Understanding the Risk to European Protected Species (bats) at Onshore Wind Turbine Sites to inform Risk Management

Final Report

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EXECUTIVE SUMMARY

Aims and objectives

This study aimed to provide information on the extent of any hazards posed by onshore wind turbines to British bats and to supply evidence that could potentially assist in managing any such risk.

Primary objectives

(a) To assess whether bats in Great Britain are killed by wind turbines.

(b) If bats are being killed, to estimate casualty rates with acceptable precision.

Secondary objectives

(a) To explore the relationships between bat mortality and potential risk factors.

(b) To assess the relationship between the intensity of bat activity at wind turbines, as measured using acoustic techniques, and potential risk factors.

Rationale

Evidence that wind turbines potentially pose a collision threat to bats has been available from the USA and some European countries since the early 2000s. However the scale of any risk in the UK was unknown. The Department for Environment, Food and Rural Affairs, Department of Energy and Climate Change, Natural Resources Wales, Scottish Natural Heritage, Natural England and RenewableUK therefore commissioned this research project in 2010. It is the first attempt, anywhere in the world, to assess the impact of wind turbines on bats at a national scale through the systematic survey of a representative sample of wind energy facilities.

The study methodology reflects the primary objective of establishing whether bats are killed by wind turbines in the UK and, if they are, estimating the likely casualty rates. The project therefore focused on late summer and early autumn, which is the period identified elsewhere in the published literature as the time of peak collision risk. It is possible that in the UK there are additional periods of high risk, and these would not have been detected in this project. Because the expected numbers of casualties was unknown, and because substantial variation between sites and regions were anticipated, a large-scale survey was used that would allow even low casualty rates to be detected with statistical rigour. It was not possible to make robust assessments of the different risks posed by all possible types of wind energy installations, and therefore the project focused strategically on large-scale commercial wind farms. Specifically, all the sites in the project had at least 5 turbines and towers of 31-81m to hub (most were >50m): it is therefore accepted that risk estimates may not apply to small-medium wind energy installations; to sites with only small numbers of turbines; or to those with unconventional rotors e.g. vertical axis designs. It is also accepted that the use of a relatively short survey window (c. 1 month) at each site means that it is impossible to detect seasonal trends within sites.

Methods

Surveys were conducted at 46 commercial wind turbine sites, approximately equally distributed across England, Wales and Scotland, from 2011 to 2013 inclusive. A wide range of habitats were included, and the distribution of sites approximated their availability across Britain. In response to the practical difficulties of locating bat carcasses in sites with dense habitat, a formal trial was undertaken of alternative survey methodologies, and the use of trained search dogs was selected as the most efficient and cost-effective approach. Surveys took place between July and October, and at each site, carcass searches were conducted at 6-8 turbines on multiple occasions for approximately one month. The average interval between searches was 2.5 days, with the aim of minimising the extent to which bats were removed by predators and therefore the errors associated with estimating casualty rates. This interval is considerably shorter than most other wind-turbine studies. It is therefore likely that most carcasses would have been available for detection by our search dog teams. Because the species and sex of the bat carcases were not always readily apparent (due to the nature of injuries, decay and/or partial predation), confirmatory analyses were conducted using a multiplex polymerase chain reaction (PCR) developed for this project. This technique is cheaper and quicker than other available approaches, and will be applicable to future studies of wind turbine casualties.

Nightly acoustic surveys were conducted at both nacelle (the housing at the top of the turbine tower that covers the generating components of the wind turbine) and at ground level at 3 of these turbines at each site to provide an index of bat activity throughout the study period. To gain an insight into bat activity in the absence of turbines, which could potentially aid with the interpretation of pre-construction surveys at new wind farm sites, an additional acoustic survey was conducted at the study sites in similar habitat but away from the turbine. In this report, as in other studies using acoustic surveys of bats, it is not possible to determine the precise link between bat activity recorded by the detectors and the absolute abundance of bats, since a given individual may be recorded multiple times. Rather, we use bat activity as an index, and explore its links with casualty risks.

The numbers of casualties found at each site is likely to be a subset of the total number of animals killed because, even with good survey techniques, it is unlikely that all casualties will be found and a proportion are also likely to be taken by predators. Therefore, following international conventions, the true casualty rate has been estimated by taking these factors into account statistically. The necessary information for this procedure was generated by conducting trials at each study site of the rates at which dead bats were removed by predators and the proportions that were overlooked – in the habitats present at that particular site – by the dog-handler teams. The bats used in these trials were animals that had died of natural causes and which were supplied to the study by bat rehabilitators

Results and interpretation

In total, 120 bat carcasses were retrieved. No casualties were identified at a third of sites, but it is important to note that zero observations does not necessarily imply that no casualties occurred. Most fatalities were common pipistrelle bats (*Pipistrellus pipistrellus*), soprano pipistrelle bats (*P. pygmaeus*) and noctule bats (*Nyctalus noctula*). In addition, single carcasses of Brown long-eared bat (*Plecotus auritus*), Nathusius' pipistrelle bat (*P. nathusii*) and Natterer's bat (*Myotis nattereri*) were found – the latter is the European first record of a fatality at a wind turbine site. The numbers of casualties found at the study sites, and the species involved, are compatible with reports from elsewhere in Northern Europe.

The estimated casualty rates, which correct for predator removals and the efficiency of the searches, ranged from 0 to 5.25 bats per turbine per month, and from 0-77 bats per site per month, during the period of the survey. There was considerable variability between sites, but within sites there was little evidence of clustering of casualties at particular turbines (though it must be noted that since all turbines were not surveyed at each site, it is possible that geographical clustering of casualties within a particular region of the wind farm has been overlooked). The percentages of soprano pipistrelle bat, common pipistrelle bat and noctule bat casualties were 40.6%, 48.6% and 10.7%. For soprano pipistrelle and noctule bats, these proportions are higher than their estimated relative national abundance. They are also higher than the relative proportions of their calls recorded in ground-level acoustic surveys, where only 16% of recorded calls were soprano pipistrelle and 4% were noctule, but are more similar to the species distributions found in recordings made at the nacelle.

Approximately equal numbers of male and female casualties were found, and there was no evidence for an excess of juveniles. It therefore does not appear that young bats are more at risk of collision during July-October, perhaps due to poorer navigational abilities, as has sometimes been suggested. When considering the impacts on populations, this finding is important as population modelling on a wide range of bat species has indicated that whilst juvenile survival rates have little impact on the viability of populations, the adult survivorship is extremely important (Schorcht et al. 2009). However, we caution that the sample size was relatively low for this analysis, and therefore type II error (failing to detect a true association when one exists) is a possibility. In addition, different results may be obtained where wind turbines are sited close to maternity roosts.

The risk to bats from wind farms increased with the number of turbines: there was no evidence to suggest that the risk from an individual turbine at a large site was any different from that at a small site. There was also an increasing risk to bats with larger rotor sizes: each metre increase in blade length was associated with an increase of approximately 18% (95% confidence intervals 5% to 32%) in the probability of a casualty (of any species) occurring. Similar relationships were apparent for the numbers of bat casualties. In contrast, the height of the nacelle, and the period for which the wind farm had been operational were not independently linked with the risk to bats.

Weather conditions were also linked with bat fatalities, as had been expected from previous research in other countries. Most nights where casualties occurred (81.5%, 95% Confidence Interval[*] 69.2, 89.6) had low mean wind speeds (≤5 m/s measured at the ground) and maximum night-time temperatures of >10°C. It is possible to be confident that most nights with wind speeds >5m/s will have no casualties: formally, we can estimate that 95.3% (95% CI 91.5, 97.4) of nights with mean wind speeds >5m/s will have no casualties. However, it must be noted that most nights in the study had low wind speeds, and only 3.6% (95% CI 2.7-4.8) of these had casualties. It can

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[*] The 95% confidence interval gives the boundaries within which the estimate would be expected to fall 95% of the time were the project repeated multiple times. Wide confidence limits therefore indicate a high degree of uncertainty about the size of the effect, whereas narrow confidence limits indicate that the mean has been estimated with high precision.

therefore be concluded that whilst curtailing wind turbines in low winds would be extremely effective in minimising the collision risk to bats, it would also mean that turbines would be curtailed on most nights and, on average, only 3.6% of these nights would present a risk to bats. It may therefore be necessary to apply additional measures to identify that a substantial risk exists at a particular site or during a particular time-period (for example by finding casualties during post-construction monitoring) before considering curtailment as a mitigation strategy. A simple strategy that should be considered at all sites where technically feasible is to restrict the rotation of turbine blades as much as possible below the cut-in speed (e.g. by feathering the blades). This will have a positive outcome for bats, as the amount of time the blades are turning at low wind speeds will be reduced, whilst also involving no loss of energy generation.

For all bats collectively, the number of bat casualties declined with the area of broad-leaf woodland within a 1.5km radius of the centre of the wind farm, possibly through the provision of alternative foraging habitat. This relationship was also evident when soprano pipistrelle bats were analysed individually (p = 0.108 and p = 0.002 respectively). For common pipistrelle and noctule bats, the associations were in the same direction, but were not statistically significant (p=0.108 and p=0.336 respectively). In contrast, the total area of coniferous woodland (including recent clear-fell) was associated with increased risks to noctules (p<0.001). At a smaller spatial scale, sites with broadleaf and mixed woodland within a 200m buffer had an 86% probability of no soprano pipistrelle casualties. In contrast, sites *without* broadleaved and mixed woodland in a 500m radius had a 94% probability of no noctule bat casualties (coniferous woodland gave similar results to those for broadleaved and mixed woodland). However, whilst woodland was a good predictor of where casualties would *not* occur, it was of much less value in predicting where fatalities were likely: most sites without woodland had no pipistrelle casualties, and most sites with woodland had no noctule casualties. The presence or absence of woodland is therefore most likely to be useful as a means of "ruling in" areas potentially suitable for wind farms (the direction of this association depending on species) rather than for identifying areas likely to be problematic.

As expected there were also positive associations between bat activity indices and casualty rates. These were present for both ground and height recordings, but for soprano pipistrelles, the confidence intervals were wide, possibly because their activity patterns were much more variable than for common pipistrelles. Sites were categorised according to whether the activity of all pipistrelles at ground level was high, medium or low (the maximum pass rate recorded across the site each night was averaged across all survey nights and divided into thirds). Those sites in the lowest third had significantly fewer casualties than those in the upper two thirds. However, it is important to note that none of the models explained most of the variability in collision risk, and there is considerable uncertainty associated with the estimates. This partly reflects the difficulties of modelling rare events, but also appears to reflect a high level of inherent variability in collision risk that is not explained by bat activity or the other features we explored. For example, sites studied in more than one year had different fatality patterns in each year; and whilst many fatalities occurred at sites with high activity levels, some occurred at sites with very low bat activity rates.

Bat activity at control locations (away from turbines) was also examined as an index of background bat activity at the site. This measure will be similar to pre-construction survey data in that there are no repellent or attractive effects from turbines. However it is clearly not an exact equivalent as our control data are collected contemporaneously with fatality monitoring, whereas pre-construction data are temporally separated and would therefore be expected to predict fatalities less well. Activity at the control locations was not a useful predictor of the number of bat casualties, but it was a predictor of whether or not *any* casualties occurred (i.e. a binary yes/no categorisation). Sites in the highest third of pipistrelle bat activity at control sites had a 75% probability (95% CI 46.8, 91.1) of pipistrelle casualties, though only approximately a third of sites in the top third of overall bat activity had bat casualties of any species (33.5%; 95% CI 19.7, 57.0). Nevertheless, bat activity may be useful in identifying sites at low risk: 93.3% (95% CI 70.2, 98.8) of sites classified as having low-medium bat activity at control sites had no casualties. In addition, it may be valuable in identifying sites with unusually high levels of bat activity where the development of wind farms would be best avoided. Consideration must now be given to whether classifying a site as being simply at risk of any casualties is useful, or whether predictions must be better able to quantify the scale of risk in order to be useful. If it is, then further species-specific research, preferably using preconstruction data, is warranted.

This research project has identified standardised methodologies which could be applied to future surveys of wind turbine sites. Specifically we have found that bat activity is highly variable and an increase in the duration of static surveys would provide more robust assessments of bat activity. Our data are derived from post-construction surveys, but data from the control locations (away from turbines) also suggest that it is likely that the same variability would be present in preconstruction data. Although ground-based monitoring alone is likely to detect whether species at risk of collision are present at a site, caution is warranted in interpreting indices of abundance. Our surveys show that that the relative abundance of noctule and soprano pipistrelle bats varies between acoustic recordings made simultaneously at the ground and at the nacelle. Use of the ground-level data alone would underestimate the relative abundance of these species, presumably because some bat passes from these species are made only at heights beyond the acoustic range of the ground-based detector. Given that both noctule and soprano pipistrelle bats are at risk of collision with wind turbines, consideration should be given to the use of acoustic monitoring at height.

Conclusions

1. This study has shown that bat casualties occur at British wind energy installations at rates similar to those reported elsewhere in Europe. The collision rate per turbine ranged from 0

to 5.25 bats turbine^{-1} month^{-1} during the survey period (July-October). At a third of sites, no casualties were found, and at 15% of sites, the rate exceeded 1 bat tubine^{-1} month^{-1}. From this project it is not possible to conclude whether or not there is an impact on local or national bat populations.

2. The species identified as being at highest risk of collision are common pipistrelle, soprano pipistrelle and noctule bats. This finding also aligns with evidence collected elsewhere in Europe. Single casualties of Nathusius' pipistrelle, brown long-eared and Natterer's bats were also found. Because our work was not conducted within its strongholds, it is difficult to make inferences about the scale of the risk to Nathusius' pipistrelle bat. For the same reason, the lack of casualties of greater or lesser horseshoe bats, barbastelle or Leisler's bats in this project, also does not imply an absence of localised risk. Some areas have high concentrations of *Myotis* bat activity during swarming periods, and any risk associated with movements to these sites would not have been captured during this project.
3. Casualty rates are highly variable. Most of this variability appears to be due to site-specific factors, and is not simply explained by differences in bat activity levels. Collision risk is generally lowest at locations with low bat activity, but risks rise very rapidly with increasing activity, and not all sites with high risk had high bat activity. Further work is required to determine whether preconstruction surveys can usefully predict future risk of collision at wind turbine sites: initial evidence from this study using data collected away from turbines suggests that low bat activity may be useful in identifying low risk sites, but that high bat activity does not necessarily indicate high collision risk. Nevertheless, the precautionary principle would indicate that sites with unusually high levels of bat activity would be best avoided as sites for wind energy installations.
4. The size of the wind energy installation had no link with the per-turbine casualty rate. This means that there is a simple increase in risk for each additional turbine. Turbine number

had a greater effect on the risk a site posed to bats than any other feature identified in the project.

5. Turbines with larger blade lengths pose an increased risk to bats, and this is a stronger predictor than the height of the nacelle.
6. Most fatalities occur on nights of relatively low mean wind speed (≤5m/s at ground level). However, most turbines that are operational on nights of mean low wind speed have no bat casualties. The link between ground and rotor-level wind speed is difficult to model, but may equate to a speed of 7-9 m/s, based on the broad relationship between average wind speeds observed at ground and height for a subset of 27 sites were paired data were available. Using the subset of 18 sites where wind speed data from the nacelle were available and where casualties were considered fresh, all casualties occurred on nights with mean wind speed <10m/s.
7. The presence of woodland within a 1500m radius of wind farms in the vicinity of wind turbines appears to reduce the risk to pipistrelles but increase the risk to noctule bats. However, noctule bat casualties are relatively uncommon events and therefore most of the sites with woodland within this radius will experience no casualties.
8. Trained search dog and handler teams are the most effective and efficient way of identifying dead bats at wind turbine sites.
9. Acoustic recordings made at the ground and nacelle give different estimates of the relative abundance of species on site, with ground-level recordings underestimating the abundance of soprano pipistrelle and noctule bats within the ‘at risk’ zone of the turbine rotor sweep.
10. Bat activity shows extremely high variability. The day-to-day variation in bat passes is similar to that observed between different sites, and in some cases – notably for common pipistrelle, soprano pipistrelle and *Myotis* bat activity at height – can even exceed it. Much longer monitoring periods than are currently used as standard practice are therefore required for robust estimation of bat activity.

SECTION 1: CONTEXT

Overview of Wind Energy Development

Great Britain has some of the most favourable conditions for wind energy generation in the world (Asif and Muneer 2007). In the UK, installed onshore wind capacity at the end of 2014 was 7,994MW, with a further installed capacity of 4,049MW offshore (RenewableUK 2015), making the UK the third largest European generator of wind power (European Wind Energy Association 2015, Figure 1.1). At the end of 2014, there were 692 onshore wind projects (with a total of 4,812 turbines) in operation across the UK; and three of the five largest wind farms in Europe have been built, or are under construction, in the UK (as reported mid-2013, (European Wind Energy Association 2013). In contrast to many other European countries, economic conditions appear to have had less of a restrictive effect on wind energy expansion in recent years (Table 1.1)(European Wind Energy Association 2015): in 2014, 59.5% of all new installed capacity was in Germany and the UK (European Wind Energy Association 2015).

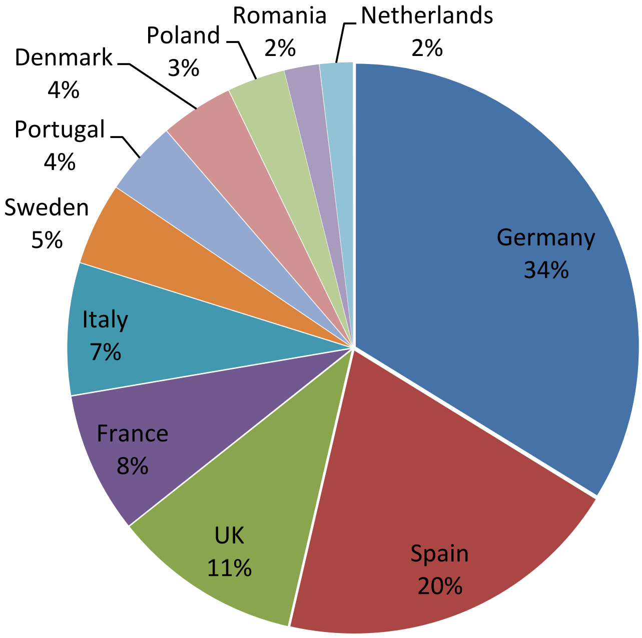

Figure 1.1. Contribution of EU member states to total installed onshore and offshore wind energy capacity (2014).(European Wind Energy Association 2015)

Table 1.1. Newly installed and total capacity in the UK (onshore and offshore) compared with the other leading generators of wind energy in Europe (European Wind Energy Association and 2014, European Wind Energy Association 2015)

	Newly Installed 2012 (MW)	Newly Installed 2013 (MW)	Newly installed 2014 (MW)	Total Installed Capacity (MW)
UK	2,064	1,883	1,736	12,440
Spain	1,110	175	27.5	22,987
Germany	2,297	3,238	5,279	39,165
France	814	631	1,042	9,285
Italy	1,239	444	108	8,663

Potential for conflict between wind turbines and bats

Bat casualties have been identified at European wind farms for more than a decade (for summary see Table 1.2) (Rydell et al. 2010b). Although 27 species have been identified, the majority have been common pipistrelle (*Pipistrellus pipistrellus*, 18%), noctule (*Nyctalus noctula*, 14%), Nathusius' pipistrelle (*P. nathusii*, 13%), soprano/common pipistrelle (*P. pipistrellus/pygmaeus*, 11%) and Leisler's bats (*N. leisleri*, 8%)(EUROBATS 2014a). These data cannot be taken to represent directly the actual relative mortality risks across species, as the data were not derived from systematic survey effort. Nevertheless, they suggest that species in the *Pipistrellus* genus and *Nyctalus* genus are likely to be most at risk from wind turbines. It is difficult to draw inferences for rare species such as barbastelle (*Barbastella barbastellus*) or greater horseshoe bats (*Rhinolophus ferrumequinum*), as encounter rates would be expected to be very low and further research is needed within areas which are their strongholds: some such sites are currently in the planning system. It is notable that very few casualties have been found of common and widespread species such as Brown long-eared bats (0.09%) or of all *Myotis* spp. (0.6%).(EUROBATS 2014a). It would therefore be reasonable to conclude that these species are at lower risk than species in the *Nyctalus* and *Pipistrellus* genus, at least within the open environments from which most of the data are derived. However, as recommended by EUROBATS, further work is warranted for wind turbines ‘key-holed’ into woodlands (EUROBATS 2014).

Table 1.2. Reported bat fatalities in Europe 2003-11 (EUROBATS 2014a)

Species	AT	BE	CH	CR	CZ	DE	ES	EE	FI	FR	GR	IT	LV	NL	NO	PT	PL	SE	UK	Total
Nyctalus noctula	24				3	716	1			12	10					1	5	1		**773**
N. lasiopterus							21			6	1					8				**36**
N. leisleri			1		1	108	15			39	58	2				206				**430**
Nyctalu spp.							2									16				**18**
Eptesicus serotinus					7	43	2			14	1			1			3			**71**
E. isabellinus							117									1				**118**
E. serotinus / isabellinus							11									16				**27**
E. nilssonii						3		2	6				13		1		1	8		**34**
Vespertilio murinus				5	2	89				6	1		1				3	1		**108**
Myotis myotis						2	2			2										**6**
M. blythii							4													**4**
M. dasycneme						3														**3**
M. daubentonii						5										2				**7**
M. bechsteinii										1										**1**
M. emarginatus							1			1										**2**
M. brandtii						1														**1**
M. mystacinus						2					2									**4**
Myotis spp.						1	3													**4**
Pipistrellus pipistrellus		5			3	431	73			278		1		14		243	1	1		**1050**
P. nathusii	2				2	565				87	34	2	23	7			12	5		**739**
P. pygmaeus						46				121			1			31	1	1	1	**202**
P. pipistrellus/ pygmaeus			1				483			24	54					35	1			**598**
P. kuhlii				51			44			81						37				**213**
P. pipistrellus/ kuhlii																19				**19**
Pipistrellus spp.				13	2	36	20			85	2		2			85			3	**248**
Hypsugo savii				24		1	44			30	28	10				43				**180**
Basbastella basbastellus						1	1			2										**4**
Plecotus austriacus	1					6														**7**
Pl. auritus						5														**5**
Tadarida teniotis							23			1						22				**46**
Miniopterus schreibersii							2			4						3				**9**
Rhinolophus ferrumequinum							1													**1**
R. mehelyi							1													**1**
Chiroptera spp.		1		46		46	320	1		189	8	1				102	2	30	7	**753**
Total	**27**	**6**	**2**	**139**	**20**	**2110**	**1191**	**3**	**6**	**983**	**199**	**16**	**40**	**22**	**1**	**870**	**29**	**47**	**11**	**5722**

The potential for wind turbines to cause substantial numbers of bat casualties and affect the local population, has been highlighted in both Europe and the USA (Arnett et al. 2008, Rydell et al. 2010b, Hayes 2013, Hedenström and Rydell 2013). Concern has also been raised about the potential of wind turbine casualties to influence populations outside their national boundaries, due to effects on migrating bats (Voigt et al. 2012, Lehnert et al. 2014). However, no scientific study has ever established the actual impact of wind-turbine linked deaths on local populations (EUROBATS 2014a). This does not imply that no effect exists, but rather that estimation of the proportion of the population of bats killed is extremely problematic: baseline data on population size and structure are frequently lacking (EUROBATS 2014a, Huso and Dalthorp 2014). Even where local population data are available, it is very difficult to disentangle the effects of the wind turbine from those of other impacts such as alterations in land use, loss of daytime roosts or climatic fluctuations (EUROBATS 2014a, Huso and Dalthorp 2014).

Why bats are killed at wind turbines

Several hypotheses have been proposed to explain why bats are killed by wind turbines (Kunz et al. 2007, Cryan and Barclay 2009, Rydell et al. 2010a). These include: accidental encounter, particularly by migrating or juvenile animals; deliberate foraging around the blades; and deliberate use of tall structures as display sites by bats in the breeding season. In North America, casualties of migrating and tree-dwelling species are of primary concern (Jameson and Willis 2014). Recent research has shown that migrating bats preferentially visit tall structures in the landscape, potentially explaining their high turbine collision rates (Jameson and Willis 2014). In addition, the use of thermal imaging has shown tree-dwelling bats preferentially orientating towards turbines and approaching turbines from the leeward side (Cryan et al. 2014). It has been suggested that tree bats use streams of air flowing downwind from wind turbines while searching for roosts and insect prey, similar to those produced around trees at night (Cryan et al. 2014).

In North America, an excess risk to breeding males has been reported, possibly resulting from males investigating tall structures in the landscape for use as display sites (Cryan et al. 2012). However, this pattern is not apparent among casualties in Europe (Rydell et al. 2010b). In contrast with N. America, peak fatality periods in Europe do not correspond with peak migration times, and in addition, there is no evidence that species which use trees as day-roosts or mating sites are at higher risk (Rydell et al. 2010b). It has therefore been suggested that in Europe fatality risks are more likely to be linked with foraging behaviour rather than migration (Rydell et al. 2010a). Nevertheless, it is clear that at least some species in Europe are killed during migration, and therefore the impacts of turbine-induced fatalities may extend beyond the immediate local population (Voigt et al. 2012, Lehnert et al. 2014).

In addition to direct collision with moving turbine blades, a significant proportion of fatalities may be due to barotrauma (Baerwald et al. 2008), that is, damage to tissues, particularly in the lungs and ears, by the rapid changes in air pressure near turbine blades. The relative importance of these two mechanisms is unclear; recent research suggests that most bats with barotrauma also have evidence of direct collision (Rollins et al. 2012). It is likely that the numbers of bats affected by barotrauma is under-recorded, due to (1) bats killed as a results of barotrauma need to be examined by post-mortem immediately after death (Rollins et al. 2012) and (2) if there is a delay between an encounter with the turbine and fatality the casualty may not be located under the turbine (which has been noted with some large raptors which continue to soar even after collision). This could affect overall estimates of casualty rates. However, further research into the effect of this is extremely difficult due to the cost and practicalities of increasing the search area and searching turbines daily, first thing in the morning.

