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Increasing neonicotinoid use and the declining butterfly fauna of lowland California

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2   **Increasing neonicotinoid use and the declining butterfly fauna of lowland California**

3

4   Short title: Neonicotinoids and butterflies

5

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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

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38   **1. INTRODUCTION**

39   Understanding cumulative effects of multiple anthropogenic stressors on wild populations of

40   plants and animals is of prime importance for twenty-first century ecology [1]. With one recent

41   exception [2], the effects of deliberate application of insecticides have not been described for

42   non-target taxa for which effects of other stressors, such as changing climate and land conversion

43   (e.g., urbanization), have also been characterized. Here we address this knowledge gap by

44   examining the use of neonicotinoid insecticides in Northern California, a region with a well-

45   studied butterfly fauna.

46   Neonicotinoids are a relatively new class of synthetic nicotine-like insecticides that have

47   increased in use during the last 20 years [3], partly because of ease of application: they are water

48   soluble, relatively stable, and can be applied to seeds, soil or growing plants, with systemic

49   uptake as the result [4]. Because they are systemic, effects on insects are not restricted to a

50   particular plant tissue (e.g. leaf surfaces) or to a narrow post-application window. Moreover,

51   runoff containing neonicotinoids from agricultural fields can be incorporated into tissues of

52   plants growing nearby, which might include host plants and nectar resources for non-target

53   insects [5]. Research into neonicotinoid exposure on honey bees and bumblebees has revealed a

54   range of lethal and sublethal effects [6], but little is known regarding effects of neonicotinoids on

55   other non-target insects.

56   Northern California is home to a rich butterfly fauna that has been monitored biweekly

57   (every other week) for over 40 years [7]. Monitoring has revealed a decline in butterfly

58   populations occurring at low elevations (less than 25 meters) within this region, especially since

59   the late 1990s [8]. Previous analyses have implicated changing patterns of land use and warming

60   fall and summer temperatures [9]. Notably, neither land conversion, nor shifting temperatures

61   show evidence of increased rate of change concomitant with the butterfly declines beginning in

62   the late 1990s. However, neonicotinoid use in the region began to increase dramatically at that

63   time. Here we analyze county neonicotinoid application records in relation to both the total

64   number of butterfly species observed per year, and in relation to occupancy records for

65   individual species at individual sites, while controlling for land use and climatic effects.

66

2. MATERIALS AND METHODS

(a) *Butterfly and insecticide data*

69   Butterfly data were generated with biweekly Pollard walks along fixed transects for all species of

70   butterfly (52 spp.) at four sites: Suisun Marsh (studied since 1972), West Sacramento (since

71   1988), North Sacramento (since 1988), and Rancho Cordova (since 1975); see [7,9] for site

72   descriptions and additional details on data collection. These sites are embedded in a matrix of

73   land use types that includes developed land (urban and suburban) and open spaces (agricultural

74   lands, public recreational areas and others) [9]. For each site, the total number of species

75   observed per year was represented as an effective number of species by taking the exponential of

76   the Shannon diversity index, which combines richness and evenness [10]. Evenness for each

77   species is informed by variation in the number of days observed in a given year out of the total

78   number of visits to a site.

79   Data describing annual use of insecticides by county were compiled for five common

80   neonicotinoid insecticides, as well as for the four most widely used non-neonicotinoid insecticide

81   classes. These data, originating from the California Department of Pesticide Regulation, were

82   obtained from the US Geological Survey National Pesticide Use database (details in the

83   electronic supplementary material), but do not include all types of use, and thus likely

84   underestimate total application.

85

86   **(b)** *Faunal analyses*

87   We developed two linear mixed models, one focused on neonicotinoids and a second

88   encompassing other factors of interest, particularly land conversion. Both models included site

89   (N=4) as a random (intercept) effect, the numbers of visits (a control for sampling effort), and the

90   effective number of butterfly species as the dependent variable. The first model also included

91   year, while the second model included average minimum daily summer temperature [9] and

92   "converted land", a county level index (available every other year) of the amount of land that has

93   been converted to urban or suburban spaces. For more information on the index of land

94   conversion, the choice of climatic data, and other details of analyses see the electronic

95   supplementary material. Finally, change through time in the butterfly fauna was visualized with

96   the aid of a spline with a single inflection point as implemented in the R package SiZer [11].

97

98   **(c)** *Species-specific analyses*

99   In order to investigate species-specific sensitivities to neonicotinoids, we used a hierarchical

100   Bayesian binomial regression that estimates population-level beta coefficients, as described in

101   detail elsewhere [12,13]. The model included annual neonicotinoid totals (kg.) for each county,

102   as well as year, with the response variable being the number of days butterflies were observed

103   (for each species) out of the total number of days that each site was visited. Posterior probability

104   distributions were used to calculate species-specific beta coefficients summarizing associations

105   with neonicotinoid use (further details in electronic supplementary material). Beta coefficients

106   were then examined in simple linear models with the following predictors: wingspan, geographic

107   range, number of broods per year, resident status, overwintering mode, number of host genera,

108   and ruderal status (a composite natural history variable encompassing variation in dispersiveness

109   and association with disturbed habitats [8]). We also considered the relationship between

110   neonicotinoid sensitivities and beta coefficients for year (from the same Bayesian models) to ask

111   if species in more severe decline were estimated to have greater sensitivity to neonicotinoids.

