



## Research

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## Conservation biology

# Increasing neonicotinoid use and the declining butterfly fauna of lowland California

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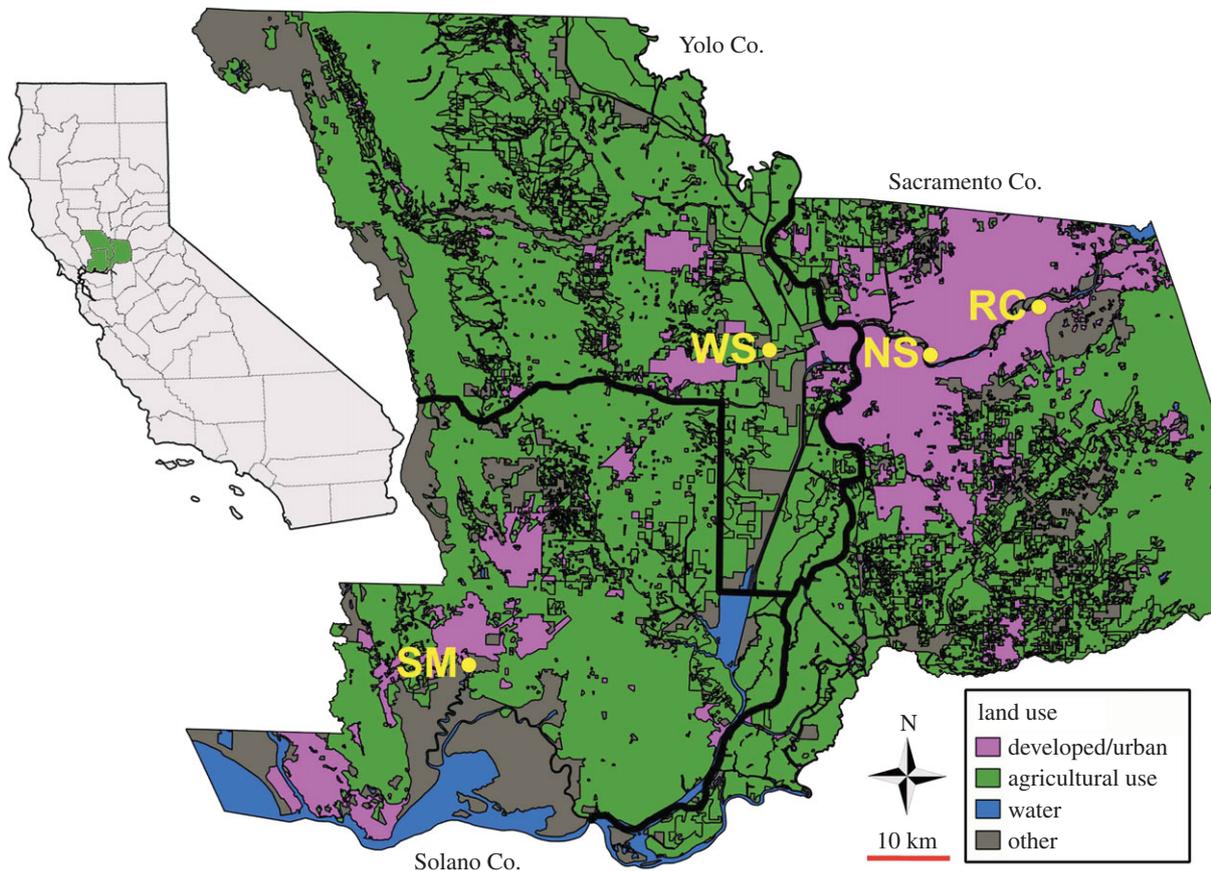
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The butterfly fauna of lowland Northern California has exhibited a marked decline in recent years that previous studies have attributed in part to altered climatic conditions and changes in land use. Here, we ask if a shift in insecticide use towards 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 highlights the need for mechanistic work to complement long-term observational 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, have also been quantified with long-term data. Here, we examine the use of neonicotinoid insecticides in Northern California, a region with a well-studied butterfly fauna.

Neonicotinoids are a relatively new class of nicotine-like insecticides that have increased in use during the last 20 years (<https://water.usgs.gov/nawqa/pnsp/usage/maps/>). They are water soluble, relatively stable, and can be applied to seeds, soil or growing plants, with systemic uptake [3]. 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, run-off containing neonicotinoids from agricultural fields or urban areas can be incorporated into tissues of nearby plants, which might include host plants and nectar resources for non-target insects [4]. Research into neonicotinoid exposure on honeybees and bumblebees has revealed a range of lethal and sub-lethal effects [5], but little is known regarding the effects of neonicotinoids on other non-target insects, especially under field conditions.