History of work on bat-wind turbine conflicts and legal context for the UK

The first commercial wind farm in the UK opened in 1991. However, little attention was paid to the potential impacts on bats until 2008, when, amid concerns about bat fatalities reported in the USA, Germany and elsewhere, parties to the EUROBATS Agreement were urged to draw up national monitoring strategies (Rodrigues et al. 2008). In the European Union, the Environmental Impact Assessment (EIA) Directive 85/337/EEC legally requires an assessment to be carried out for all wind farms with 5 or more turbines, or which are over 5MW capacity. Also, all member states are required to restore or maintain their bat populations in favourable conservation status (Council Directive 92/43/EEC); translation of the directive into domestic legislation[†] makes it an offence to deliberately ('recklessly' in Scotland) kill or injure a bat; or to deliberately ('intentionally or recklessly' in Scotland) disturb bats in a way that would (significantly in England Wales and Scotland) affect their local distribution or abundance. A scoping report was therefore commissioned jointly by the British government and the industry body (RenewableUK) (Jones et al. 2009). This reported two cases where bat casualties had been found (n=8 in total; all pipistrelles), and suggested that a formal study was required.

Interim guidance was issued by the Statutory Nature Conservation Organisations in 2009, with minor updates being published in 2012 and 2014 (Natural England 2009, 2012, 2014). This guidance categorises likely risks to different bat species as 'high', 'medium' or 'low' according to flight and foraging characteristics. Additionally, population risks are evaluated by joint consideration of collision risk and estimates of population sizes (Table 1.3). The EUROBATS guidance proposes that turbines should not be placed closer than 200m to woodland (Rodrigues et al. 2008), whereas the current recommendations for the UK are that the blade tips should be at least 50m from woodland

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[†] In England and Wales, the relevant legislation is the Wildlife and Countryside Act (1981) (as amended); the Countryside and Rights of Way Act, 2000; the Natural Environment and Rural Communities Act (NERC, 2006); and by the Conservation of Habitats and Species Regulations (2010). In Scotland, the key legislation is the Conservation (Natural Habitats &c.) Regulations 1994 (as amended). In Northern Ireland, bats are listed under Schedule 2 of the Conservation (Natural Habitats &c.) Regulations (Northern Ireland) 1995.

or hedgerows (EUROBATS stand-off distances may be used in some circumstances). It is argued that a smaller buffer size is acceptable in the UK, because the activity of bats found here tends to decline rapidly with increasing distance from linear landscape features and woodlands.

Table 1.3. Assessments of collision risk and likely impacts on the conservation status of bat populations found in the UK (Natural England 2009, 2014).

Risk of collision		
Low	**Medium**	**High**
Myotis species Long-eared bats (*Plecotus spp.*) Horseshoe bats (*Rhinolophus spp*.)	Common pipistrelle (*Pipistrellus pipistrellus*) Soprano pipistrelle (*P. pygmaeus*) Serotine (*Eptesicus serotinus*) Barbastelle (*Barbastella barbastellus*)	Noctule (*Nyctalus noctula*) Leisler's (*Nyctalus leisleri*) Nathusius's pipistrelle (*P. nathusii*)
Risk to populations		
Low	Medium	High
Myotis species (*Myotis spp*.) Long-eared bats (*Plecotus spp*.) Horseshoe bats (*Rhinolophus spp*.) Common pipistrelle (*P. pipistrellus*) Soprano pipistrelle (*P. pygmaeus*)	Serotine (*E. serotinus*) Barbastelle (*B. barbastellus*)	Noctule (*N. noctula*) Leisler's (*N. leisleri*) Nathusius's pipistrelle (*P. nathusii*)

It is acknowledged that risk assessments for UK bats are restricted by a lack of evidence in crucial areas (Natural England 2014). Population estimates are uncertain, therefore even if collision rates are known inferences about the impact of turbine collisions on population viability, are also uncertain. Evidence is also lacking on the behaviour of bats around turbines. It might be anticipated that species which tend to fly at low heights and close to vegetation, such as brown long-eared bats (*P. auritus*) would be at lower risk than those which regularly fly in the open and at greater heights, such as noctules (*N. noctula*). These differences are reflected in the current classifications of risk (Table 1.3). However, it is unclear whether the presence of a turbine would alter bat behaviour. Such alterations could either be attractive or repellent. Work on very small wind turbines in the UK has suggested that bats avoid areas with turbines (Minderman et al. 2012), whilst other authors have suggested that turbines could be attractive (Kunz et al. 2007, Cryan et al. 2014).

Migratory activity, a known risk factor for wind turbine collisions, particularly North America, was, until very recently, thought to be absent among bats in Britain and Ireland. However, evidence of bats occurring mid-way between Plymouth and Roscoff (from recordings made on cross-channel ferries) (Mathews *pers. obs*), new banding data (Hargreaves and Mathews *pers. obs*. 2013), and seasonal peaks in acoustic records (Russ et al. 2001) now provide evidence that at least some of the British *Pipistrellus nathusii* population migrates. This species appears to be expanding its range into the UK (Lundy et al. 2010) and further work on its migratory habits is currently being undertaken by The Bat Conservation Trust, The University of Exeter, and The Vincent Wildlife Trust using approaches including stable isotope analysis and acoustic surveys.

At present, most work on wind turbines and bats is focused on preconstruction assessments: the principle is that accurate assessment of risk will minimise conflicts as high risk sites will be avoided and/ or appropriate mitigation will be initiated. Proposed wind energy developments are therefore required to assess the likely risks posed to bats. The use of the site by bats must be evaluated, with a focus on identifying whether the site is used by significant numbers of bats, especially those species identified as being at high risk. Emphasis is also placed on identifying bat roosts on, or adjacent to, the site; and on determining whether flight paths currently used by bats are likely to place them at risk of collision. However, there is no standardised prescriptive guidance on survey methods. Therefore surveys range from desk studies of historical records, to roost searches and acoustic surveys. The amount of effort deployed is roughly guided by the size of the development and the risk of significant numbers of bats using the site (Table 1.4). In practice, most sites proposed for the generation of more than >500kW are subject to a combination of these strategies, with at least one acoustic survey per month between April and October. Separate ‘Good Practice’ guidance produced by The Bat Conservation Trust (Hundt 2012), and followed by most ecologists within the Chartered Institute of Ecology and Environmental Management (CIEEM) recommends:

- investigating roost sites (of high/medium risk species or of district importance and above);
- transect surveys seasonally (low risk sites), monthly (medium risk sites) or twice a month (high risk sites);
- static surveying for five consecutive nights in each season (low risk), five consecutive nights per month (medium risk sites) or two lots of five consecutive nights per month (high risk sites);
- survey at height where justified.

Updated bat survey guidance has been produced, but pending the results of this project, has not specifically included recommendations for wind energy sites. However, for assessing the impact of other types of development, it recommends up to 5 consecutive nights of survey, with repetition frequency (per season or per month) depending on habitat suitability (Collins 2016).

Table 1.4. Criteria for pre-construction survey effort at site (adapted from Hundt 2012)

Quality of habitat and number of habitat features likely to affect bat mortality rates if altered by development	Species likely to use site	Importance of roosts, of species likely to use site, which may be affected by development	Potential risk level of development
No potential habitat for roosting, foraging or commuting bats	None	Local	Lowest
	Low number, single slow risk species High number, several low risk species	Parish	Low
	Low number, medium risk species High number, medium risk species	District County	Medium
High foraging and/or roosting potential. Good connectivity within landscape	High number, single high risk species High number, several high risk species High number, all high risk species	National International	High

Standardised post-construction monitoring, including acoustic and carcass surveys, is recommended for sites identified as ‘high risk’ (Natural England 2014). In reality, little post-construction monitoring occurs. This is at least partly because responsibility for requiring and enforcing survey conditions lies with Local Planning Authorities and other Competent Authorities, which are reluctant to impose conditions that could potentially be open to legal challenge. A further particular difficulty is that while generic guidance on survey designs is available, the lack of standardised methodology makes it extremely difficult to judge whether a particular level of reported bat activity would place a site as being in a 'high risk' category: this point has already been raised by at least one Planning Inspector at Public Enquiry (2009 Planning Appeal re. Hempnall, Norfolk; appeal reference no. APP/L2630/A/08/2084443). Further, there has been no assessment of the relationship between pre-construction and post-construction acoustic surveys (or collision risk). It is therefore unclear how pre-construction data can be used to predict post-construction risk. A final difficulty for Local Authorities is that the legal basis of bat protection relates to the conservation status of local populations (except potentially in Scotland, where recklessly killing a bat is also an offence). Given that local population sizes are very poorly characterised, it is unclear how or whether mitigation (such as specifying turbine cut-in speeds) could be triggered on the basis of reduced bat activity or bat fatalities.

Current research

To address some of the identified knowledge shortfalls, a large project funded by The Department for Environment, Food and Rural Affairs (Defra), The Department of Energy and Climate Change (DECC), Scottish Natural Heritage, Natural Resources Wales, Natural England, and the industry body RenewableUK was commissioned in 2010. The primary aims were to:

1. Determine whether bats are killed by wind turbines in the Great Britain.
2. Establish the species and sex distribution of any bat casualties at wind turbines.
3. Assess the relationships between casualty rates and bat activity (acoustic data), habitat and local weather in order to improve future mitigation strategies.

SECTION 2: BAT ACTIVITY AND CASUALTY RATES AT BRITISH WIND FARMS

Introduction

EUROBATS Resolution 7.5 (Eurobats 2014b), recognises the need for standardised survey methodologies and risk assessments when studying the risk to bats from wind turbines. However, most assessments have been based on either incidental casualty reports (Rydell et al. 2010b, EUROBATS 2014a), or from detailed longitudinal studies of individual or small numbers of wind farms conducted as part of legal obligations (e.g. (Brinkmann 2005, Barclay et al. 2007, Niermann et al. 2007, Arnett et al. 2008, Bach et al. 2013, Bio3 2013). Whilst these approaches have provided useful data, they also have significant limitations. The sites studied are therefore not a representative sample of all those available, making extrapolations about national or regional fatality rates problematic. In addition, it is difficult to separate out the effect of predictors, such as turbine height, from potential confounding variables such as turbine age or local habitat. Finally, differences in observer effort and monitoring protocols at different facilities make it difficult to combine data from different study sites. The current study is the first attempt, anywhere in the world, to assess the impact of wind turbines on bats at a national scale through the systematic survey of a representative sample of wind energy facilities.

Given that the primary objective of the research was to establish the extent and nature of fatalities occurring at British wind farms, rather than to assess temporal variations in casualty rates at particular focal sites, the project used a cross-sectional sampling of large numbers of wind farms. Recognising the difficulties likely to be encountered with identifying potentially small numbers of bat carcasses, particularly in moorland and arable habitats, where vegetative cover is likely to conceal carcasses, attention was given to identifying a robust methodology that could be applied consistently at all sites. Consideration was given to the use of search dogs, a method found to be successful on a small scale in the USA (Arnett 2006), given that improvement of search conditions by habitat removal were unlikely to be acceptable. We conducted a large, blinded, randomised trial and found that search dogs were considerably more effective in finding carcasses, identifying 73% of carcasses compared with 20% for human observers. In addition, search dog-handler teams were considerably more efficient, completing searches in approximately 40 minutes, which was less than a quarter of the time taken by humans (Mathews et al. 2013). Therefore, a team of 5 search dogs and handlers were specifically trained to undertake this project (full details are provided in Section 4). In addition to developing a novel approach to carcass identification, attention was also paid to the choice of technique for the acoustic monitoring of bat activity. Static detectors able to record in real-time for prolonged periods were used because of their ability to provide continuous information from the study turbines. We selected Songmeter2 (SM2 and later newer versions, SM2+) detectors (Wildlife Acoustics USA) on the basis of their omni-directional microphone, longer acoustic range, greater reliability and ease of deployment compared with other similarly-priced passive detectors: this project was their first use in the UK. Whilst more sensitive bat detectors are available (for example Avisoft Ultrasound Gate, Avisoft Bioacoustics and Batlogger (Elekon AG) (Adams et al. 2012), they would have been prohibitively expensive for this project where the application of a consistent methodology at a large number of sites was a primary consideration.

Methods

Study sites

The primary aim of the project was to establish, with a reasonable degree of confidence, whether or not bats were being killed at wind energy installations in the UK: this was not known at the start of the project and other research questions were of lower priority. We therefore conducted power calculations to establish a robust sampling protocol for the project. These calculations indicated that a cross-sectional sample size of 46 sites would allow us to distinguish whether casualties occurred at 3% or more of wind energy installations (compared with a null-hypothesis of casualties occurring at 0.01% of sites, a level considered to be of negligible importance) with a power[‡] of 0.8 and the significance level set at 0.05. We accepted from the outset that the power to detect links between potential risk factors and collision rates would be somewhat lower, and that some potential risk factors such as seasonality would be better addressed with a different type of study design (for example, longitudinal versus cross-sectional monitoring). To avoid bias in the sample, randomised sampling, stratified by country/region (Scotland, Northern England, Southern England and Wales) and habitat type (arable, Upland/Moorland, and mixed) was conducted to identify an initial target list of wind energy installations to include in the project. To be eligible for inclusion, sites had to have at least 6 operational turbines with towers of at least 50m tall (9 exceptions were made due to local unavailability of sites with towers >49m). Participation in the project was voluntary and, particularly in the early years, many sites were not made available for study.

[‡] The threshold number of installations with casualties set as being important (here 3.3%) is necessarily arbitrary. In this case, we aimed to detect even low casualty rates, and therefore set the cut-off point to a small value. A power of 0.8 indicates that the test has the ability to detect differences as large as this (or larger) 80% of the time. It therefore follows that a sample size of 46 would have a 20% chance of failing to detect casualties even though they in fact occurred at 3.3% of sites.

The number of turbines searched per site ranged from 6-8, with the exception of site 41 where access was available to only 5 turbines. Most sites were studied for approximately one month in a single year. Data from Europe and North America indicate seasonality in fatality risks (Brinkmann et al. 2006, Niermann et al. 2007, Arnett et al. 2008, Rydell et al. 2010a). The survey window was therefore set as 1st July – 18th October, to coincide with periods of peak mortality reported elsewhere in Europe (Rydell et al. 2010). The precise survey periods were 18th July-21st October 2011; 9th July – 13th October 2012; and 1st July-18th October 2013. One site (Site 9) was studied in all three years of the project, and three sites were studied in 2012 and 2013 (Sites 20, 26, 33) following discussions with the project Steering Committee, to provide additional information about the temporal variation in casualty rates. With the exception of site 26, which was included due to being in very close proximity to site 33, these sites had particularly high fatality rates in the first year of study. The frequency of survey at these repeated sites was increased in 2013, so that the mean inter-search interval was < 2 days.

The extent of different habitats around the wind farms were quantified in ArcMap 10.1 ((ESRI) using the CEH Land Cover Map 2007 (Morton et al. 2011). Two spatial scales were examined: a buffer of 1500m and a buffer of 2500m, centred on the middle of the turbine site. The same procedure then repeated but using the outside edge of the wind farm, rather than the centre, as the start of the buffer. The features examined were: total broadleaf and yew woodland (hereafter 'broadleaf'), total coniferous woodland, total woodland, recent clear-fell (within 10 years), improved grassland, and total dwarf shrub heath (km^2 areas in each case). It is accepted that some habitat types may have changed between the time the Land Cover Map was created, and the time of the survey. This may particularly affect new clear-fell areas of woodland. However, resource constraints meant it was not possible to perform new habitat surveys across the entirety of each study site (though the habitat beneath each turbine was mapped).

Casualty surveys

The turbines included in the project were randomly selected from the operational turbines available at each site, subject to any constraints imposed by landowners. A 100mx100m search area, centred on the turbine was marked out (encompassing the rotor-swept area). The habitat beneath each turbine was recorded on a standard pro-forma (available on request). An automated weather station (Wireless Weather Station N25FR, Maplins, UK) was set up at one turbine per site. The analyses shown here present night-time weather data only.

The turbines were searched using trained dogs according to a methodology developed specifically for this study (see Section 4). The surveys began early in the morning in an effort to minimise carcass removal rates by diurnal scavengers (notably corvids). The location and condition of any carcasses found were recorded, the carcass was photographed, and an estimate of the date of death was made where possible. Wing samples were taken and stored in 90% ethanol for subsequent genetic examination. Fur samples were also taken and stored for potential future analysis of stable isotope composition, which could provide evidence of whether the casualties were migratory. The remainder of the specimen was stored in neutral buffered formalin (in 2011 and 2012) and in 90% ethanol (2013) to permit subsequent *post-mortem* examination. On their return to the laboratory, *post-mortem* examinations of the casualties were conducted. Presumptive species identifications were made on the basis of morphometric characteristics, and the carcass condition, together with the sex and age (adult or juvenile) of the animal was noted. Molecular methods were developed to identify the species and sex of the carcass and were used to validate the *post-mortem* examination results (see Section 5). In 12 cases, species identifications were available only by genetic means because the carcass was decayed or damaged beyond recognition. There were 39 cases where sex identification was based on genetic analysis only (this higher number reflected the fact that predators frequently removed the body cavity contents and genitals first).

Predator removal and observer efficiency trials

As in most studies of wind turbine casualties, it was not possible to conduct surveys daily, and instead searches were conducted at 2-3 day intervals (mean 2.5). Whether conducted by dogs, as in this case, or by humans, the number of individuals found will be a minimum estimate of the true casualty rate. This is primarily due to two factors: observer efficiency (the probability that the observer finds a carcass if it is present); and carcass removal (by scavengers, decay and weather if the carcass if washed away or covered by blown debris). In our survey, the entire search area was usually surveyed by the dogs, and therefore there was no need to adjust the estimates for the proportion of the target area surveyed. However, estimates of casualty-rates at the scale of the whole wind farm, rather than the turbine, accounted for the proportion of turbines that were searched. The degree of influence exerted by carcass removals and observed efficiency will vary between sites, and across time, and will be influenced by environmental conditions such as topography, vegetation cover, and local weather conditions. The need to account for these sources of error when estimating true casualty rates is now well-recognised. Approaches range from relatively simple adjustments (e.g. (Johnson et al. 2003), to more sophisticated models (e.g. (Bispo et al. 2010, Floud et al. 2011, Huso 2011), including some which account for the persistence of undetected carcasses between subsequent surveys (e.g. (Erickson et al. 2004, Korner-Nievergelt et al. 2011). A difficulty with all of these adjustment techniques is that it is unlikely that carcass removal rates and observer efficiency are consistent for the duration of a study.

At each study site, dead bats supplied to the project by bat carers were used for observer efficiency and carcass-removal trials. These were performed at a control turbine that was not part of the main areas surveyed: if there were no additional turbines, then the trial was conducted in a nearby area of similar habitat. An independent observer placed between 3 and 14 bats in positions beneath the turbine randomly selected from a grid. The carcasses were dropped rather than placed to simulate as closely as possible field conditions, and the carcasses were handled with gloves and/or forceps to avoid introducing fresh human scent. The bats were of a range of species, but were predominately pipistrelles, and most had been frozen prior to use. The field worker, blinded to their locations, as well as to the total number of bats used, then attempted to locate the carcasses with the aid of their search dog. After the completion of the observer efficiency trial, the carcasses were left in position and the field worker recorded whether they remained on each visit to the site. Observations stopped only if all carcasses were removed, or when the project finished at a particular site. Care was taken to ensure that location markers were placed away from the carcass itself, in order to avoid artificially drawing the attention of predators to the carcass.

For the purposes of our analyses, we used the carcass removal rate trials to produce empirical estimates of carcass loss specific to each site. The actual number of bats found was therefore multiplied by the removal rate observed for the mean inter-search interval at the particular site. This figure was then adjusted for observer efficiency. It is recognised that the possibility of recording false zero carcass observations is present, this will reflect a combination of true zeros (no dead bats were present) and false zeros (bats were present but were removed by predators, missed by observers, or fell outside the survey area). This is a difficulty that affects all similar studies, and it must be recognised that there is currently no accepted way of adjusting zeros to produce a more realistic estimate of casualty rates (Huso 2011), though Bayesian approaches have recently been developed (Huso et al. 2014). In this project we were able to conduct only single predator removal/observer efficiency surveys at each site due to the cross-sectional design of the project. Further, we had limited availability of bat carcasses with which to evaluate predator removals and observer efficiency. This makes it difficult to apply the more sophisticated casualty estimators currently available (Bispo et al. 2010, Korner-Nievergelt et al. 2015), as the sample sizes were relatively low and repeated trials of predator removal/observer efficiency were not possible. However, we are working with one of the developers of these modelling procedures (Fraenzi Korner-Nievergelt) to explore new methods of adjustments, and will report these values, and the associated credibility intervals, in due course. In the interim, we can be confident that the current estimates are sufficiently robust for decision-making, since the project had high levels of observer efficiency, all of the search area beneath the turbines was usually accessible for searching, and the inter-search interval was short. Therefore whilst there may be some under-estimation of true casualty rate, it is unlikely that the revised estimates will be sufficiently different from those reported here to materially change the conclusions of the report.

Acoustic Surveys

Full spectrum acoustic recorders for bats (SongMeter2 (SM2 and SM2+), Wildlife Acoustics USA, hereafter referred to as SM2s), equipped with a minimum of 32GB SD cards were set up at the base of 3 turbines per site, usually on the steps leading up to the tower, or occasionally on 2-metre high tripods. The SMX-US omnidirectional microphones were orientated horizontally. In addition, recorders were installed in the nacelle of the same turbines. For these ‘at height’ recordings, the microphone was protruded from the nacelle by approximately 1.5 metres on an extension cable, and was held in position with plastic conduit tubing. This procedure reduced the amount of non-target noise from the nacelle that was recorded by the SM2 detector. Logistical constraints on the timing of installations at height meant that at some sites there were slightly shorter recording periods at height than at the ground. At 4 sites, it was not possible to make recordings at the nacelle because turbine technicians were not available to install the equipment. Each detector was programmed to make daily automatic recordings from 30 minutes before sunset until 30 minutes after dawn. Bat activity was monitored for an average of 27 (±SD 8) continuous nights at each site. Recordings were stored when triggered by the signal to noise ratio exceeding a pre-set threshold (here: 36db in year 1 and 2; 48db in year 3, with the increase in threshold reflecting revised advice from the manufacturer). Only sounds above 16kHz were recorded, and once triggered, the recording continued until the threshold was not reached for a period of at least two seconds. Preliminary tests in the field (using bats in the hand and an ultrasonic sound generator) suggested a detection distance of approximately 30m for pipistrelle bats under conditions of low wind and no rain. This is compatible with sensitivity tests reported elsewhere (Adams et al. 2012), and it should be noted that it is somewhat less than the length of many turbine blades. Each recording was tagged with a time-stamp. The clocks of all SM2s were set to within a second of each other, initially synchronised with the world clock, and resynchronised at the start of each new site. SM2s were programmed to save continuous acoustic data as compressed Wildlife Acoustic Compression (WAC) files and saved every hour to an SD card, thereby minimising data loss in the event of technical failures. Data were downloaded from the SD cards at weekly intervals for the ground-level detectors. For the nacelle-level detectors, data were usually downloaded only at the completion of the survey and it was extremely difficult to check for any technical difficulties during the recording period. New microphones were used at each study site to minimise the loss of sensitivity over time.

The data were transferred to the University of Exeter and duplicate back-up copies were made onto 2 dedicated NAS-RAID drives. Checks of the data were made for evidence of microphone failure or other technical difficulties. Data were excluded where this occurred, except for 5 locations where data would have been lost for whole nights (4 cases in the nacelle, 1 case at ground level) by this approach, and so all available data were included. Comparisons were made of models obtained with and without the inclusion of these data: there was no evidence that their inclusion affected the results.

At each site, an SM2 bat detector was also installed at ground level at a control location in similar habitat to, but away from, the turbines. These control locations were between 173 and 1939m away from the nearest 'matched' turbine and were within the land to which the turbine company had access (the 'red line' of the site). In 2013, additional surveys were conducted at the request of the project Steering Committee to reflect the data collection strategies frequently used by ecological consultants working at potential wind turbine sites. At each study site, an additional SM2 detector was also placed at a linear feature close to a turbine (distances ranged from 55-608m) , and 3 transect surveys were conducted at approximately weekly intervals at each study site with bat activity being recorded for approximately 2 hours from sunset using full-spectrum EM3 bat detectors (Wildlife Acoustics, USA).

The compressed WAC files were later converted to Waveform Audio Format (WAV) files using the Kaleidoscope Pro (KPro) programme (version 1.2.0, Wildlife Acoustics, Maynard, USA). During this process, the triggered events were extracted from the hour-long files, and non-bat (noise) signals were filtered out. Further noise was filtered out by selecting only triggered events which had individual pulse durations of between 2 and 500ms, and with a minimum of 2 pulses per triggered event. The advanced signal enhancement was also selected, as per the manufacturer recommendations (see Wildlife Acoustics http://www.wildlifeacoustics.com/), and the signals were either allocated to a species, or to a no-identification category, using the programme's automated algorithm.

Following extensive preliminary trialling, the acoustic data were analysed using Kaleidoscope Pro software (Wildlife Acoustics, USA). Automated recognisers were used to identify potential bat sounds, and all calls were then subsequently analysed manually using KPro viewer using the parameters described by Russ (Russ 2012). The manual check also enabled more than one pass per file to be detected, which would otherwise have been missed by the automatic recognition system.

A pass was defined as a continuous run of pulses separated by gaps of less than one second (Fenton et al. 1973), and bat activity is defined in terms of passes throughout this report (in contrast to some reports which use a 'file' as equivalent to a bat pass).

For a random sample of 21 sites (42% of all sites), all passes were counted. In all other cases multiple passes were recorded as 'two or more passes' for each species, to reduce analysis time. Whilst this slightly under-recorded the actual number of passes, it represents only a very small proportion (4%) of all files manually identified (4%). The total number of passes for sites where each pass was counted was only 2% higher compared to counting only the first two, and therefore we assume that our methods give a reasonable approximation of true bat pass rates. Note that it is not possible to infer the numbers of bat present at a site on the basis of acoustic recordings, since a given individual may be recorded once or many times. However, it is valid to compare the relative activity levels, and to use the index as a potential predictor of casualty risk.

All bats were identified to species with the exception of *Myotis* spp. which were identified to genus because of the similarity of the calls of many species and because previous research has not identified *Myotis spp*. as being at appreciable risk from wind turbines (Rydell et al. 2010c). Screening of noise files also showed that a very small proportion (<0.1%) contained bat calls, and these were generally of poor quality: no formal analysis of the noise files has therefore been conducted. In total 240,344 call files were screened, identifying 180,062 passes from the study turbines.

