



Technical Report

The Bugs Matter Citizen Science Survey: counting insect 'splats' on vehicle number plates reveals a 58.5% reduction in the abundance of actively flying insects in the UK between 2004 and 2021.

Lawrence Ball¹, Robbie Still¹, Alison Riggs², Alana Skilbeck¹, Matt Shardlow³, Andrew Whitehouse³, & Paul Tinsley-Marshall¹

¹Kent Wildlife Trust, Tyland Barn, Sandling Lane, Maidstone, Kent, ME14 3BD

²Diocese of Oxford, Church House Oxford, Langford Locks, Kidlington, Oxford, OX5 1GF

³Buglife, G.06, Allia Future Business Centre, London Road, Peterborough, PE2 8AN

Address for correspondence: info@bugsmatter.app

Abstract

In recent years, scientists and the media have drawn attention to global declines in insect abundance, the consequences of which are potentially catastrophic. Invertebrates are critical to ecosystem functions and services, and without them life on earth would collapse. However, there has been insufficient data to make robust conclusions about trends in insect abundance in the UK, because standardised insect sampling approaches are not widely applied to all insect groups or at a national scale. Here, we demonstrate the use of an innovative and scalable invertebrate sampling technique conducted by citizen scientists, to examine the difference in invertebrate abundance in the UK over a 17-year timeframe. The 'windscreen phenomenon' is a term given to the anecdotal observation that people tend to find fewer insects squashed on the windscreens of their cars now, compared to in the past. This observation has been ascribed to major declines in insect abundance. In this study, citizen scientists were asked to record the numbers of squashed insects and other invertebrates on their vehicle number plates following a journey, having first removed any residual insects sampled on previous journeys. We compared the number of insects sampled by vehicles in 2019 (n = 599 journeys in Kent) and 2021 (n = 3,348 journeys nationwide) with the results of a nationwide survey using this methodology led by the RSPB ('Big Bug Count') in 2004 (n = 14,466 journeys). The results show that the number of insects sampled on vehicle number plates in the UK decreased by 58.5% between 2004 and 2021, and that these differences were statistically significant. A comparison of the 2004 national data with the 2019 data from Kent showed a 53.7% decrease. The greatest decreases in splat rate between 2004 and 2021 occurred in England (65%) whilst journeys in Scotland recorded a comparably smaller decrease (27.9%), with intermediate decreases in Wales (55%). These results are consistent with the declining trends in insect populations widely

reported by others, and informs a growing requirement for conservation research, policy and practice targeted at invertebrates in the UK. However, our results are based on data with low temporal resolution and consequently we interpret this change between two points in time with caution. Furthermore, inter-annual variation in a range of unmeasured factors, such as wind speed, predation or land-use change, could significantly influence the observed pattern. To draw robust conclusions about long-term trends in insect populations in the UK, scientists require data from multiple years, over long time periods, and over large spatial scales – the Bugs Matter citizen science survey has demonstrated that it has the potential to generate such data.

1 Introduction

A growing body of evidence (Fox et al., 2013; Hallmann et al., 2017; Goulson, D. 2019; Sánchez-Bayo et al., 2019; Thomas et al., 2019; van der Sluijs, 2020; Macadam et al., 2020; Outhwaite, McCann and Newbold, 2022) highlights population declines in insects and other invertebrates at global scales (herein referred to collectively as ‘insects’). These declines, which are evident across all functional groups of insects (herbivores, detritivores, parasitoids, predators and pollinators) could have catastrophic impacts on the earth’s natural systems and human survivability on our planet. Invertebrates are functionally of greater importance than large-bodied fauna, and in terms of biomass, bioabundance and species diversity, they make up the greatest proportion of life on earth.

Invertebrates are critical to ecosystem functions and services. They pollinate most of the world’s crops, provide natural pest control services, and decompose organic matter and recycle nutrients into the soil. Without them we could not grow onions, cabbages, broccoli, chillies, most tomatoes, coffee, cocoa, most fruits, sunflowers, and rapeseed, and demand for synthetic fibres would surge because bees pollinate cotton and flax. Invertebrates underpin food chains, providing food for larger animals including birds, bats, reptiles, amphibians, fish and terrestrial mammals. Almost all birds eat insects, and many of those that eat seeds and other food as adults must feed insects to their young – it is thought to take 200,000 insects to raise a single swallow chick (Chapman et al., 2013). Without insects, life on earth would collapse, millions of species would go extinct, and we would be surrounded by the carcasses of dead animals.

Evidence of insect declines comes from targeted surveys using specific sampling techniques aimed at specific target groups. Many of these have generated long-term data sets, such as the Rothamstead Insect Survey of aphids and larger moths, since 1964 (Taylor, 1986), the UK Butterfly Monitoring Scheme, since 1976, (Brereton et al., 2020), and the National Moth Recording Scheme, since 2007 (Fox et al., 2021), and they provide a good indication of trends for those target taxa. However, generalising national and global trends from surveys of a limited number of insect groups could be inaccurate. Patterns and trends for specific species or species groups are nuanced, and while trends in some insect groups are well understood, there is a paucity of data for many others. Whilst some survey techniques such as moth trapping and butterfly transects are discriminate in terms of what species they record, there are very few established methods for large-scale monitoring of insect abundance across a broad range of insect groups. Both discriminate and indiscriminate approaches have advantages and disadvantages. Here we present the results from a survey that used an innovative method for large-scale indiscriminate monitoring of flying insect populations, which has potential to provide an efficient, standardised and scalable approach to monitor trends in insect abundance across local, regional and global scales.

The ‘windscreen phenomenon’ (Wikipedia, 2021) is a term given to the anecdotal observation that fewer insects tend to get squashed on the windscreens of cars now compared to a decade or several decades ago. These observations have served as an indication of the major global declines in insect abundance,

and have been reported from empirical data (Møller, 2019). Flying insects are inadvertently sampled when they become squashed on vehicle windscreens and number plates when they are impacted. We implemented an invertebrate sampling technique based on the ‘windscreen phenomenon’. Data were collected by citizen scientists to assess invertebrate abundance over a 17 year timeframe (Tinsley-Marshall et al., 2021a, 2021b). The aim was to quantify insect abundance in the UK using a standardised approach and to make comparisons with pre-existing baseline data from 2004, which was collected as part of a national survey using the same sampling method led by the RSPB (‘Big Bug Count’). By repeating the survey in 2019 and 2021 it was possible to compare the numbers of insects sampled between these points in time.

We aimed to test the null hypothesis H0: there is no evidence of variation in the numbers of insects sampled on vehicle number plates in the UK between 2004, 2019 and 2021 and to determine whether an alternative hypothesis H1: there is evidence of variation in the numbers of insects sampled on vehicle number plates in the UK between 2004, 2019 and 2021, could be accepted. This report summarises the results of an analysis of the insect abundance and participation data from the Bugs Matter survey in the UK, and adds to the evidence base for patterns in invertebrate abundance.

2 Materials and Methods

Study area and survey design

The parameters of the study landscape were defined as the whole of the UK. For some parts of the analysis we provide country-specific results for England, Scotland, Wales and Northern Ireland separately, accepting that some data was collected from journeys that spanned the country borders (Figure 1). It was not possible to isolate at which point in each journey insects were sampled, therefore each complete journey was included where journeys crossed country borders. Survey design was informed by a list of desirable attributes of monitoring programmes, ordered from most elemental to most aspirational (Pocock et al., 2015) and aimed to ensure that all relevant attributes were adopted.

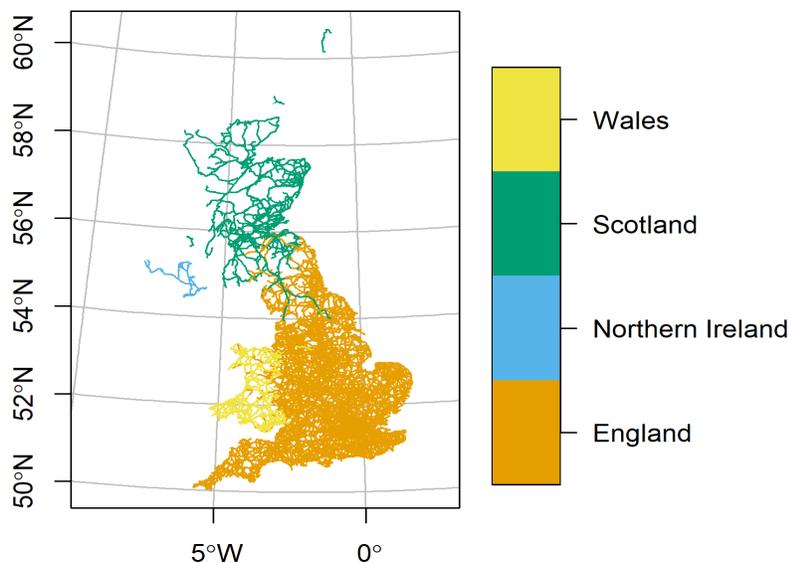


Figure 1. A map showing the distribution and extent of journeys in 2004, 2019 and 2021 included in this analysis of Bugs Matter survey data on insect numbers sampled by vehicle number plates in the UK.

Insect sampling method

Prior to commencing a journey, citizen scientists cleaned the front number plate of their vehicle to remove any residual insects. Insects were then sampled when they collided with the number plate throughout the duration of a journey. Whilst the sampling method was not designed to identify species, or groups of species, insects sampled will have been predominantly the adult forms of flying species from the following taxonomic groups: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Megaloptera, Neuroptera, Plecoptera, Trichoptera and Thysanoptera. Citizen scientists were asked to participate only on essential journeys and not to make journeys specifically to take part in the survey. Using a standardised sampling grid, termed a 'splatometer', citizen scientists recorded the number of insects squashed on the number plate of their car (Figure 2). Only insects within the cut-out portions of the splatometer were counted to ensure all counts were made from within a standardized area on each number plate. In 2019 and 2021, data was collected on journeys undertaken between 1st June and 31st August, and in 2004 data was collected in June. In 2004 and 2019, the start and end times and locations of the journeys were recorded, along with the journey distance using vehicle odometer readings. In 2019, data was only collected from journeys starting in Kent. In 2021 the precise route of the journey was recorded in real-time using the Bugs Matter mobile app.



