Article

EVALUATING THE EFFECTS OF OBSERVATION PERIOD, FLORAL DENSITY, AND WEATHER CONDITIONS ON THE CONSISTENCY AND ACCURACY OF TIMED POLLINATOR COUNTS

Neil Mahon*, Simon Hodge

School of Agriculture and Food Sciences, University College Dublin, Dublin, Ireland

Abstract—Insect pollinators are experiencing substantial declines as a result of habitat loss, agricultural intensification, invasive pests, and climate change. To investigate factors causing pollinator declines, evaluate the success of conservation measures, and institute long-term monitoring schemes, it is essential to validate and standardize pollinator sampling techniques. This study investigated how sampling duration, weather conditions, and abundance of floral resources influenced the results of timed pollinator counts by repeatedly sampling the same pollinator assemblage in an Irish meadow. The likelihood of detection of *Apis mellifera*, *Bombus* spp, solitary bees, and Syrphidae was strongly associated with the density of floral units or floral cover in the observation plot. Also, even though protocol criteria restricted pollinator counts to the middle of the day and benevolent weather, pollinator counts were strongly influenced by factors such as cloud cover, light levels, wind speed and relative humidity. Increasing the duration of the timed counts from 5-minutes to 30-minutes considerably increased the probability of detection of each pollinator group. Additionally, the perceived diversity of the pollinator assemblage at the meadow was markedly affected by sampling duration and floral abundance. To improve the consistency or comparability of studies using timed pollinator counts, we recommend that criteria are set restricting surveys to narrow ranges of weather conditions and floral density when possible. Additionally, pollinator field investigations or monitoring programs would benefit from a systematic evaluation of how erroneous non-detection of target taxa can be reduced to acceptable levels by modifying sampling duration.

Keywords—Ecosystem services; insect declines; non-detection errors; pollinator monitoring; bees; Syrphidae

Journal of Pollination Ecology, 32(13), 2022, pp 124-138
DOI: 10.26786/1920-7603(2022)699
Received 12 April 2022, accepted 2 August 2022
*Corresponding author: neil.mahon@ucdconnect.ie

INTRODUCTION

Insect pollinators are experiencing substantial declines in abundance and diversity, primarily driven by habitat loss, agrochemical toxicity, exposure to pests, and impacts of invasive species (Brittain et al. 2010; Potts et al. 2010; Dicks et al. 2021). These pollinator declines are of major concern because they represent a fundamental loss of biodiversity and because of the immediate implications for food production. Consequently, much research has been generated to demonstrate and clarify relationships between pollinator communities and the 'health' of agro-ecosystems, along with repeated calls to initiate long-term monitoring programs of pollinator abundance and diversity (Lebuhn et al. 2013; Dicks et al. 2021; Hodge 2020; Hutchinson et al. 2022; Krahner et al. 2021).

Successful ecological monitoring programs require that sampling methods are standardized and optimized as far as possible, especially to ensure the data collected allow for meaningful comparisons among locations and clearly illustrate patterns over time. It is also valuable to establish what sampling effort is required to record all, or at least most, taxa present, and reduce the probability of erroneous non-detection of taxa to acceptable levels (Hodge et al. 2017). Increasingly, the

weaknesses of pollinator monitoring schemes that represent a misallocation of resources, or advocate the use of inappropriate or sub-optimal collecting methods, or employ methods that strongly bias collections in favour of certain taxa or body sizes, are being strongly critiqued in the ecological literature (e.g. Tepedino et al. 2015; Prendergast & Hogendoorn 2021; Saunders et al. 2021; Thompson et al. 2021; Tepedino & Portman 2021).

Several passive and active sampling methods are used to study insect pollinators, and several previous papers have compared and contrasted the value and weaknesses of these different techniques (e.g. Westphal et al. 2008; O'Connor et al. 2019; Prendergast et al. 2020; Hutchinson et al. 2022; Krahner et al. 2021; Thompson et al. 2021; Leclercq et al. 2022). The sampling method adopted in any given study often reflects the prior experiences of the researchers, as well as the scale of the project and the research aims, so the data set obtained is fit for purpose (Westphal et al. 2008). Additionally, consideration is often given to which and how many pollinator groups are being investigated, the level of taxonomic resolution required, and the time, labour, and resources available to carry out the research (Westphal et al. 2008; Hutchinson et al. 2022).

Large numbers of pollinators can be collected by passive trapping methods such as pan traps and Malaise traps, although there are often concerns that such methods bias collections towards certain taxa and may not be appropriate when studying species of conservation concern (Hutchinson et al. 2022; Krahner et al. 2021; Thompson et al. 2021). Additionally, these methods do not allow the identification of interactions connecting flowering plants and specific pollinator species. Non-lethal sampling methods such as timed quadrat counts, timed observations of individual plants or flowers, transects, and recording flower or blossom occupancy, have been suggested to provide a more representative sample of pollinator assemblages and allow direct identification of specific plant-pollinator interactions (Hodge et al. 2017; Krahner et al. 2021; Prendergast & Hogendoorn 2021). In turn, these observational methods are criticised because they are prone to inter-observer error and often preclude fine taxonomic resolution or separation of similar looking taxa in the field (Prendergast et al. 2020; Krahner et al. 2021; Saunders et al. 2021).

When employing observational methods such as timed plot counts and transects, several factors external to the actual pollinator assemblage present, such as time of day and weather conditions, can influence the data obtained. To reduce the effects of these nuisance factors, researchers will often impose certain criteria or rules restricting when pollinator counts should be attempted. For example, as some pollinating insects show clear circadian trends in activity (e.g., Gilbert 2005; Prasad & Hodge 2013) researchers often restrict surveys to time windows centred around the middle of the day to minimise temporal variation among samples (Hodge & Stout 2019). Similarly, because the foraging activity of flower-visiting insects can be influenced by a range of weather parameters, such as temperature, relative humidity, wind speed, and light intensity (Burrill & Dietz 1981; Peat & Goulson 2005; Hennessy et al. 2021; Sühs et al. 2021), protocols for comparative pollinator assessments often include weather-based criteria that must be met before surveys are considered valid (Hodge & Stout 2019). The density and diversity of floral units present in the observation area can also influence which and how many pollinators are recorded during a timed count or transect survey (Ohashi & Yahara 2002; Byrne & DelBarco-Trillo 2019; Brunet et al. 2021). This can be of high relevance if the locations of transects or timed counts are assigned using some randomisation procedure, as is often recommended to avoid systematic sampling biases: if, by chance, different surveys are performed across locations with high variation in floral density then this may add additional variation to the final pollinator data set.