Statistical Analysis

All analyses were undertaken using R (v.3.1.1) (R Development Core Team 2013) and CIA (Bryant 2011). Comparisons of bat activity were made between control sites (no turbine but in similar habitat) and the nearest turbine; and in 2013 between a recorder placed at a linear feature and the nearest turbine. Very strong over-dispersion was detected and therefore these data were modelled using a negative-binomial model with a random variable for site (using the R package GLMMADMB) (Skaug et al. 2012).

Comparison of assessments of bat activity at ground and height

Since bat activity is usually monitored from ground level, we compared recorded activity at ground with paired recorded activity at height. The probability of detecting a species at height but not at ground level was assessed using presence/absence data for species with sufficient data to make meaningful comparisons (pipistrelle and noctule bats) using a Generalised Linear Mixed Model (GLMM) with a binomial error structure and logit link function. Sites were included for the analysis of a particular species only if they were within the species' geographical range (IUCN 2008); and only nights which had activity at either ground or height were considered. Site and night were specified as random effects in order to account for spatial and temporal autocorrelation. In addition to using height/ground as a binomial predictor, we included the height of the detector above the ground (m) (for the detector in the nacelle) a fixed effect to test whether there was any change in recorded bat activity as distance to the ground increased.

To assess whether bat activity differed between paired ground and height detectors, we modelled total nightly species activity per detector using a GLMM with a Poisson error structure and a log-link function. Data that were unpaired (due to technical issues with the detector at either height or ground) were excluded. We fitted site and night as random effects, and also added a random variable with a distinct level for each observation in the dataset to account for over-dispersion in the data (Bolker et al. 2009). An interaction term between height (a fixed factor with 2 levels: ground and height) and species (a fixed factor with 6 levels: common pipistrelle; soprano pipistrelle; Nathusius' pipistrelle, long-eared, noctule and *Myotis* bats, excluding species with no or insufficient data at height to enable model convergence) was fitted. Due to the significant interaction between species and height ($\chi^2 = 394.53$, n = 5, $P < 0.001$), the analyses were then repeated for each species separately.

To assess whether the ratio of activity at ground compared to height was consistent between species for which sufficient data were available (pipistrelle and noctule bats), the nightly ground to height ratio was modelled using a GLMM. The ability to predict total activity at height, using the total activity measured at ground level, was then investigated for each species. Because the activity of bats measured at the nacelle is likely to depend in part on the height of the nacelle, an interaction between the height of the nacelle and ground level activity was included in the models. Site was not included as a random effect since all turbines within site were the same height, therefore, site and detector height were confounded. The fit of the fixed terms in the models was evaluated by calculating marginal R^2 ($R^2_{GLMM(m)}$) and the combined fixed and random terms by calculating conditional R^2 ($R^2_{GLMM(c)}$) (Nakagawa and Schielzeth 2013).

Results

Site Characteristics

Forty six wind farms out of the 204 operational wind farms in Great Britain were included in the project. These were operated by 10 different companies. The distribution of sites by country is given in Table 2.1. The schematic map (Fig. 2.1a) shows the kernel densities (based on 50km buffers) of wind farms included in the project alongside a comparable kernel density map of all operational onshore wind farms in Great Britain (data derived from RenewableUK website database, 2014). Figure 2.1b shows the current distribution of operational wind farms in the UK (RenewableUK website database, 2016). The study succeeded in being broadly representative of the distribution of turbines within each country, with the possible exception of the Scottish Borders and West Yorkshire/East Lancashire, which were under-represented. However, the study sites in year 1 (and to a much lesser extent in years 2 and 3) deviated substantially from the original randomised selection. The extent and implications of any resulting bias is extremely difficult to quantify.

Table 2.1 Numbers of sites studied by country.

	2011	2012	2013	Total
Scotland	6	5	5	**16**
Wales	2	3	4	**9**
England	6	5	10	**21**
TOTAL	**14**	**13**	**19**	**46**

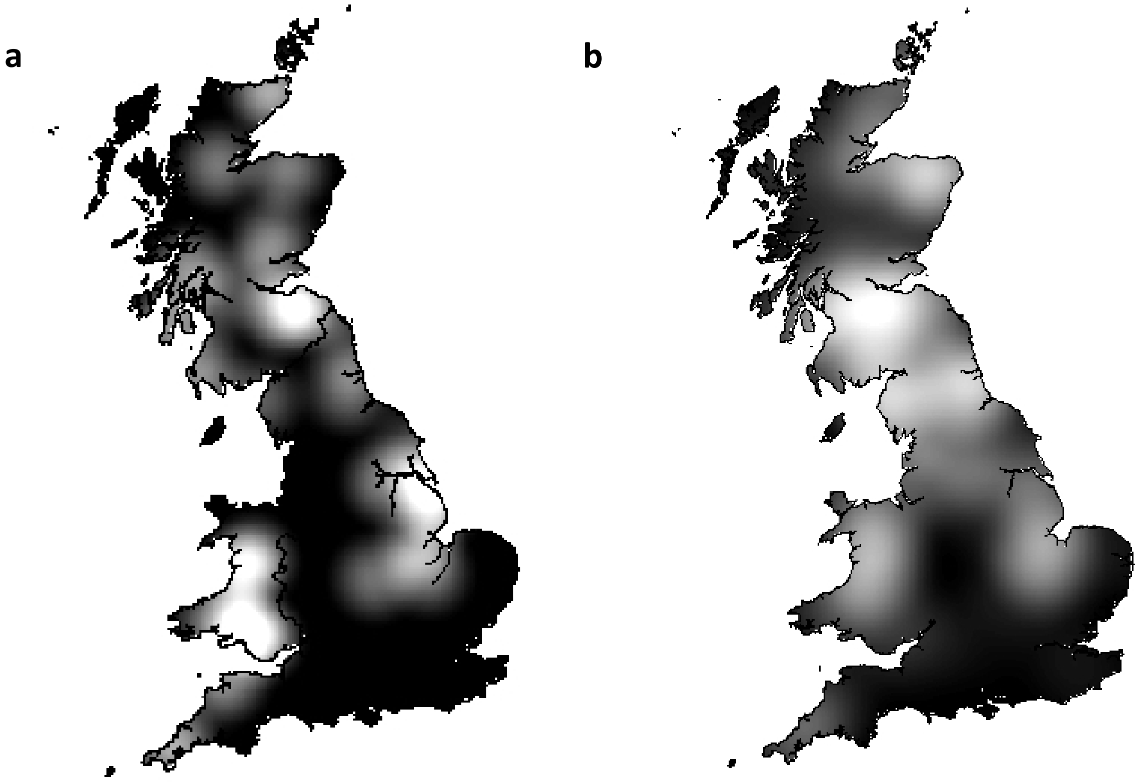

Fig 2.1a. Kernel density maps of (a) wind farms included in the study, and (b) density of operational wind farms meeting the selection criteria at the time of the study. Black indicates absence of wind farm and lighter shades indicate areas with highest density. Map based on wind farm densities within 100km study site densities within 100km zones.

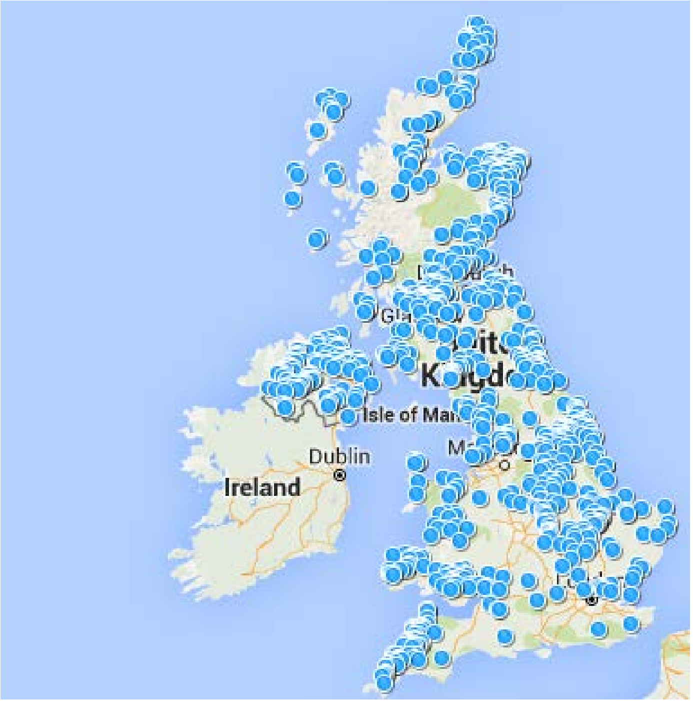

Fig 2.1b. Distribution of operational wind farms in the UK (Feb 2016, RenewableUK).

The mean numbers of turbines present at the study sites was 13 (SD=7; range 6-45); note that at some sites, extensions or contiguous sites meant that the numbers of turbines were higher. The mean hub height was 62m (SD 16), and distributions of turbine heights to hub and tip are shown in Fig. 2.2.

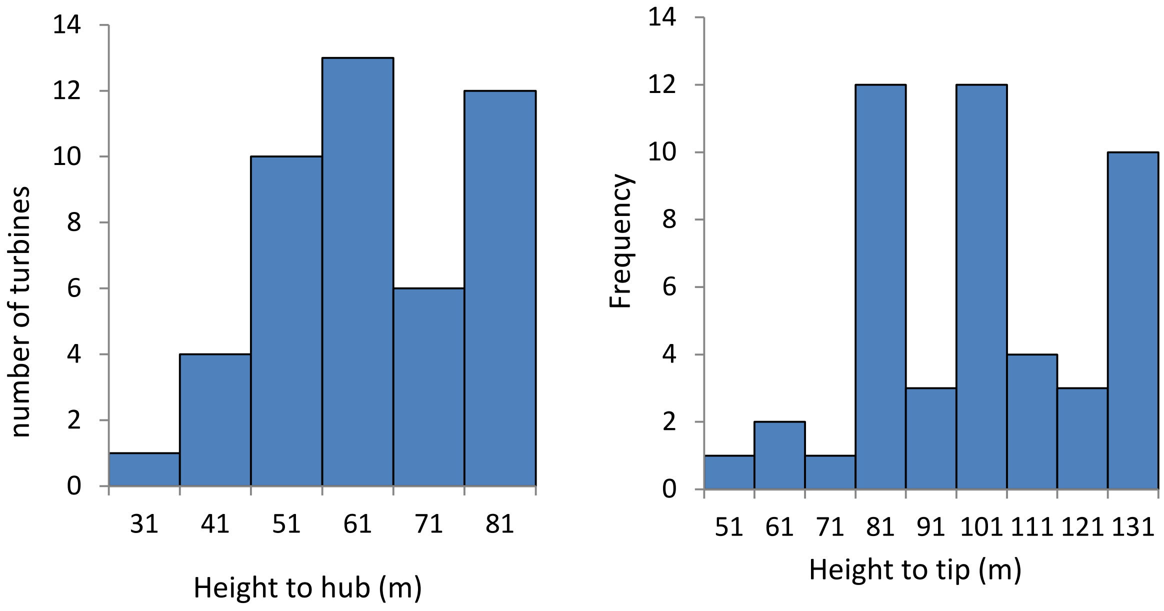

Fig 2.2. Distribution of turbine heights to (a) hub and (b) tip.

The total number of turbines studied was 290. The repeated surveys (4 sites) involved 30 turbines. The mean number of casualty searches per site (searches x turbines searched) was 63.6 (SD 8.7). The dominant habitats at the sites studied were farmland (arable (n = 11), pastoral (n = 6), mixed (n = 7)), woodland (n = 16), upland moorland (n = 16) and a brown field site (n = 1). There were 1545 site-nights of observation across the project.

Description of casualties: species, sex, age-structure and distribution across sites

In total, 120 bat carcasses were found, and most were within 30m of the turbine tower (Fig 2.3). The distribution of species is shown in Fig 2.4. Noctule bat casualties were found at 7 sites: of these, 1 site had 3 casualties, 2 sites had 2 casualties, and the remainder had 1. Single fatality cases were found for Natterer's bat (the first record for Europe), Brown long-eared bat and Nathusius's pipistrelle bat. The Natterer's bat was found at a site which also had common and soprano pipistrelle casualties; and the Brown long-eared bat was found at a site which also had common pipistrelle casualties. The Nathusius' pipistrelle was the only casualty found at the site.

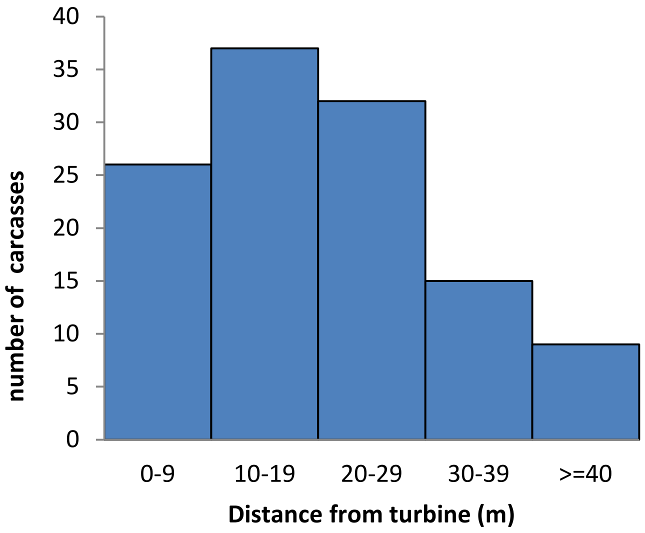

Fig 2.3. Distance between the casualties and the turbine towers.

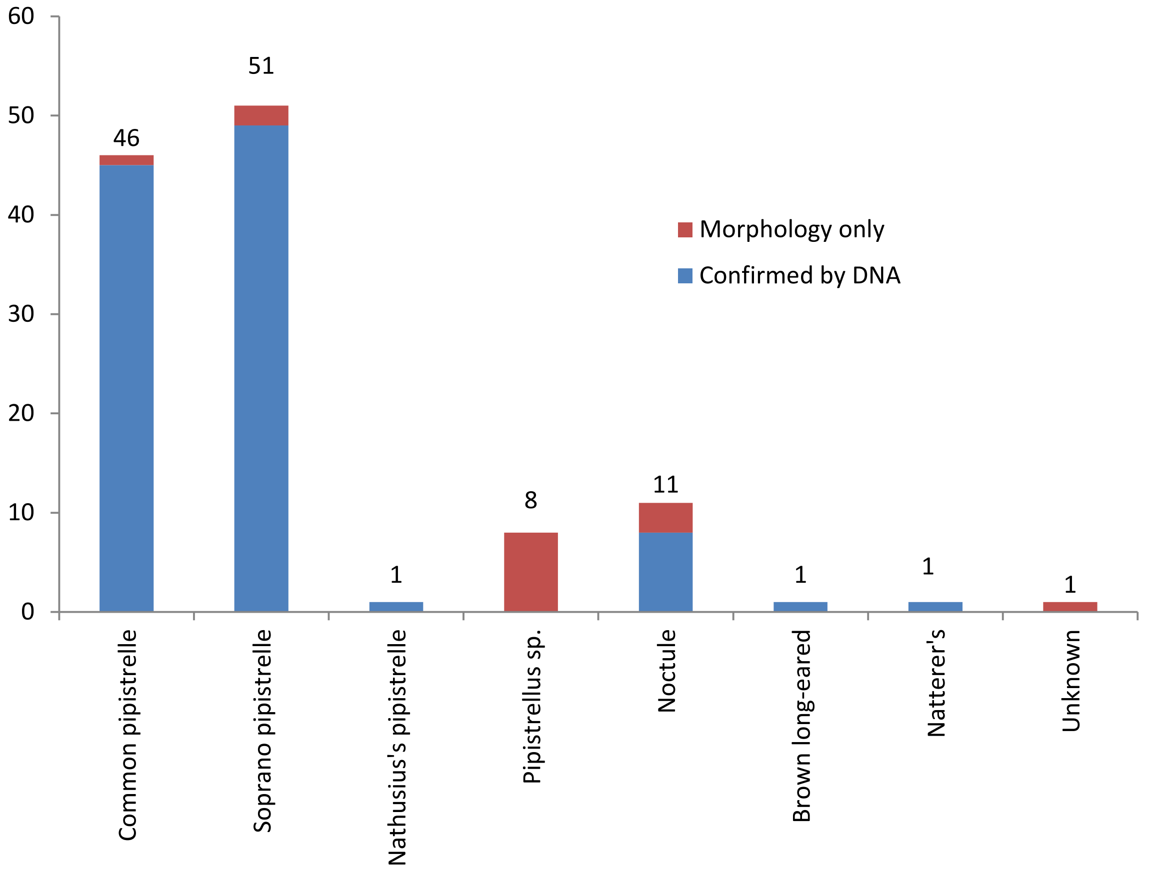

Fig 2.4. Frequency distribution of the observed bat casualties by species (numbers shown above each bar).

Although there was considerable variability in the numbers of bat casualties retrieved at each site, there was no indication that casualties were clustered at particular turbines within sites. Twelve of the 29 sites with casualties had 3 or more fatalities observed within a year (one of these sites was visited three times, giving a total of 14 occasions on which clustering could be explored). The expected number of searched turbines with zero casualties, given the total number of casualties observed at the site, was calculated: in no case did this number exceed that expected by chance (Fisher’s Exact Test, $p>0.182$ in each case). Further analyses to explore potential clustering of fatalities relative to key topographical features, such as hill-crests, are ongoing. It is important to recognise that the study had low statistical power to detect clustering effects. The deposition of data on the location of casualties to a central data storage facility in future monitoring work would permit more detailed analysis in the future.

The sex distribution of casualties is shown in Table 2.2. For common and soprano pipistrelles, the only species for which sample sizes permitted formal exploration, there was no evidence that the sex ratio deviated from 1:1 (common pipistrelle male proportion mean = 0.57 (95% CI Wilson Method 0.42-0.70; soprano pipistrelle male proportion mean = 0.44 (95% CI 0.31-0.59). Most of the bats were adults (mean proportion 0.84, 95% CI 0.75-0.89, age unknown for n = 14). It is reasonable to expect that the behaviour of predators and observers would not differ for bats of different sexes or age classes, and therefore that the observed casualties reflect the true proportions in each class. However, it is possible that the assumption may be violated for differences between size-classes of bats. For example, larger species, may be more attractive to vertebrate predators than smaller ones, and therefore be removed more quickly; but conversely, it may take longer for larger carcasses to disappear through other processes, such as decay or being buried by invertebrates. In our study, the supply of carcasses for the predator removal trials was heavily weighted towards pipistrelle bats, as these are the species most commonly received by bat carers. We therefore have insufficient data to permit exploration of potential biases in removal rates. Other studies elsewhere have found that the probability of carcass removal is influenced by the guild of predators present and weather conditions (Paula et al. 2015); but we are not aware of any work examining the relative removal rates of bats of different sizes. Observer efficiency does not appear to vary between bat species (Mathews et al. 2013).

Table 2.2. Distribution of casualties by sex

	P. pipistrellus	*P. pygmaeus*	*P. nathusii*	*Pipistrellus sp.*	*N. noctula*	*M. nattereri*	*Pl. auritus*	**Unknown**	**TOTAL**
Male	25	20			6				**51**
Female	19	25	1		4	1	1		**51**
Unknown	2	6		8	1			1	**18**
Grand Total	46	51	1	8	11	1	1	1	**120**

Carcass detection rates across Europe are reported to range from 0 to 0.11 bats turbine^{-1} day^{-1} (these are raw data, with no adjustments for observer efficiency or carcass removals) (Rydell et al. 2010b). Our raw data are consistent with this range (0-0.18 observed bats turbine^{-1} day^{-1}).

No casualties were found at approximately a third of the study sites (17/46). However, it is important to emphasise that zero observations does not necessarily imply zero casualties; and it is difficult to produce estimates of true rates for sites where no carcasses were found. The 95% confidence intervals[§] for the proportion of wind farms with observed casualties are 48.6%-75.5%. At sites where at least one casualty was found, the casualty rates were variable: estimated total bat casualty rates (adjusted for predator removal and observer efficiency) ranged from 0 to 5.25 bats turbine^{-1} standard month^{-1}, and from 0 to 77 bats site^{-1} standard month^{-1}. At 15% (7/46) of sites, rates exceeded 1 bat turbine^{-1} standard month^{-1}. The estimated total casualty rates per turbine are shown in Fig 2.5a, and the estimated total rates per site are shown in Fig 2.5b. In sites studied in more than one-year, casualty rates were not consistent across time (Table 2.3). The estimates for each of the primary species affected are shown, for each country separately, in Fig 2.5c (expressed as casualties^{-1}turbine^{-1}standard month^{-1}). Across all study sites, the relative percentage occurrence of soprano pipistrelle, common pipistrelle and noctule casualties was 40.6%, 48.6% and 10.7%.

[§] Wilson Method

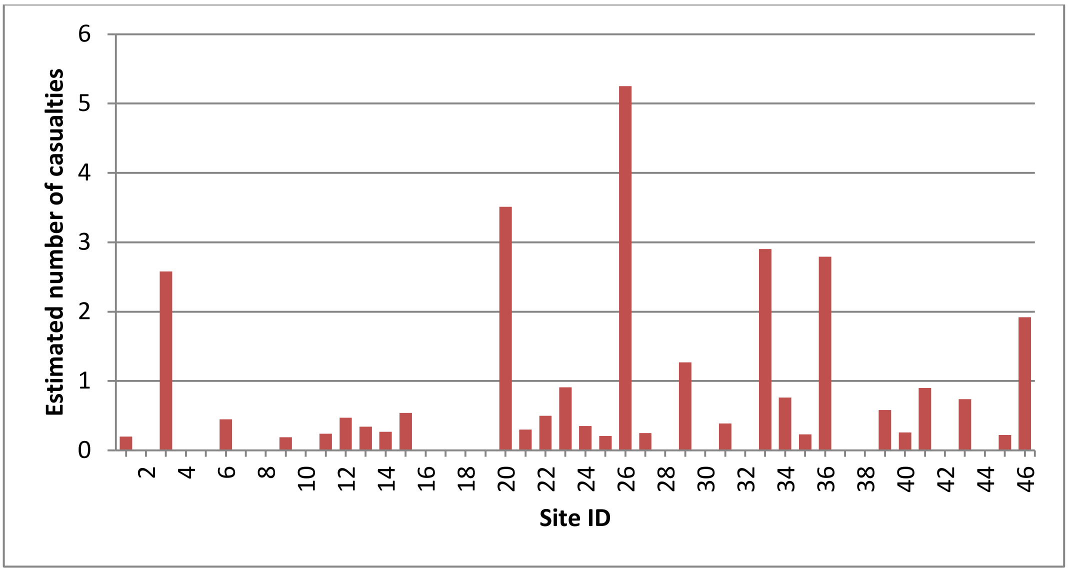

Fig 2.5(a). Estimated total numbers of bat casualties month^{-1} turbine^{-1} in the 4-month survey.

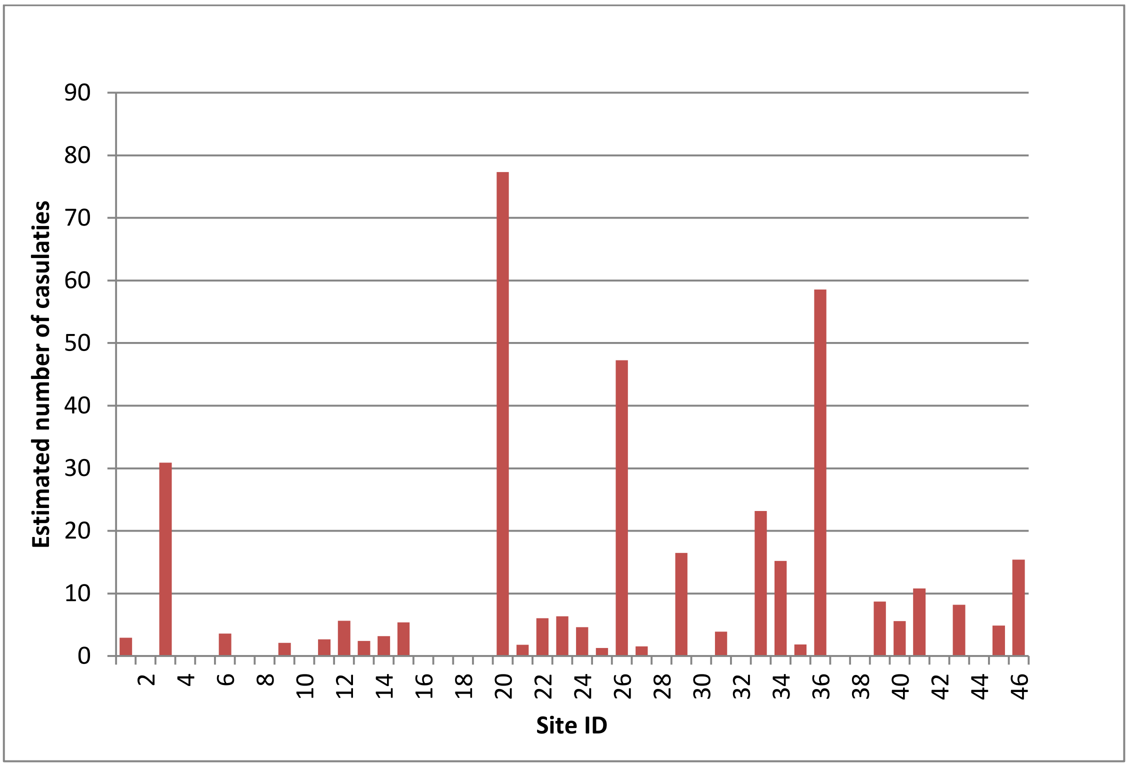

Fig 2.5(b). Estimated total numbers of bat casualties month^{-1} per site during 4-month survey.