Figure 2. Photograph showing the splatometer positioned over a number plate.

Bugs Matter mobile app

In 2021, data were submitted by citizen scientists via the Bugs Matter mobile app (Figure 3). The app provided a platform to record counts of insects on number plates, track the journey route using GPS, and collect information on the length, duration, and average speed of each journey undertaken as part of the survey. It also used an Application Programming Interface (API) number plate look-up service to collect information about vehicles involved in the survey. This data was used in the analysis to determine whether and how vehicle specifications influence insect sampling.



Figure 3. Screenshots of the Bugs Matter mobile app.

Collating explanatory variables

Time of day was calculated for each journey as the intermediate time between the start and end times. As 97% of journeys occurred during daytime hours (05:00-21:00), we treated time as a continuous variable in the statistical modelling, rather than converting to a factor variable or sin/cos time. The 'sf' package (Pebesma, 2018) in R was used to calculate journey length. The average speed of the journey was calculated by dividing the journey distance by the journey duration. The vehicle type, acquired via the API, was classified to align with the analysis conducted by the RSPB in 2004. These categories were car, heavy goods vehicle (HGV), multi-purpose vehicle (MPV), sports car, sports utility vehicle (SUV) and van. Data collected prior to 2021 contained only start and end postcodes, and so journey routes were obtained from the Google Directions API, through the R 'mapsapi' package (Dorman, 2022). Mean temperature was calculated for each journey by averaging the intersecting raster cell values from 0.1 degree E-OBS gridded daily mean temperature (Cornes et al., 2018).

Maximum greenest pixel composites of normalized difference vegetation index (NDVI) values were generated in Google Earth Engine (Gorelick et al., 2017) from MODIS Terra Vegetation Indices 16-Day Global 250 m data (Didan, 2015) for each survey year. NDVI describes the difference between visible and near-infrared reflectance of vegetation cover based on chlorophyll content, and can be used to estimate vegetation productivity. Artificially-surfaced areas such as roads and buildings show as low values, whilst vegetated areas show as high values. The NDVI values were averaged within a 500 m buffer of each journey route to approximate the suitability of the habitat for insects surrounding each journey route. The NDVI values were rescaled to a -10-10 range to aid interpretation of the model coefficients.

Finally, the proportion of each journey that was conducted on 'primary', 'secondary', 'tertiary' and 'other' roads were extracted for each journey by snapping the journeys to OpenStreetMap roads data and extracting the road type information. Journeys mostly followed primary, secondary, and to a lesser extent tertiary roads, with very few on other road types. Only data on the proportions of secondary and tertiary

roads were included as variables in the model because including additional variables in the model would lead to perfect collinearity, as the proportions of each road type sum to a whole (100%).

Statistical analysis

Data cleaning and preparation

To make the data comparable between journeys, insect counts recorded by citizen scientists, were converted to a 'splat rate', by dividing the insect count by the journey distance, expressed in a unit of 'splats per mile'. This important metric is easily defined as the number of insects sampled on the number plate every mile. Differences in insect splat rate (splats per mile) between years were visualized in a boxplot. In addition, relationships between other variables, such as how journey distance or the types of vehicles used in the surveys varied between years, were examined visually in boxplots and correlation plots, and tested using Kruskal-Wallis tests or Spearman correlation tests.

Prior to the analysis, some steps were taken to clean the data and remove outliers. Journeys with GPS errors were removed from the 2021 data. These errors were caused by a drop-out of background tracking due to GPS signal being lost by the device, and they appeared as long straight lines between distant locations. All journeys with a 1 km or greater gap between route vertices were omitted. Of the 4834 journeys collected in 2021, 825 (17%) had GPS errors and were removed from the analysis. Some journeys were very short with extremely high splat rates. Therefore, very short journeys of less than 0.3 miles were removed, as they are highly likely to be the result of GPS errors or incorrect use of the app, for example by the user forgetting to press the start journey button at the appropriate time. Similarly, all journeys that lasted less than one minute and journeys with an average speed of less than 1 mph or over 80 mph were omitted. In addition, all journeys during which rainfall occurred were omitted from the dataset due to the risk that rainfall could dislodge insects from numberplates, leading to bias in the data. After data cleaning, 18,413 of 22,364 journeys were retained.

Modelling

We performed a statistical analysis to examine the relative effects of survey year, time of day of the journey, average journey temperature, average journey speed, journey distance, vehicle type, local NDVI, and road type, on insect splat rate. The response variable in our analysis was the insect count which showed a right-skewed distribution due to the high number of zero and low values, as is typical for count-derived data (Appendix 1). Therefore we tested several modelling approaches suited to over-dispersed and zero-inflated count data and compared their performance, to identify the optimum model to use (Yau, Wang and Lee, 2003).

Journey distance was included in the models as an offset term. Offset terms are included in models of count-derived data to deal with counts made over different observation periods, which in this case was journey distance. This is preferable to using the precalculated splat rate because by adding the denominator of the ratio (distance) as an offset term, it makes use of the correct probability distributions. It can be thought of as explicitly modelling the expected rate of sampling an insect as distance driven changes. The model with offset does model the splat rate (splats per mile), but in a way that is likely to be much more compatible with the data (Coelho et al., 2020).

We performed a Poisson generalized linear model (Poisson), a negative binomial generalized linear model (NB), a zero-inflated Poisson model (ZIP), and a zero-inflated negative binomial generalized linear model (ZINB) and compared their Log Likelihood, AIC, BIC and Likelihood ratio test statistics (Table 1). Overdispersion was confirmed using a test for overdispersion on a Poisson model (Cameron and Trivedi,

1990), which resulted in a test statistic of $c = 11.664$, indicating overdispersion ($c = 0$ for equidispersion). The ZINB model provided the best fit and was therefore used for the main analysis.

Table 1. Summary statistics from fitting several different models to the data from the Bugs Matter citizen science survey of insect abundance. Based on the evaluation metrics, the ZINB model was found to provide the most accurate fit.

Model	Log.likelihood	AIC	BIC	Likelihood ratio test, DF diff.
Poisson	-130198.13	260426.3	260543.3	149481.51 , -14
NB	-56021.80	112075.6	112200.4	10659.28 , -14
ZIP	-125627.81	251315.6	251549.7	29174.8 , -28
ZINB	-55956.73	111975.5	112217.3	2802.61 , -28

The ZINB model, akin to the ZIP model, is designed for data that includes excess zeros. The model accepts that there could be additional processes that are determining whether a count is zero or greater than zero and models this explicitly. Whilst the importance of submitting data for zero-count journeys was explained to citizen scientists in all survey years, there may be other unknown processes that result in zero count journeys, for example associated with journey speed or location. The ZINB has two parts. The first is a binomial model which models the relationship between the independent variables and a binary outcome of zero or greater than zero insect splats. The second part is a negative binomial model to model the count process. The analysis was performed using the MASS package (Venables and Ripley, 2002) and the pscl package (Zeileis, Kleiber and Jackman, 2008) in RStudio (R Core Team, 2021) following established techniques (Sokal & Rolf, 1995; Crawley, 2007).

After running the model, variance inflation factor (VIF) scores were calculated to check for multicollinearity between independent variables. A VIF score greater than 10 indicates high collinearity, which means two or more independent variables are correlated with one another. This can cause unreliable predictions and weaken the statistical power of the model. A likelihood ratio test was used to compare a model with only survey year included as an independent variable, with the full model, to evaluate the contribution of the other independent variables to the model fit.

The results of the ZINB zero-inflated model show the change in the odds of a zero-count journey occurring given a one-unit change in the independent variable. The results of the ZINB negative binomial model show the quantity of change (a multiplier) in the response variable given a one-unit change in the independent variable, while holding other variables in the model constant. These values are called incidence rate ratios and they can be visualized effectively in a forest plot.

To examine country-specific trends, we repeated the analysis on the data for each country separately. We used NB models because there was perfect separation between the binomial outcome of zero or greater than zero and one or more independent variables in the these country-specific datasets.

We also performed a regression tree analysis (RTA) in the R 'rpart' package (R Core team, 2019; Therneau and Atkinson 2019b) which implements methodologies of Breiman et al. (1984). Regression tree analysis partitions a dataset into smaller subgroups through recursive partitioning. The binary splits occur at nodes based on true/false answers about the values of predictors, and each split is based on a single variable. The rule generated at each step maximizes the class purity within each of the two resulting subgroups (Breiman et al. 1984; Miska and Jan 2004). This machine learning classification approach enabled us to detect any important non-linear relationships between our independent variables and splat rate and also provides a measure of variable importance.

3 Results

Flying insect abundance

In 2004, 196,448 insects were sampled over 14,466 journeys comprising 867,595 miles. In 2019, 1,063 insects were sampled over 599 journeys comprising 9,960 miles. In 2021, 11,712 insects were sampled over 3,348 journeys comprising 121,641 miles. The average splat rate in 2004 was 0.238 splats per mile, in 2019 it was 0.098, and in 2021 it was 0.104 splats per mile. The spread of the insect splat rate data is shown in Figure 4. The proportion of journeys in which zero insects were sampled was 7.8% in 2004, 54.3% in 2019, and 39.5% in 2021. The majority of journeys (85%) were undertaken in a conventional car with the remainder being undertaken in HGVs, MPVs, sports cars, SUVs, and vans (Appendix 2). The average time of day of journeys in 2004 was 13:40, in 2019 it was 12:48 whilst in 2021 it was 13:33 (Appendix 3). The mean average journey speed in 2004 was 37.2 mph, in 2019 it was 21.7 mph, whilst in 2021 it was 29.3 mph (Appendix 4). The average journey temperature in 2004 was 16°C, in 2019 it was 17°C, whilst in 2021 it was 16.7°C (Appendix 5). The average journey distance in 2004 was 60 miles, in 2019 it was 16.6 miles, and in 2021 it was 36.3 miles (Appendix 6). The average NDVI surrounding journeys in 2004 was 4.975, in 2019 it was 5.423, and in 2021 it was 5.428 (Appendix 7). The mean proportion of journeys conducted on primary roads was 71.6% in 2004, 39.8% in 2019, and 47.2% in 2021. The mean proportion of journeys conducted on secondary roads was 25.1% in 2004, 48.6% in 2019, and 42.6% in 2021. The mean proportion of journeys conducted on tertiary roads was 3.3% in 2004, 11.5% in 2019, and 10.1% in 2021 (Appendix 8). A positive correlation was observed between journey distance and count of splats (Appendix 9). A positive correlation was also observed between journey distance and splat rate (Appendix 10). A weak positive trend was found between vehicle registration year and splat rate (Appendix 11). The VIF scores (max VIF = 1.49) showed very low collinearity between independent variables.