In addition to sampling method, several studies have examined how sampling effort, generally expressed as the number of samples obtained or individuals collected, influences the probability of taxa being recorded and the perceived diversity of the pollinator assemblage (e.g., Tikoca et al. 2016; Wheelock et al. 2016; Hodge et al. 2017). For observational sampling methods, the duration of individual monitoring events can differ widely among studies. Although, in general, individual transect surveys or timed pollinator counts tend to be between 10-30 minutes in duration (Hutchinson

et al. 2022), they can also be of much shorter periods: six minutes (Westphal et al. 2008), five minutes (Russo et al. 2020), three minutes (Tamburini et al. 2016) and 30 seconds (Prasad & Hodge 2013). There can be an inherent issue with short observation periods in that the final data can be highly zero inflated (e.g., Russo et al. 2020) and/or be prone to non-detection errors where designation of a species as absent may be due to inadequate sampling effort rather than actual non-occurrence (MacKenzie 2005; Royale et al. 2012; Hodge et al. 2017; Blasco-Moreno et al. 2019). If this latter situation is extended to multispecies data, surveys of short duration may tend to record only the commoner species present in the pollinator assemblage, leading to erroneous inferences regarding pollinator diversity (Hodge et al. 2017). It is often necessary, however, to balance the desired duration of each survey with other practical aspects of the field study, such as the number of samples required that day, the number of distinct sites that are to be visited, and the time limitations imposed by the weather and diurnal time-window criteria outlined above. Additionally, after a point, increasing the length of observations may provide only minor improvements in the final data set, and, in the extreme, be totally superfluous if there really are no insects present to record (Hodge & Vink 2016; Tikoca et al. 2016).

The situation in Ireland with regard to insect pollinators largely mirrors that seen in other countries. Of the approximately 100 wild bee species present, half have undergone major declines since the 1980s, 30 species are threatened with extinction in Ireland, and three of these species are threatened with extinction at a European level (AIPP 2021). The main causes of Irish pollinator declines are thought to be primarily associated with land use changes, agricultural intensification, and loss of semi-natural habitats such as florally rich grasslands and hedgerows. The All-Ireland Pollinator Plan (AIPP; www.pollinators.ie), a multi-stakeholder organisation involving academics, local councils, businesses, and community groups, has recently announced its objectives for 2021-2025 which include the need to increase pollinator monitoring on farmland in line with European Pollinator Monitoring Scheme recommendations (2020). The latest AIPP objectives also include recommendations for additional research into the development and testing of methods for non-lethal monitoring of pollinators and further optimization of Flower-Insect Timed counts (FIT Counts) as a means of obtaining standardized data from research initiatives and citizen science schemes (AIPP 2021).

The primary aim of the current study was to gauge the level of variation that can occur in timed pollinator counts even when using a standardized method and adhering to strict protocol criteria pertaining to weather conditions and time of day. We evaluated three aspects of timed pollinator counts that can systematically influence the data obtained, namely: (i) the effects of immediate weather conditions on pollinator visitation to study plots, (ii) the effects of floral density and richness on pollinator visitation to study plots, and (iii) how the duration of timed counts influences the likelihood of pollinators being recorded and how this in turn affects the diversity of the pollinator assemblage that is inferred to be present. To meet these objectives, we repeatedly sampled the same pollinator assemblage occurring in an Irish wildflower meadow over 20 samplings days. With these results, we offer recommendations as to how pollinator monitoring based on timed counts might be improved and highlight potential confounding variables that should be recorded as part of the data collection process.

MATERIALS AND METHODS

POLLINATOR SURVEYS

Surveys were carried out in a wildflower meadow (1.4 ha) at Rosemount Environmental Research Station, University College Dublin, Ireland (53.305712, -6.232129). To provide measures of floral density, the floral units within each 2 x 2 m survey quadrat were counted, and the percentage cover of open blossoms was estimated. The primary aim of the study was to investigate the effects of floral density on pollinator counts rather than floral richness. Therefore quadrats were restricted to containing a maximum of four flowering species from the ten most commonly occurring species in the meadow (common bird's-foot trefoil (*Lotus corniculatus* L.); meadow buttercup (*Ranunculus acris* L.); red clover (*Trifolium pratense* L.); white clover (*Trifolium repens* L.); dandelion (*Taraxacum vulgare* ((L.)

Weber ex F.H.Wigg.); common field-speedwell (*Veronica persica* Poir.); common mouse-ear (*Cerastium fontanum* Baumg.); cat's-ear (*Hypochaeris radicata* L.); common vetch (*Vicia sativa* ssp. *Segetalis* L.); daisy (*Bellis perennis* L.)].

Pollinator surveys were performed between May 19th and July 1st, 2021, over 20 sampling days. On each sampling day, six 2 x 2 m quadrats were placed so that two quadrats contained high floral density (>150 floral units), two quadrats contained medium floral density (50-150 floral units), and two quadrats contained low floral density (<50 floral units). Thus, each floral density category was replicated 40 times in total. The order in which plots of different floral densities were observed was randomised each day to avoid confounding floral density with time of day. At each quadrat, the number of insect pollinators belonging to four main groups [bumblebees (*Bombus* spp.), honeybees (*Apis mellifera* L.), solitary bees (Anthophila), hoverflies (Syrphidae)] that interacted directly with any part of the open flowers were recorded every five minutes over a 30-minute observation period.

At the start of each five-minute observation period temperature (°C) and relative humidity (%) were recorded at a height of 1 m off the ground using a portable electronic meter [HT-86, Donguan Xintai Instruments Ltd., China). Additionally, light intensity [Klux; MT-912 Light Meter, Shenzen Plus Tech Ltd, China], wind speed (ms^{-1}) and air pressure (mbar) [Digital Anemometer 866B-WM, Infuridor, China] were also recorded. Cloud cover (%) in an overhead view was estimated as assigning the proportion of sky covered by cloud to broad percentage intervals (eg. 0%, 10%, 25%, 50%, 100% etc).

Pollinator counts were restricted with respect to environmental conditions using criteria typically recommended in pollinator research (e.g., Kleijn et al. 2015; Hodge & Stout 2019) so that no surveys were performed when there were high wind speeds (> 8 ms^{-1}), low temperatures (< 10°C), or during rain fall, and all counts were performed between 11am and 4pm.

Statistical analyses

All statistical analyses were conducted using Genstat (v21, VSN International Ltd., UK). To assess the effect of weather and flora on the counts of pollinators in each 30-minute survey, the mean values of the weather variables (temperature, relative humidity (RH), cloud cover, light, wind speed, air pressure, cloud cover) were obtained from the measurements taken at the start of each 5-minute observation period. Rank correlation coefficients were then calculated using all 120 of the 30-minute surveys to provide information on the strength and direction of relationships between pollinator counts and the different weather and floral measurements.

To examine the effect of weather and flora on pollinator counts in more detail, a separate generalized linear mixed model (GLMM) was performed for each pollinator group, treating the 30-minute count data as Poisson distributed and using a log link function. To account for any over dispersion of the data, these GLMMs estimated the model dispersion parameter and included sampling day as a random factor. Statistical significance of effects was estimated by dropping each term from the full model. Finally, a step-wise generalized linear modelling (GLM) procedure was performed for each pollinator group to identify the best model based on the Akaike Information Criteria (AIC). These step-wise GLMs also used a Poisson data structure with log link function, but did not include sampling day as a random factor.