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113   **3. RESULTS**

114   Our four study sites exhibited a dramatic decline in the numbers of butterfly species observed

115   annually starting in the late 1990s: the breakpoint estimated by spline inflection was 1997 (figure

116   1a). Neonicotinoid use began in the region in 1995 and has been increasing dramatically (figure

117   1b) in comparison with other insecticide classes showing largely static or declining usage (with

118   the exception of a recent increase in pyrethroids; figure 1b). A negative relationship between

119   neonicotinoid use and annual variation in butterfly species observations was readily detectable

120   (likelihood ratio 7.16, $P = 0.0075$; table 1, figure 1c), which was true while controlling for year

121   as an independent variable. Although a less powerful approach, we also considered a simple

122   correlation between detrended variables: with the annual trend in both neonicotinoids and

123   butterfly richness removed prior to analysis, the negative relationship is still detected (Pearson

124   correlation coefficient -0.25, $P = 0.066$).

125   A relationship between neonicotinoid application and the number of butterfly species was

126   also successfully modeled while accounting for effects of summer temperature and land

127   conversion, with the effect of the latter roughly equal to the effect of neonicotinoids (table 1b).

128   At the level of individual species, those with the strongest negative association with

129   neonicotinoid use also experienced more severe declines (see the year effect in table 2). They

130   also tended to be smaller-bodied species (figure 1d) with fewer generations per year (table 2): the

131   mean (± s.e) neonicotinoid effect for single brooded species was negative (-0.05 ± 0.078), and

132   positive for multiple-brooded species (0.013 ± 0.072).

133

134   **4. DISCUSSION**

135   California is a hotspot of biological diversity, as well as an area of rapid human population

136   growth and land development [14]. The Central Valley of California has also seen some of the

137   most intense use of neonicotinoids in the country [3]. Here, we find that neonicotinoid

138   application is negatively associated with butterfly populations in the region. Furthermore, the

139   effect of neonicotinoids is detectable while accounting for land conversion, and effects of the

140   two factors are roughly equal in magnitude. The species most negatively associated with

141   neonicotinoids are smaller bodied and have fewer generations per year, traits that may confer a

142   reduced capacity for response to stressors.

143   Our results derive from observations aggregated at a broad spatial scale, specifically at

144   the county level (for insecticide and land use data), which should limit our ability to detect

145   associations between stressors and butterfly declines. However, detection of associations even at

146   this crude spatial scale raises the possibility that neonicotinoid insecticides are having a negative

147   effect on butterfly populations occurring in areas undergoing insecticide application.

148   Experimental work documenting non-target effects of neonicotinoids on honey bees and

149   bumblebees has been extensive [15,16], and while only one experimental study on butterflies has

150   been reported [5], many studies have documented negative effects of neonicotinoids on pest

151   moths [e.g., 17]. The findings reported here should encourage researchers to broaden the scope

152   of investigations beyond narrow temporal and spatial windows of application to understand

153   spillover effects on non-target species and possible indirect effects on other species, including

154   bats and insectivorous birds.

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156   *Data accessibility*. Butterfly data are available at AMS's site (http://butterfly.ucdavis.edu/), and

157   insecticide data are publically available, as explained in text.

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198 **Figure legend**

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200 Figure 1. *(a)* The number of observed butterfly species at four sites (SM: Suisun Marsh; WS:

201 West Sacramento; NS: North Sacramento; RC: Rancho Cordova). The response variable (in *a*

202 and *c*) is the exponential of Shannon diversity, i.e., the effective number of species; the spline

203 knot in *a* is 1997 (95% confidence interval: 1990-2001). *(b)* Insecticide application for

204 neonicotinoids in focal counties (colored lines), and for the four most commonly-applied non-

205 neonicotinoid classes (gray lines). The non-neonicotinoids are, in decreasing order of line

206 elevation in 1995, organophosphates, carbamates, pyrethroids, and organochlorines (lines are

207 county averages). Note the different range of years in the first two panels, as *(b)* starts in the

208 year in which neonicotinoids are first reported. *(c)* Relationship between number of butterfly

209 species and neonicotinoids (values of the latter at zero jittered for visualization). *(d)* Response of

210 individual species to neonicotinoids as predicted by wingspan; more negative values on the y-

211 axis indicate species with more negative associations with neonicotinoids. Gray polygons in

212 panels *(a)*, *(c)*, and *(d)* are 95% confidence intervals. *Pyrgus scriptura* (in *d)*, is one of the

213 smallest species in the fauna; drawing by MLF.

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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

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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)

Factor	Estimate (± SE)	P	F_{df}	R^2
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

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Fig. 1