The results of the ZINB negative binomial model showed a 53.7% (95% CI [46.7%, 59.7%]) reduction in insect splat rate in 2019 (35.8%/decade), and a 58.5% (95% CI [56.2%, 60.8%]) reduction in 2021 (34.4%/decade), compared with 2004 (Figure 5). The differences were statistically significant ($p < 0.001$). The Likelihood Ratio test statistic was 2,802.6, and in a model with only year as a predictor it was 1,449.9. This shows that the goodness of fit of the model almost doubled with the addition of the other independent variables.

Regarding the other independent variables, the results showed that compared to conventional cars, splat rate was 48% higher for HGVs, 15% higher for sports cars, and 26% lower for MPVs, and these relationships were statistically significant. Splat rates of vans and SUVs did not differ significantly from conventional cars. On average, splat rate increased by 6% with each hour of the day, splat rate increased by 2% with each one degree increase in mean daily temperature, and splat rate increased by 3% with each one unit increase in NDVI, and these relationships were statistically significant. There was a significant but very slight change in splat rate with journey distance, whereby splat rate decreased by 0.1% with each mile driven. There was no significant relationship between splat rate and average journey speed (Figure 5).

The results of the ZINB zero-inflated model showed that the odds of a zero-count journey occurring increased by 2.9 times between 2004 and 2021. The odds of a zero-count journey occurring increased by 1.01 times with each 1% increase in the proportion of a journey that was conducted on secondary roads. Furthermore, the odds of a zero-count journey occurring increased by 1.94 times if the vehicle was a HGV rather than a car and 3.28 times if the vehicle was a SUV rather than a car. The odds of a zero-count journey occurring decreased by 1.15 times with each hour in the day, decreased by 1.17 times with each

one degree increase in temperature, and decreased by 1.3 times with each unit increase in NDVI. In addition, the odds of a zero-count journey occurring decreased by 1.02 times with each mile increase in journey distance. These relationships were statistically significant (Appendix 12).

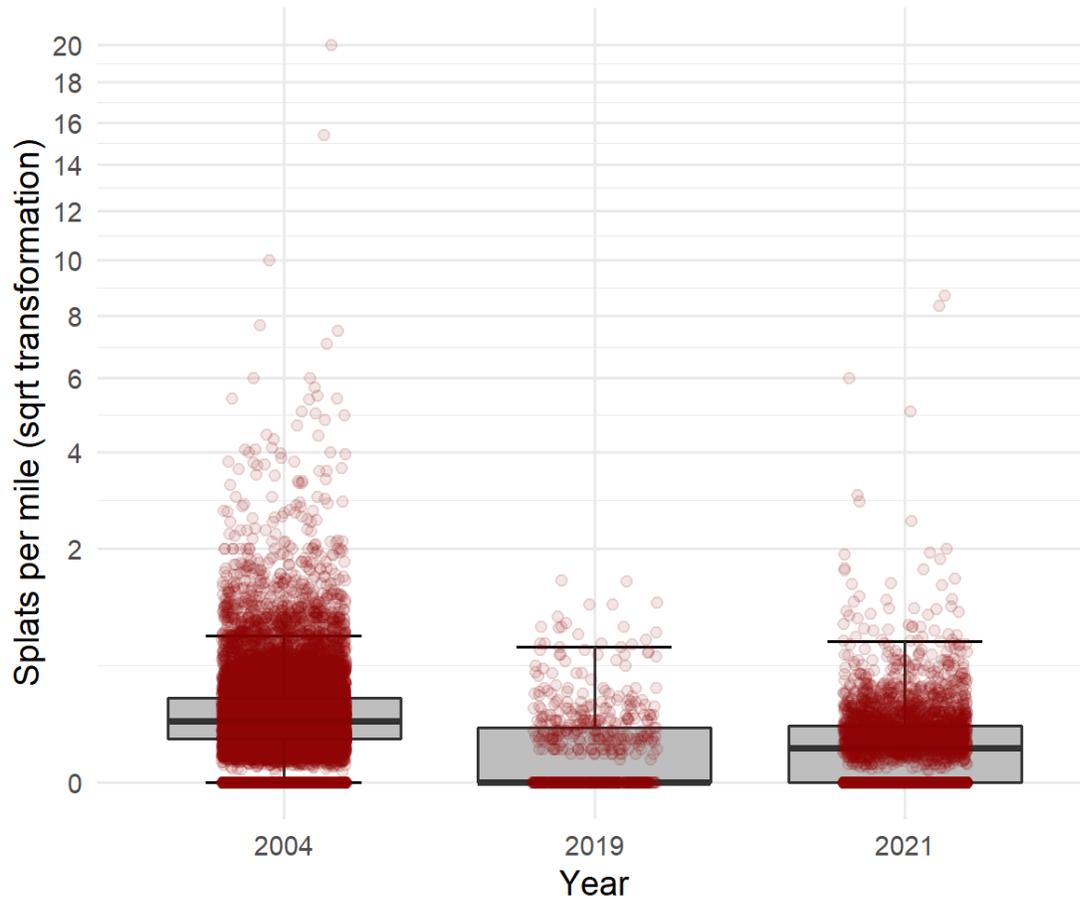


Figure 4. Box and whisker plot with jittered data points showing the spread of insect splat rate data (splats per mile) from the Bugs Matter survey of insects on car number plates in the UK in each of the survey years. The boxes indicate the interquartile range (central 50% of the data), either side of the median splat rate which is shown by the horizontal line inside the box. The vertical lines extend out by 1.5 times the interquartile range, and the data points themselves are added with a 'horizontal jitter' so they do not overlap to improve visualization of the data distribution. The thick line at $y = 0$ for each year are data points for journeys with a count of zero splats per mile. If splat rate on every journey was identical, we would only see the line across the middle of the box, with the data points on top of it.

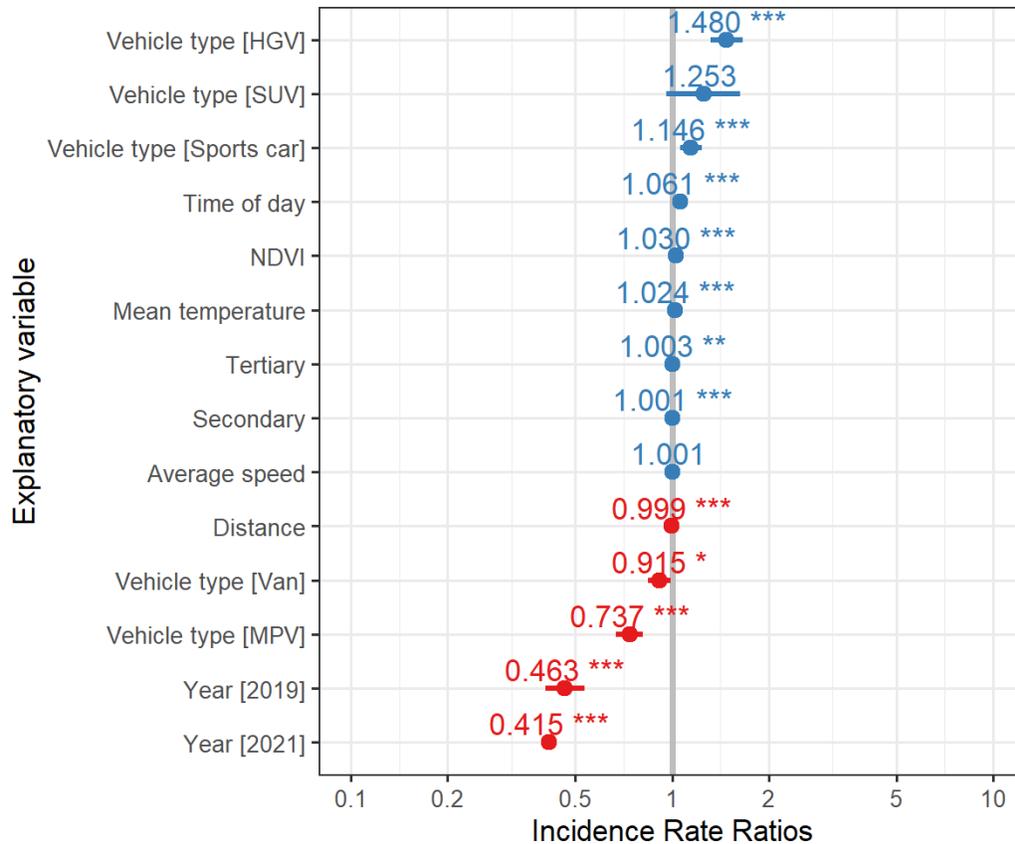


Figure 5. Forest plot of incidence rate ratios from the ZINB negative binomial model of Bugs Matter survey data of insects on car number plates in the UK, showing the quantity of change (a multiplier) in splat rate (splats per mile) given a one-unit change in the independent variable, while holding other variables in the model constant. Significant relationships between splat rate and independent variables are shown by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Vehicle types are compared to the reference category of 'conventional cars'. The reference year is 2004.

The regression tree describing splat rate (Appendix 13) had two splits, three terminal nodes and a cross-validated error of 0.918. It showed that splat rate was, on average, over three times as high after 8 pm, and highest in 2004. The complexity parameter plot shows the reduction in the cross-validated error with decreasing complexity parameter values and increasing tree size (Appendix 14). We would see diminishing returns if we continued to grow the tree. A cross-validated error of 0.918 shows that the tree could only explain a small amount of the variance in the data. Variable importance is calculated as the sum of the goodness of split measures (Gini index) and considers both primary and surrogate splits. Time of day of the journey and the journey year were the two most important variables (Appendix 14).