The effect of observation period

The effect of observation duration on the probability of recording each pollinator group was initially examined using a binomial expansion. The probability of recording each pollinator group in a 5-minute period was estimated for high, medium, and low floral density quadrats by determining the number of 5-minute observation periods (from a total of 240) that each group was present. Then, if the probability of a pollinator group being present in any 5-minute period was taken as $P_{present}$, the probability of that group not being recorded would equal $(1\text{-}P_{present})$. The probability of the pollinator group not being recorded in n successive 5-minute periods can then be estimated as $(1\text{-}P_{present})^n$, so conversely the probability of detection in the same number of 5-minute periods would be equal to $1\text{-}(1\text{-}P_{present})^n$.

To examine the effect of extending the actual sampling duration by 5-minute increments, we used the raw data from the 40 surveys at each floral

density to calculate the proportion of surveys of increasing duration (in terms of 5-minute periods) that had recorded each pollinator group. We then subjected these binomial proportion data to probit analysis to estimate the observation period (OP) required for 90% (OP90) and 50% (OP50) of surveys to detect each pollinator group at each floral density.

RESULTS

INFLUENCE OF WEATHER CONDITIONS AND FLOWERS ON POLLINATOR OBSERVATIONS

In total, 876 individual pollinators were observed visiting flowers over the 120 30-minute observation periods. This total consisted of 467 *Bombus* spp., 64 *Apis mellifera*, 64 solitary bees, and 281 syrphids (Table 1). Some weather variables, such as air pressure (1,009 to 1,020 mbar), showed relatively little variation during the study period, whereas other variables, including cloud cover (0-100%), wind speed (0-6.6 m/s) and RH (28-82%) exhibited more extreme values (Table 2). The number of floral units in each 2 x 2 m^2 quadrat ranged between 4 and 300, and floral cover ranged between 1% and 90% (Table 2). There was significant collinearity among the floral variables and among the weather variables. All three floral measurements (cover, units, species richness) were positively correlated with each other ($r_s > 0.2$, $P < 0.03$; Supplementary File S1). Wind speed and air pressure were not correlated with any of other weather variables ($r_s < |0.14|$, $P > 0.13$), whereas all six pairwise correlations between light, cloud cover, temperature and RH were statistically significant ($r_s > |0.3|$, $P < 0.001$; Supplementary File S1).

When examining each of the explanatory variables individually using rank correlations, several significant relationships between the pollinator counts and explanatory variables were identified (Table 2; Supplementary Fig. S2, S3). *Apis mellifera* and solitary bees were both negatively associated with high RH, with *A. mellifera* also showing a negative association with

Table 1. Summary of timed surveys of four pollinator groups (*Bombus* spp., *Apis mellifera*, solitary bees, Syrphidae) at Rosemount Orchard, Dublin in 2 x 2 m plots with low, medium, and high densities of floral units. Counts were obtained over forty 30-minute timed pollinator counts at each floral unit density. Proportion of surveys of duration 5-minutes (n = 240 per density) and 30-minutes (n = 40 per density) when each pollinator group was recorded is also provided.

		Floral density			
	Pollinator group	Low	Medium	High	All
Total counts	*Bombus* spp.	39	91	337	467
	Apis mellifera	17	19	28	64
	Solitary bees	4	10	50	64
	Syrphids	37	70	174	281
	All pollinators	97	190	589	876
Proportion	*Bombus* spp.	0.150	0.313	0.700	0.388
5-minute counts	*Apis mellifera*	0.054	0.063	0.083	0.067
Present	Solitary bees	0.017	0.042	0.171	0.076
	Syrphids	0.142	0.229	0.500	0.290
	All pollinators	0.333	0.513	0.842	0.563
Proportion	*Bombus* spp.	0.550	0.850	1.000	0.800
30-minute counts	*Apis mellifera*	0.175	0.150	0.300	0.208
Present	Solitary bees	0.100	0.175	0.425	0.233
	Syrphids	0.500	0.800	0.875	0.725
	All pollinators	0.800	0.975	1.000	0.925

Table 2. Summary of weather and floral unit parameters occurring in 120 30-minute timed pollinator counts in 2 x 2 m plots at Rosemount Orchard, Dublin. Relationships between explanatory variables and counts of four pollinator groups (*Bombus* spp, *Apis mellifera*, solitary bees, Syrphidae) are given as pairwise rank correlation coefficients, effects in GLMMS (with sampling day included as a random factor) and effects in GLMs obtained by stepwise modelling using AIC to indicate best model. Figures highlighted bold indicate statistically significant effects (* P < 0.05; ** P < 0.01; *** P < 0.001). Parameters tested are: air pressure (AP), cloud cover, light intensity, relative humidity (%), temperature, wind speed, floral unit density (FD), floral cover (FC), and floral species richness (FS).

		AP (mbar)	Cloud (%)	Light (Klux)	RH (%) (%)	Temp (°C)	Wind (m/s)	FD	FC (%)	FS
Summary	Mean	1015	54	55	51	21.5	1.5	106	34	1.7
	Minimum	1009	0	4	28	14.3	0.0	4	1	1
	Maximum	1020	100	79	81	27.0	6.6	300	90	4
Rank Correlations (r_s)	*A. mellifera*	-0.011	**-0.325*** **	**0.285** **	**-0.213***	-0.030	**-0.183** **	0.121	0.150	0.012
	Bombus spp	0.003	-0.137	0.161	0.055	0.029	-0.072	**0.732*** **	**0.727*** **	0.102
	Solitary bees	-0.111	**-0.214***	0.133	**-0.240** **	-0.026	0.015	**0.322*** **	**0.345** **	-0.139
	Syrphidae	-0.143	**-0.250** **	**0.252** **	-0.114	0.005	-0.103	**0.542*** **	**0.533*** **	0.057
GLMMs (effects)	*A. mellifera*	0.034	-0.003	0.071	-0.042	-0.058	-0.758	-0.008	**0.040***	-0.089
	Bombus spp	0.011	-0.001	0.005	0.005	0.021	0.032	**0.001***	**0.020***	**-0.170** **
	Solitary bees	-0.067	-0.010	0.012	-0.050	**-0.259***	-0.265	0.002	**0.030***	**-0.601** **
	Syrphidae	-0.031	-0.005	0.012	-0.010	-0.055	-0.101	0.004	0.010	-0.129
Stepwise GLM (effects)	*A. mellifera*			**0.105*** **	**-0.054***	**-0.445*** **	**-0.523***		**0.010***	
	Bombus spp					0.053	-0.133		**0.029*** **	**-0.196** **
	Solitary bees		**-0.014** **		**-0.046***	**-0.217** **			**0.038*** **	**-0.635*** **
	Syrphidae		**-0.009*** **					0.003	**0.013***	-0.137

wind speed. With the exception of *Bombus*, the remaining three pollinator groups showed a negative relationship with cloud cover, this relationship being reinforced for *A. mellifera* and Syrphidae which both showed a positive relationship with light intensity (Table 2). *Bombus*, solitary bees, and Syrphidae all exhibited highly significant positive correlations with floral cover and with the density of floral units in the observation plots (Table 2). Based on these rank correlations, air pressure, temperature and floral species richness showed no significant relationships with the timed counts of any pollinator group (Table 2; Supplementary Figures S2, S3).