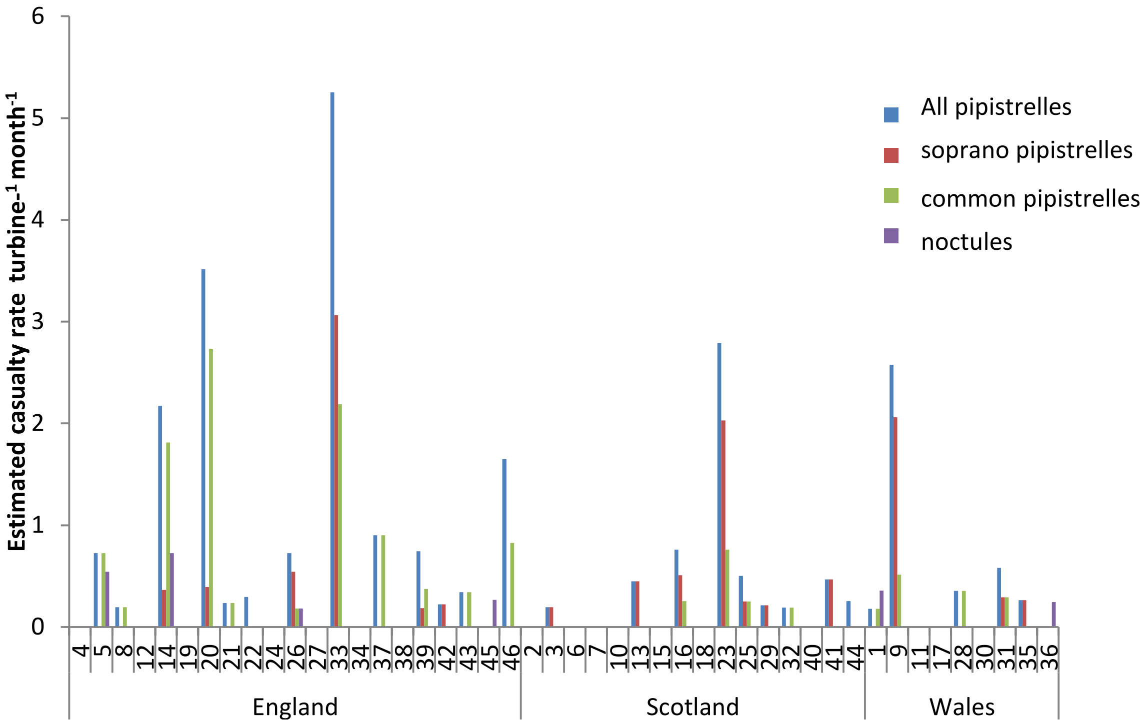

Fig 2.5(c). Estimated casualty rates month^{-1} turbine^{-1} shown separately by country. The category 'all pipistrelles' includes pipistrelle bats identified to genus only. Numbers on the x-axis are the site ID numbers.

Table 2.3. Estimated casualty rates for sites studied in more than one year.

	turbine^{-1}month^{-1}			site^{-1}month^{-1}		
Site ID	**2011**	**2012**	**2013**	**2011**	**2012**	**2013**
9	2.6	3.3	0.39	30.9	40.1	4.7
20	no survey	3.5	0.24	no survey	77.3	5.4
26	no survey	0.91	0	no survey	6.3	0
33	no survey	5.3	0	no survey	47.3	0

The geographical ranges and known distributions of bats in the UK are presented in Appendix 1. It is acknowledged to be extremely difficult to determine risks to rare species (for example the bats listed on Annexe II of the Habitats Directive) because the low probability of encounter means that error rates are extremely large. However, even small numbers of casualties may have very significant effects on local populations (Huso 2011, Huso et al. in press). It therefore cannot be concluded for rare species that a zero recorded collision rate in this study implies a zero or negligible risk.

This relative distribution of casualties may be compared with estimates of the relative abundance of species in Great Britain (Fig. 2.6 and Table 2.4; see also Appendix 1). Whilst some caution must be exercised when interpreting absolute population estimates, since these are widely acknowledged to be poor, there is more confidence in the accuracy of relative measures. Both soprano pipistrelle and noctule bat casualties appear more frequent than expected from their relative population estimates, whereas Brown long-eared bat casualties are less frequent.

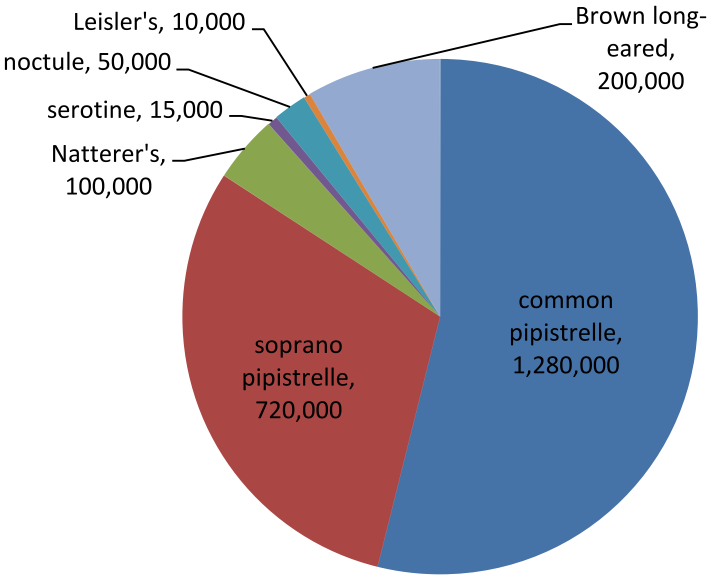

Fig 2.6. Best available estimates of bat abundance in Great Britain (based on (Harris et al. 1995) *op cit* (Battersby 2005). Figure is designed to illustrate relative abundance rather than absolute population size.

Table 2.4. Summary of the number of sites within the IUCN (2008) range of each British bat species, and the number of sites that the species was detected. Total population estimates for Great Britain derived from (Harris et al. 1995) with updates where available (Battersby 2005).

Species	Number of sites within a species' range	Number (and %) of sites species detected	GB population estimate*
common pipistrelle	46	44 (96)	1,280,000
soprano pipistrelle	44	42 (95)	720,000
Nathusius' pipistrelle	39	35 (90)	4,000
noctule	35	30 (86)	50,000
Leislers'	31	6 (19)	10,000
serotine	10	4 (40)	15,000
Myotis spp**.**	46	40 (87)	291,500
long eared spp.	46	37 (80)	200,000
barbastelle	26	8 (31)	5,000
lesser horseshoe	12	5 (42)	18,000
greater horseshoe	12	1 (8)	6,600

*figures are provided to illustrate relative abundance rather than absolute population size

Bat activity

Distribution of bat activity across the sites

Bat activity levels were highly variable at both turbines and the two types of control examined (similar habitat control, and linear feature control) (Fig. 2.7). Overall, there was no difference in total mean bat activity at the similar habitat control and turbine sites (estimate 0.146, z=0.13, n=1816 observations, 41 sites, p=0.26). However, it was much greater at the linear features compared with the similar habitat control (estimate -1.607, z=-6.19, n=484 observations, 14 sites, *p* < 0.001).

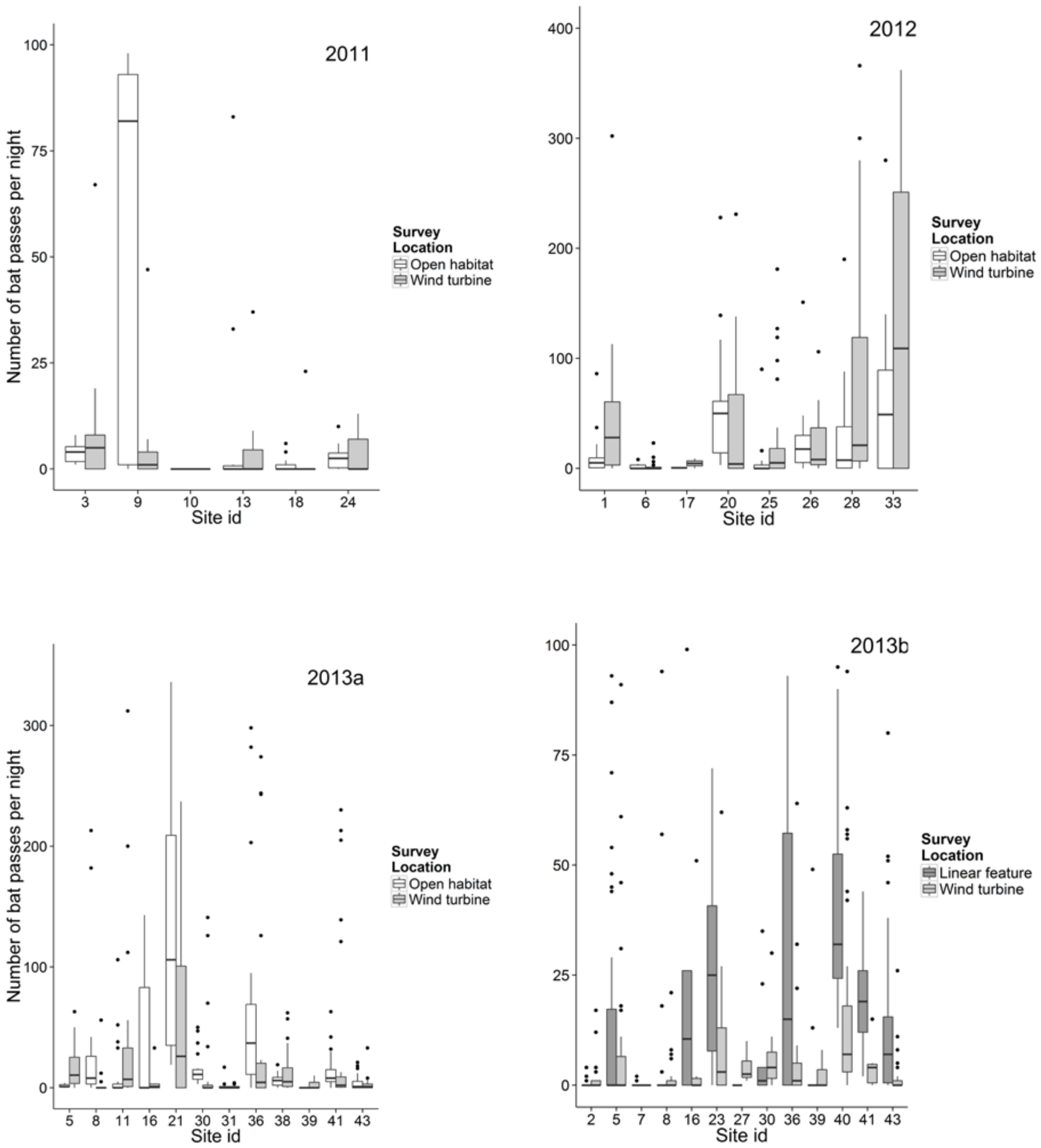

Figure 2.7. **Yearly comparison of bat activity at wind turbines, open habitat (2011, 2012, 2013a), and linear features (2013b).** Note that only one detector per site was deployed in ‘open habitat’ and ‘linear feature’. Therefore to permit fair comparisons, data from only one turbine per site (that most closely matching the ‘open habitat’) are shown. The upper and lower hinges (ends of rectangles) correspond to the first and third quartiles, whereas the upper and lower whiskers extend to the value that is within 1.5 times of the interquartile range of the hinge. Outlying values are shown as dots.

The transect surveys also revealed variable activity patterns, with some sites having high levels of activity close to turbines whereas the opposite was true at other sites (see Appendix 2 for table).

Bat activity at height and ground level

At both ground and nacelle, most calls were from common pipistrelle, soprano pipistrelle and noctule bats (Fig 2.8). However much higher levels of bat activity were recorded at ground level compared with the nacelle for all species (Fig 2.9), despite it being likely that a greater amount of 3-dimensional space was surveyed at height (at ground, the omnidirectional microphone would be limited to detecting in a hemisphere, compared with a sphere at height). Data for each species, at ground and height, are shown separately for each country in Table 2.5.

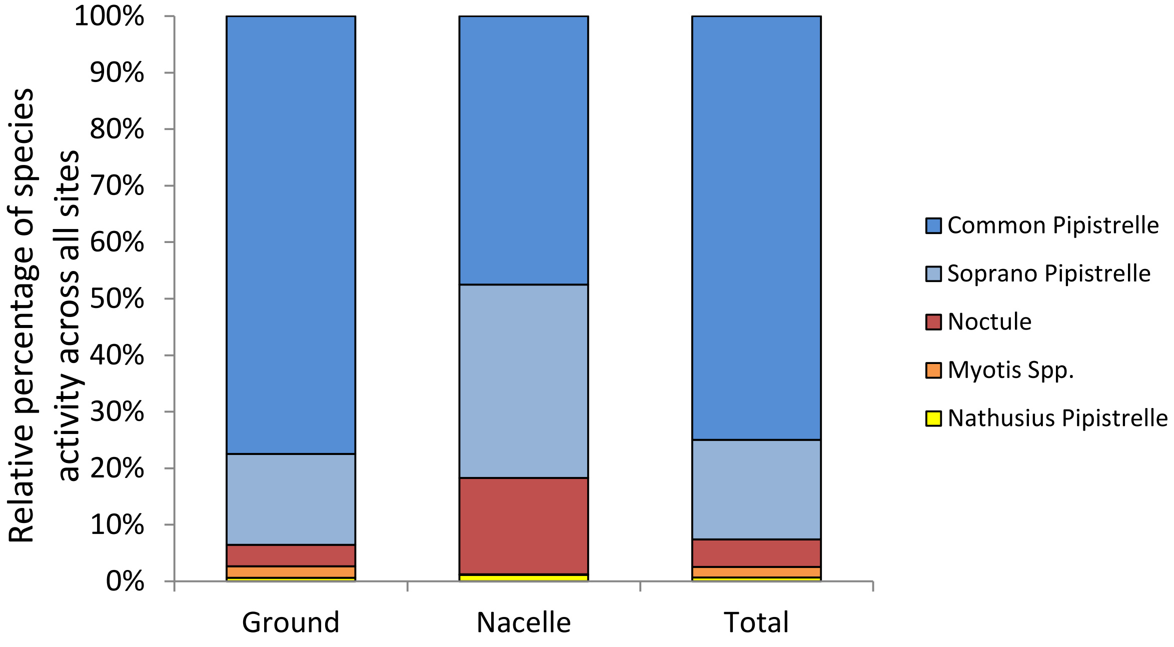

Figure 2.8. Overall distribution of bat activity

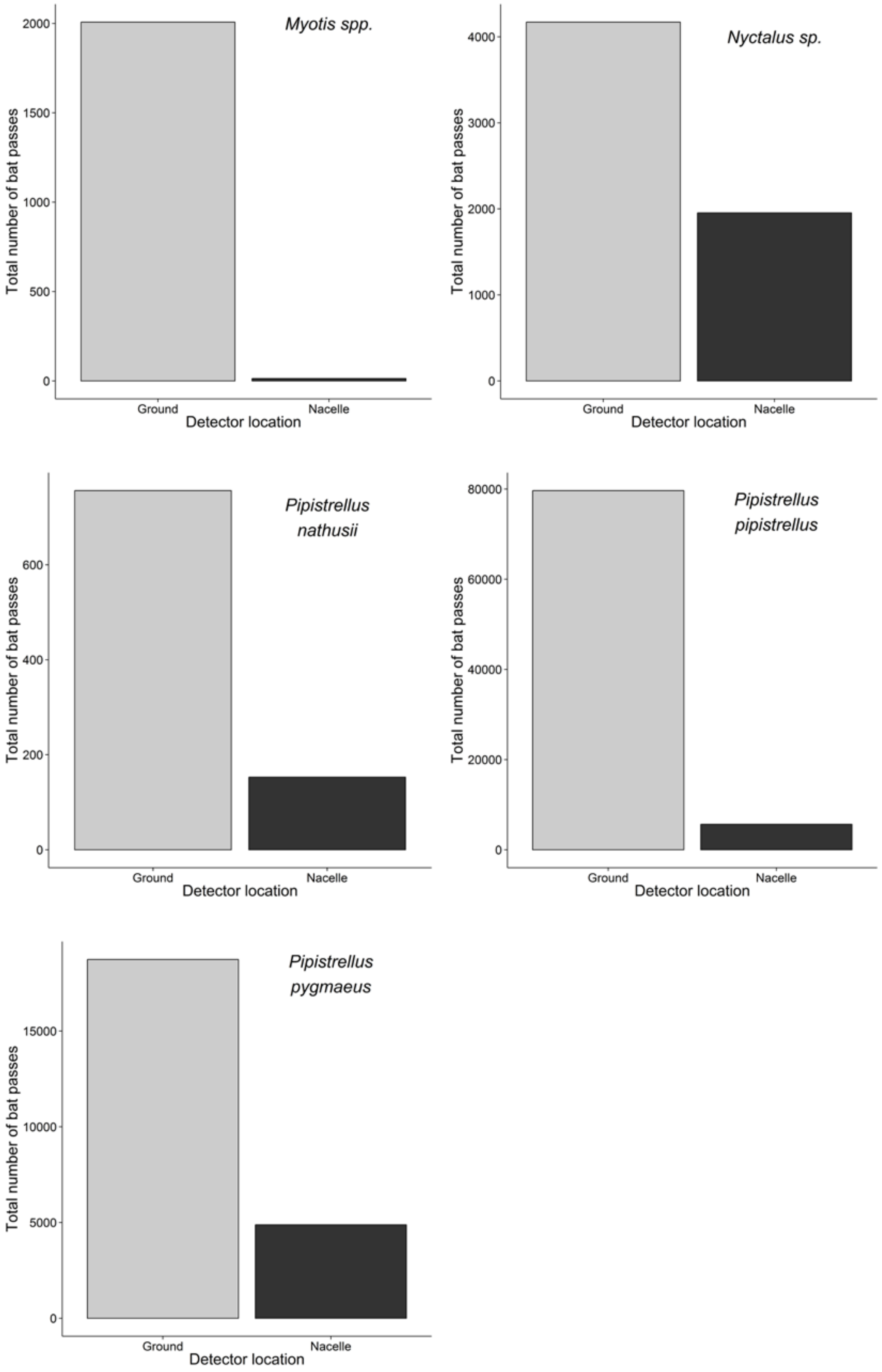

Figure 2.9. Total number of bat passes by species/genus recorded across all sites. A paired design is used, matching the numbers of detectors and survey nights at ground and height for each site, so that survey effort is equal in both locations.

Among the low flying specialists, horseshoe bats were only detected at ground level and *Myotis* spp. and long-eared bats were rarely detected at height. For bats considered to fly at medium height, barbastelles were only detected at ground level, whereas *Pipistrellus* species and high flying specialists were frequently detected at higher elevations. Further consideration of the implications of varying detection probabilities at height and ground is provided in Section 4.

Table 2.5. Total recorded calls (%) at ground and height by country

	England		Scotland		Wales		TOTAL
SPECIES	ground	nacelle	ground	nacelle	ground	nacelle	
barbastelle	88 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	**89**
Myotis spp.	1473 (0.01)	18 (0)	396 (0.03)	2 (0)	1341 (0.03)	0 (0)	**3,230**
long-eared	341 (0)	12 (0)	154 (0.01)	5 (0)	194 (0)	0 (0)	**706**
serotine	11 (0)	1 (0)	0 (0)	0 (0)	95 (0)	6 (0)	**113**
noctule	2443 (0.02)	1260 (0.11)	185 (0.01)	196 (0.19)	3841 (0.07)	1082 (0.35)	**9,007**
Leisler's	52 (0)	4 (0)	5 (0)	20 (0.02)	7 (0)	0 (0)	**88**
Nathusius' pipistrelle	725 (0.01)	140 (0.01)	81 0.01)	9 (0.01)	349 (0.01)	32 (0.01)	**1,336**
common pipistrelle	92873 (0.85)	5797 (0.49)	6860 (0.47)	322 (0.32)	37258 (0.7)	1345 (0.44)	**144,455**
soprano pipistrelle	10009 (0.09)	4457 (0.38)	6646 (0.45)	352 (0.35)	9574 (0.18)	573 (0.19)	**31,611**
greater horseshoe	6 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**6**
lesser horseshoe	5 (0)	0 (0)	0 (0)	0 (0)	5 (0)	0 (0)	**10**
TOTAL*	**108,957**	**11,689**	**14,327**	**906**	**52,655**	**3,038**	**190,651**

*Total includes calls which could only be identified to genus, and therefore the species shown do not sum exactly to the total.

Discussion

The project achieved its intended aim of undertaking a systematic survey of bat activity and casualty rates at a representative sample of wind farms in Great Britain. It is the largest survey of its kind in the world, and provides a unified assessment of the risks to bats across sites of different sizes and in varying habitat conditions.

Whilst the overall casualty rates fall within the ranges reported elsewhere in Europe (Rydell et al. 2010b, EUROBATS 2014a), there is clearly considerable variation between sites. Whilst approximately a third had no observed casualties, there are also a significant number where casualty rates are high. As with studies elsewhere in the world, it is currently difficult to draw inferences about risks to local or national populations, and further research is urgently required to resolve this issue. For marginal populations, even relatively low casualty rates may present a significant threat, whereas in core areas (central to range, where recolonization is more likely and/or where population sizes are higher), it is possible that populations may be robust to higher casualty rates.

The overall picture is one of considerable variability in casualty rates. Within each country, at least one of the study sites had high casualty rates (>40 bats per month, during the period of peak risk), but there are also many sites where casualty rates are low. The limited data available from the sites visited in more than one year emphasise this variability. However, it is unclear whether the lower casualty rates observed in 2013 among the sites with repeated monitoring reflect changes in risk factors, alterations in the local population, or variation due to unknown causes.

Section 3: Prediction of bat fatalities

Introduction

Minimising the risk to bats from wind energy installations relies on two principles: (1) avoidance of risk by, for example, placing turbines in low risk areas or avoiding turbine characteristics linked with bat fatalities; and (2) implementation of mitigation strategies should the risks remain above acceptable levels.

Perhaps the most intuitive predictor of future risk at a potential wind turbine site is the amount of bat activity. In the UK and elsewhere, considerable effort is therefore put into preconstruction acoustic surveys for bats. However, bat activity is highly variable, both spatially and temporally (see also Section 4), and it is currently unclear whether measurements repeated at the same site in subsequent years are strongly related, even if other features of the site remain constant. The erection of a wind turbine *per se* could also alter bat behaviour, further reducing the link between pre- and post-construction measurements. There has been only one study to date investigating the potential value of assessing bat activity as a means of predicting the risk to bats from future wind turbines. This was conducted using data from 12 wind farm sites in the USA (Hein et al. 2013) where pre-and post-construction data were available, albeit with inconsistent methodologies. Although there was a slight trend towards higher fatalities at sites with greater bat activity, this relationship was not significant and explained only a low proportion of the variability in the data (Hein et al. 2013). The authors concluded that current evidence did not support the use of preconstruction data to predict future risk, however, more research is warranted as the original data were of variable quality.

It has also been suggested that characteristics of the turbine may be important predictors of the risk to bats: fatality rates have been reported to increase with the height of the turbine tower, raising concerns about the trend towards the use of taller turbines in repowering operations (Barclay et al. 2007, Rydell et al. 2010b); and a positive link with the rotor diameter has been reported in Europe (Rydell et al. 2010b) but not North America (Barclay et al. 2007). Habitat features may also be linked with risk, but there are no available studies with a sufficiently large sampling frame to have a reasonable probability of separating the effects of habitat from other factors. In Portugal, in the absence of direct evidence, habitat suitability modelling was used for the species most at risk from turbines in that country (*Hypsugo savii, Nyctalus leisleri*, *P. kuhlii* and *P. pipistrellus*) to develop predictive models of the locations likely to be most at risk (sites in humid areas with mild temperatures, <5km to forest and within 600m of steep slopes) (Santos et al. 2013). Using a non-random sample, (Rydell et al. 2010b) noted that mortality rates in North-western Europe appear to be lowest in flat arable areas, higher in more complex agricultural landscapes, and highest in forested regions and close to coasts, though sample sizes did not permit formal statistical analysis.

The current study was designed to allow some analysis of potential predictor variables. However, as recognised from the outset of the project, a sample of 46 sites gives limited statistical power, particularly in the context of the very large numbers of potential predictors, and the information presented here should therefore be considered exploratory. This project was not designed to assess pre-construction activity. Whilst some insights into the likely behaviour of bats in the absence of wind turbines can be derived from the 'control' site surveys, further work in this area is urgently required.

The mitigation strategies considered to reduce the risk from operational wind farms include the reduction in turbine activity during low wind speeds (curtailment), the use of acoustic deterrents (Arnett et al. 2013b) and radar (Nicholls and Racey 2009). However, the latter two strategies have not yet proven effective and there are concerns about non-target effects on other wildlife (Amorim et al. 2012), and therefore the current focus is on the use of curtailment. This is achieved through alteration of the cut-in speed, or by feathering the blades (pitched 90° and positioned parallel to the wind so that the turbine is prevented from freewheeling, or spins at only a very low rate – generally below 1 revolution per minute). A recent review of 10 operational mitigation studies in North America concluded that increasing the cut-in speed by 1.5m/s above the manufacturers cut-in speed generally reduced fatalities by at least 50% (Arnett et al. 2013a). There are also several examples of curtailment strategies being used in Europe (EUROBATS 2014a). To minimise economic loss, it may be possible to undertake focused curtailment when the risk of collision has been shown to be highest, for example in warm weather, at low wind speeds, during migratory periods and/or when bat activity levels are high (Arnett et al. 2011, Arnett et al. 2013a, Korner-Nievergelt et al. 2013). Seasonal variation in risk, with a peak in fatalities in late summer and early autumn, is consistently reported in both Europe and North America (e.g. (Niermann et al. 2007, Arnett et al. 2008, Rydell et al. 2010b), with a smaller peak sometimes being reported in late spring (Rydell et al. 2010b), and mitigation strategies therefore usually focus curtailment in these periods. In Germany and France, automated systems have been trialled where bat detectors housed in the turbine nacelle trigger curtailment once activity levels pass particular thresholds (using an algorithm which includes weather variables and site-specific data on activity) (Behr et al. 2011, Lagrange et al. 2013). These systems are reliant on detailed longitudinal monitoring of bat activity at individual sites to establish activity patterns: it is important to note that data of this kind were not collected in the present study.