The country-specific results show that the greatest decreases in splat rate occurred in England (65% between 2004 and 2021) whilst journeys in Scotland recorded a comparably smaller decrease in splat rate between 2004 and 2021 (27.9%) (Table 2 and Figure 6).

Table 2. The results from country-specific NB models of insects sampled on vehicle number plates gathered by the RSPB Big Bug Count in 2004 and by the Bugs Matter survey in 2019 and 2021, showing the estimates and confidence intervals (95%) of the percentage decrease in splat rate between years.

Country (years)	% decrease in splat rate			
	Estimate	Per decade	2.50%	97.50%
England (2004-2019)	56.19	37.5	61.36	50.31
England (2004-2021)	64.96	38.2	66.78	63.02
Scotland(2004-2021)	27.85	16.4	41.07	11.32
Wales (2004-2021)	54.95	32.3	62.28	46.11

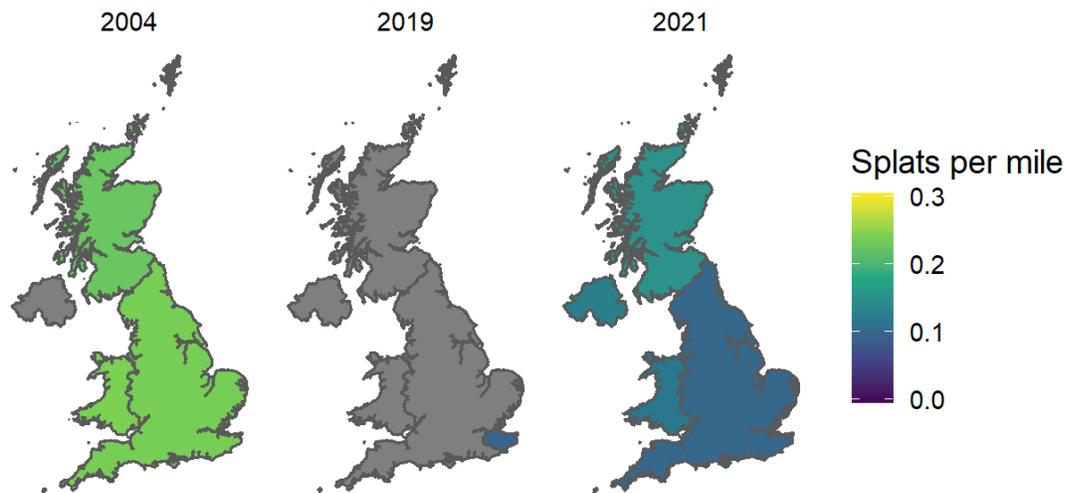


Figure 6. Heat map of splat rate of insects on car number plates from the Bugs Matter survey in the UK in each of the survey years, 2004, 2019 and 2021.

Participation

In the 2021 survey season, 5,215 users signed up to Bugs Matter via the mobile app. The majority signed-up in the initial launch period between mid-May and early-June, although there were considerable spikes in signups around key dates (Figure 7). For example, an increase in early June coincides with Bugs Matter featuring on BBC Springwatch. There was a slight lag between launch and sign-up spikes in Wales - this may have been due to delays in translating communication materials into the Welsh language.

Of the 5,215 individuals who signed up to the Bugs Matter app in 2021, 710 participated in the survey, the criteria for which was submitting data for at least one journey. We calculated a conversion rate as the number of participants who submitted one or more journeys (710) divided by the number of sign-ups (5,215). This gives a conversion rate of 13.6%. At the end of the survey season, these users had recorded a total of 4,778 journeys. The average number of journeys recorded by each surveyor was 4.7. In 2021, 4,053 journeys were completed in England, 36 journeys were completed in Northern Ireland, 283 journeys were completed in Scotland, and 403 journeys were completed in Wales (Figure 8).

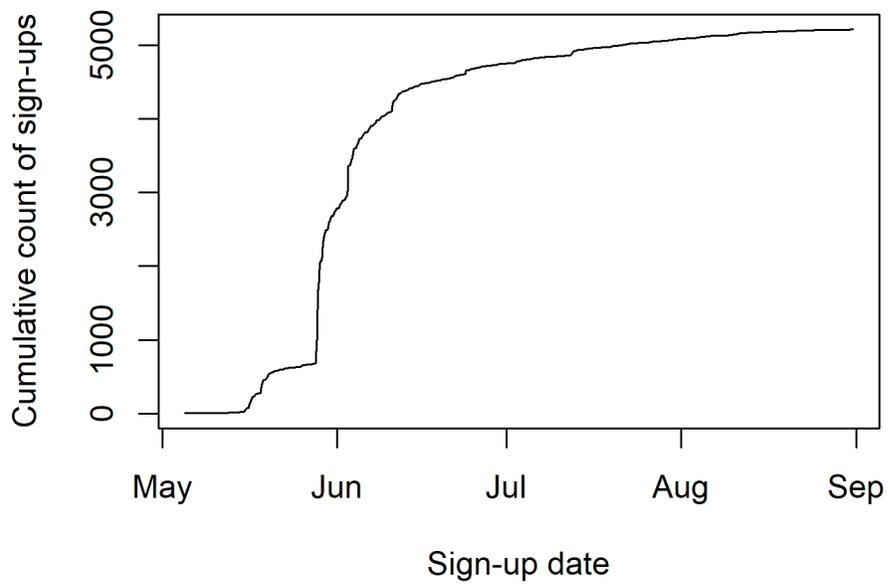


Figure 7. Number of signups to the Bugs Matter app during the 2021 survey season.

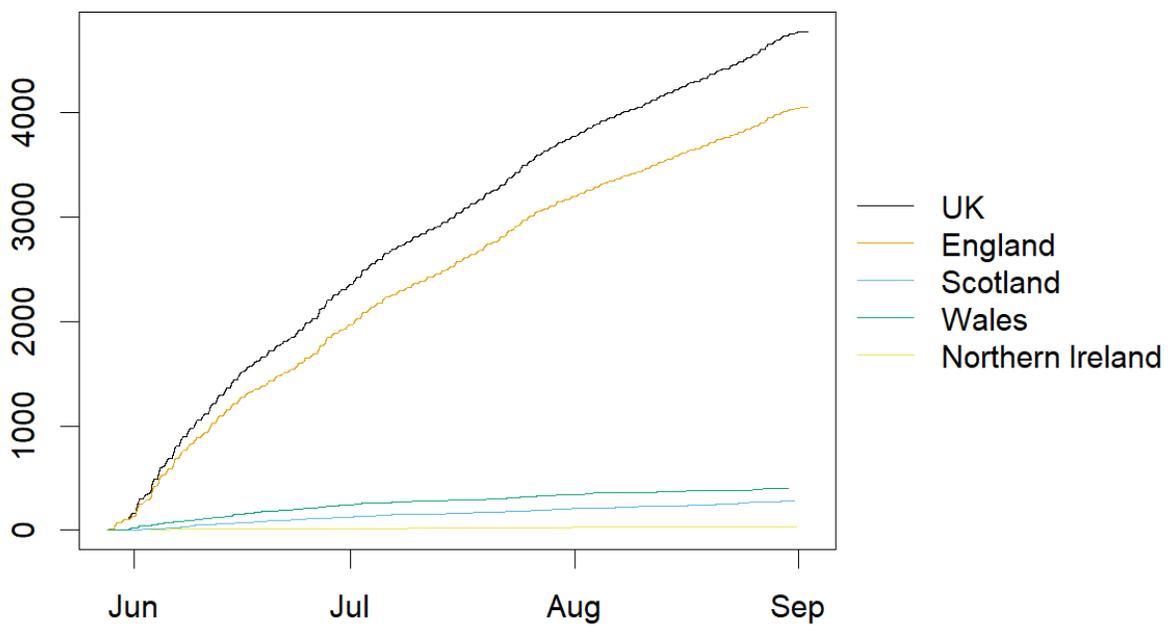


Figure 8. Number of journeys submitted via the Bugs Matter app for the UK and each country during the 2021 survey season.

4 Discussion

Insect abundance

The results of this study show a reduction in numbers of insects sampled on vehicle number plates, consistent with insect abundance decline rates reported by others (Fox et al., 2013; Goulson, D., 2019; Hallmann et al., 2017). The estimate of change in splat rate between 2004 and 2021 (a decrease of 58.5%) has a lower confidence interval of 56.2% and an upper confidence interval of 60.8%, at a 95% confidence level. This means that if we repeated the study, 95% of the time we would expect the estimate of change in splat rate to fall between these values. However, it should be noted that the observations reported here are based on data from only three points in time with a skewed temporal distribution, and consequently do not constitute a trend. With such a low temporal resolution, there is a risk of uncharacteristically high or low insect abundances during these sampling years showing an apparent change in abundance that is unrepresentative of actual insect abundance trends. To accurately estimate change in insect abundance over time, the population needs to be monitored comprehensively at regular intervals over an extended timeframe to reveal the direction and scale of genuine trends. However, the pattern observed in this study is consistent with examples of insect decline reported elsewhere and informs a growing requirement for conservation research, policy and practice targeted at invertebrates in the UK. Similar declining trends were recorded in a study that sampled insects splatted on vehicle windscreens every year between 1997 and 2017 in Denmark (Møller, 2019). However, when windscreen splats in Denmark and Spain in just 1997 and 2018 were compared there was no significant difference due to year (Møller, et al. 2021).