The GLMM analyses identified considerably fewer significant associations between the weather explanatory factors on pollinator counts, and only one combination, the effect of ambient temperature on solitary bee counts, was statistically significant (Table 2). However, counts of *A. mellifera*, *Bombus*, and solitary bees were all positively related to floral cover, and *Bombus* counts were also positively related to floral density (Table 2).

The stepwise GLM analysis of pollinator counts generally identified that a mixture of weather and floral variables should be included in the optimal model as identified by AIC values, the exception being for *Bombus* where only floral factors were included (Table 2). In these GLMs, floral cover was the only explanatory factor to be included in the optimal models for all four pollinator groups (Table 2).

Effect of floral density and observation period on probability of pollinator detection

When considering all pollinators, the probability of observing at least one individual in a 5-minute of observation period was 0.33 for low density floral patches, 0.51 for medium density floral patches, and 0.84 for the high-density floral patches (Table 1). Using these probabilities to estimate the likelihood of detection after different observation periods indicated that in the high-density floral patches it was highly probable (> 97%) that at least one specimen would be recorded in a 10-minute observation period (Fig. 1). For the medium floral density patches, 15-20 minutes

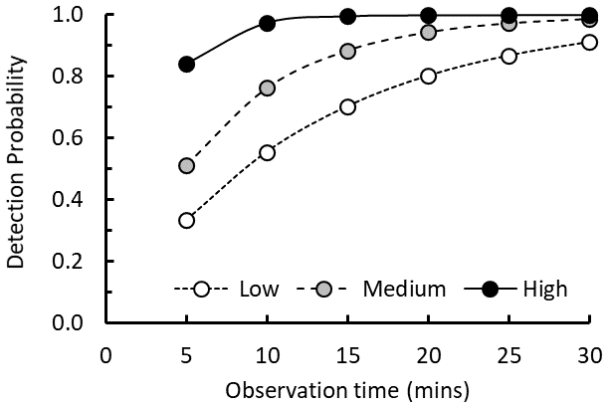

Figure 1. Probability of observing at least one pollinating insect in 2 x 2 m patches of low, medium, or high floral density as a function of observation time. Probability was calculated by binomial expansion using the mean probability of pollinators being observed in any one 5-minute observation period (see Methods for details).

observation would be required to achieve a 90% likelihood of recording a pollinator, and for the low-density floral patches an observation period of around 30 minutes would be required (Fig. 1).

In general, the likelihood of a pollinator group being present in a timed count of a given duration was strongly related to the total count of that group in plots of each floral density (Supplementary File S4). The patterns for the separate pollinator groups also indicated that the probability of detection was positively related to the density of floral units and the duration of the observation period (Table 1; Fig. 2). For the commoner pollinators such as *Bombus* and syrphids, the probability of detection in the high floral density plots was over 90% after 20 minutes and approached 100% after 30 minutes. For the less common pollinator groups, such as solitary bees and *A. mellifera*, the calculated probability of detection did not approach 100% even after a 30-minute observation period (Fig. 2). These latter two groups also highlighted the variability in the effect of floral density on pollinator counts. The probability of detection of solitary bees was much higher in the high-density plots compared with the medium and low-density plots, whereas the probability of detection of *A. mellifera* was relatively similar in plots of all three floral densities.

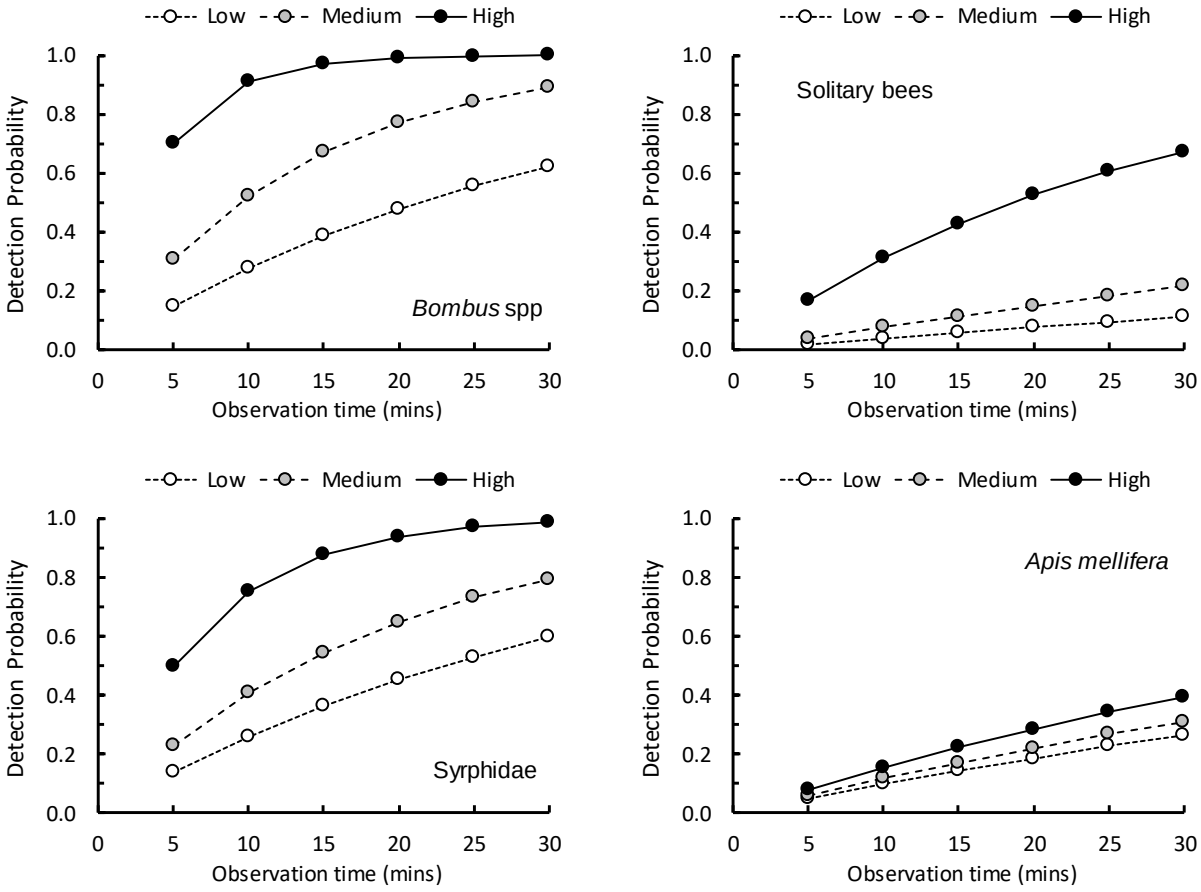

Figure 2. Probability of observing at least one *Bombus* spp, *Apis mellifera*, solitary bee or syrphid in 2 x 2 m patches of low, medium, or high floral density as a function of observation time. Probability was calculated by binomial expansion using the mean probability of pollinators being observed in any one 5-minute observation period (see Methods for details).