In exploring the prediction of bat fatalities, it is important to distinguish two, operationally different, scenarios. In the first, the aim is to draw conclusions about the value of alternative variables in predicting risks for the entire 'population' of wind farms. This scenario is relevant in drawing up guidance about minimising the risk to bats through better siting of future wind energy facilities, avoiding turbines with features that increase risk, or drawing up general rules about curtailment to be applied to all wind energy facilities. In the second, the aim is to identify the features strongly linked with risk at individual sites where a substantial risk to bats has already been established, in order to develop a maximally efficient curtailment strategy. Hence, the 'Chriotech ©' automated turbine switch-off system has been developed at French sites known to have extremely high fatality rates (Lagrange et al. 2013). In Germany, the killing of individual bats is considered illegal and therefore operators must reduce the risk to below a low threshold (usually taken as 1 bat per species per wind turbine per year), and therefore single fatalities at a site are sufficient to trigger detailed site-specific monitoring of activity and casualty levels. In these situations, the sensitivity of the predictions – that is their usefulness in preventing further collisions – is of primary concern. In contrast, when drawing up *a priori* guidance for wind turbines more generally, there is a trade-off between model sensitivity and specificity (their usefulness in keeping turbines operational when the risk to bats is low). For example, a particular model indicating that turbines should be switched off at winds of below 6m/s would have good sensitivity if this prevented 99% of collisions. However, if the model also meant that the turbines would be switched off on 95% of summer nights, even though casualties would only occur on two nights were the turbines operational, then it would have poor specificity. Where the balance between sensitivity and specificity is placed depends on the legislative context of the country (so in Germany, models with high sensitivity and low specificity are acceptable), and also on the conservation status and local population size of the species.

In the current study, the value of turbine characteristics, region, local habitat, weather and bat activity in predicting the risk to bats across wind turbine sites generally is explored. In addition, the sensitivity and specificity of alternative thresholds for key risk factors are examined. No information on seasonality in risk is available due to the cross-sectional design of the survey. The project was intended as a representative survey of Great Britain and therefore deliberately focused on surveying a large number of sites for a limited period, over the months likely to have the highest bat activity and therefore potential risk to bats. The creation of site-specific algorithms, comparable with those used in France and Germany, was therefore also not possible because this study did not gather the detailed longitudinal data necessary to establish site-specific activity patterns at each site.

Methods

Analysis of risk factors across wind farms generally

Casualties that were found incidentally or during the follow up surveys to a subset of sites (see p 24) were excluded (the true casualty rate could not be estimated for the former; and the latter were not comparable with the rest of the project due to differing search intervals). A sample of 98 bats was therefore available for analysis. Models were first constructed for all species collectively. Recognising that the behaviour and casualty risk is likely to vary between species, we also conducted separate species-specific analyses. The statistical power of these species-level analyses is much lower than those based on all species collectively, due to the relatively low numbers of replicates (both in terms of sites, and in terms of casualties per site): the lack of significant effects should therefore not be taken to imply the lack of biologically important relationships with the predictor variables. Insufficient data were available to permit the analysis of Brown long-eared, Natterer's or Nathusius' pipistrelle bats separately.

Following initial exploration of the data, generalised linear models were built to examine the links between casualty rates and potential predictor[**] variables. The presence or absence of casualties at a site overall, or on a particular day, was modelled by logistic regression. The estimated numbers of casualties per site per standard 30-day month were modelled as counts in generalised linear models (GLMs) with a Poisson error structure. The results were compared to those achieved from the same models but which included an offset to account for the size of the wind farm (defined as $\log_e$ (total number turbines)): there were no material differences in the results from the two approaches, and the latter are presented here (essentially giving casualty rates per month per turbine as the outcome measure). As a final check to ensure that there were no unexpected relationships between wind farm size and the per turbine risk of collision which might compromise the logic of using a simple offset – such as might be generated if multiple turbines induced greater behavioural alterations in bats than single turbines – the relationship was plotted, and a model was constructed using a the per-turbine casualty rate (rounded to an integer) as the outcome and the number of turbines as the site as a predictor. Temporal variables and turbine characteristics were also included as covariates (see Table 3.1 below). The fit of all models was assessed by inspection of the residuals and comparison of the residual deviance and degrees of freedom. For species other than noctule bats, over-dispersion was detected and standard errors were therefore corrected using a quasi-GLM model where the

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[**] In this report, 'predictor' is considered synonymous with 'explanatory'. It is important to recognise that the relationships are derived from associative analysis of observational data, and may therefore the relationships modelled may be affected by unknown confounding factors. However, in the absence of experimental evidence (which in the case of many features, such as habitat and weather would be impossible to gather) the approach offers the best opportunity of understanding the risk factors for bat collisions with wind turbines.

variance is given by Ø x µ, where µ is the mean and Ø is the dispersion parameter. For turbine-level analyses binomial mixed effects models were fitted (see (3) below for full details).

To keep the numbers of predictors included in the models within reasonable limits, each predictor type (weather, turbine characteristics etc.) was initially modelled separately, before more complex analyses were conducted. Correlation matrices were examined and variables with very high collinearity were not included within the same model (e.g. rotor sweep and blade length): instead the variable that made the greatest change to the model's AIC value was included for further analysis. Model selection for binomial models was based on examination of the deviances and AICc values. Starting with full models (see details below of the variables included in each section of the analysis), terms were removed backwards stepwise until the minimum adequate model was achieved (Zuur et al. 2009). For the logistic regression analyses, an Information Theoretic approach to model selection was used (Burnham and Anderson 2002, Burnham et al. 2011), using the R package MuMin (Barton 2014), and averaged results for the top-ranking models ($\Delta AICc<4$) are presented. For the quasi-Poisson GLMs, this approach was not used because AICc values are not reliable on their own, as an approach to model ranking, and therefore backwards sequential deletion was used (models with and without the target variable were compared using an F test with an empirical scale parameter, and those variables for which $p<0.05$ were removed (Crawley 2012)).

A hierarchical approach was taken to the data analysis:

1. *Site-level analyses across whole survey period.* Estimated casualty numbers were derived for the entire site across the whole survey period.

   The predictor variables were tested in sets encompassing similar kinds of predictors (e.g. turbine characteristics, wind speed etc.) as shown below. All the variables listed within the set were included in a global model, unless stated otherwise. As a second step, the duration of the survey period at each site, and the numbers of turbines searched were added to each of the global models, to test whether the intensity of survey effort at each site influenced the results. Finally, those variables found to predict casualty risk in each of the separate analyses (with relaxed inclusion criteria of $p<0.1$) were included in an overall global model. However, it was not possible to include bat activity data and weather data together because of the high degree of collinearity between these features.

Temporal variables: year of study (to account for potential differences in local bat colony breeding conditions etc. between years) and time since 1st July: (days). The latter variable was used to account for the known temporal variation in casualty risk and bat activity.

Turbine Characteristics: hub height (m); blade length (m); interval between wind farm commissioning and survey (years). Note that preliminary analysis showed that the variables rotor swept area; clearance between blade and ground and height to tip were all highly correlated with either hub height or blade length (Pearson's correlation coefficient >0.9). Those variables were therefore excluded and hub height and blade length were used as features that are well understood and for which data are readily available;

Weather: Temperature (°C): mean temperature across each survey night averaged (mean) across the survey period ('average temperature'); minimum temperature during each survey night averaged (mean) across all study nights ('minimum average temperature'); minimum temperature at any time across the study period ('minimum temperature'); *Wind speed (m/s measured at ground level)*: mean wind speed across each study night averaged (mean) across the survey period ('average wind speed'); minimum wind speed for each night averaged (mean) across the survey period ('minimum average wind speed');

Bat activity: mean number of passes per night across nights and the three study turbines ('average passes')[††]. Each of these variables was computed for ground-level and nacelle-level activity data for each of the following species and groups: total bats (all species); all pipistrelle species; common pipistrelle; soprano pipistrelle; all *Nyctalus* species; and all big bats (Nyctaloid i.e. noctule, Leisler's and serotine). The bat activity data were modelled in relation to the relevant fatality data (i.e. casualties of all species were compared with activity of all species; casualties of all pipistrelles were compared with activity of all pipistrelles etc.). Separate models were built for each species and at ground and height (as missing data would prevent valid comparison of models). In addition, the maximum pass rate across all turbines and nights was analysed for all bat species combined (the maximum pass rate was not explored for each species/species group separately to reduce the statistical issues associated with conducting multiple significance tests). The mean nightly pass rate for all species combined at the control sites (in similar habitat but away from turbines) was examined to give some insight into the likely behaviour of bats in the absence of turbines. The four sites without acoustic data at height were excluded from analyses involving height-level data, and no control site data were available for 9 sites.

Habitat: habitat composition (% cover) within 1500m, 2500m and 5000m buffers of the centre of the wind farm was extracted from the CEH land-cover map (Morton et al. 2011) using ArcMap 10.1 (ESRI). Analyses included the following categories: conifer; clear-felled conifer; new clear-felled conifer; total conifer; broad-leaf woodland (includes subclasses deciduous, mixed and scrub); total arable; improved grassland; rough grassland; and inland water (includes standing water and larger moving water bodies, but under-records small features such as streams).

[††] A bat pass defined as a continuous run of pulses recorded on an acoustic recorder that are separated by gaps of less than one second

A second analysis was conducted using the proportions of each habitat within buffers of 1km, 2.5km and 5.0km from the edge, rather than the centre, of the wind farm. The minimum adequate model derived from this analysis was compared with that obtained above (in terms of model fit and parameter estimates) to check whether the results differed materially. A separate analysis was then conducted to assess the potential importance of distance (km) between possible risk factors and the centre of the wind farm. The features included were total woodland, broadleaf woodland, inland water, buildings and coast. To investigate the importance of features at a smaller spatial scale, the presence/ absence of these feature within buffers of 200m, 500m and 1500m of the wind farm were also investigated in a separate model.

Table 3.1. Summary of models tested for site-level analyses

Predictor type	Variables included in global models[1]
Temporal	year; time since 1st July (days)
Turbine characteristics	Hub height (m), blade length (m), operational time (years)
Weather	average temp (°C), minimum average temp (°C); minimum temp (°C); average wind speed (m/s), minimum average wind speed (m/s)
Bat activity[2]	*Ground*: mean activity across turbines and nights (passes): total bats (all species); all pipistrelle species; common pipistrelle; soprano pipistrelle; all *Nyctalus* species; and all big bats (Nyctaloid) *Height:* mean activity across turbines and nights (passes): total bats (all species); all pipistrelle species; common pipistrelle; soprano pipistrelle; all *Nyctalus* species; and all big bats (Nyctaloid) *Maximum pass rate (Ground, all species*) across nights and turbines Mean pass rate (all species) at control sites
Habitat	*1500m buffer from centre of wind farm (% cover)*: conifer; clear-felled conifer; new clear-felled conifer; total conifer; broad-leaf woodland (includes subclasses deciduous, mixed and scrub); total arable; improved grassland; rough grassland; and inland water. *2500m buffer from centre of wind farm (% cover)* (variables as above) *5000m buffer from centre of wind* farm (% cover) (variables as above) *Distance (km) to nearest patch of*: total woodland; broadleaf woodland; inland water; buildings; coast *Presence/absence of features within 200m buffer of centre of wind farm:* total woodland; broadleaf woodland; inland water; buildings; coast *Presence/absence of the features within a 500m buffer of centre of wind farm:* (variables as for 200m buffer) *Presence/absence of the features within a 1500m buffer of centre of wind farm:* (variables as for 200m buffer)

[1] Italics indicate that separate models were built using subsets (variables shown in plain type) within each predictor type. [2] Separate models were built for each species.

2. *Site-level analyses across short observation periods*. The predictor variables examined were as described for (1) with the following modifications. The date of collection for each casualty was identified, and the weather and acoustic data for the preceding three nights, rather than the whole survey period, were summed across the whole site. The features of these summarised nights were then compared with those where no bat fatalities were detected using multiple logistic regression. This period was slightly longer than the average inter-search interval, and therefore reflects the fact that the precise date of death is unknown and the fatality could therefore have occurred on any of the previous three nights. Searcher-efficiency in the project was approximately 70% and therefore there is a small chance that the casualty actually occurred earlier but was missed by previous searches. This would tend to weaken any observed associations with activity or weather patterns rather than generate spurious relationships, and is ignored for the purpose of analysis. If fatalities were found on the second day of survey at a site, then the predictor mean of the predictor variables were computed from the first two days of observation. Fatalities found on the first day of observation were excluded as relevant data for the predictors were not available.

Because of the very small numbers of individual fatalities within each observation period, and the fact that specific data on scavenger removal rates within each study period were not available, raw data rather than casualty rates adjusted for observer-efficiency and predator removals were used for these analyses. It should also be noted that the numbers of turbines searched for casualties was greater than the numbers surveyed acoustically (6-8 casualty searches per site, *versus* 3 acoustic surveys). The power of the analysis was maximised by comparing the numbers of casualties observed across the whole site with bat activity at height summarised across the 3 study turbines. However, a disadvantage of the approach is that the models were not able to link local variability in bat activity within a site to casualty events at a particular turbine. The predictors used were mean bat activity at height (per 3 turbines), maximum activity, habitat and weather. The acoustic data were logged to reduce the leverage of outlying values. Total bat passes from all species were used in the analysis of total casualty rates (of all species), whereas species-specific activity levels were used in the analysis of individual species. All analyses were repeated using nacelle and ground-level activity data separately. Mixed effects regression models (LMERs) were used, which included a random effect to account for the repeated measures (multiple nights of observation) within sites.

3. *Turbine-level analyses across short observation periods*. These analyses made use of information on the location of the casualties within a site. Models were based on a subset of carcasses where the date of death could be estimated with reasonable accuracy using the condition of the carcass (general condition, invertebrate infestation, state of eyes) and the date of the preceding casualty search. As in the previous analysis, carcasses found on the first day of the survey were excluded. Acoustic data were derived from the same turbine (or where the turbine did not have acoustic data, from the nearest turbine). In addition, because of uncertainty in this estimate, the averaged acoustic data from each period between searches was used. Binomial mixed models were fitted, assigning a value of one if at least one fatality occurred on a night and zero where no fatality occurred. This avoided problems with over-dispersion which is a common problem when modelling rare events. Due to a very low number of nights with multiple fatalities (13% of nights when considering the dataset with the fatality night estimated and the nearest turbine used) this should not have biased results. In all models site was entered as a random effect with varying intercept to account for the spatial autocorrelation in the data. In addition the proportion of the night for which the turbine was turning and the number of turbines searched (or number of turbine nights depending on the dataset) was also entered into the model. As with the previous analyses, the fatalities were linked with the relevant acoustic data (common pipistrelle fatalities were examined in relation to total nightly common pipistrelle activity etc.). For these analyses there were too few casualties to allow meaningful separate analyses of noctule, long-eared or Natterer's bat fatalities.

The high correlation between weather conditions and bat activity also means that it is not possible to explore separately the influence of weather conditions.

Finally, the ability of different variables to predict casualties in ways useful to stakeholders was explored by cross-tabulating the presence or absence of casualties occurring under different conditions. The positive predictive value[‡‡] and negative predictive value[§§] of the these binary categories were examined using CIA (Bryant 2011). The approach is more simplistic than analyses modelling exposures as continuous variables or more than one variable; nevertheless they can provide useful insights into whether simple 'rules of thumb' might be available.

The analyses of habitat used site as a replicate, with the presence or absence of deciduous and mixed woodland within 1500m, 500m and 200m buffers being used as predictors of fatalities. For weather variables, each night was considered a replicate, and casualties were only included if they were considered likely to have occurred within 2 days of the search (very few casualties were judged to have occurred with certainty on the night before the search). There was no material difference to the findings according to whether averaged weather conditions over the previous two nights were used, or, as presented here, the weather on the night preceding the search.

Data on wind speed at the nacelle was not available from many of the turbine operators. Unfortunately, prediction of nacelle-level speeds from our equipment based at ground level is not straightforward because of the influence of local topography and habitat features, and so varies between sites. Nevertheless, because international discussions on wind speed in relation to curtailment usually report speeds at nacelle-

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[‡‡] The chance that casualties occur among sites/nights where the test is positive
[§§] The chance that casualties do not occur among sites/nights where the test is negative

rather than ground-level, some kind of conversion is necessary. Figure 3.1 illustrates the data available from 27 sites where paired observations of average wind speeds measured at ground and height were available. Whilst between-site variation is evident, a mean nightly speed of 4m/s on the ground might approximate a mean nightly speed of 8 m/s at the nacelle.

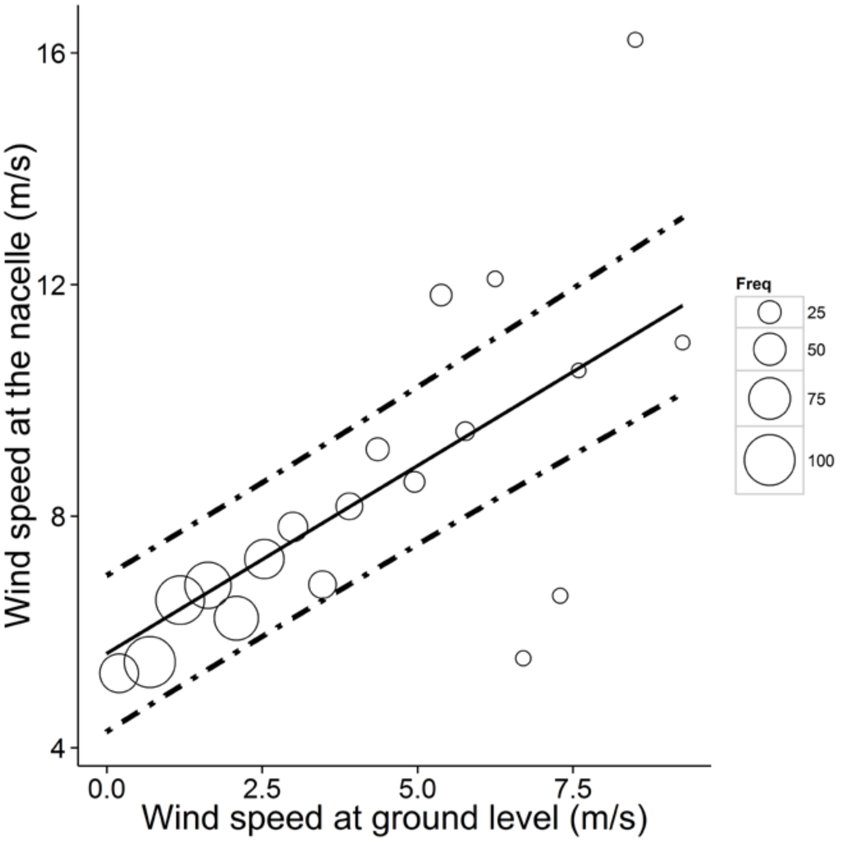

Fig. 3.1. Relationship between mean wind nightly speed measured at ground level and paired mean wind speed at the nacelle at 27 sites. Confidence intervals are shown as dotted lines, and circle size is proportional to the number of nights of paired records available.

Results

1. Site level analyses

Turbine characteristics and Timing

Analyses were first conducted for all bat species combined and considered simply whether casualties were absent or present at a site (rather than their number). Increasing time since 1st July (Importance index[***] I= 1.0), but not the year of the project, was linked with a greater probability of bat casualties. Subsequent analyses in this section therefore include the timing of the surveys in the models. There was a highly significant increase in the probability of casualties being present as blade lengths increased: each metre increase in length being associated with an increase in risk of approximately 18% (Importance index[†††] I= 1.0; odds ratio (OR)[‡‡‡] 1.18, 95% CI[§§§] 1.05, 1.32). The height of the nacelle, the year of study, date of study (days since 1st July), number of searches at the site, or the duration of the wind farm's operation did not add any further useful information. Essentially the same results were found for all pipistrelle species collectively (blade length, I=1, OR=1.19, 95% CI 1.06, 1.34). When separate analyses were conducted for each species, similar patterns with blade length were seen for common and soprano pipistrelle bats, though the associations were now of borderline statistical significance, probably reflecting the smaller sample size (common pipistrelle bats I=0.66, OR 1.046, 95% CI 0.99, 1.17; soprano pipistrelle bats (I=0.58, OR 1.036, 95% CI 0.98, 1.15). No link with blade length was evident for noctule bats (I=0.19, OR 1.01, 95% CI 0.91, 1.16). The duration of wind farm operation and hub height were not important predictors of fatalities for any

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[******] The importance index, I, ranges from 0 to 1; 1 indicates the variable occurred in all the top-ranking models.

[††††††] The importance index, I, ranges from 0 to 1; 1 indicates the variable occurred in all the top-ranking models.

[‡‡‡] The odds ratio (OR) can be interpreted as the change in probability of a fatality occurring for each unit increase in the predictor variable. An OR of 2.0 would represent a doubling of risk, an OR of 1.0 would mean no change in risk, and an OR of 0.5 would mean that the risk halved. In other words, the coefficients can be roughly interpreted as indicating the % change in odds of a casualty, if all other predictors are held constant: a coefficient of 1.1 indicates a 10% increase in risk; 1.5 a 50% increase in risk, and 2.0 a 200% increase in risk. For this project, all of the coefficients are close to 1, indicating small changes in risk are associated with each unit increase in the predictors.

[§§§] The confidence intervals indicate the range of values within which the estimate would be expected to fall 95% of the time were the work repeated multiple times: where the confidence intervals exclude zero, the result is considered 'statistically significant', p<0.05. Wider intervals indicate increasing uncertainty.

species; and nor did the inclusion of the survey duration and the number of turbines searched alter any of the findings.

The analyses were repeated to assess the links with the numbers of bats killed, rather than simply whether sites were classified as having at least one casualty or none. The relationships were similar to those obtained for the presence/absence of casualties. The total casualty rate increased with the length of the turbine blades (OR=1.05, 95% CI 1.00, 1.11), but was not influenced by the other turbine characteristics. The results for all pipistrelles collectively, and for the species individually were also virtually identical to the results for the presence/absence of bat casualties (all pipistrelle bats OR 1.05, 95% CI 1.00, 1.11; common pipistrelle bats OR 1.05, 95% CI 1.0, 1.12; soprano pipistrelle bats OR 1.04, 95% CI 0.97, 1.12; noctule bats OR 1.05, 95% CI 1.00, 1.09).

There was no relationship apparent between the number of turbines present at a site and the casualty rate *per turbine* (Fig 3.2, F=2.07, p=0.158). Further, whilst the relationship between the number of casualties *per site* and the number of turbines was, as expected, positive and highly significant (F=13.27, p<0.001) there was no evidence of any non-linearity in this relationship (i.e quadratic terms were not significant). It is therefore reasonable to conclude that there is a simple relationship of increasing risk from a wind farm as the number of turbines increases. The use of a simple offset function when modelling the casualty rate per turbine is therefore justified.

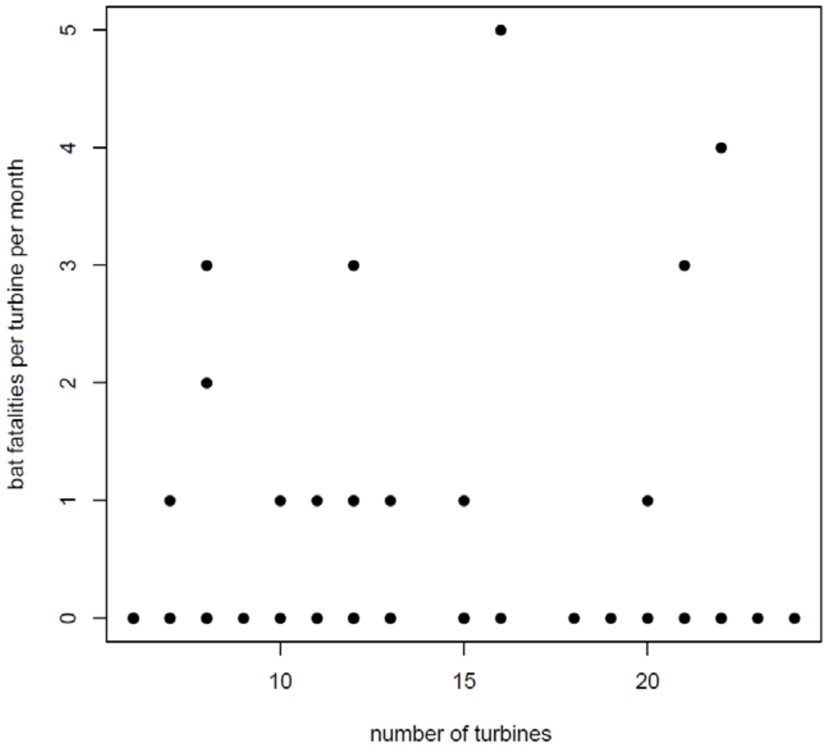


Fig 3.2. Relationship between the number of casualties per turbine and the number of turbines present at each wind farm.

Weather

No associations were seen between the presence/absence of casualties and the weather variables examined. This was also true for when the numbers of bats killed was used as the outcome measure. The only exception was for the number of noctule bats killed. For these bats, the minimum adequate model included the average temperature, where the risk increased by approximately 62% for each 1°C increase in average temperature (OR 1.62, 95% CI 1.29, 2.04) and a non-significant negative relationship with minimum nightly wind speed.

Bat activity measured at the turbines

No links were found between total casualty rates and total bat activity levels. However, at a species-specific level, recordings made at ground level were linked with bat casualties (Table 3.3). There were also positive associations between bat activity at height and fatality rates, but these had larger confidence intervals, probably reflecting the smaller numbers of recordings made at height. It should be noted that the detection distance of the microphone is unlikely to encompass the entire rotor-swept area, and therefore bats may still be within the zone of risk despite having not been recorded on the detector.

Table 3.3. Relationship between acoustic indices of bat activity and bat casualties.

	Species	presence/absence casualties		number of casualties	
		Odds Ratio	95% Confidence Interval	Odds Ratio	95% Confidence Interval
GROUND	noctule	1.39	1.10, 2.27	1.15	1.09, 1.20
	common pipistrelle	1.01	1.10, 1.03	1.01	1.0, 1.02
	soprano pipistrelle	1.15	1.04, 1.33	1.05	1.03,1.07
HEIGHT	noctule	1.35	0.93, 1.93	1.29	1.07, 1.47
	common pipistrelle	1.01	0.95, 2.09	1.46	1.19, 1.78
	soprano pipistrelle	1.17	1.15, 15.14	1.86	0.93, 3.19

Bat activity at the control sites

There was a positive link between the total number of passes recorded at the open control sites (all of which were ground level) and probability of any casualties occurring. However, the confidence limits were very wide (Figure 3.3), and the relationship was of borderline statistical significance (OR=1.01, *p*=0.064). No relationship was apparent between total pipistrelle passes and the presence/absence of pipistrelle bat casualties. There were also no links between the number of bat passes and the number of bat casualties.