Insect population dynamics and activity are influenced by a range of natural factors that vary inter-annually and across spatial and temporal scales (Figure 9). These factors add noise to longer-term trends in insect abundance but can be partly controlled for in our modelling. For instance, the inclusion of mean temperature and NDVI in our models controls for inter-annual differences in temperature and spatial variation in vegetation cover, both of which may naturally influence insect abundance and activity. Whilst insect populations vary spatially and temporally, so did our insect sampling approach. The time of day and date of the journey, the vehicle type, the vehicle speed and the journey distance all create sampling bias, which we have attempted to control for in our methods, by measuring these variables and including them in our models (Figure 9). By controlling for these effects we obtain more accurate estimates of change in insect splat rate between survey years. However, there are other important variables that are not yet included in the models. For example, environmental variables with demonstrated lethal and sub-lethal influence on insect population ecology such as pesticide use (Møller, et al. 2021a), pollution, land-use change and climate change could explain a further proportion of the unexplained variation in the data. Our model also lacks data on a number of other influential factors on insect abundance and activity such as variation in habitat type and management, disease and predation of insects, other weather conditions including humidity or wind, and natural variation in insect lifecycles or flight periods. Finally, there may be subtle differences in survey methods and/or approaches between journeys and/or years which were not recorded or communicated to subsequent survey managers.

By including a range of variables in the statistical model, it was possible to examine how specific variables affected insect splat rate while controlling for the effects of the other variables in the model. This was important for a more robust estimation of change in splat rate between years, but also allowed us to examine the effects of other factors on insect splat rate. HGVs and sports cars sampled more insects than conventional cars. This may be due to their typical travel speed or aerodynamic properties. Insect splat rate increased by 6% as each hour in the day passed. This could be due to the fact that insects are more

active at higher ambient air temperatures (Mellanby, 1939). Indeed, insect splat rate increased by 2% with each one degree increase in mean daily temperature. Splat rate was found to increase by 3% with each one unit increase in NDVI and the odds of a zero-count journey occurring decreased by 1.3 times with each unit increase in NDVI. These results most likely reflect the fact that insects are more abundant in more vegetated rural areas compared to urban areas, due to the relative suitability of habitats. However, it should also be noted that certain crops will show high NDVI values, but insect abundance may be low in these locations due to pesticide use, the negative influence of crop monocultures on insect abundance, a lack of habitat attributes that provide nesting or overwintering habitats, and a lack of undisturbed habitat and habitat continuity due to intensive management for crops. In future analyses we aim to include data on broad habitat types surrounding journey routes which might help to reveal further information about how insect splat rates vary between land use types. There was no significant relationship between splat rate and average journey speed. Average journey speed is a very low resolution measure of journey speed, and it is likely that a range of other factors such as the spatial distribution of insects and road type interact with the vehicle speed differently along different sections of the journey route. The weak positive relationship between vehicle registration year and splat rate suggests that newer vehicles are more efficient at sampling insects than older vehicles. This is contrary to a suggestion that finding fewer insects on number plates in recent years might be attributed to increasing streamlining of vehicle aerodynamics over time. Our data show that newer vehicles sample more insects than older vehicles, and we have observed pattern of fewer insects on number plates more recently than in the past in spite of this effect of vehicle age, which is assumed to be correlated with aerodynamics.

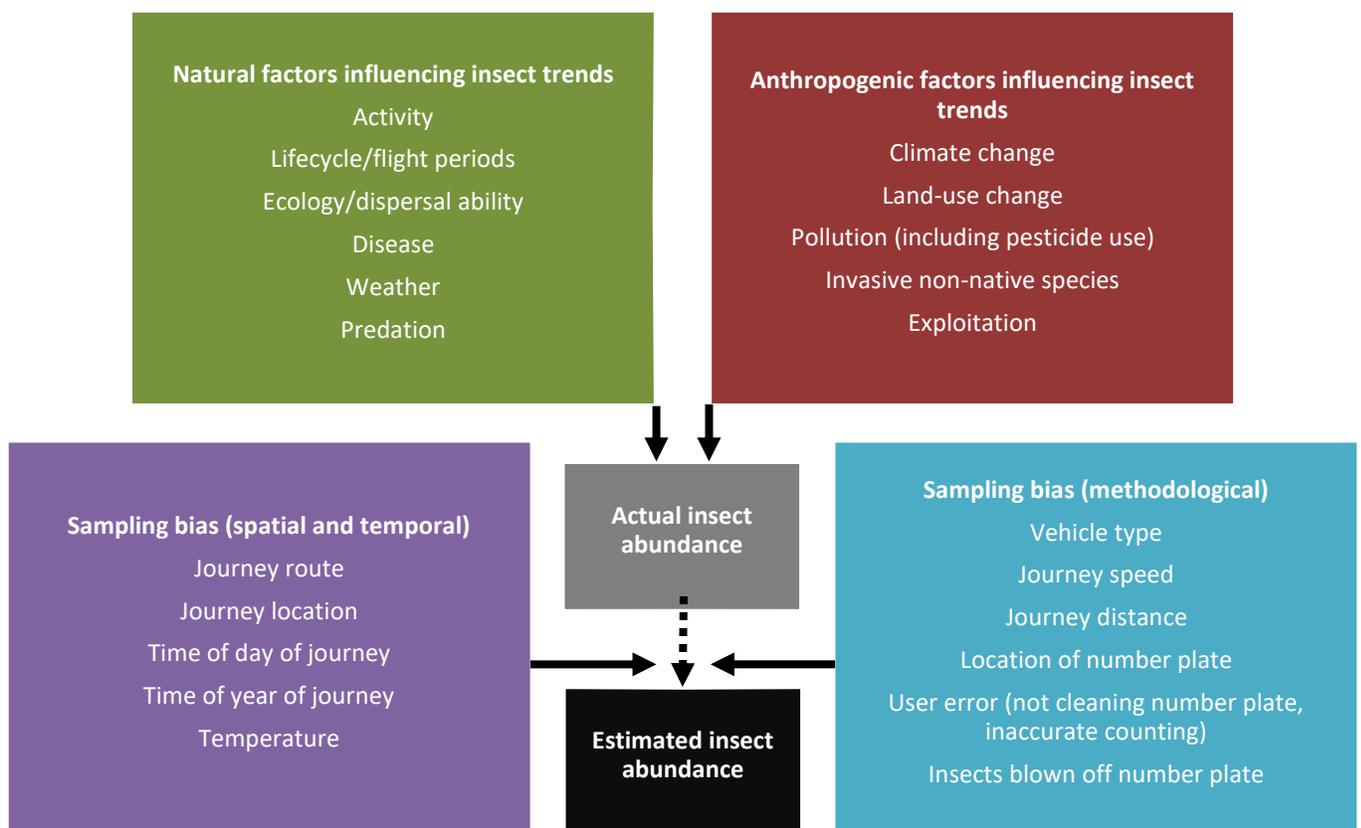


Figure 9. A conceptual diagram showing the range of variables potentially influencing actual insect abundance and estimates of insect abundance using the Bugs Matter app and insect sampling using vehicle number plates conducted by citizen scientists.

Splat rate showed little correlation with journey distance, as shown by a significant but very slight change in splat rate with journey distance. This is somewhat expected as the splat rate is normalized over journey distance, however we might have expected to see more insects sampled over longer journeys due to the increased chances of encountering areas with higher densities of flying insects. Conversely, longer journeys tend to follow motorways where insect abundance may be lower and it is possible that sampled insects could be blown off the number plate on long journeys, especially if the average journey speed is high. The correlation plots showing the relationship between journey distance and splat count and rate (Appendix 8) show some long journeys with very few insect splats or low overall splat rates, which could be partially explained by this phenomenon. Interestingly, the ZINB zero-inflated model determined that the odds of a zero-count journey occurring decreased by 1.02 times with each mile increase in journey distance, suggesting a threshold distance might exist, above which one or more insects are sampled. The average journey distance in 2004 was 60 miles, in 2019 it was 16.6 miles, and in 2021 was 36.3 miles, perhaps reflecting the 2019 survey being focused only in Kent, and changes in traveling behaviour influenced by the global COVID-19 pandemic.

The results of the ZINB zero-inflated model showed that the odds of a zero-count journey occurring increased by 2.9 times between 2004 and 2021. The importance of submitting data for journeys during which zero insects were sampled was communicated to citizen scientists during all survey years, yet there was still a considerably higher proportion of journeys with zero insect splats in 2019 (54.3%) and 2021 (39.5%) compared to 2004 (7.8%). In 2004, the primary method of engagement with citizen scientists was a printed leaflet. With the rise in the use of social media and digital communications it was possible for engagement with citizen scientists in 2019 and 2021 to be more frequent, targeted and specific. This may have resulted in more effective communication of the importance of submitting zero-count journeys, and therefore greater frequency of their occurrence in the data. Another limitation of the survey was that citizen scientists may have forgotten to clean their numberplate prior to conducting a survey, although the risk of this is very low for the 2021 Bugs Matter survey, where the app required a checkbox confirmation that the number plate had been cleaned, the risk may have been higher in 2004, resulting potentially in an elevated count in that year.

Differences in participant behaviour between the two surveys cannot however explain the fact that there were significantly different changes in splat rates in 2004 and 2021 between the different countries of the UK. Most notably while the splat rate was 27.9% lower in Scotland in 2021, it was over twice as reduced in England - 65% lower. Annual counts of moths caught in Rothamsted moth traps were analysed by Fox et al. (2021), they revealed declining trends in moth abundance in traps in Northern and Southern Britain between 1968 and 2017, however while the reduction was -22% in northern Britain, it was nearly twice that, -39%, in southern Britain. Rothamsted moth trap data is itself a proxy for moth abundance, and the time period of the decline is much longer, but the similar pattern of greater rates of loss in the south reinforces concerns that the factors responsible for recent insect declines are acting more strongly on populations in England or Southern Britain.

The national rate of change in flying insect abundance that may be inferred by this study, -34.4%/decade, is much higher than the longer term -6.6%/decade rate of annual moth change calculated by Fox et al. (2021), however the figures are similar to more recent trends, such as the change in insect numbers sampled on vehicle windscreens recorded by Møller (2019), on two transects in Denmark between 1997 and 2017, -38.0%/decade and -46.0%/decade, and are slightly higher than the -28.0% decadal change in the biomass of flying insects in malaise traps on nature reserves in Germany between 1990 and 2011 revealed by Hallmann et al. (2017).