The times required to achieve a 90% detection rate (OP90) for each pollinator group in the plots of different floral densities are given in Table 3, and were inversely related to the total counts obtained (r_s = -0.96, P < 0.001; Supplementary File S4). This process suggested that for *Bombus* in high floral density plots, 90% detection could be achieved using observation periods of just 5 minutes. At the other extreme, for *A. mellifera* in low floral density plots, observation periods of around 1 hour 18 minutes would be needed to achieve a similar 90% detection rate. Indeed, when surveys were performed in plots of low floral density, the OP90 for all four pollinator groups was close to, or over, one hour (Table 3).

If the detection criteria were relaxed and only a 50% detection rate was used, then the required observation periods (OP50s) for each pollinator group at each floral density naturally decreased (Table 3). For the commoner pollinators, *Bombus* and syrphids, a 50% detection rate could be achieved with surveys less than 30 minutes in plots in all three floral density categories. However, for the less abundant groups in this system, *A. meliifera* and solitary bees, a similar OP50 of around 30 minutes (36 and 28 minutes respectively) could only be achieved if sampling was performed in the high floral density plots (Table 3).

Effect of observation time and floral unit density on pollinator richness

The average number of the four target pollinator groups recorded in a survey was positively related to the observation period and to density of floral units in the sampling area (Fig. 3). However, only at the highest floral density was there some indication that, even after the full 30-minute observation period, the average number of groups observed was starting to level off (Fig. 3). Additionally, the asymptote of the high floral density curve was approximately 2.6 groups (± 0.3

Table 3. Observation periods (OP) estimated to achieve 90% (OP90) and 50% (OP50) detection rates of four pollinator groups in an Irish meadow in 2 x 2 m quadrats assigned as having low, medium, and high density of floral units. *assigned a nominal value of 1 minute as calculated value was negative.

		Floral density		
	Pollinator group	Low	Medium	High
OP90 (mins)	*Bombus* spp.	55 (50-61)	35 (31-39)	5 (2-11)
	Apis mellifera	78 (70-88)	74 (67-84)	64 (58-71)
	Solitary bees	76 (68-86)	73 (65-82)	56 (51-62)
	Syrphids	55 (50-62)	44 (40-49)	29 (25-33)
OP50 (mins)	*Bombus* spp.	27 (24-31)	8 (4-11)	1*
	Apis mellifera	50 (43-58)	47 (41-54)	36 (32-42)
	Solitary bees	49 (42-56)	45 (40-52)	28 (25-32)
	Syrphids	28 (24-32)	17 (14-20)	1 (0-5)

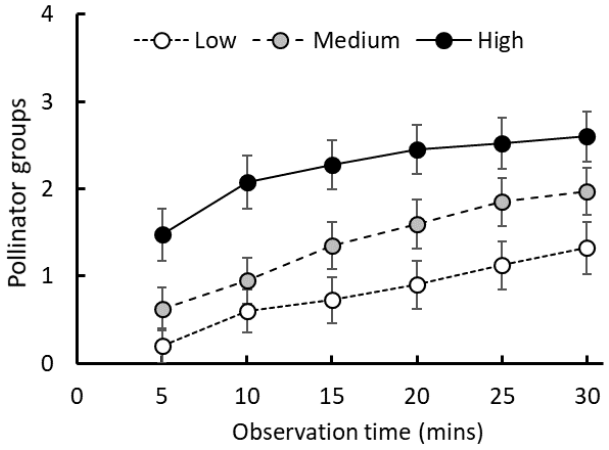

Figure 3. Number of pollinator groups (*Bombus* spp., *Apis mellifera*, solitary bees, syrphids) recorded in observation periods extending from 5 to 30 minutes in 2 x 2 m plots having low, medium, and high flower density (mean ± 95% CI; n = 40).

95% CI) per survey, strongly suggesting that, on average, any single 30-minute sample period was unlikely to record all four pollinator groups (Fig. 3).

Further insight into the effects of floral density and sampling period on observed pollinator richness can be gained by looking at frequency distributions for the 5-minute and 30-minute surveys (Fig. 4). In the low floral density plots, 80% of the 5-minute counts recorded no pollinators, with the remaining 20% only recording one of the four target pollinator groups (Fig. 4). By extending the observation period to 30-minutes, only 20% of counts in low floral density plots resulted in zero records, and 42.5% of samples recorded two or three of the four pollinator groups (Fig. 4). Of note, is that in the low floral density plots, no single 30-minute survey recorded all four pollinator groups.

Compared with the low-density plot, in the high floral density plots the situation changed noticeably. Only 12.5% of the 5-minute surveys recorded zero pollinators, and one 5-minute survey recorded all four pollinator groups. None of the 30-minute counts at the high floral density plots resulted in zero pollinators being recorded, while over half of the 30-minute samples (55%) recorded three or four of the four pollinator groups (Fig. 4).

DISCUSSION

This study illustrates the considerable variation that can occur in the data collected by timed pollinator counts of the same system due to variability in environmental conditions, density of floral resources, and duration of the sampling period. Several previous studies have shown that pollinator activity is related to multiple aspects of the immediate weather conditions, such as temperature (e.g. Comba 1999), wind speed (e.g. Hennessy et al., 2021), humidity (e.g. Peat & Goulson 2005) and light (e.g. Primack and Inouye 1993). Yet equally there often appear counter