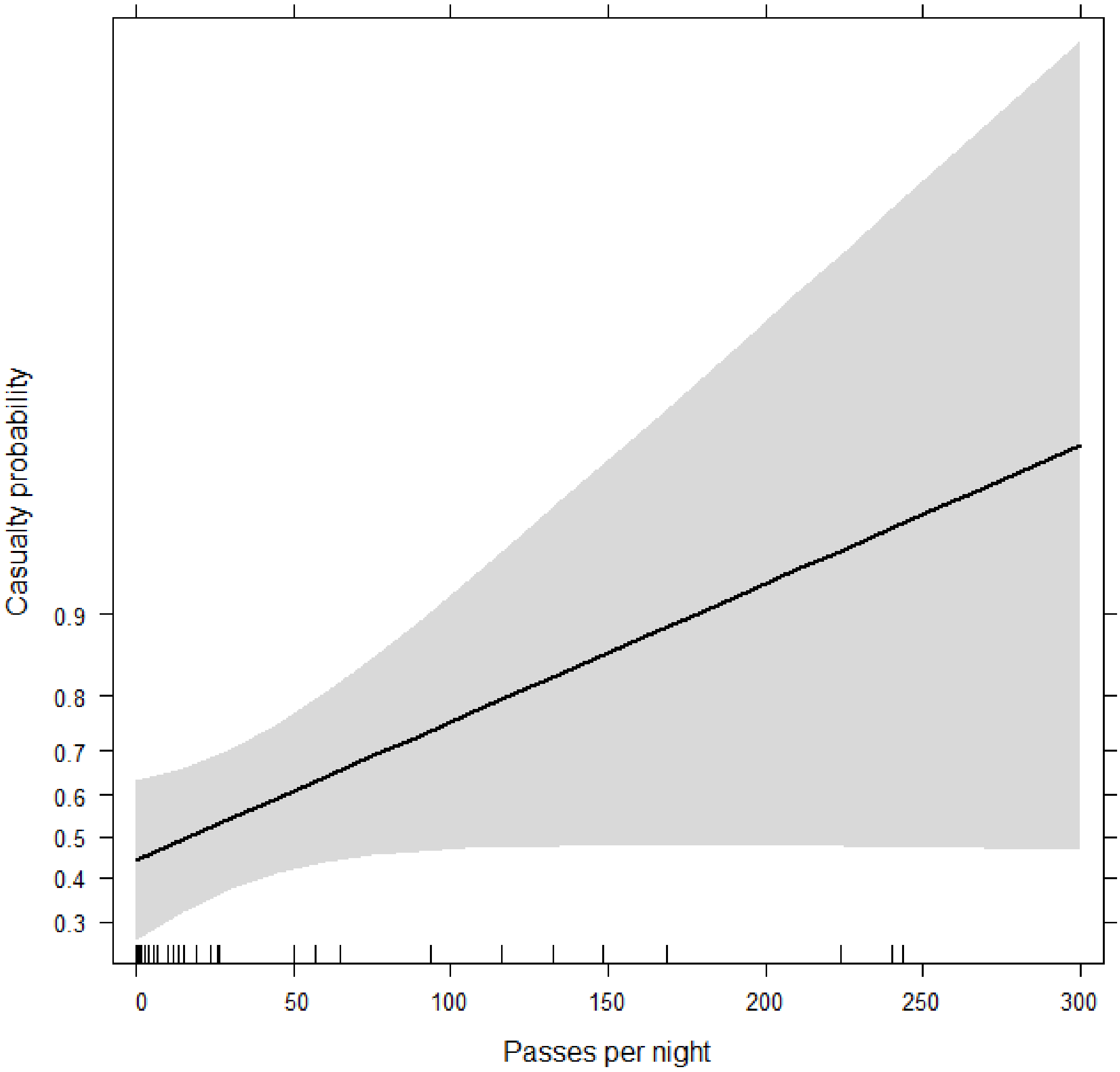


Figure 3.3. Relationship between total bat passes per night at control sites (away from turbines) and the probability of a casualty occurring (all species). 95% Confidence intervals are shown as grey bars.

Habitat

Analyses were conducted separately for the land cover data at the 1500m and 2500m buffer scales. For all bats collectively, and also for individual species, there were no significant associations between the presence/absence of casualties and any habitat variable. There were also no links with the habitat composition of the buffers of 1km, 2.5km or 5km constructed from the edge of the wind farm. Considering the number, rather than the presence/absence of casualties, the minimum adequate model for all species contained the category “broadleaved and mixed woodland” only. For all bats collectively, there was a negative relationship with the number of individuals killed for the 1500m scale buffer: each % increase in the cover of broadleaved and mixed woodland was associated with a 14% fall in risk (OR 0.86, 95% CI 0.66, 1.00), with a trend in the same direction for the 2500m scale (OR 0.88, 95% CI 0.73, 1.02). For common pipistrelles there was a similar (non-significant) association (OR 0.89, 95% CI 0.66, 1.05). The trend was particularly marked for soprano pipistrelles (OR 0.65, 95% CI 0.29, 0.93). For noctule bats, the minimum adequate model contained only the total amount of coniferous woodland (including clearfell), and this relationship was positive (OR 1.04, 95% CI 1.02, 1.06). The addition of turbine characteristics made no improvement to any model that included habitat.

Further analyses were then conducted at a greater spatial resolution for features considered likely to present a risk. The presence of inland water within buffers of 200, 500 and 1500m had no significant associations with the presence of bat casualties (all bats, common pipistrelle, soprano pipistrelle or noctule bats), with the exception of noctule bats where there was a positive significant association at the 200m level (p=0.00235). There was a non-significant positive tendency of the presence of water to be linked with fatalities of noctule bats at the other spatial resolutions, and also for all bats collectively; for pipistrelles, the trend was reversed. There was no association between distance to the coast, or whether the site was considered ‘coastal’ or not, and fatality risks for any bats.

2. Site-level analyses across short observation periods.

The night that the fatality occurred was estimated for 91 of the 98 fatalities (93%), of which 42 (43%) occurred at the same turbine that the acoustic data was gathered.

Weather

There was no significant association between temperature and either the probability of a casualty or the number of casualties occurring. This was true for all species combined, as well as for the groups separately. However, there was a small inverse relationship between minimum wind speed and casualty risk. For casualties of any species, the risk of a casualty occurring declined by approximately 6% for each 1 m/s increase in wind speed (OR=0.94, 95% CI 0.88, 0.99). Similar results were obtained when the number of casualties was used as the outcome measure. This link was also demonstrated for common pipistrelles (OR=0.94, 95% CI 0.90, 0.99) but not for other species or groups individually, probably reflecting the lower statistical power available for other comparisons.

Bat activity

There was a significant positive association between the presence of a bat casualty (any species) and total bat activity recorded at the nacelle ($\log_e$ mean passes per night) (OR 1.54, 95% CI 1.20, 1.96). There was a similar relationship for the number of all pipistrelle casualties and the ($\log_e$) activity of all pipistrelle bats recorded at the nacelle (OR 1.43, 95% CI 1.19. 1.71); and also for the number of common pipistrelle bat casualties and mean common pipistrelle bat activity (OR 1.54, 95% CI 1.20, 1.96). However, there were no associations detected for noctule or soprano pipistrelle bats, possibly due to a lack of statistical power.

3. *Turbine level analysis across short time periods*

The average number of nights that a fatality was estimated to have occurred previous to the search was 2.62 (±SE 0.18 range 1 to 8 nights), counting the night previous to a search as 1.

Total activity was a significant predictor of the risk of a casualty (any species) occurring. Soprano pipistrelle activity was a significant predictor of soprano pipistrelle fatalities, but common pipistrelle species models were often not significant. However, in most models the conditional R^2 values were low (Table 3.3), showing that activity is a poor predictor for fatalities at the turbine and nightly level. An exception was models where activity was averaged across each search period and acoustically monitored turbines. Using this dataset, most models were significant and R^2 values were higher for common pipistrelle fatalities using acoustic data from both ground and nacelle, and for total fatalities and soprano pipistrelle fatalities when using activity data measured at the nacelle. Predictions are not shown for models derived from activity recorded at height because these have higher conditional R^2 values than those derived from ground-level data. Caution needs to be taken in using these models as many of the R^2 values are low and most variability in fatality is explained by individual characteristics of the site as opposed to the number of bat passes.

Table 3.3. Results of models predicting the probability of a casualty occurring of any species (all), common pipistrelle (C pipistrelle), or soprano pipistrelle (S pipistrelle). Models shown in light type have poor fit, and are considered unlikely to be reliable.

Species	Method[1]	Turbine[2]	Height	Estimate[3]	Marginal R^2 (%)[4]	Conditional R^2 (%)[5]
all	**estimated**	**nearest**	**ground**	**Int*†**	**15**	**40**
all	estimated	nearest	nacelle	0.105**	3	29
C pipistrelle	estimated	nearest	ground	0.086	*NA*	*NA*
C pipistrelle	estimated	nearest	nacelle	0.010	*NA*	*NA*
S pipistrelle	**estimated**	**nearest**	**ground**	**0.229****	**18**	**49**
S pipistrelle	estimated	nearest	nacelle	0.236*	4	43
all	**estimated**	**averaged**	**ground**	**Int*****	**29**	**48**
all	estimated	averaged	nacelle	0.087*	3	24
C pipistrelle	estimated	averaged	ground	0.152*	8	51
C pipistrelle	estimated	averaged	nacelle	0.022	*NA*	*NA*
S pipistrelle	**estimated**	**averaged**	**ground**	**Int****	**16**	**38**
S pipistrelle	**estimated**	**averaged**	**nacelle**	**0.275*****	**12**	**53**
all	SP	nearest	ground	0.096†	*NA*	*NA*
all	SP	nearest	nacelle	0.037†	*NA*	*NA*
C pipistrelle	**SP**	**nearest**	**ground**	**0.174*†**	**10**	**54**
C pipistrelle	SP	nearest	nacelle	0.117†	*NA*	*NA*
S pipistrelle	**SP**	**nearest**	**ground**	**0.408*****	**41**	**62**
S pipistrelle	SP	nearest	nacelle	0.247**	6	69
all	SP	averaged	ground	0.163†	8	33
all	**SP**	**averaged**	**nacelle**	**Int*†**	**45**	**56**
C pipistrelle	**SP**	**averaged**	**ground**	**0.350†**	**38**	**56**
C pipistrelle	**SP**	**averaged**	**nacelle**	**0.183†**	**8**	**30**
S pipistrelle	SP	averaged	ground	Int*	2	43
S pipistrelle	**SP**	**averaged**	**nacelle**	**Int***	**18**	**73**

1. Indicates whether the date of death was estimated or whether the whole search period was used.
2. Indicates whether acoustic data were derived from the turbine where the casualty occurred (or the nearest turbine), or averaged across all three turbines.
3. Estimate is the regression coefficient linking bat activity with the probability of a casualty. Larger coefficients indicate a greater increase in the casualty probability for each unit increase in bat activity. 'Int' shows that the interaction between activity and the proportion of the time the turbine was turning was significant i.e. the effect of bat activity on the risk of collision varies depending on the proportion of the night for which the turbine was operational. Significance levels are shown as *** (p <0.001), ** (p <0.01) and * (p <0.05). †indicates the number of turbines (or turbine nights) searched was also a significant predictor in the model.
4. Marginal R^2 gives an estimate of model fit (higher % indicating better fit) based on fixed factors only.
5. Conditional R^2 gives an estimate of model fit based on fixed and random effects (i.e. accounting for variability

4. Prediction of casualty rates by categories of bat activity (high/medium/low) measured at the similar habitat control sites

Simple explorations of the potentially non-linear relationships between fatalities and bat activity, models were conducted by splitting bat activity into three categories. Figure 3.4 shows for all bats collectively, and also for all pipistrelles, the proportion of sites with low, medium or high levels of activity at their control sites that also have at least one casualty (cut-off points were defined as the following number of passes per night low <3; medium 3-49; high ≥50 which split the data into three equally sized groups[****]). A statistically significant positive association was found between the probability of a bat casualty occurring at the site and average activity levels (recorded as a continuous variable) of all species recorded at the control site (OR 1.01, 95% CI 1.002, 1.03; 37 sites with control data available for analysis). However this acoustic measure was not a significant predictor of the numbers of casualties occurring. Further analyses of non-linear patterns in the data (using generalised additive models) may be warranted for each species individually.

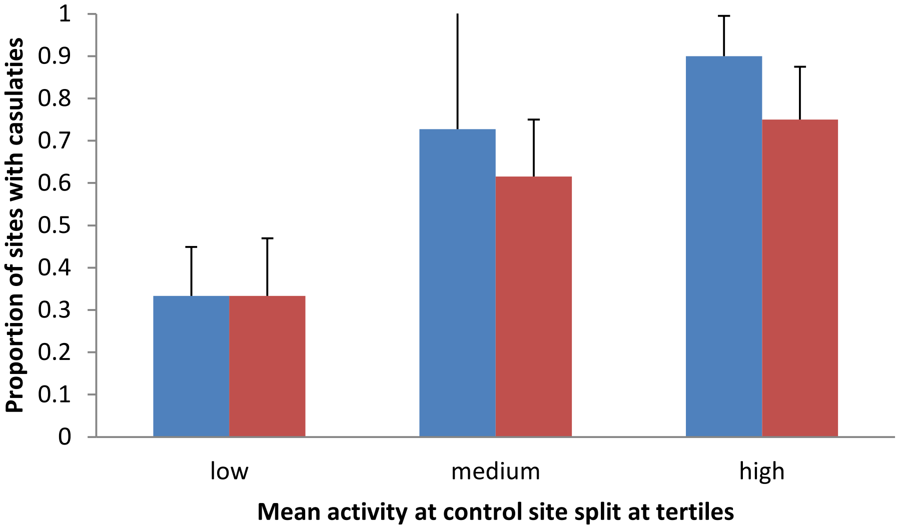


Fig. 3.4. Proportion of sites with casualties by bat activity at control site. Blue bars show mean activity of all bats; red bars show mean activity of all pipistrelle bats. Bars show standard errors.

[****] Note that the cut-off points used in Section 4 to divide the data from the whole project into three equally sized groups were not useful here as none of the 37 control sites would have been defined as having 'high bat activity'.

5. *Positive and negative predictive values*

The presence of broad-leaf woodland (hereafter referred to simply as woodland) close to the turbine site was linked statistically with casualty risk in the models presented earlier. Sites with noctule bat casualties more commonly had woodland present than absent within the buffers examined (true for all buffer distances), whereas the reverse was true for sites with common pipistrelle or soprano pipistrelle bat casualties. Therefore in the exploration of the value of woodland as a predictor, the presence of woodland is treated as a positive risk factor for noctule bats, whereas the absence of woodland is treated as a risk factor for pipistrelles.

For noctule bats, 17 out of the 18 sites without deciduous or mixed woodland within a 1500m radius had no casualties, and the negative predictive value (i.e. the % of sites without nearby woodland that also had no casualties) was therefore very good; however noctule casualties were rare events (only 9 were found during the main part of this project) whereas the presence of woodland within a 1500m radius of a wind farm site is not. Hence only 18% of sites with woodland within this radius had casualties in this project, meaning that the presence of woodland cannot necessarily be interpreted as risky (Table 3.4). For common and soprano pipistrelle bats, the apparent protective effect of woodland was particularly apparent at the smallest spatial resolution: 13 out of the 16 sites with common pipistrelle casualties, and 13 out of the 15 sites with soprano pipistrelle casualties, had no woodland within 200m. The sensitivity of ‘woodland absence’ as a predictive test was therefore high (86%). The negative predictive value was again high, particularly for soprano pipistrelles at close proximity: 86% of sites with woodland within a 200m buffer had no casualties. However, around a third of sites that had woodland also had casualties: the presence of woodland therefore clearly does not preclude collisions.

Table 3.4. Positive and negative predictive value of using presence/absence of broad-leaf woodland at distances of 1500m, 500m and 200m from the wind farm as a predictor of fatality risk. Note that these analyses treat the *presence* of woodland within each buffer area as a risk factor for noctule bat, whereas the *absence* of woodland is considered a risk factor for pipistrelle bats.

	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
1500m		
noctule	17.9 (7.9, 35.6)	94.4 (74.2, 99.0)
common pipistrelle	32.1 (17.9, 50.7)	44.4 (24.6, 66.3)
soprano pipistrelle	25.0 (12.7, 43.4)	50.0 (29.0, 71.0)
500m		
noctule	13.3 (3.7, 37.9)	93.9 (80.4, 98.3)
common pipistrelle	27.3 (15.1, 44.2)	60.0 (35.7, 80.2)
soprano pipistrelle	30.3 (17.4, 47.3)	73.3 (48.0, 89.1)
200m		
noctule		
common pipistrelle	31.7 (19.6, 47.0)	71.4 (35.9, 91.8)
soprano pipistrelle	31.7 (19.6, 47.0)	85.7 (48.7, 97.4)

In contrast to habitat variables, which are not readily modified post-construction even should important numbers of casualties be found, weather and bat activity variables are used in other countries to trigger curtailment. The distribution of total fatalities relative to weather conditions were therefore explored (Table 3.5). Most nights where casualties occurred (81.5%, 95% CI 69.2, 89.6[††††]) had low mean wind speeds (≤5 m/s measured at the ground). However, low wind speeds occurred on the majority of nights. The negative predictive value of the test was therefore very good (95.3%; 95% CI 91.5, 97.4) – meaning that most nights with mean wind speeds >5m/s had no casualties – but the positive predictive value was only 3.6% (95% CI 2.7-4.8) since most nights with lower wind speeds had no casualties. Temperature at dusk yielded similar results. Most nights where casualties occurred (94.4%[‡‡‡‡], 95% CI 81.9, 98.5) had dusk temperatures >10°C. However, most nights fell within this category. The negative predictive value was therefore high (98.6%,95% CI 95.1, 99.6), but the positive predictive value was poor (3.8%, 95% CI 2.7, 5.2) since most nights with warm dusk temperatures had no fatalities.

[††††] Also known as the sensitivity of the test (44 fatalities on nights with low wind speed/54 nights with fatalities)

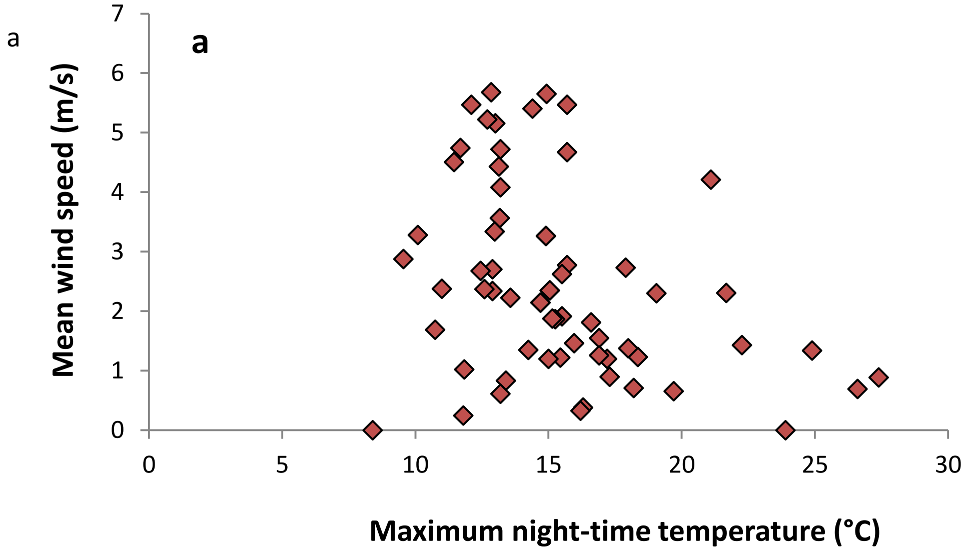
[‡‡‡‡] Also known as the sensitivity of the test (34 fatalities on nights with warm dusk temp/36 nights with fatalities)

Table 3.5. Number of nights and proportion of fatalities which occur on nights under a range of weather conditions. Fatalities were only included if they were estimated to have occurred in either of the previous two nights before a search. **Note that wind speeds are measured at ground level.**

Mean Rain mm/hr	0	>0≤2.5	>2.5≤7.5	>7.5		Totals*
Fatalities	91.84%	0.00%	0.00%	8.16%		49
No.nights	50.98%	33.03%	3.71%	12.27%		1,320
Mean Wind m/s	**≤4**	**>4≤5**	**>5≤6**	**>6≤7**	**>7**	
Fatalities	66.67%	14.81%	9.26%	7.41%	1.85%	54
No.nights	77.45%	7.79%	6.96%	3.27%	4.52%	1437
Min. Wind m/s	≤4	>4≤5	>5≤6	>6≤7	>7	
Fatalities	85.96%	14.04%	0.00%	0.00%	0.00%	57
No.nights	93.74%	3.95%	1.58%	0.33%	0.40%	1,518
Max. Wind m/s	≤4	>4≤5	>5≤6	>6≤7	>7	
Fatalities	38.60%	12.28%	8.77%	8.77%	31.58%	57
No.nights	50.13%	13.70%	9.22%	8.37%	18.58%	1,518
Mean Temp Dusk °C	**≤5**	**>5≤10**	**>10≤15**	**>15**		
Fatalities	2.78%	2.78%	63.89%	30.56%		36
No.nights	0.19%	13.69%	59.79%	25.67%		1052

* Totals are not equal due to missing weather data on some nights.

These data are also illustrated by plotting the wind and temperature conditions for the nights on which fatalities were estimated to have occurred (each point represents one bat fatality) (Fig 3.4a &b). In the cases where the date of death could be estimated with reasonable precision, it can be seen that fatalities occurred across a range of wind speeds, but always less than 6 m/s (ground level), whereas there was considerable spread in temperature ranges, and no suggestion that different wind-speed thresholds might be appropriate for curtailment depending on temperature. Wind speed data were available from the nacelle at a subset of 14 sites where fresh casualties were found. No night prior to finding a casualty had mean wind speeds >10m/s at the nacelle, but all had mean wind speeds >6m/s (Fig 3.5).



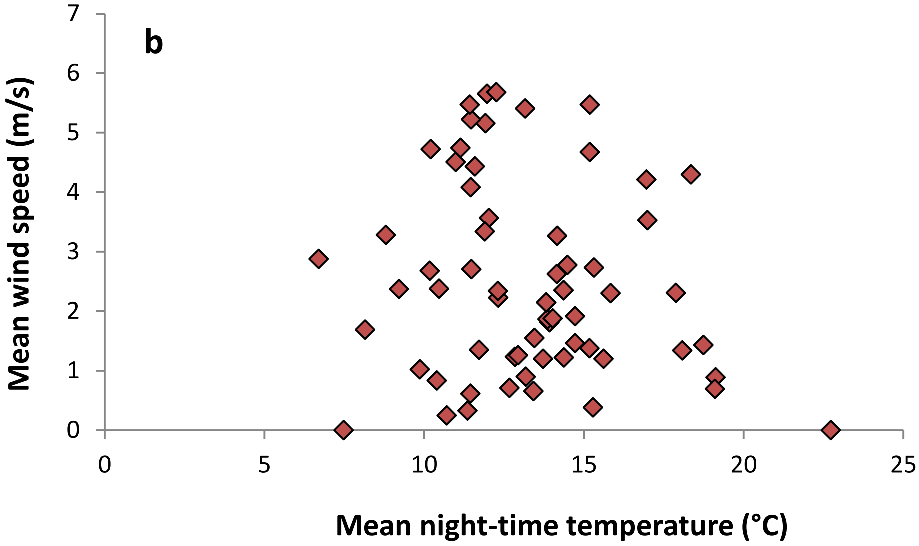


Fig 3.4. Distribution of bat fatalities according to the combination of (a) maximum night-time temperature and (b) mean night-time temperature and wind-speed.