While this data firms up a picture of widespread and severe modern declines in insects, caution is required in extrapolating conclusions from this apparent decline, and in particular in drawing conclusions about insect abundance itself as this is not the only factor affecting the splat rate of insects on number plates. Insect sampling was restricted to transects along the road network, and therefore the spatial coverage of the surveys is inherently limited and may be in part dependant on specific changes to roadside verge management. Whilst this design serves to provide a robust measure of change in the number of insects sampled by cars, by comparing one year to the next, we caution against the use of this data to directly infer insect abundance. Indeed, our method is an activity-density measure and it is conceivable that insects are just as abundant between years, but are less active. We can see this in our results at shorter timescales, where splat rate increases with temperature and after 8pm, not because there are more insects, but because the same number of insects are active in a different way.

Reduced frequency and distances of flying is a scenario that occurs when habitats become so fragmented that dispersal becomes evolutionarily disadvantageous for a species (Hill et al., 1999). There is evidence that when habitats become fragmented there is a tipping point beyond which dispersal is more likely to decrease genetic resources than give genes the chance to proliferate in an under-exploited habitat.

Eventually the high probability of failure outweighs the benefits if successful, so wings shrink, wing muscles atrophy, dispersal reduces (Davies and Saccheri, 2013) and we assume, long-distance dispersal eventually stops. The relationship between increasing habitat fragmentation, increasing temperature and reduced wing functionality has been shown in most groups of butterflies including swallowtails (Dempster et al., 1976; Dempster, 1991), skippers (Fenberg et al., 2016), blues (Dempster, 1991; Wilson et al., 2019), and a white and nymphalid (Bowden et al., 2015). Shrinking wing-size is a phenomenon that has been recently observed in many smaller animals that are likely to be more vulnerable to the effect of fragmentation, such as Spanish wasps (Polidori et al., 2019), German craneflies, where wing size increased but wing loading increased by 26.9% in males (Jourdan et al., 2019), and Bornean moths (Wu et al., 2019). While in South American birds in primary forest body size is reducing but wing size is increasing (Jirinec et al., 2021) indicating that dispersal or at least flight is still evolutionarily beneficial to birds in less fragmented habitats. It may be that reductions in the occurrence of insects in traps or on numberplates is being caused, at least in part, by reduced activity, flight and dispersal of insects, which may be a response to combinations of climate change, habitat fragmentation and pesticide contaminated landscapes that reduce the occurrence of genes associated with long distant flight. Of course, reduced activity of flying insects would itself be indicative of reduced pollination rates for plants at a distance from quality habitats, reduced prey availability for flying insectivores, reduced ability of species to respond to climate change and reduced ability to recolonize after an extinction event, and may be associated with declines in insect populations at a landscape scale.

Synthesis and Application

The Bugs Matter survey successfully quantified a difference in the number of insects sampled on vehicle numberplates over time from baseline data established in 2004. The approach has the potential to provide an efficient, standardised and scalable approach to monitor insect population trends across local, regional and global scales, to add to the growing body of evidence for trends in insect populations and to provide a coarse metric of the functional provision by insects within ecosystems.

We are currently investigating how we could use AI algorithms to automatically count the number of insects on number plates. This would use a virtual template within the app., similar to those used to automatically read credit card details, and return the count in real-time to the user. This would negate the requirement for a splatometer making it quicker and easier for citizen scientists to count and record

data. In 2021, a high proportion of people who downloaded the app. did not submit any data. The need for a physical splatometer is thought to be one barrier to participation, and removing this requirement may help to increase numbers of participants in future years and to reduce the operating costs of the survey.

An increasing number of studies are accumulating evidence of insect declines, and associated consequence for ecosystem functions, including the reductions in genetic diversity, β -diversity and species evenness that are associated with the failure of species to disperse and colonise or recolonise habitats in a fragmented landscape (Vasiliev et al., 2021). It is important to recognise that these patterns and trends are often nuanced, and that local conditions and choice of analytical approach may mean that results reported locally or regionally may not reflect patterns everywhere. Over-simplified reporting by the media of negative trends from short time series data such as those presented here, risks missing some of the nuances and limitations of research. Whilst there is growing evidence of potentially catastrophic declines in insect diversity and abundance, care must be taken to not extrapolate too far, with potential consequences for undermining public confidence in research. We recognise and stress that the results we have reported here do not constitute a trend, and advocate strongly for data collection over extended timeframes to enable conclusions about trends in insect populations to be drawn. We believe that the widespread adoption of the Bugs Matter survey facilitated by the Bugs Matter app can provide a replicable and scalable approach for the generation of an enhanced evidence-base that can be used to assess trends and drive positive action for insects and other invertebrates.

Increasing sample size both by increasing the number of citizen scientist participants and the number of journeys undertaken would provide greater confidence in the reliability of our data as a robust indicator of patterns in insect abundance. Similarly, cross-validating our results with other monitoring schemes for insect abundance, such as the Rothamsted Insect Survey (RIS) (Fox et al., 2013) or the UK Pollinator Monitoring Scheme (<https://www.ceh.ac.uk/our-science/projects/uk-pollinator-monitoring-scheme>), or the results of long-term Malaise trapping studies (Hallmann et al., 2017), would provide another means to calibrate and critique the patterns in our data. There is potential for the survey method to have global application and relevance, and deployed at a national scale, it can provide data at resolutions appropriate to the scale at which the ecosystem services provided by insects operate. By continuing to promote participation in the survey in subsequent years, insect conservationists can capitalise on the opportunity to gather long-term data and build the evidence base for insect abundance at UK county and national scale.

Acknowledgements

Bugs Matter is a partnership between Kent Wildlife Trust and Buglife, with Gwent, Essex and Somerset Wildlife Trusts, and is supported by the RSPB. PTM, LB, RS and AS are employed by, and AR formerly employed by Kent Wildlife Trust and AW is employed by Buglife – The Invertebrate Conservation Trust. We sincerely thank all of the citizen scientists who took part in the survey and made this analysis possible, and the wider project team: Evan Bowen-Jones, Ashton Dreyer, Chloe Edwards and Paul Hadaway of Kent Wildlife Trust, and Paul Hetherington and Matt Shardlow of Buglife. We also thank Guy Anderson, Richard Bashford and Richard Bradbury of the RSPB for facilitating our use of the ‘splatometer’ method, providing the data from 2004 and for helpful discussion. Mick Crawley and Simon Leather provided helpful feedback on an earlier iteration of the analysis presented in this report. Craig Macadam of Buglife provided helpful comments on the manuscript. The Bugs Matter app. was developed by Natural Aptitude, and was funded by two anonymous donors.

Author contributions

This project was led and managed by PTM, who contributed much of the text for this report along with guidance for the statistical analyses. LB led on the collation of variables, statistical analysis and report writing, whilst RS performed initial data cleaning and formatting and the analysis of the participation data. AR and AS were part of the project team in 2019, AR leading on GIS and data assimilation, and AR on citizen scientist participation. PTM and AR designed the original brief and specification for the Bugs Matter app., and MS and AW provided helpful comments and input on an earlier draft of the manuscript.

References

- Bowden J.J., Eskildsen A., Hansen R.R., Olsen K., Kurle C.M. & Høye T.T. (2015) High-Arctic butterflies become smaller with rising temperatures. *Biology Letters* 11(10), 1–4. doi: 10.1098/rsbl.2015.0574
- Brereton T.M., Botham, M.S., Middlebrook, I., Randle, Z., Noble D., Harris, S., Dennis, E.B., Robinson A., Peck, K. & Roy, D.B. 2020. United Kingdom Butterfly Monitoring Scheme report for 2019. UK Centre for Ecology & Hydrology, Butterfly Conservation, British Trust for Ornithology and Joint Nature Conservation Committee.
- Breiman, L., Friedman J.H., Olshen R.A., and Stone C.J. (1984) *Classification and regression trees*. Taylor & Francis, Monterey.
- Cameron S.A., Lozier J.D., Strange J.P., Koch J.B., Cordes N., Solter L.F. and Griswold, T.L. (2011) Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*. 108, 662-667. doi: 10.1073/pnas.1014743108
- Chapman R.F., Simpson S.J., and Douglas, A.E. (2013) *The Insects: Structure and Function*. Cambridge University Press.
- Coelho R., Infante P. and Santos M.N. (2020) Comparing GLM, GLMM, and GEE modelling approaches for catch rates of bycatch species: A case study of blue shark fisheries in the South Atlantic. *Fish Oceanography*. 29, 169–184. doi: 10.1111/fog.12462
- Cornes R., G. van der Schrier, E.J.M. van den Besselaar, and Jones P.D. (2018) An Ensemble Version of the E-OBS Temperature and Precipitation Datasets, *Journal of Geophysical Research: Atmospheres*, 123, 9391-9409. doi: 10.1029/2017JD028200
- Crawley M.J. (2007) *The R Book*. John Wiley and Sons Ltd, Chichester.
- Davies W.J. and Saccheri, I.J. (2013) Maintenance of body-size variation and host range in the orange-tip butterfly: evidence for a trade-off between adult life-history traits. *Ecological Entomology* 38, 49–60. doi: 10.1111/j.1365-2311.2012.01402.x
- Dempster J.P., King M.L. and Lakhani K.H. (1976) The status of the swallowtail butterfly in Britain. *Ecological Entomology* 1(2), 71–84.
- Dempster J.P. (1991) Fragmentation, isolation and mobility of insect populations. In *The conservation of insects and their habitats* (ed. N. M. Collins & J. A. Thomas), pp. 143-154. London: Academic Press.
- Didan K. (2015). MOD13Q1 MODIS/Terra Vegetation Indices 16-Day L3 Global 250m SIN Grid V006. NASA EOSDIS Land Processes DAAC. Accessed 2022-02-08 from <https://doi.org/10.5067/MODIS/MOD13Q1.006>
- Dorman M. (2022). mapsapi: 'sf'-Compatible Interface to 'Google Maps' APIs. R package version 0.5.3. <https://CRAN.R-project.org/package=mapsapi>
- Fenberg P.B., Self A., Stewart J.R., Wilson R.J. and Brooks, S.J. (2016) Exploring the universal ecological responses to climate change in a univoltine butterfly. *Journal of Animal Ecology*, 85(3), 739–748. doi: 10.1111/1365-2656.12492
- Fox R, Dennis EB, Harrower CA, Blumgart D, Bell JR, Cook P, Davis AM, Evans-Hill LJ, Haynes F, Hill D, Isaac NJB, Parsons MS, Pocock MJO, Prescott T, Randle Z, Shortall CR, Tordoff GM, Tuson D & Bourn NAD

(2021) The State of Britain's Larger Moths 2021. Butterfly Conservation, Rothamsted Research and UK Centre for Ecology & Hydrology, Wareham, Dorset, UK.