**Figure 1.** Land use around study sites (data from ([http://www.conservation.ca.gov/dlrp/fmmp/Pages/county\\_info.aspx](http://www.conservation.ca.gov/dlrp/fmmp/Pages/county_info.aspx))), with the three counties highlighted on the California map at left. The ‘other’ category includes commercial real estate, undeveloped urban land, and non-agricultural vegetation [8].

Northern California is home to a rich butterfly fauna that has been monitored every other week for over 40 years [6]. Monitoring has revealed a decline in butterfly populations at low elevations (less than 25 m), especially since the late 1990s [7]. Previous analyses have implicated changing patterns of land use and warming autumn and summer temperatures [8]. 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 analyse 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, while controlling for land use and climatic effects.

## 2. Material and methods

### (a) Butterfly and insecticide data

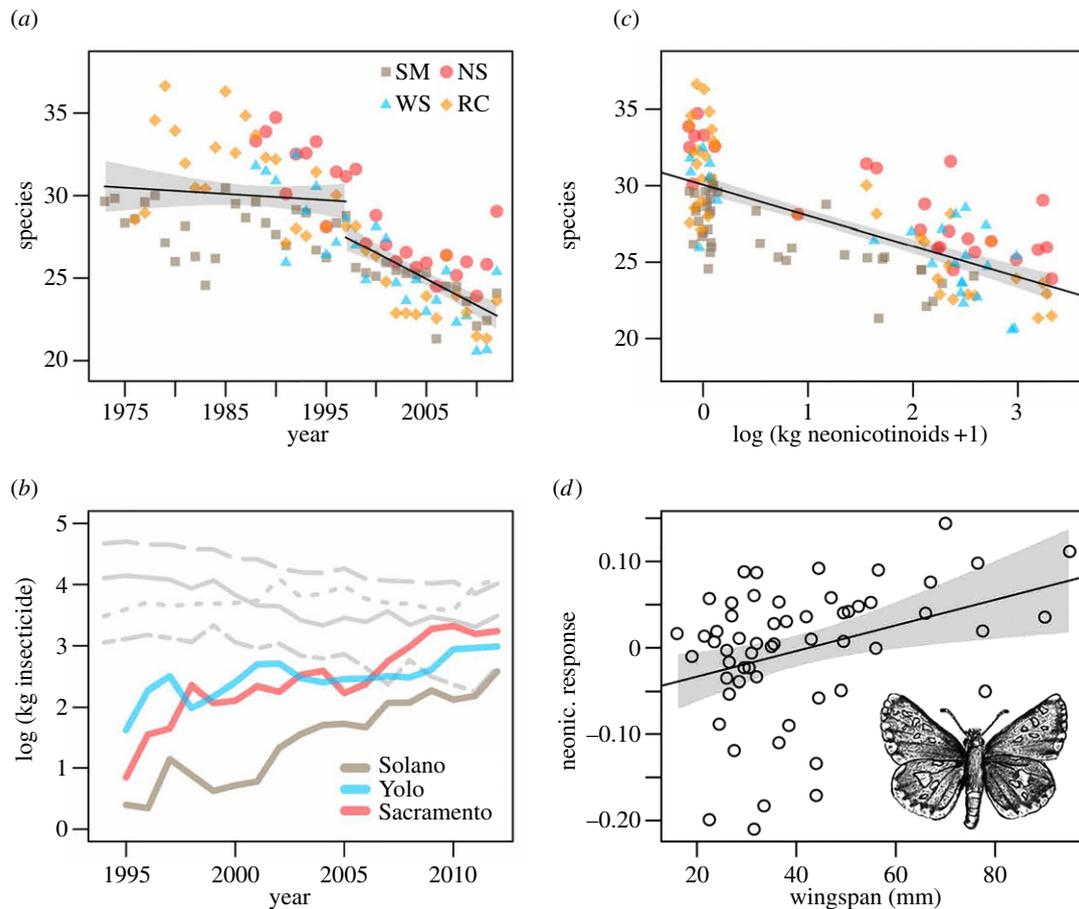
Butterfly data were generated with biweekly walks along fixed transects with the presence and absence of 67 species of butterflies recorded at four sites: Suisun Marsh (‘SM’, studied since 1972), West Sacramento (‘WS’, since 1988), North Sacramento (‘NS’, since 1988), and Rancho Cordova (‘RC’, since 1975); see [6,8], 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) (figure 1) [8]. 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 [9]. Evenness for each species derives from variation in the number of days observed per 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 (see the electronic supplementary material), but do not include all types of use, and thus underestimate total application. This scale of analysis (at the county level) is informed both by previous analyses (e.g. [6,8]), and by our natural history knowledge of the system (<http://butterfly.ucdavis.edu/>). In the vast majority of cases, butterfly populations at our study sites are part of regional metapopulations, characterized by local reproduction as well as adult movement linking study sites to reproduction in neighbouring areas (see the electronic supplementary material, for details on individual species). The observation that the populations at our focal sites are connected to regional metapopulations is supported by the effectiveness in previous analyses of regional climatic factors (e.g. the El Niño Southern Oscillation) when compared with the most local weather (from site-specific weather stations) in predicting butterfly dynamics [10,11].