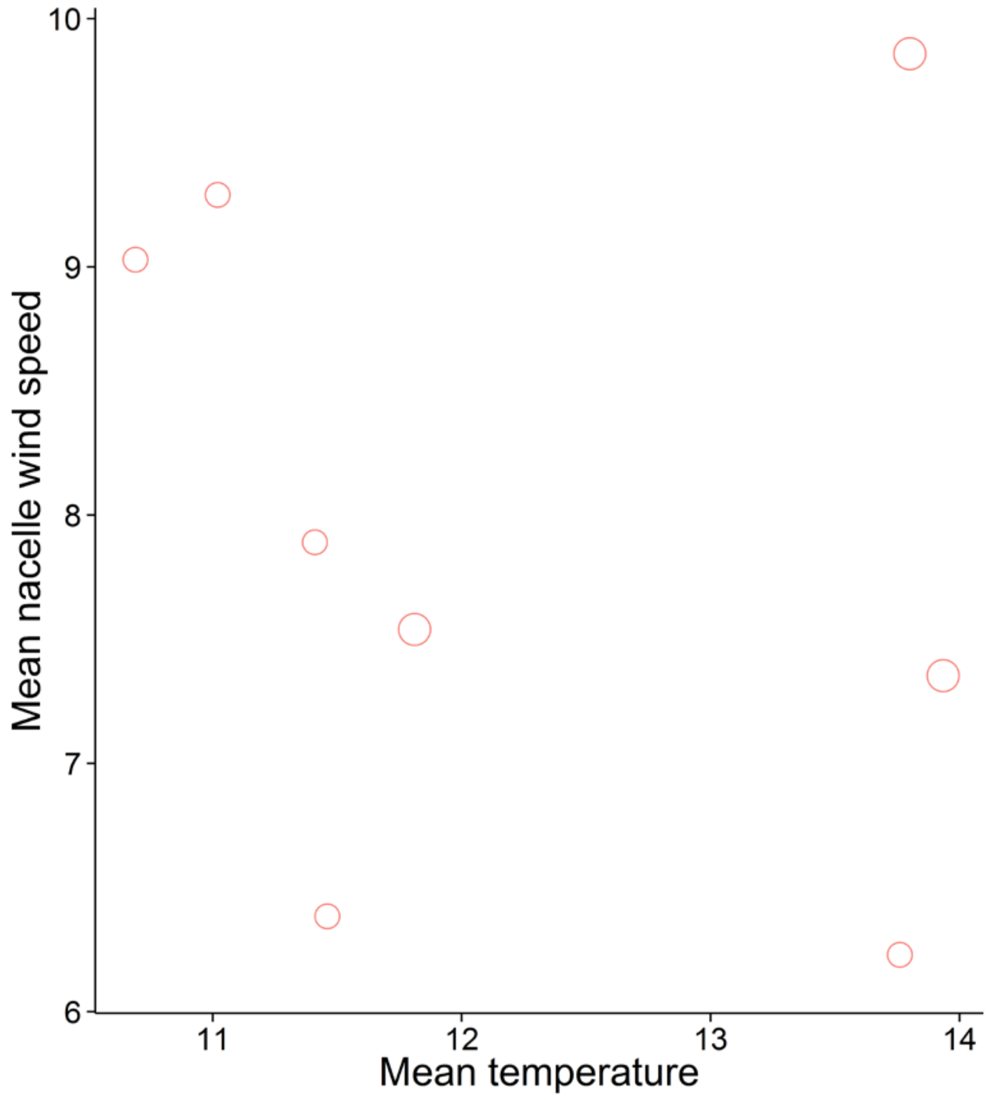
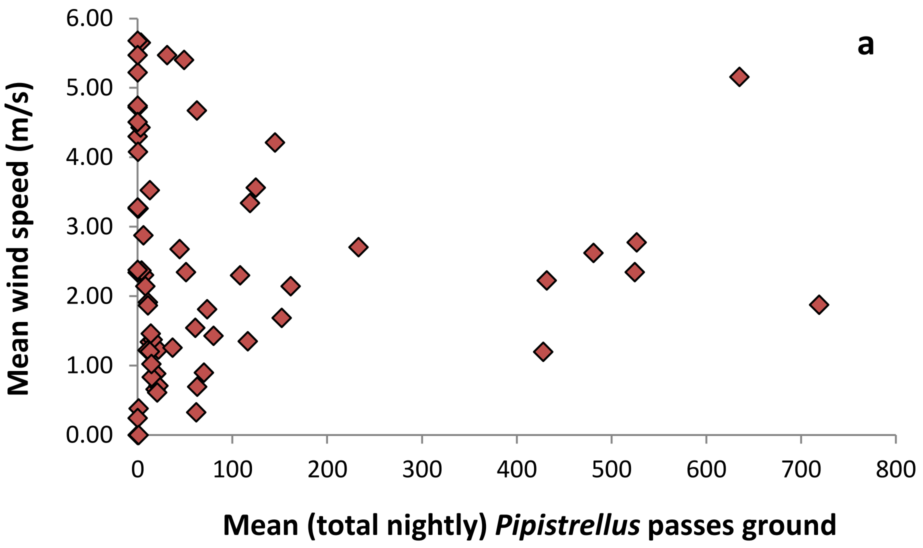


Fig 3.5. Weather conditions (mean temperature (°C) and mean wind speed (m/s) on the night preceding a fresh casualty being located. Small circles indicate one casualty; large circles indicate 3 casualties.

The possibility of using ground-level data from control sites to create simple predictions of whether a site would have fatalities was also explored. Categorising sites as ‘high activity’ (top third) had reasonably good positive predictive value for all pipistrelles (75%; 95% CI 46.8, 91.1), but a relatively poor negative predictive value:only 52% (95% CI 33.5-70.0%) of low-medium activity sites had no pipistrelle casualties. However, for all bats collectively the positive predictive value was much poorer (36%; 95% CI 19.7; 57.0), meaning that only a third of sites with high activity also had a bat casualty. Nevertheless, the negative predictive value was good:

14 of the 15 sites that were classified as having low-medium bat activity had no casualties of any species (93.3%; 95% CI 70.2, 98.8).

The overall distribution of pipistrelle fatalities in relation to the combination of ground-level wind speed and recorded activity are illustrated in Fig 3.6a and b. The data do not suggest that lower wind speed thresholds might be appropriate when recorded pipistrelle activity is low.



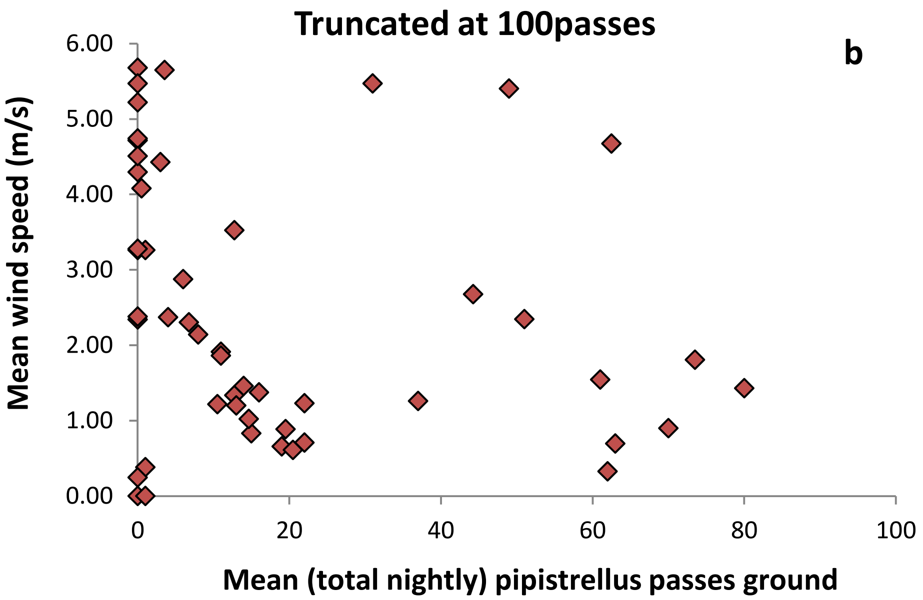


Fig 3.6. Distribution of bat fatalities according to the combination of (a) mean ground-level wind speed and (b) mean ground-level wind speed with passes at ground truncated at 100 per night.

The number of pipistrelle (all species) fatalities/turbine/month, adjusted for searcher efficiency and carcass removal, was predicted by the categorisation of sites as having low, medium or high pass rates. For each site the maximum nightly pass rate at turbines at ground level for all pipistrelle passes combined was used to categorise sites into tertiles. There was a significant association between the number of pipistrelle fatalities and the activity category of the site. Sites categorised as low activity had significantly fewer pipistrelle fatalities compared to medium and high category sites (t_{45}=-2.498, *p* = 0.016), but there was no difference between sites categorised as medium and high activity (t_{45}=-1.170, *p* =0.245) (Fig. 3.7).

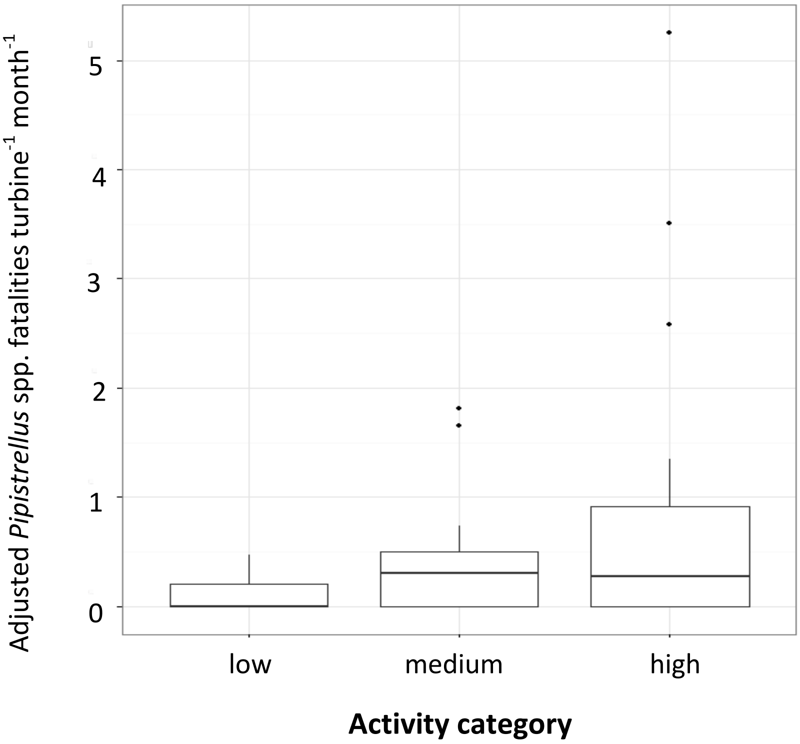


Fig 3.7. Box plot of the adjusted number of pipistrelle (all species) fatalities/turbine/month against sites categorised as having low, medium or high pass rates (data divided at tertiles of the maximum pass rate per night for each site).

Discussion

This study has shown that fatality risks are linked statistically with turbine characteristics, habitat, bat activity and weather, supporting observations reported elsewhere (Barclay et al. 2007, Arnett et al. 2008, Rydell et al. 2010b, Korner-Nievergelt et al. 2013). We found no independent link between turbine tower height and casualty rates unlike some previous reports ((Barclay et al. 2007, Rydell et al. 2010b) and the reasons for this are unclear. Height is to some extent confounded with the duration of operation (modern turbines tend to be larger), and these two previous review papers were not based on systematic surveys of a representative sample of turbine heights, so there may have been confounding effects. We did, like (Rydell et al. 2010b) but not (Barclay et al. 2007) find that casualties increased with rotor size, and this could have important practical applications in assessing future risks.

It is also evident that the prediction of fatalities with precision is extremely difficult. This is for several reasons. First, each individual fatality is a rare event, and modelling the occurrence of rare events within reasonable confidence limits is inherently challenging (Zuur et al. 2009). Second, there is clustering of the predictor variables – most study nights have rather similar weather conditions; and within each site, turbines are the same make and model, and the landscape context is constant. This makes it more difficult to observe associations than where there is good replication across a wide range of values. Finally, there are inherent errors associated with the outcome measures (casualty rates). Whilst it was possible to adjust for observer-error and carcass removal rates for analyses conducted at the site-level (sections 1 and 2), analyses based on observations of individual bats (section 3) could not be corrected in the same way. It was also not possible to adjust zero observation: the consequent presence of false-negative results in the dataset will have been a particular difficulty for those analyses based on the presence/absence of casualties. Finally, it appears that casualty patterns are inherently very variable. For example, sites studied in more than one year (n = 4), had different fatality patterns in consecutive years, however, replication was low. Whilst many fatalities do occur at sites with high bat activity rates, fatalities are also recorded at sites where bat activity was low; and in addition, some sites had high bat activity rates but few or no fatalities.

It has not been possible, to date, to identify features that could be used easily to minimise the risk to bats *a priori* through, for example, the better siting of turbines. In particular, it is notable that bat activity at control sites (the nearest proxy available to preconstruction bat activity surveys), did not predict the *number* of fatalities. However, there was some support for high pipistrelle bat activity being positively linked with the risk of a site having *any* casualties; and sites with low-medium activity rates were unlikely to have casualties of all species combined. Stakeholders must now assess whether the simple classification of a site as being at risk of having at least one casualty is useful in practice, or whether more refined risk estimates are required in practice. We recommend further analysis of these relationships using more sophisticated generalised additive modelling that would allow non-linear patterns in the data to be explored more thoroughly. We also emphasise that the assessments of the positive and negative predictive value of bat activity are intended as simple univariate guides, and do not take into account the complexities of collision risks that may affect a particular site (for example, they do not adjust for the increased risks associated with larger blade lengths).

The influence of habitat appears complex, with the direction of association with broad-leaf woodland varying between pipistrelle and noctule bats. The apparent protective effect of woodland may be because this habitat is preferred by pipistrelles foraging, whereas if no woodland or other suitable linear features are present then the bats are instead attracted to the turbine. In contrast, woodland may be associated with increased risk of noctule casualties because it provides roosting opportunities (and hence affects population density) without any corresponding attractiveness during foraging because the species is adapted to open-space feeding. Given that this study provides some evidence that pipistrelle casualties are more frequent at sites where woodland is absent, consideration may need to be given to revising the current guidance on this issue. It would also be valuable to conduct further analysis investigating the links between casualty risk and distance to other features associated with bat activity such as hedgerows and watercourses. However, it must be emphasised that whilst the negative predictive power of the habitat features were promising (absence of the feature predicts absence of casualties), the positive predictive value was fairly weak: most sites with the features also did not have casualties.

Bat activity measured at the turbines (and also at control sites) had some relationship with fatality risk, but there was much variability and hence confidence intervals are very wide. We conclude that most of the variability in bat fatalities was due to site-specific factors rather than to overall activity levels. At present therefore, the main management option that can be suggested by this project is standardised post-construction surveys (using the techniques developed in this work), followed by curtailment based on wind speed should problems be identified. Given that this remains a relatively crude approach, and for most nights where output is lost, there would have been no risk to bats from operational turbines, it would be proportionate only where risks to bats have been identified with certainty. Our work to improve the prediction of risk is ongoing, but we strongly encourage further research using daily sampling of turbines to assess the link between weather, bat activity and fatality risk more precisely.

Section 4: Design of acoustic surveys for wind energy facilities

Introduction

The acoustic monitoring of bat activity at wind farms is a fundamental component of pre- and post-construction ecological assessments. Recent technological advances mean that acoustic detectors are readily available, and therefore transect surveys and static detector deployment at ground level are now commonplace. Different species have different probabilities of detection because higher frequency calls attenuate more rapidly in space (Griffin 1971), and species with highly directional calls are more likely to be under-recorded. Seasonal variability in bat activity patterns is also well known (Catto et al. 1995, Russ 1999, Russ et al. 2003). However, very few studies have assessed how the altitudinal distribution of bats, or the temporal variability in activity within seasons affects the ability to estimate accurately species composition and relative abundance. One exception is Collins and Jones (2009) who recorded bat activity at seven sites over an average of five nights. Generally, they found significantly higher bat activity at ground level (~ 2m) compared to 30m above ground (measured from bat detectors attached to mobile phone and research masts). Although the total activity of open-high flying specialists (*Nyctalus* and *Eptesicus* species) did not vary significantly with monitor height, the calls were heard at different times illustrating that the same individuals were not being recorded simultaneously on both detectors. In addition, their proportions relative to other species did vary with detector height. Recently Bach *et al*. (Bach et al. 2013) showed that although there was a link between the activity levels measured at ground and wind turbine-nacelles for *Pipistrellus spp*. and noctules, these relationships were not consistent over time. In spring and autumn bats appeared to fly either at height or close to the ground, whereas in late summer they used the whole airspace. In other studies conducted in woodland or tropical forests, activity was generally greatest above the forest canopy, particularly for open flying bats (Kalcounis et al. 1999, Menzel et al. 2005). However, it is not clear how transferable these findings are to other habitats. For bats which fly in more cluttered environments the findings are less consistent between studies. For example Kalcounis et al. (1999) found that activity did not differ significantly within and above the forest canopy, but this finding was not replicated in other studies (Hayes and Gruver 2000, Menzel et al. 2005). Recently, work in Germany has indicated that pipistrelles, which are generally considered to fly at low-mid heights forage regularly above the forest canopy, potentially explaining their risk from wind turbines (Müller et al. 2013).

In addition to the survey problems caused by altitudinal spatial variability, temporal variability in activity also represents a major potential problem for effective monitoring. Previous assessments of temporal variability in activity within sites are very limited, and studies that have estimated the number of survey nights required to determine accurately species composition and relative abundance have only been conducted at single sites (Hayes 1997, Broders 2003). Here, bat activity varied by a factor of 6 over four nights, and the number of surveys nights required to portray mean activity accurately for *Myotis lucifugus* was suggested to be more than 20 nights.

The UK has some of the most detailed guidelines for surveying bats (Hundt 2012). Europe has some of the highest levels of protection for bats (Directive 1992), where all species and their roosts are legally protected (translated into domestic legislation in the UK via the Natural Environment and Rural Communities Act 2006, Conservation of Species and Habitats Regulations 2010), and this is reflected in the survey guidelines. These guidelines state “bat detectors can be installed at height, in order to quantify the amount of bat activity in specific locations. This method is normally only employed in specific circumstances such as at high risk wind turbine sites.” They advise a static detector survey effort of 5 consecutive nights per survey location per site per season for low risk sites, and 5 consecutive nights per month per site for moderate and high risk locations, and advise surveyors to consider the size of the survey area and the quality of the habitat; proximity to protected sites; and proximity to buildings or features providing potential roosting sites in determining the appropriate level of survey effort (Collins 2016).

In section 2 of this report, we have described the difference in bat activity patterns recorded at ground level and from the nacelle of wind turbines in this project. We now assess the number of nights needed to detect species composition and relative abundance at ground and height with acceptable accuracy.

Methods

Analyses were conducted separately for each species, and only sites that fell within the conventionally-defined geographical range for the target species (according to IUCN International Union for the Conservation of Nature, (IUCN 2008)) were considered. The analysis therefore does not consider cases where occasional passes were recorded from rare or vagrant animals. The species considered were: common pipistrelle*,* soprano pipistrelle*,* Nathusius' pipistrelle*,* noctule, Leisler's*,* and serotine (*Eptesicus serotinus)* bats. All statistical analyses were conducted in the programme R (version 3.0.3) (R Development Core Team 2013).

Data collected at ground and height were analysed separately to reflect the fact that most bat surveys are conducted at ground level – so separate results would be more useful to practitioners – and also because the measurements made at ground and height differ substantially. A Monte-Carlo approach was used to assess the minimum number of nights required to determine accurately activity levels for relative abundance estimates. The dataset was restricted to only nights which met minimum weather conditions for bats; minimum temperature of 10°C at dusk (Hundt 2012), maximum ground-level wind speed of 8 ms^{-1}, and average rainfall less than 2.5mm hr^{-1}, and the analyses for each species only included sites where the target species had been detected during the study. The maximum recorded nightly pass rate (across all detectors) was computed for each site during the total survey period. This approach was used as opposed to using other measures such as median or mean pass rates, because most median and mean values were zero, and the highest pass rate across the survey period is likely to be the time when bats are most at risk from wind turbines, and so represents the worst-case scenario. A single randomly selected survey night was then sampled from a randomly selected site (without replacement). From this sample, the maximum nightly pass rate was determined. We then replaced the data and repeated the process 1000 times. This process was repeated for an increasing number of consecutive nights (1 to 16 nights), comparing the maximum pass rate within each site and night combination to the overall maximum pass rate for the site. For each species, the proportion of the 1000 repeated samples, for each survey night, which fell within 10% of the maximum pass rate for the site, was then computed, along with the standard errors. The proportion of the samples that fell into the categories of 'high', 'medium' and 'low' bat activity was also calculated. (In the absence of any agreed functional definition of what constitutes 'high', 'medium' or 'low' activity, the cut-off points are simply based on taking the maximum night of activity for each site/species combination and splitting the data impartially into three equally-sized groups for the whole of Great Britain – see Section 2). To assess the ability of surveys to detect the presence of a species, the same analyses were also conducted using presence/absence data, including all sites within range for 1 to 14 nights. Comparisons of the between-site and between-night (within site) variability in bat activity were made using the outputs of GLMMs (with a negative binomial error distribution) predicting pass rates from a model with turbine specified as a fixed effect, site as a random effect, and night as a random effect nested within site. We performed all statistics with R (v.3.0.3), using the packages lme4 (v.1.1.7) and GLMMADMB to fit GLMMs.

Results

The data considered here relate to monitoring 138 turbines at ground and 105 turbines at height for 1,367 nights and 1,258 nights respectively). This differs slightly from the data presented in Section 2 since unpaired data (due to technical issues with a detector at either ground or height) are excluded and data from nights of good weather for bats (as previously described) are used unless otherwise stated. 97% of passes were identified to species level and the remainder to genus (Table 4.1). There were 992 passes for bats in the genus *Nyctalus* which could not be identified to species, and 219 passes where it was not possible to distinguish between *Eptesicus* and *Nyctalus*.

Species presence/absence

Over the full survey period at each site, all species detected were recorded at ground level, except for noctule, Leisler's and long-eared bats where, at one site each, detection occurred only at height rather than at ground. Table 4.1 summarises the data from paired detectors at height and ground, showing the relative distribution of species activity. Because our study was extensive in terms of time, we were also able to assess the probability of detecting at height only on a nightly basis. Species differed in the probability of being detected only at height and not at ground ($\chi^2_3 = 61.536$, $p < 0.001$), and the height of the nacelle also affected the probability of a species being detected ($\chi^2_1 = 4.604$, $p = 0.0319$). The likelihood of only detecting a species at height and not ground was highest for noctule, where the probability varied between 0.02 and 0.19 depending on turbine height. The probability of recording Nathusius' pipistrelle at height and not at ground was 0.05. The rates for common and soprano pipistrelle were 0.03 and 0.02 respectively. When considering only elevations between 30m and 50m, the probability of not detecting Nathusius' pipistrelle and noctule bats at ground level increased to 14% and 21%, respectively. Therefore, monitoring at ground level only would under record the presence of Nathusius pipistrelle and noctule bats.

The number of survey nights needed to detect the presence of species at 80% of sites (equivalent to the generally accepted level of power, (Cohen 1988)) was one night for common pipistrelle and ten nights for barbastelle bats, both at ground level (Table 4.2, Fig 4.1). After 5 survey nights, barbastelle and Nathusius's pipistrelle bats would not have been detected at ground level at 45% and 28% of sites respectively (Fig. 4.1). Therefore to detect the full composition of species at ground level, at least six nights of surveys are required for more common species and ten nights for less common species.

Table 4.1. Summary of number of sites surveyed in each species' range and recorded passes.*

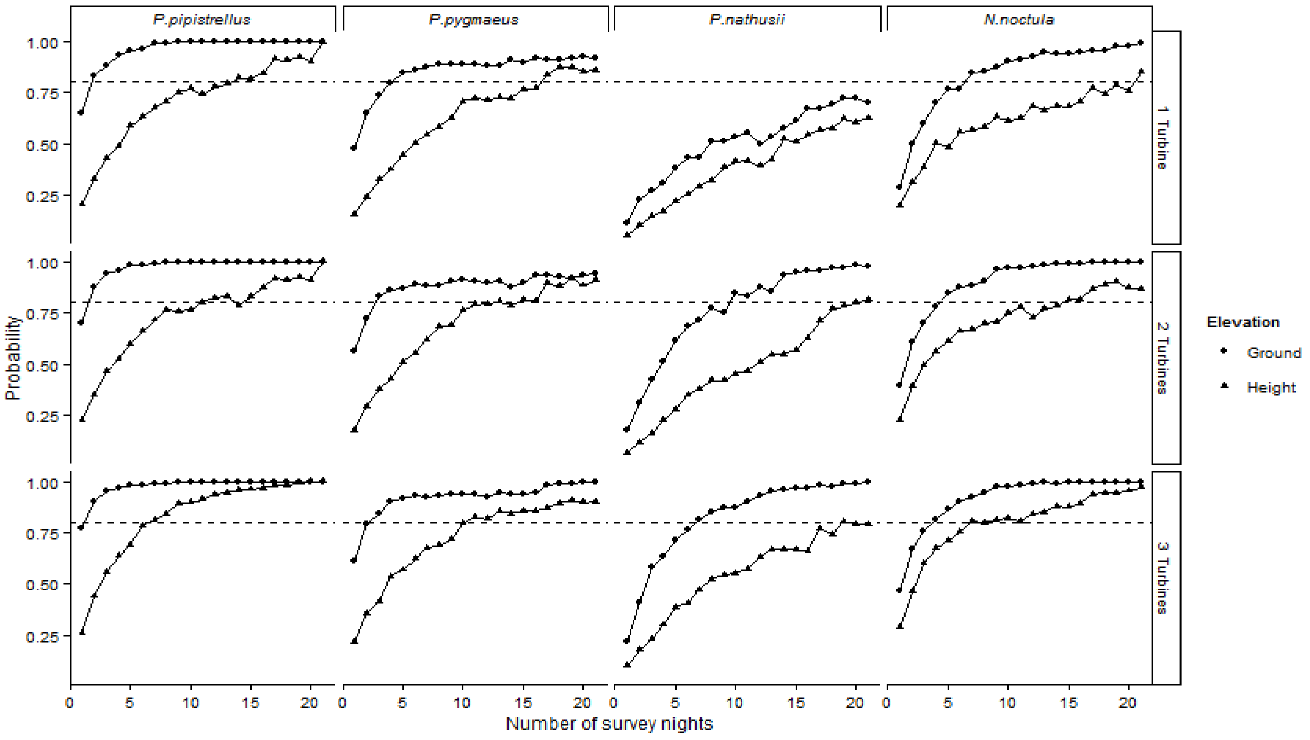
Species	Mean nightly passes per site (±SE)	Total passes	Number of passes at height (%)
barbastelle	0.03 (0.02)	89	0 (0)
lesser horseshoe	<0.001 (NA)	5	0 (0)
greater horseshoe	0.003 (0.003)	11	0 (0)
Myotis spp.	0.52 (0.10)	3,120	20 (0.64)
long-eared	0.11 (0.03)	678	17 (2)
common pipistrelle	21.66 (4.55)	138,409	6,744 (5)
soprano pipistrelle	5.16 (1.67)	30,969	5,037 (16)
Nathusius' pipistrelle	0.22 (0.05)	1,254	158 (13)
serotine	0.01 (0.003)	9	2 (22)
Leisler's	0.02 (0.02)	75	16 (21)
noctule	1.56 (0.53)	8,228	2,096 (25)

*Excluding nights with no activity and including only paired ground and height nights.

Table 4.2. Results from resampling data over increasing survey nights, showing the number of nights needed to detect species at 80% of sites and the percentage of sites where each species was detected after 5 nights of surveying at ground and height level.