Fox R., Parsons M.S., Chapman J.W., Woiwood I.P. Warren M.S. and Brooks D.R. (2013) The state of Britain's larger moths 2013. Butterfly Conservation & Rothamsted Research Wareham, Dorset

Gorelick N., Hancher M., Dixon M., Ilyushchenko S., Thau D., and Moore R. (2017) Google Earth Engine: Planetary-scale geospatial analysis for everyone. *Remote Sensing of Environment*. doi: 10.1016/j.rse.2017.06.031

Goulson D. (2019) Insect declines and why they matter. A report commissioned by the South West Wildlife Trusts. <https://www.kentwildlifetrust.org.uk/sites/default/files/2020-01/Actions%20for%20Insects%20-%20Insect%20declines%20and%20why%20they%20matter.pdf>

Hallmann C.A., Sorg M., Jongejans E., Siepel H., Hofland N., Schwan H., Stenmans W., Müller A., Sumser H., Hörrn T., Goulson D. and de Kroon H. (2017) More than 75% decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* 12(10), e0185809. doi: 10.1371/journal.pone.0185809

Hill J.K., Thomas C.D., Lewis O.T. (1999) Flight morphology in fragmented populations of a rare British butterfly, *Hesperia comma*. *Biological Conservation*, 87, 277-283.

Jirinec V., Burner R.C., Amaral B.R., Bierregaard Jr R.O., Fernández-Arellano G., Hernández-Palma A., Johnson E.I., Lovejoy T.E., Powell L.L., Rutt C.L. and Wolfe J.D. (2021) Morphological consequences of climate change for resident birds in intact Amazonian rainforest. *Science advances*, 7(46). doi: 10.1126/sciadv.abk1743

Jourdan J., Baranov V., Wagner R., Plath M., and Haase P. (2019) Elevated temperatures translate into reduced dispersal abilities in a natural population of an aquatic insect. *Journal of Animal Ecology*, 88(10), 1498-1509. doi: 10.1111/1365-2656.13054

Lovelace R. and Ellison R. (2018) "stplanr: A Package for Transport Planning." *The R Journal*, 10(2). doi: 10.32614/RJ-2018-053.

Macadam, C.R, Whitehouse, A.T. & Shardlow, M. (2020) No Insectinction - how to solve the insect declines crisis. Buglife - The Invertebrate Conservation Trust, Peterborough. <https://cdn.buglife.org.uk/2020/05/NoInsectinction2020.pdf>

Mellanby K. (1939) Low temperature and insect activity. *Proceedings of the Royal Society of London. Series B-Biological Sciences*, 127(849), 473-487.

Miska L., and Jan H. (2004) Evaluation of current statistical approaches for predictive geomorphological mapping. *Geomorphology*. 67, 299–315.

Møller A.P. (2019) Parallel declines in abundance of insects and insectivorous birds in Denmark over 22 years. *Ecology and Evolution*, 9(11), 6581–6587. <https://doi.org/10.1002/ece3.5236>

Møller A.P. (2021) Citizen Science for Quantification of Insect Abundance on Windshields of Cars Across Two Continents. *Frontiers in Ecology and Evolution*, 541. doi: 10.3389/fevo.2021.657178

Møller A.P. (2021a) Abundance of insects and aerial insectivorous birds in relation to pesticide and fertilizer use. *Avian Research*, (12)43, doi: 10.1186/s40657-021-00278-1

- Outhwaite, C.L., McCann, P. & Newbold, T. (2022) Agriculture and climate change are reshaping insect biodiversity worldwide. *Nature*. doi: 10.1038/s41586-022-04644-x
- Pebesma E. (2018) "Simple Features for R: Standardized Support for Spatial Vector Data." *The R Journal*, 10(1), 439–446. doi: 10.32614/RJ-2018-009,
- Pocock M.J.O., Newson S.E., Henderson I.G., Peyton J., Sutherland W.J., Noble D.G., Ball S.G., Beckmann B.C., Biggs J., Brereton T., Bullock D.J., Buckland S.T., Edwards M., Eaton M.A., Harvey M.C., Hill M.O., Horlock M., Hubble D.S., Julian A.M., ... Roy, D. B. (2015) Developing and enhancing biodiversity monitoring programmes: A collaborative assessment of priorities. *Journal of Applied Ecology*, 52(3), 686–695. doi: 10.1111/1365-2664.12423
- Polidori C., Gutiérrez-Cánovas C., Sánchez E., Tormos J., Castro L., and Sánchez-Fernández, D. (2019) Climate change-driven body size shrinking in a social wasp. *Ecological Entomology*, 45(1), 130-141. doi: 10.1111/een.12781
- R Development Core Team (2021) *R: A Language and Environment for Statistical Computing*. Vienna, Austria.
- Sánchez-Bayo F., and Wyckhuys, K.A.G. (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, 232(January), 8–27. doi: 10.1016/j.biocon.2019.01.020
- Sokal R.R. and Rohlf F.J. (1995) *Biometry: the principles and practice of statistics in Biological Research*. W. H. Freeman; 3rd Edition
- Taylor, L. R. (1986). Synoptic Dynamics, Migration and the Rothamsted Insect Survey: Presidential Address to the British Ecological Society, December 1984. *Journal of Animal Ecology*, 55(1), 1–38. <https://doi.org/10.2307/4690>
- Thomas, C.D., Jones, T.H., and Hartley, S.E. (2019) "Insectageddon": A call for more robust data and rigorous analyses. *Global Change Biology*, 25(6), 1891–1892. doi: 10.1111/gcb.14608
- Tinsley-Marshall P., Skilbeck A. and Riggs A. (2021a) Bugs Matter citizen science survey demonstrates temporal difference in invertebrate abundance in Kent and South East England. Kent Wildlife Trust, Maidstone doi: 10.13140/RG.2.2.15903.89768
- Tinsley-Marshall P.J., Riggs A., Skilbeck A., Ball L. and Still R. (2021b) Nature's Sure Connected: A practical framework and guidance for evidencing landscape-scale outcomes of landscape-scale conservation. Kent Wildlife Trust. doi: 10.13140/RG.2.2.10556.77447
- van der Sluijs, J.P. (2020) Insect decline, an emerging global environmental risk. *Current Opinion in Environmental Sustainability*, 46, 39–42. doi: 10.1016/j.cosust.2020.08.012
- Vasiliev D. and Greenwood S. (2021) The role of climate change in pollinator decline across the Northern Hemisphere is underestimated. *Science of the Total Environment*, 775, 145788. doi: 10.1016/j.scitotenv.2021.145788
- Venables W.N. and Ripley B.D. (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York. ISBN 0-387-95457-0
- Vuong Q.H. (1989) Likelihood Ratio Tests for Model Selection and non-nested Hypotheses. *Econometrica*. 57(2), 307–333. doi:10.2307/1912557.

Wikipedia (2021) Windshield phenomenon [Online]. [Accessed 11th November 2021]. Available from: https://en.wikipedia.org/wiki/Windshield_phenomenon

Wilson R.J., Brooks S.J. and Fenberg P.B. (2019) The influence of ecological and life history factors on ectothermic temperature–size responses: analysis of three Lycaenidae butterflies (Lepidoptera). *Ecology and Evolution*, 9(18), 10305–10316. doi: 10.1002/ece3.5550

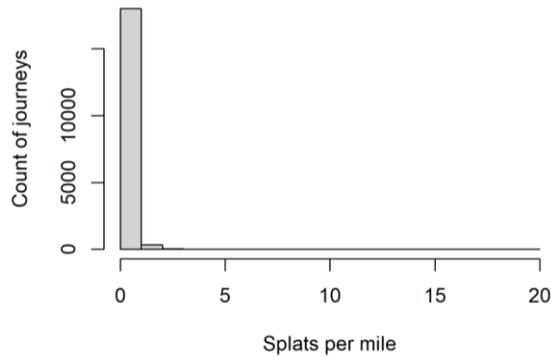
Wu C.H., Holloway J.D., Hill J.K., Thomas C.D., Chen I., and Ho C.K. (2019) Reduced body sizes in climate-impacted Borneo moth assemblages are primarily explained by range shifts. *Nature communications*, 10(1), 1-7. doi: 10.1038/s41467-019-12655-y

Yau K.K., Wang K., & Lee A.H. (2003) Zero-inflated negative binomial mixed regression modeling of over-dispersed count data with extra zeros. *Biometrical Journal: journal of mathematical methods in biosciences*, 45(4), 437-452. doi: 10.1002/bimj.200390024

Zeileis A., Kleiber C., and Jackman S. (2008) Regression Models for Count Data in R. *Journal of Statistical Software* 27(8). URL <http://www.jstatsoft.org/v27/i08/>.

Appendices

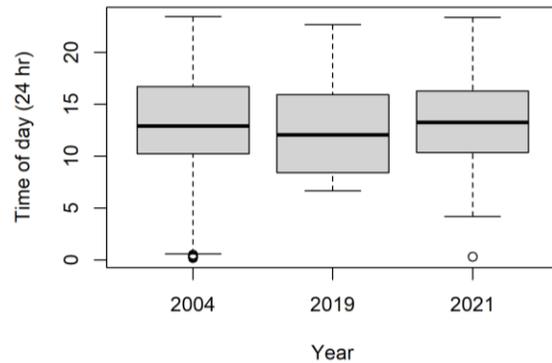
Appendix 1. A histogram of the splat rate (splats per mile) data.