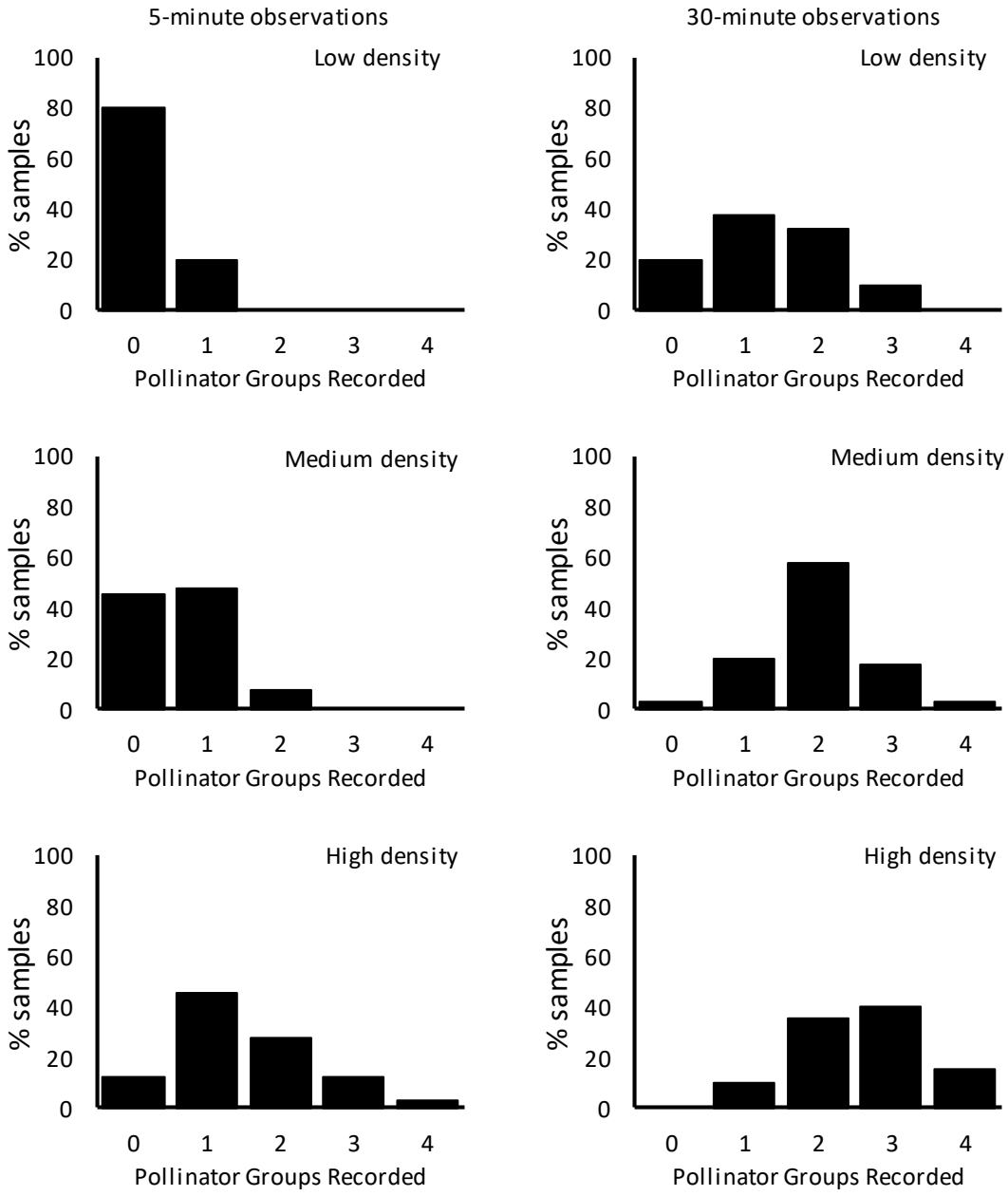

Figure 4. Number of pollinator groups (*Bombus* spp., *Apis mellifera*, solitary bees, syrphids) recorded in 5-minute or 30-minute observation periods in 2 x 2 m plots classified as having low, medium, and high density of floral units.

examples where no relationships between environmental conditions and pollinator counts are identified, and/ or these relationships are taxon-specific (e.g. Clarke & Robert 2018; Byrne & DelBarco-Trillo 2019).

Our study also found that factors such as cloud cover, wind speed, and humidity could negatively affect pollinator counts, but these effects were not universal across all pollinator groups. The collinearity among weather variables results in some surveys being performed in conditions that would tend to promote pollinator activity (i.e., warm, bright, dry) and other surveys being performed when the combination of weather variables is less conducive to pollinator foraging (i.e., cold, dark, humid; see also Prasad & Hodge 2013). Thus, even when strict criteria were applied regarding the time of day when pollinator surveys could be performed and the permissible weather conditions, the immediate environmental conditions can still have significant effects on the data obtained.

Optimal foraging theory predicts that areas with high resource density will support more foragers than sparser patches (Pyke et al., 1977). So, densely flowered areas can support more individual pollinators even if simply maintaining the forager to flower ratio (Dreisig, 1995; Tregenza, 1995), and pollinators can further optimize their foraging strategies by focusing on densely flowering areas which minimize the cost of inter-floral travel (e.g. Waddington, 1980; Zimmerman, 1981). As with several previous studies, all four groups of pollinators in our wildflower meadow setting were observed in greatest numbers in areas with a high density of floral units or high floral cover (Comba, 1999; Hegland & Boeke, 2006; Ebeling et al., 2008; O'Connor et al., 2019).

There were no positive relationships between pollinator counts and floral species richness, which may have resulted from capping the maximum number of species in any given quadrat to four. However, in most instances, especially in the GLMM and GLM analyses, floral richness was generally found to have negative relationships with pollinator counts. So, although floral richness was positively related to floral unit density, and floral unit density was positively related to pollinator counts, the expected extension of these relationships, a positive relationship between pollinator counts and floral richness, did not persist. In terms of the floral resources available, we considered only floral density as an explanatory factor, and did not examine which flowering species were present, their relative proportions, taxonomy, or their physical traits. Variation in these additional floral parameters, both within and among sites, and how they influence visitation rates of different pollinator groups requires further clarification.

The above example serves to highlight some of the issues that can occur when using complex GLMMs and step-wise regression models to identify statistically significant effects from suites of covarying explanatory factors. Relationships between pollinator counts and explanatory factors that were clearly depicted by visual inspection of the data and simplistic rank correlations were often not apparent in the more complex GLMMs or GLMs (Whittingham et al. 2006). Additionally, for the GLMs, we found that statistical significance of explanatory factors was often determined by whether terms were dropped from fully fitted models or added stepwise to empty models. In terms of our study, these discrepancies among statistical approaches are important because we are attempting to determine which of these factors are significant covariates to our pollinator counts. It should be remembered, however, that in different circumstances, factors such as temperature or floral density are not nuisance factors as such, but actually part of systematic environmental differences among seasons, sampling locations (e.g. latitudinal or altitudinal gradients) or experimental interventions (e.g. sowing of wildflower strips; fallow meadows). In these instances, rather than causing an undesirable increase in the within-group variance of a single data set, these factors are now potential causal explanatory factors, often being investigated by assessing their effects on between-group variance.

In addition to floral cover or density of floral units, extending the duration of the observation period from five to 30 minutes had substantial effects on the probability of observing each pollinator group and the number of pollinator groups. If we accept that all four pollinator groups were present at the study site during the full sampling period, then the likelihood of erroneous non-detection in any one survey could be decreased by extending the observation period (MacKenzie 2005; Royale et al. 2012; Hodge et al. 2017). Additionally, the study highlighted that the estimated time required to achieve detection in a given proportion of samples was dependent upon which pollinator group was being considered and density of floral units in the study plot. As a result of the above phenomena, the perceived diversity or structure of the actual pollinator assemblage was also highly dependent upon the duration the timed count and the density of floral units in the survey space. Depending on the aims of the investigation, the results for our pollinator system would indicate that surveys of 30-minutes would likely result in at least some pollinators being observed regardless of the floral density of the study plot, but that the minimum duration of surveys could be reduced if studying the most abundant taxa, such as bumblebees, or restricting the surveys to areas with high floral density.

As a reminder, the aims of this study were not implicitly to investigate the effects of weather or

floral resources on pollinator abundance or activity, but rather evaluate how these factors influenced the data collected when repeatedly sampling the same pollinator assemblage. Our results highlight that survey duration, ambient weather conditions and floral density can have considerable effects on the results obtained from otherwise standardized timed pollinator counts. In this study we have only examined the effects of extending the duration of single surveys, as opposed to investigating the effects of multiple sampling events on pollinator detection and taxon accumulation. It is possible that multiple short surveys, under varying conditions, may mitigate some of the issues caused by performing a single survey of longer duration under extreme conditions. In addition to imposing environmental criteria restricting when pollinator counts should be performed, we would advocate the recording of environmental variables, such as light intensity, temperature, wind speed and humidity. This additional data collection would then allow the effects of these variables to be assessed within each study system, and their inclusion as covariates into statistical models if so warranted.

