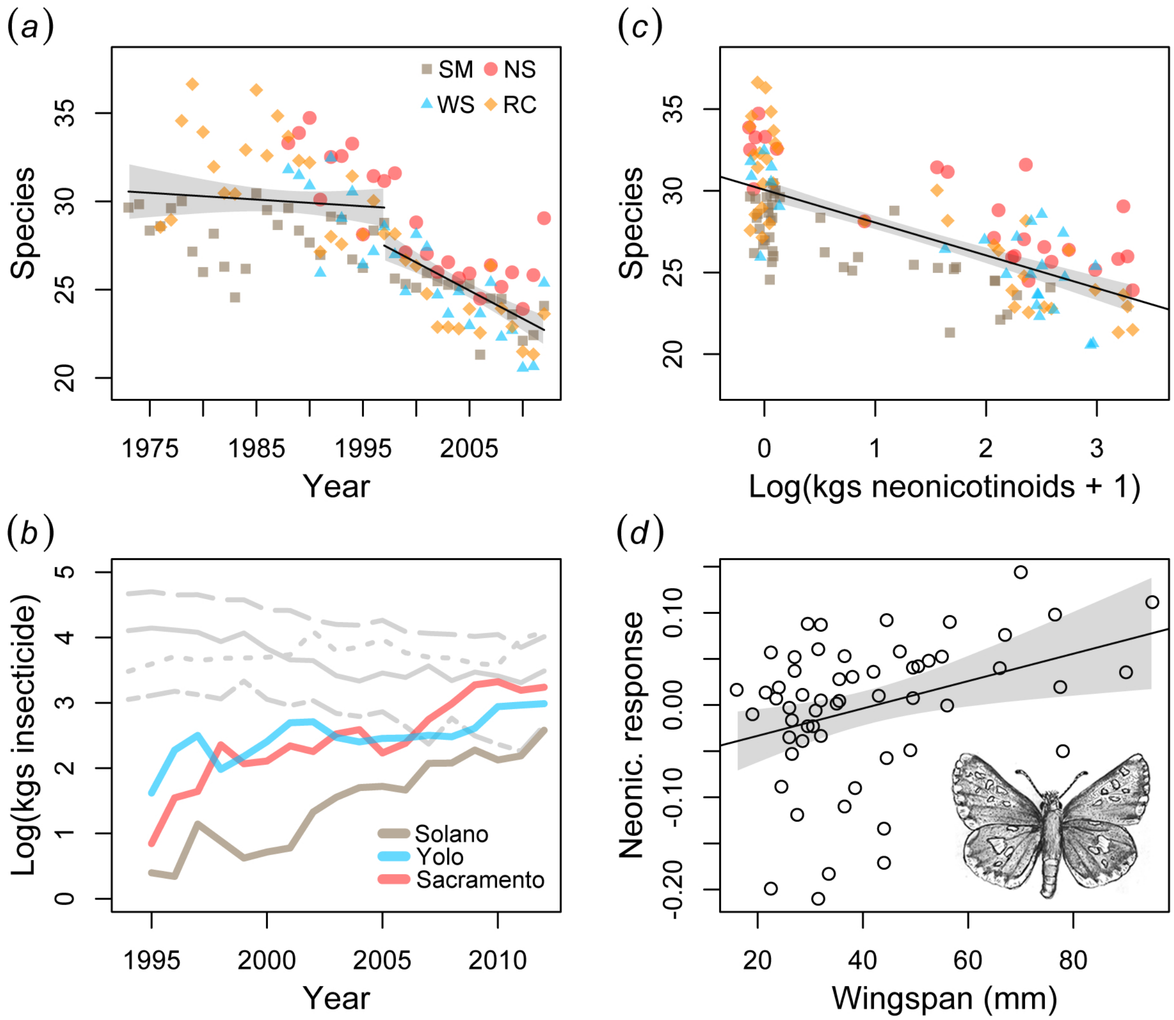
(a)

Factor	Estimate (± SE)	P	F_{df}	R^2
Year	0.037 (0.0091)	0.00014	$16.79_{1,55}$	0.23
Wingspan	0.027 (0.0098)	0.0080	$7.58_{1,55}$	0.12
Geographic range	0.0048 (0.0099)	0.63	$0.24_{1,50}$	0.0047

(b)

Number of broods		0.026	$3.91_{2,54}$	0.13
Resident status		0.099	$2.42_{2,51}$	0.087
Overwintering mode		0.28	$1.33_{3,34}$	0.10
Ruderal status		0.21	$1.58_{1,55}$	0.028
Number of host genera		0.54	$0.79_{4,36}$	0.080

Fig. 1


(a)
SM
NS
WS
RC
Species
Year
1975
1985
1995
2005
20
25
30
35
(c)
Species
Log(kgs neonicotinoids + 1)
0
1
2
3
(b)
Log(kgs insecticide)
Year
1995
2000
2005
2010
0
1
2
3
4
5
Solano
Yolo
Sacramento
(d)
Neonic. response
Wingspan (mm)
20
40
60
80
-0.20
-0.10
0.00
0.10