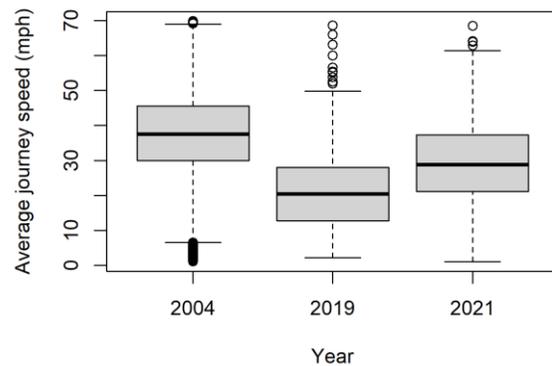
Appendix 2. The number of journeys conducted by each vehicle type in each survey year.

	2004	2019	2021
Car	12547	307	2812
HGV	257	89	75
MPV	338	13	318
Sports car	619	41	50
SUV	33	149	10
Van	672	0	53

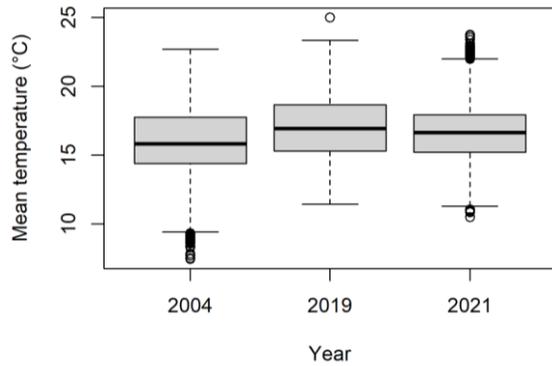
Appendix 3. Box and whisker plot showing the spread of the time of day of journey data from the Bugs Matter survey of insects on car number plates in in each of the survey years. A Kruskal-Wallis test showed a significant difference in the time of day at which journeys were undertaken between the survey years ($H(1) = 33.253$, $p = < 0.001$).



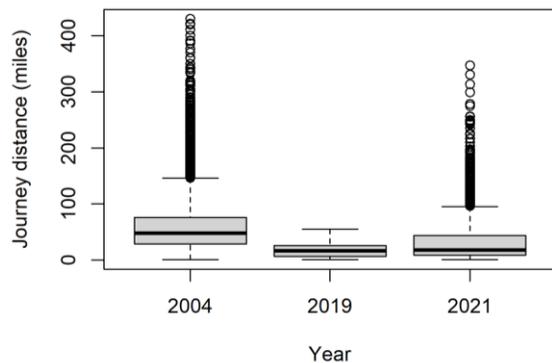
Appendix 4. Box and whisker plot showing the spread of the average journey speed data from the Bugs Matter survey of insects on car number plates in in each of the survey years. A Kruskal-Wallis test showed a significant difference in the average journey speed between the survey years ($H(1) = 1677.517$, $p = < 0.001$).



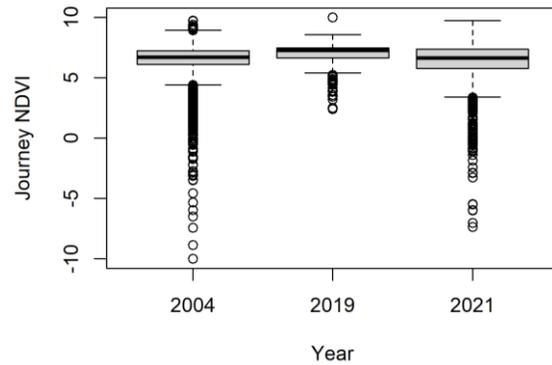
Appendix 5. Box and whisker plot showing the spread of the mean journey temperature data from the Bugs Matter survey of insects on car number plates in in each of the survey years. A Kruskal-Wallis test showed a significant difference in the mean journey temperature between the survey years ($H(1) = 274.594, p = < 0.001$).



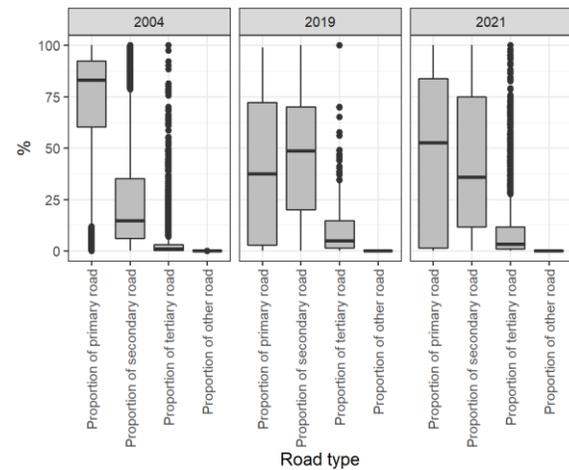
Appendix 6. Box and whisker plot showing the spread of the journey distance data from the Bugs Matter survey of insects on car number plates in in each of the survey years. A Kruskal-Wallis test showed a significant difference in the journey distances between the survey years ($H(1) = 2794.17, p = < 0.001$).



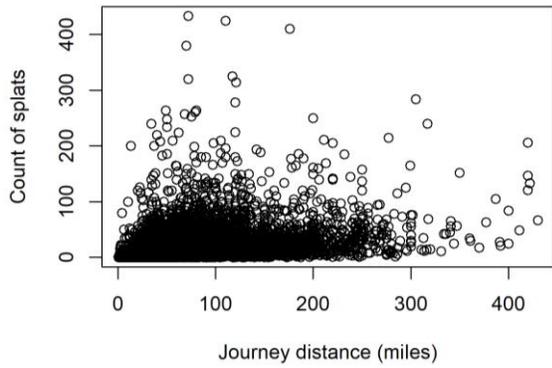
Appendix 7. Box and whisker plot showing the spread of the NDVI data from the Bugs Matter survey of insects on car number plates in in each of the survey years. A Kruskal-Wallis test showed a significant difference in the journey NDVI between the survey years ($H(1) = 144.134, p = < 0.001$).



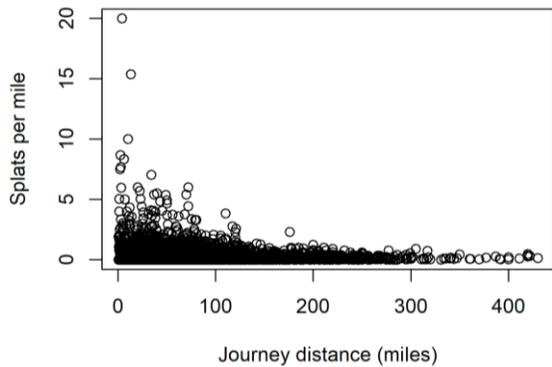
Appendix 8. Box and whisker plot showing the spread of the road type data from the Bugs Matter survey of insects on car number plates in in each of the survey years.



Appendix 9. Correlation plot showing the relationship between journey distance (x-axis) and count of splats (y-axis). A Spearman correlation test showed a significant positive correlation between journey distance and count of splats ($\rho = 0.636, p = < 0.001$).

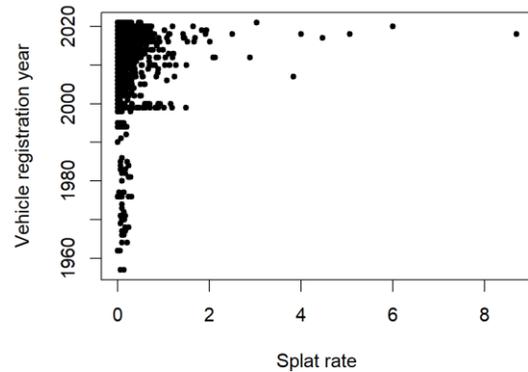


Appendix 10. Correlation plot showing the relationship between journey distance (x-axis) and splat rate (y-axis). A Spearman correlation test showed a weak but significant positive correlation between journey distance and count of splats ($\rho = 0.198$, $p < 0.001$).

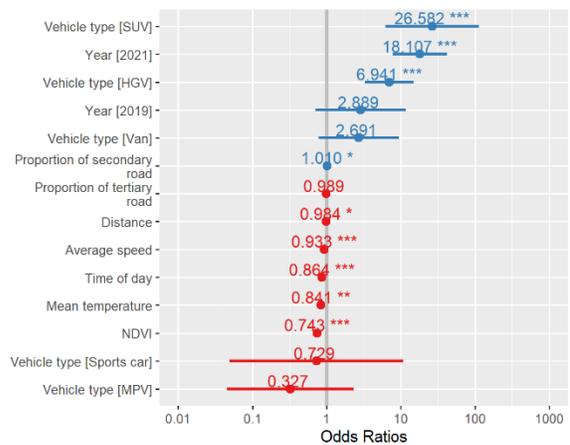


Appendix 11. Correlation plot showing the relationship between splat rate (x-axis) and vehicle registration year (y-axis) (data available only from 2019 and 2021). A simple linear regression on log-transformed splat rate showed a weak positive trend (coef 0.00072, $p = 0.015$) between vehicle

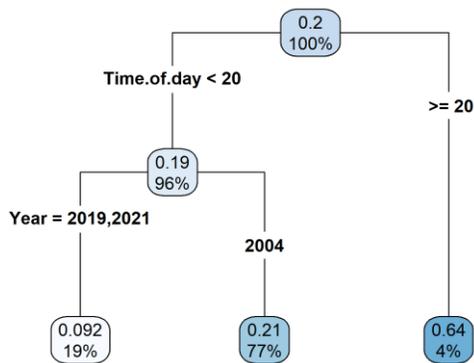
registration year and splat rate.



Appendix 12. Forest plot of odds ratios from the ZINB zero-inflated model of Bugs Matter survey data of insects on car number plates in the UK, showing the change in the odds of a zero-count journey occurring given a one-unit change in the independent variable, while holding other variables in the model constant. Significant relationships between splat rate and independent variables are shown by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Vehicle types are compared to the reference category of 'conventional cars'. The reference year is 2004.



Appendix 13. The regression tree describing splat rate had two splits, three terminal nodes and a cross-validated error of 0.918.



Appendix 14. Complexity parameter plot and variable importance for the regression tree describing splat rate. Complexity parameter plots show the reduction in the cross validated error with decreasing complexity parameter and increasing tree size. We would see diminishing returns if we continued to grow the trees. Variable importance is calculated as the sum of the goodness of split measures (Gini index) and considers both primary and surrogate splits.