### (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) as a fixed effect and the effective number of butterfly species as the dependent variable. The first model



**Figure 2.** (a) The number of observed butterfly species at four sites. 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 (coloured lines), and for the four most commonly applied non-neonicotinoid classes (grey 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. Grey 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 M.L.F.

included year, while the second model did not include year but instead included average minimum daily summer temperature from the previous year [8] and ‘converted land’, a county level index of land converted to urban or suburban spaces. Collinearity between land use and year prevented the inclusion of both factors in a single model. Additional details on analyses and model assumptions are provided in the electronic supplementary material. Finally, change through time in the butterfly fauna was visualized with the aid of a single inflection point spline as implemented in the R package SiZer [12].

### (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 [13,14]. The model included annual neonicotinoid totals (kilograms) 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 the electronic supplementary material). Beta coefficients were then examined in simple linear models with the following predictors: wingspan, geographical range, number of broods per year, resident status, overwintering mode, number of host genera and ruderal status (a composite variable encompassing variation

**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 ( $\pm$ s.e.)	lik. ratio	p-values
<i>(a)</i>			
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
<i>(b)</i>			
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

in dispersiveness and association with disturbed habitats [7]). We also considered the relationship between neonicotinoid sensitivities and beta coefficients for year (from the same Bayesian

**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.)

factor	estimate ( $\pm$ s.e.)	p-values	$F_{d.f.}$	$R^2$
<i>(a)</i>				
year	0.037 (0.0091)	0.00014	16.79 <sub>1,55</sub>	0.23
wingspan	0.027 (0.0098)	0.0080	7.58 <sub>1,55</sub>	0.12
geographical range	0.0048 (0.0099)	0.63	0.24 <sub>1,50</sub>	0.0047
<i>(b)</i>				
number of broods		0.026	3.91 <sub>2,54</sub>	0.13
resident status		0.099	2.42 <sub>2,51</sub>	0.087
overwintering mode		0.28	1.33 <sub>3,34</sub>	0.10
ruderal status		0.21	1.58 <sub>1,55</sub>	0.028
number of host genera		0.54	0.79 <sub>4,36</sub>	0.080

models) to ask if species in more severe decline were estimated to have greater sensitivity to neonicotinoids.

### 3. Results

A dramatic decline in the numbers of butterfly species observed annually is evident, starting in the late 1990s: the breakpoint estimated by spline inflection was 1997 (figure 2*a*). Neonicotinoid use began in the region in 1995 and has been increasing dramatically in comparison with other insecticides showing largely static or declining usage (with the exception of a recent increase in pyrethroids; figure 2*b*). 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 and figure 2*c*), which was true while controlling for year as an independent variable.

A relationship between neonicotinoid application and the number of butterfly species was also successfully modelled while accounting for effects of summer temperature and land conversion, with the effect of the latter roughly equal to the effect of neonicotinoids (table 1*b*). 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 (figure 1*d*) with fewer generations per year: the mean ( $\pm$  s.e.) neonicotinoid coefficient for single brooded species was  $-0.05$  ( $\pm 0.078$ ).

### 4. Discussion

California is a hotspot of biological diversity, as well as an area of rapid human population growth and development [15]. The region that includes our study sites is dominated by agriculture as well as a large urban centre, the city of Sacramento (figure 1), which has been found to contain high levels of insecticide accumulation in surface waters [16]. Here, we find that neonicotinoid application is negatively associated with butterfly populations, consistent with a previous European study

that included broader geographical sampling and focused on fewer, wide-ranging species [2]. 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 at a broad spatial scale, specifically at the county level, which might limit our ability to detect associations between stressors and butterfly declines. However, butterflies are mobile and individuals observed at focal sites have potentially developed on host plants many kilometres away, in areas that include agricultural margins or spaces in the urban matrix. Experimental work documenting non-target effects of neonicotinoids on honeybees and bumblebees has been extensive [17], and while only one experimental study on butterflies has been reported [4], many studies have documented negative effects of neonicotinoids on pest moths (e.g. [18]). 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 insectivorous species, including bats and birds.

**Data accessibility.** Butterfly data are available at A.M.S.'s site (<http://butterfly.ucdavis.edu/>), and insecticide data are publically available.

**Authors' contributions.** A.M.S. collected the butterfly data; B.C. and H.v.V. extracted, compiled and interpreted the neonicotinoid annual usage data, with input from M.L.H. and M.D.P.; M.L.F., C.C.N. and J.G.H. conducted analyses; all authors contributed to interpretation of results; M.L.F. wrote the manuscript with input from all authors, who agree to be held accountable for the content therein and approve the final version of the manuscript.

**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 US Government.

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