Although researchers must accept weather conditions when surveys are being performed, the selection of flower patches of different densities is something researchers can control. If survey areas are selected randomly, there is a risk that areas of low floral cover or density will be chosen which will consequently reduce pollinator counts. As above, this issue might be resolved if multiple surveys are performed which would, by chance, mean that pollinator counts are performed at a range of floral densities centered around the average. However, if the study design only allows for one or low number of surveys to be performed at any one location or at any timepoint, researchers may benefit from imposing additional criteria regarding floral cover to maintain standardization and allow for more meaningful comparisons. For example, areas may be selected based on 'typical' floral cover, or with a minimum number of floral units, or within a given range of floral units. Regardless, floral units and/ or floral cover should be recorded so that this variable can also be included as a covariate in future statistical analysis.

We concede that the specifics of our evaluation of survey duration on pollinator detection and diversity may only apply to our particular study system, or similar ecological systems in Irish/ European landscapes. However, we believe the analytical process we have applied would be valuable to other studies of insect pollinators involving timed counts or transect surveys, and might be extended to investigate specific pollinator-plant interactions, or determine the observation effort required where pollinator-plant networks are considered to be (more-or-less) complete. Timed counts that are too short in duration run high risks of not recording taxa that are actually present and, as a consequence, underestimate actual taxonomic richness. For comparative studies, this under recording may result in real effects or differences among sites or treatments not being identified (Type II statistical errors) because of an excess of erroneous non-detection events (Ebeling et al., 2008; Fijen & Kleijn, 2017; Russo et al. 2020). As such, when time permits, pollinator field investigations or monitoring programs would benefit from some systematic evaluation of how sampling duration and total sampling effort influence results, and how erroneous non-detection of target taxa could then be reduced to acceptable levels.

ACKNOWLEDGEMENTS

The authors thank Rosemount Glasshouse staff Bredagh Moran and David Brogan for technical assistance, and Thomas J. Butler for statistical advice.

APPENDICES

Additional supporting information may be found in the online version of this article:

Supplementary file S1. Rank correlations between weather variables and floral variables used in models of timed pollinator counts.

Supplementary file S2. Scatterplots showing relationship of counts of pollinators in a 30-minute period with environmental and weather conditions.

Supplementary file S3. Scatterplots showing relationship of counts of pollinators in a 30-minute period with floral units present, floral cover, and floral species richness.

Supplementary file S4. Relationship between total counts of pollinators and the calculated OP90, and the proportion of 5-minute (P5) and 30-minute (P30) surveys in which each group was present.

REFERENCES

AIPP 2021. All-Ireland Pollinator Plan 2021-2025 (2021) National Biodiversity Data Centre Series No. 25, Waterford. March 2021. pp63

Blasco-Moreno A, Pérez-Casany M, Puig P, Morante M, Castells E (2019) What does a zero mean? Understanding false, random and structural zeros in ecology. Methods in Ecology and Evolution, 10:949–959

Brittain CA, Vighi M, Bommarco R, Settele J, Potts SG (2010) Impacts of a pesticide on pollinator species richness at different spatial scales. Basic and Applied Ecology, 11(2), 106–115. https://doi.org/10.1016/j.baae.2009.11.007

Brunet J, Flick AJ, Bauer AA (2021) Phenotypic selection on flower color and floral display size by three bee species. Frontiers in Plant Science, 11, 2244. doi.org/10.3389/fpls.2020.587528

Burrill RM, Dietz A (1981) The response of honeybees to variations in solar radiation and temperature. Apidologie, 12(4), 319–328. https://doi.org/10.1051/apido:19810402

Byrne F, DelBarco-Trillo J (2019) The effect of management practices on bumblebee densities in hedgerow and grassland habitats. Basic and Applied Ecology, 35, 28–33. https://doi.org/10.1016/j.baae.2018.11.004

Carvell C, Meek WR, Pywell RF, Goulson D, Nowakowski M (2007) Comparing the efficacy of agri-environment schemes to enhance bumble bee abundance and diversity on arable field margins. Journal of Applied Ecology, 44(1), 29–40. https://doi.org/10.1111/j.1365-2664.2006.01249.x

Clarke D, Robert D (2018) Predictive modelling of honey bee foraging activity using local weather conditions. Apidologie, 49(3), 386–396. https://doi.org/10.1007/s13592-018-0565-3

Comba L (1999) Patch use by bumblebees (hymenoptera apidae): Temperature, wind, flower density and traplining. Ethology Ecology and Evolution, 11(3), 243–264. https://doi.org/10.1080/08927014.1999.9522826

Corbet SA (1990) Pollination and the Weather. Israel Journal of Botany, 39(1–2), 13–30. https://doi.org/10.1080/0021213X.1990.10677131

Corbet SA, Fussell M, Ake R, Fraser A, Gunson C, Savage A, Smith K (1993) Temperature and the pollinating activity of social bees. Ecological Entomology, 18(1), 17–30. https://doi.org/10.1111/j.1365-2311.1993.tb01075.x

Dicks LV, Breeze TD, Ngo HT, et al. (2021) A global-scale expert assessment of drivers and risks associated with pollinator decline. Nature Ecology and Evolution, 5, 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Dreisig H (1995) Ideal Free Distributions of Nectar Foraging Bumblebees. Oikos, 72(2), 161. https://doi.org/10.2307/3546218

Ebeling A, Klein AM, Schumacher J, Weisser WW, Tscharntke T (2008) How does plant richness affect pollinator richness and temporal stability of flower visits? Oikos, 117(12), 1808–1815. https://doi.org/10.1111/j.1600-0706.2008.16819.x

Fijen TPM, Kleijn D (2017) How to efficiently obtain accurate estimates of flower visitation rates by pollinators. Basic and Applied Ecology, 19, 11–18. https://doi.org/10.1016/j.baae.2017.01.004

Gilbert FS (1985) Diurnal activity patterns in hoverflies (Diptera, Syrphidae). Ecological Entomology, 10(4), 385–392. https://doi.org/10.1111/j.1365-2311.1985.tb00736.x

Hegland SJ, Boeke L (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. Ecological Entomology, 31(5), 532–538. https://doi.org/10.1111/j.1365-2311.2006.00812.x

Hennessy G, Harris C, Pirot L, Lefter A, Goulson D, Ratnieks FLW (2021) Wind slows play: increasing wind speed reduces flower visiting rate in honey bees. Animal Behaviour, 178, 87–93. https://doi.org/10.1016/j.anbehav.2021.05.022

Hodge S (2020) When I'm sixty-four: long-term monitoring and the (missing?) New Zealand insect apocalypse. The Wētā, 54, 1-11

Hodge S, Curtis N, Vink CJ, Marris J, Brown SDJ (2017) Native arthropods on exotic sand dune flowers: consideration of sample size and number for investigating rare species and sparse communities. Arthropod-Plant Interactions, 11, 691-701. doi 10.1007/S11829-017-9521-9

Hodge S, Stout J (2019) Protocols for Methods of Field Sampling. Deliverable D1. 1 PoshBee Project, Grant agreement No. 773921. Available online: Poshbee.eu

Hodge S, Vink CJ (2016) Evidence of absence is not proof of absence: the case of the New Brighton katipō. New Zealand Journal of Zoology, 47: 14-24 doi10.1080/03014223.2016.1227343

Hutchinson LA, Oliver TH, Breeze TD, O'Connor RS, Potts SG, Roberts SPM, et al. (2022) Inventorying and monitoring crop pollinating bees: Evaluating the effectiveness of common sampling methods. Insect Conservation and Diversity, 15(3), 299 311. https://doi.org/10.1111/icad.12557

Kleijn D, Winfree R, Bartomeus I, et al. (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature

Communications, 6(1), 36. https://doi.org/10.1038/ncomms8414

Krahner A, Schmidt J, Maixner M, Porten M, Schmitt T (2021) Evaluation of four different methods for assessing bee diversity as ecological indicators of agro-ecosystems. Ecological Indicators, 125, 107573. https://doi.org/10.1016/j.ecolind.2021.107573

Leclercq N, Marshall L, Weekers T, et al. (2022) A comparative analysis of crop pollinator survey methods along a large-scale climatic gradient. Agriculture, Ecosystems and Environment. 329: 107871

Lebuhn G, Droege S, Connor EF, Gemmill-Herren B, Potts SG, Minckley RL, Griswold T, Jean R, Kula E, Roubik DW, Cane J, Wright KW, Frankie G, Parker F (2013) Detecting Insect Pollinator Declines on Regional and Global Scales. Conservation Biology, 27(1), 113–120. https://doi.org/10.1111/j.1523-1739.2012.01962.x

MacKenzie DI (2005) What are the issues with presence-absence data for wildlife managers? The Journal of Wildlife Management, 69(3), 849-860. https://doi.org/10.2193/0022-541X(2005)069[0849:WATIWP]2.0.CO;2

O'Connor RS, Kunin WE, Garratt MPD, Potts SG, Roy HE, Andrews C, Jones CM, Peyton JM, Savage J, Harvey MC, Morris RKA, Roberts SPM, Wright I, Vanbergen AJ, Carvell C (2019) Monitoring insect pollinators and flower visitation: The effectiveness and feasibility of different survey methods. Methods in Ecology and Evolution, 10(12), 2129–2140. https://doi.org/10.1111/2041-210X.13292

Ohashi K, Yahara T (2002) Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. Functional Ecology, 16(4), 492–503. https://doi.org/10.1046/j.1365-2435.2002.00644.x

Peat J, Goulson D (2005) Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. Behavioral Ecology and Sociobiology, 58(2), 152–156. https://doi.org/10.1007/s00265-005-0916-8

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: Trends, impacts and drivers. In Trends in Ecology and Evolution 25:345–353. Elsevier Current Trends. https://doi.org/10.1016/j.tree.2010.01.007

Prasad AV, Hodge S (2013) Factors influencing the foraging activity of the allodapine bee Braunsapis puangensis on creeping daisy (Sphagneticola trilobata) in Fiji. Journal of Hymenoptera Research, 35, 59-69. doi 10.3897/JHR.35.6006

Prendergast KS, Hogendoorn K (2021) Methodological shortcomings and lack of taxonomic effort beleaguer Australian bee studies. Austral Ecol. https://doi.org/10.1111/aec.12998.

Prendergast KS, Menz MHM, Dixon KW, Bateman PW (2020) The relative performance of sampling methods for native bees: an empirical test and review of the literature. Ecosphere 11(5):e03076. 10.1002/ecs2.3076

Primack RB, Inouye DW (1993) Factors Affecting Pollinator Visitation Rates - a Biogeographic Comparison. Current Science, 65(3), 257–262. https://www.jstor.org/stable/24095126

Pyke GH, Pulliam HR, Charnov EL (1977) Optimal Foraging: A Selective Review of Theory and Tests. The Quarterly Review of Biology, 52(2), 137–154. https://doi.org/10.1086/409852

Royle JA, Chandler RB, Yackulic C, Nichols JD (2012) Likelihood analysis of species occurrence probability from presence-only data for modelling species distributions. Methods in Ecology and Evolution, 3(3), 545-554. https://doi.org/10.1111/j.2041-210X.2011.00182.x

Russo L, Buckley YM, Hamilton H, Kavanagh M, Stout JC (2020) Low concentrations of fertilizer and herbicide alter plant growth and interactions with flower-visiting insects. Agriculture, Ecosystems & Environment, 304, 107141. https://doi.org/10.1016/j.agee.2020.107141

Saunders ME, Hall MA, Lentini PE, Brown J, Cunningham SA (2021) Scholarly shortcomings and a lack of evidence beleaguer bee sampling critique: A response to Prendergast and Hogendoorn (2021). In Austral Ecology. Blackwell Publishing. https://doi.org/10.1111/aec.13042

Sühs RB, Somavilla A, Giehl ELH (2021) Weather variables affecting the behaviour of Insect flower visitors and main pollinators of *Erythroxylum myrsinites* Martius (Erythroxylaceae). Sociobiology, 68(1), 5451.

Tamburini G, Berti A, Morari F, Marini L (2016) Degradation of soil fertility can cancel pollination benefits in sunflower. Oecologia, 180(2), 581–587. https://doi.org/10.1007/s00442-015-3493-1

Thompson A, Frenzel M, Schweiger O, Musche M, Groth T, Roberts SP, Kuhlmann M, Knight TM (2021) Pollinator sampling methods influence community patterns assessments by capturing species with different traits and at different abundances. Ecological Indicators, 132, 108284.

Tikoca S, Hodge S, Tuiwawa M, Brodie G, Pene S, Clayton J (2016) An appraisal of sampling methods and effort for investigating moth assemblages in a Fijian forest. Austral Entomology, 55(4), 455–462. https://doi.org/10.1111/aen.12209

Tepedino VJ, Durham S, Cameron SA, Goodell K (2015) Documenting bee decline or squandering scarce resources. Conservation Biology 29: 280-282

Tregenza T (1995) Building on the Ideal Free Distribution. Advances in Ecological Research, 26(C), 253–307. https://doi.org/10.1016/S0065-2504(08)60067-7

Waddington KD (1980) Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar. Oecologia, 44(2), 199–204. https://doi.org/10.1007/BF00572680

Westphal C, Bommarco R, Carré G, Lamborn E, Morison N, Petanidou T, Potts SG, Roberts SPM, Szentgyörgyi H, Tscheulin T, Vaissière BE, Woyciechowski M, Biesmeuer JC, Kunin WE, Settele J, Steffan-Dewenter I (2008) Measuring bee diversity in different European habitats and biogeographical regions. Ecological Monographs, 78(4), 653–671. https://doi.org/10.1890/07-1292.1

Wheelock MJ, Rey KP, O'Neal ME (2016) Defining the insect pollinator community found in Iowa corn and soybean fields: implications for pollinator conservation. Environmental Entomology, 45(5), 1099-1106.https://doi.org/10.1016/j.ecolind.2021.108284

Whittingham MJ, Stephens PA, Bradbury RB, Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, *75*(5), 1182-1189.

Zimmerman M (1981) Optimal foraging, plant density and the marginal value theorem. Oecologia, 49(2), 148–153. https://doi.org/10.1007/BF00349181