	80% sites species detected		% sites species detected after 5 nights (±SE)		80% sites within 10% max. relative abundance		% sites within 10% max. activity index after 5 nights (±SE)	
Species	Ground	Height	Ground	Height	Ground	Height	Ground	Height
barbastelle	10	-	54.5 (0.16)	-	13	-	34.0 (0.05)	-
***Myotis* spp.**	3	4	93.4 (0.19)	89.5 (0.21)	13	13	48.0 (0.72)	49.1 (0.12)
long-eared	6	6	77.0 (0.17)	74.8 (0.19)	12	12	51.4 (0.12)	52.6 (0.14)
common pipistrelle	1	1	99.9 (0.20)	99.7 (0.22)	16	14	38.7 (0.09)	42.2 (0.11)
soprano pipistrelle	2	3	92.5 (0.19)	92.5 (0.22)	13	13	44.3 (0.10)	47.0 (0.12)
Nathusius' pipistrelle	6	7	72.5 (0.16)	70.9 (0.17)	16	13	37.9 (0.09)	46.4 (0.12)
noctule	3	5	90.8 (0.21)	81.6 (0.22)	16	14	37.5 (0.10)	41.5 (0.13)

Figure 4.3. Comparison of using 1, 2 or 3 detectors placed at ground or height in order to detect species presence with varying degrees of precision.



Relative abundance

The maximum pass rate (per turbine per night) was highly variable, and this variability was particularly marked for common and soprano pipistrelles. The maximum nightly pass rates in each category were highest when all nights were included compared to when only nights which met minimum weather conditions were included: for noctules, and common and soprano pipistrelles, the highest pass rates occurred on nights which were not classified as having favourable weather conditions for bats.

Across all sites, pipistrelles were the most frequently recorded bats (94%), with common pipistrelle being the most abundant (mean 22 ± SE 5 passes per night). Activity was always significantly higher at ground compared to height for each species (Table 4.3). This difference between species in abundance at height was significant (χ^2_3 = 3734.4, $P < 0.001$); and the height of the nacelle also predicted abundance (χ^2_1 = 4.913, P = 0.027). A lack of consistency in relative abundance at ground compared to height positively biases abundance estimates of low flying specialists and common pipistrelles*,* and negatively biases abundance of the medium flying specialist soprano pipistrelle and the higher flying specialist noctule bats if monitoring occurs only at ground level.

Table 4.3. Relationship of total nightly activity at ground to that at height (Model 1), and prediction of total nightly activity at height by ground-level activity and the nacelle height (Model 2). Table shows z scores and associated significance values *** p<0.001; ** p<0.01; * p<0.05

	Model 1	Model 2		
Species	Ground vs Height	Ground	Elevation of detector at height	$R^2_{GLMM(m)}$
***Myotis* spp.**	-16.499^{***}	1.41	-0.80	*NA*
long-eared	-4.77^{***}	1.25	-0.28	*NA*
common pipistrelle	-35.77^{***}	2.29^{*}	-2.96^{**}	0.8%
soprano pipistrelle	-27.63^{***}	7.17^{***}	-1.98^{*}	2.4%
Nathusius' pipistrelle	-3.81^{***}	1.38	-1.04	*NA*
noctule	-10.92^{***}	-2.00^{*}	6.90^{***}	2.3%

Under ideal weather conditions, between 13 and 16 survey nights are required to give an 80% probability that surveys will be within 10% of the maximum recorded bat abundance at height. (The range of 13 to 16 is due to a variation in detector height and species sampled (Table 4.2)). Only a third of the sites we studied would detect a relative abundance within 10% of the maximum recorded activity using five survey nights.

The number of nights of monitoring required to classify bat activity with acceptable accuracy into ‘high’, ‘medium’ and ‘low’ activity classes (based on maximum recorded activity as defined in Table 4.4) was also explored (Table 4.5). The number of nights required for ground level recordings were slightly higher than those required to classify the maximum pass rate within 10%; whilst for recordings made at height, approximately 3 weeks of survey effort were required.

Table 4.4. The lower and upper limits for the maximum nightly pass rates used to categorise sites using data from 1, 2 and 3 turbines. Sites were separated into tertiles based on their maximum nightly pass rates within the survey period.

		Ground			**Height**		
Species	No. turbines	Low	Medium	High	Low	Medium	High
B. barbastellus	1	0	na	1-6	0	na	na
***Myotis* spp.**	1	0-1	2-3	4-88	0	na	1-4
N. noctula	1	0-1	2-7	8-271	0	1-21	23-396
Nyctalus/Eptesicus	1	0	1-5	6-272	0	1-2	3-405
P. nathusii	1	0	1	2-47	0	na	1-20
P. pipistrellus	1	0-15	16-162	232-3,324	0	1-8	12-682
P. pygmaeus	1	0-2	3-30	31-813	0	1-2	3-802
Plecotus spp.	1	0	na	1-16	0	na	1
B. barbastellus	2	0	na	1-6	0	na	na
***Myotis* spp.**	2	0-2	3	4-88	0	na	1-4
N. noctula	2	0-1	2-12	13-271	0	1-2	3-396
Nyctalus/Eptesicus	2	0	1-8	9-272	0	1-2	3-405
P. nathusii	2	0-1	2-3	4-47	0	na	1-20
P. pipistrellus	2	0-34	35-387	388-3,324	0	1-7	8-682
P. pygmaeus	2	0-3	4-45	46-813	0	1-2	3-802
Plecotus spp.	2	0	1	2-16	0	na	1-3
B. barbastellus	3	0	na	1-6	0	na	na
***Myotis* spp.**	3	0-2	3-6	7-88	0	na	1-4
N. noctula	3	0-2	3-12	13-272	0	1-9	10-396
Nyctalus/Eptesicus	3	0	1-8	9-271	0	1-2	3-405
P. nathusii	3	0-1	2-5	6-91	0	na	1-20
P. pipistrellus	3	0-47	48-454	455-3,324	0	1-8	9-682
P. pygmaeus	3	0-7	8-46	47-813	0	1-2	3-802
Plecotus spp.	3	0-1	2	3-27	0	na	1-3

.

Table 4.5. The minimum number of nights to categorise 80% of sites correctly as having maximum activity rates that fell into low, medium or high categories (defined by maximum nightly activity).

	All data (ground)	All data (height)	Good weather (ground)	Good weather (height)
common pipistrelle	15	20	12	12
soprano pipistrelle	12	19	10	9
Nathusius' pipistrelle	15	21	12	14
noctule	15	21	11	19

In summary, when considering all the ‘at risk’ species, and restricting nights to only those that met the minimum weather conditions, a minimum of 12 nights at ground and 19 nights at height should be considered for static monitoring.

We also explored the effect of varying the numbers of detectors used at each site. Our survey protocol surveyed 3 turbines per site, and therefore it was possible to assess the impact of using 1, 2 or 3 detectors on the duration of survey effort required. In general, increasing the number of detectors from 1 to 2 increased survey precision, but increases from 2 to 3 made little improvement if the surveys were of short duration. It therefore appears that increasing the number of detectors is unlikely to compensate for short survey effort, primarily because of the very high night-to-night variability in bat activity.

Predicting activity at height

Recent high bat fatalities at wind farm sites have highlighted the need to record accurately bat abundance indices within the rotor sweep area. This area is often well beyond the maximum recording range for detectors used at ground level. Detector height and total nightly ground level activity were significant predictors for determining total nightly activity at height for common and soprano pipistrelle and for noctule bats, but these predictors only explained between 0.8% and 2.4% of the variability in recorded activity (Table 4.3). However, the R^2 value for the combined fixed and random terms explained between 83.9% and 86.8% of the variability, highlighting the considerable amount of random variability between sites. There were no clear linear relationships between the elevation of the detector and the ratio of passes recorded (ground to height), and there was considerable variability between sites (Fig 4.4).

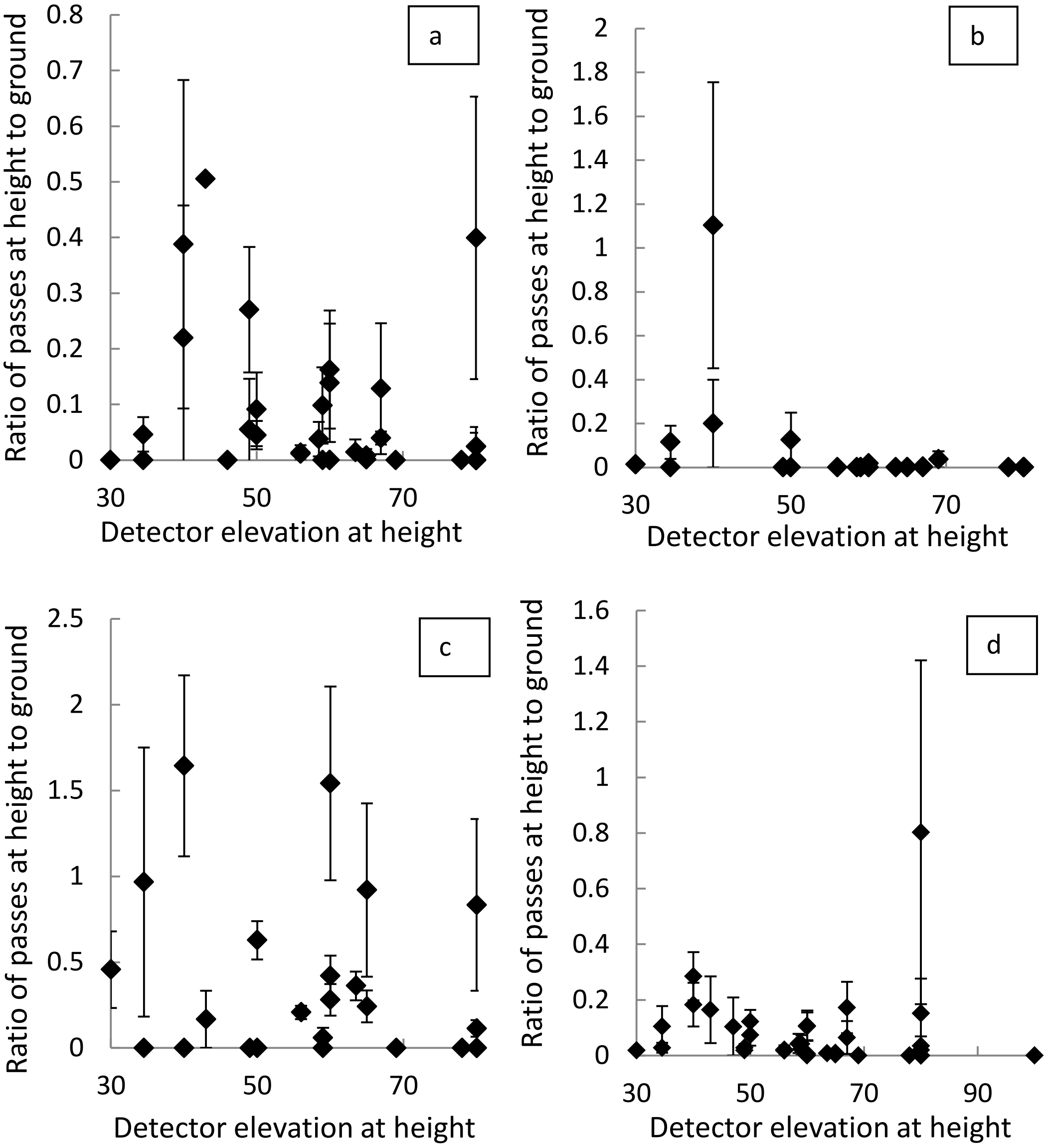


Figure 4.4. Nightly paired ratio of total passes at height compared to ground for each site (±SE) against detector elevation for (a) common pipistrelle bats, (b) soprano pipistrelle bats, (c) Nathusius' pipistrelle bats and (d) noctule bats.

Comparison of variability between sites and nights

There was considerable variability between nights at the same site in bat activity. Frequently the between-night SD was of similar magnitude to that between sites. It was also notable that for the recordings made at height for common pipistrelle, soprano pipistrelle and *Myotis* bats, the between night variability was greater than that between sites (Table 4.5).

Table 4.5. SDs for night and site measured at ground and height for different species

	Ground		Height	
	Site	**Night**	**Site**	**Night**
common pipistrelle	2.42	2.00	0.25	7.83
soprano pipistrelle	1.94	1.94	0.71	8.58
Nathusius' pipistrelle	1.35	1.53	1.95	0.01
noctule	2.14	1.61	3.02	2.08
Nyctalus & Eptesicus spp.	2.77	1.58	3.48	2.19
Myotis spp.	1.58	1.13	1.90	10.95
long-eared	1.43	1.43	NA	NA

Discussion

Species composition and relative abundance were not consistent between ground and height level. When monitoring is only conducted at ground level there is probability of between 0.02 (2%) and 0.19 (19%) of not detecting noctule bats (depending on turbine height) and a probability of 0.05 (5%) of not detecting Nathusius' pipistrelle bats. This increased to 0.21 (21%) and 0.14 (14%) respectively when the analysis was restricted to include only elevations of 30m-50m in the 'height' category. However, there was only very low chance of not detecting common and soprano pipistrelles if they were truly present at a site. Barbastelle and horseshoe bats were only detected at ground level (although the number of sites where these species were detected was low (n = 9 and n = 7, respectively) and within these sites, encounter rates were low.

Estimates of the activity levels of common bat species was significantly positively biased towards common and Nathusius' pipistrelle bats and negatively biased towards soprano pipistrelle and noctule bats*,* when using monitoring data from ground level*.* This therefore raises concerns about assessments of the relative abundance of some medium and high flying specialists if monitoring is conducted only at ground level. In addition further bias may be introduced as a result of bat surveys being too short in duration. Excluding those species not expected to be detected in more open environments such as horseshoe species, our study shows there is high night-to-night variability in bat activity, and that at least a third of surveys conducted to current specifications are not detecting the full composition of species at a site. Their estimates of activity levels are even poorer.

The conservation of threatened populations is based on accurate recordings of distributions and population sizes and the ability to detect their changes (Yoccoz et al. 2001; Lancia et al. 2005). Due to the high temporal variability in measured activity levels within seasons, and the under-recording of medium and high flying species, care is needed in interpreting data on abundance and distribution. Our research shows that ground level monitoring is not able to predict accurately activity within the full rotor sweep area for all species. Acoustic monitoring at height in addition to ground at wind farm sites is therefore recommended, especially for sites within the distribution range of high-flying specialists, such as noctule bats. Combining these results with the models predicting fatality, we cannot confirm that monitoring at height is a better predictor of fatality for higher flying specialists (noctule bats), due to low fatalities for this species, however, activity data shows that detection and relative abundance would be less accurate when only monitoring at ground. Ground level monitoring may be sufficient when only common and soprano pipistrelles are at risk, because activity at ground rather than height was a better predictor of fatality.

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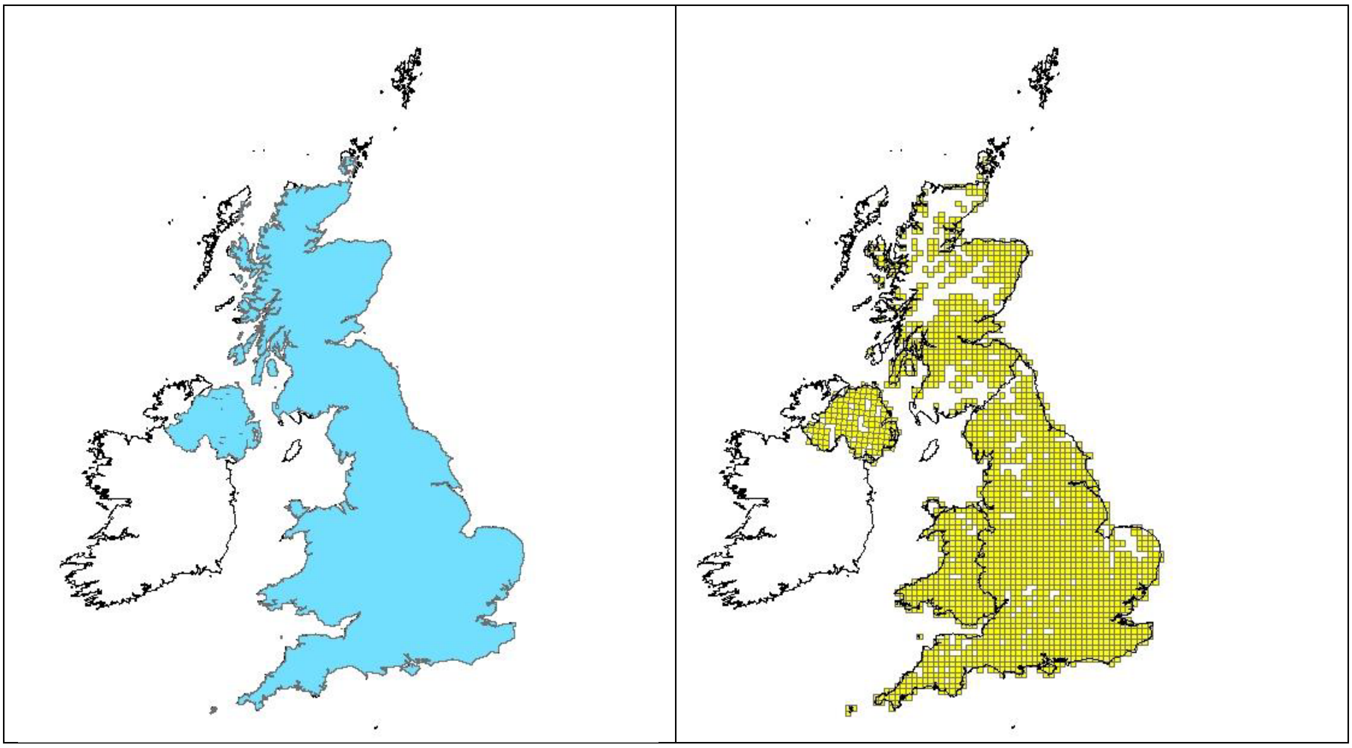
SECTION 5: PRIORITIES FOR FUTURE RESEARCH

In approximate order of urgency:

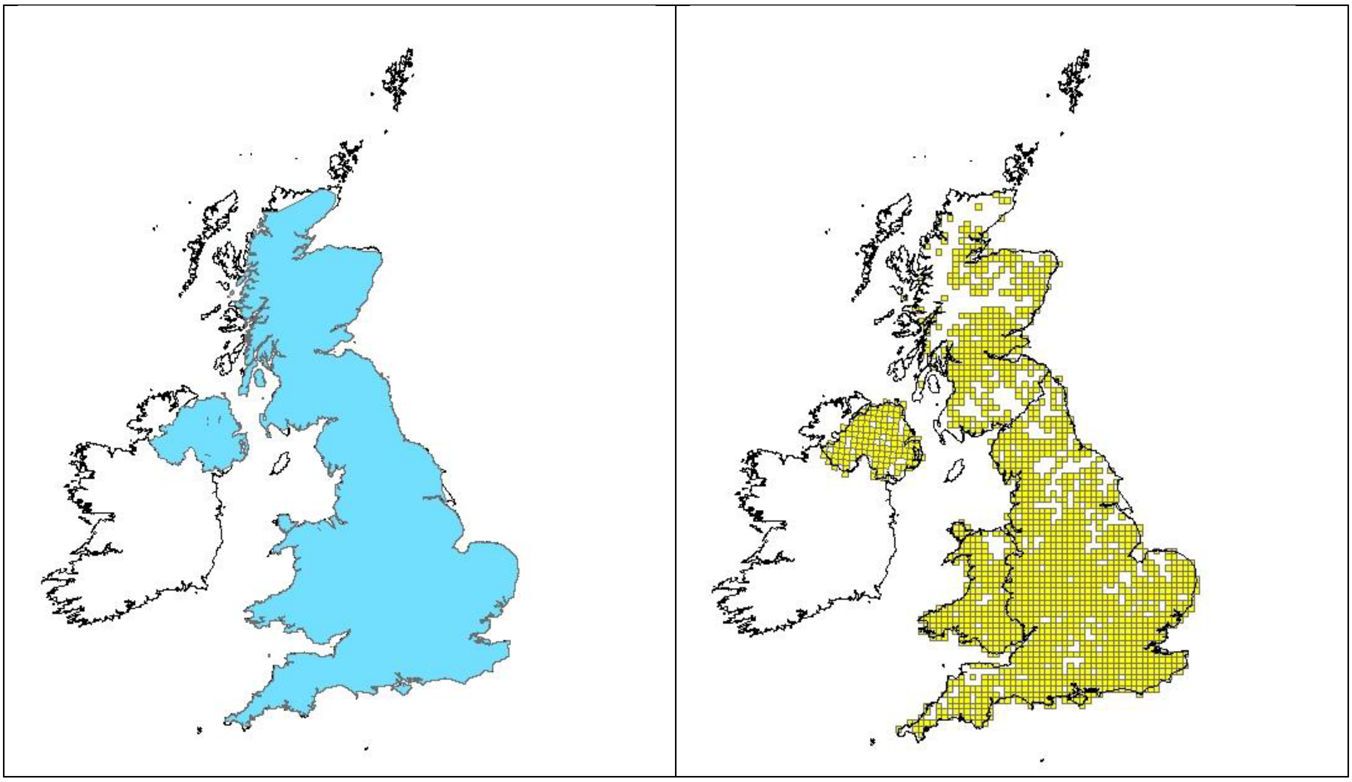
1. Assessment of the risk to bats from turbines key-holed into woodland, or placed very close to woodland.
2. Quantification of the risk to bats from turbines in coastal and offshore environments.
3. Assessment of the risk to rare bats from turbines placed in the vicinity of known roosts or swarming sites.
4. Assessment of the risk to bats from single or double turbine installations, particularly in areas of high habitat suitability for bats.
5. Investigation of the predictive value of preconstruction surveys in identifying sites of high collision risk for bats.
6. Construction of a central repository for data (acoustic monitoring and carcass surveys) collected in future monitoring of wind turbines. It is critical that these surveys are conducted using standardised methodologies. The repository will allow data synthesis and facilitate the future investigation of risk factors for collision and seasonality of collision risk.
7. Assessment of the effectiveness of curtailment as a means of minimising the risk to bats in the UK, including site-specific identification of cut-off speeds; and review the applicability of automated curtailment systems used elsewhere in the world for use in the UK.
8. Quantification of local and national bat population sizes in order to permit a sensible assessment of the likely impact on local populations.
9. Assessment of the cumulative impacts of turbines and other development pressures on local populations.
10. Characterisation of behavioural characteristics of noctule bats likely to place them in conflict with turbines (including identification of home range size).

APPENDIX 1. Bat range and distribution maps for the UK.

Maps for 1) bat species that were found during carcass searches at wind turbines, and 2) rare species that are unlikely to have been detected in this study. Range maps (left) indicate the geographical limits, whereas distribution maps (right) show locations where species has been recorded. Maps from Joint Nature Conservation Committee 2013. Third Report by the UK under Article 17 on the implementation of the Habitats Directive from January 2007 to December 2012. Peterborough: JNCC. www.jncc.gov.uk/article17



1a) *Pipistrellus pipistrellus* – Common pipistrelle (JNCC species code: S1309)



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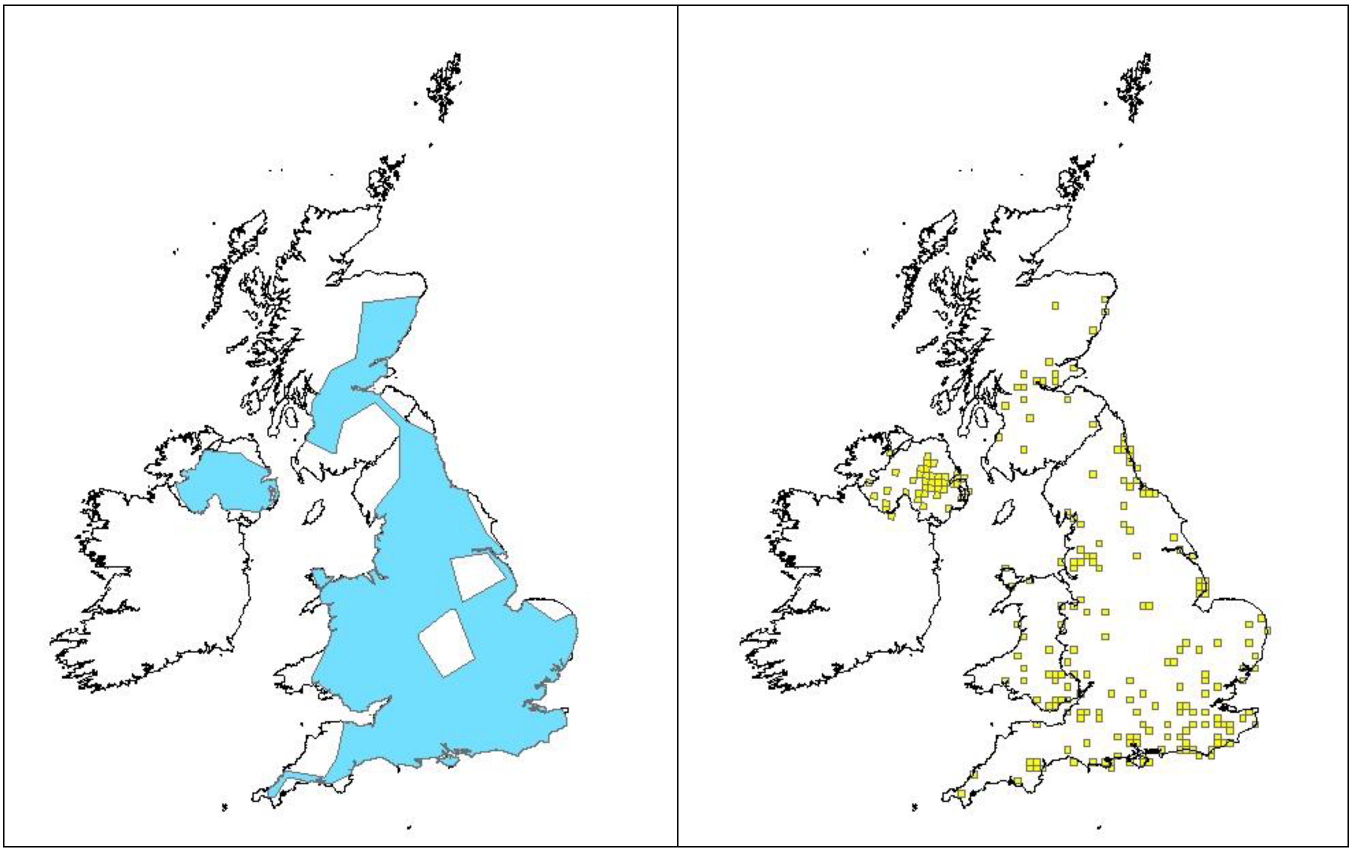
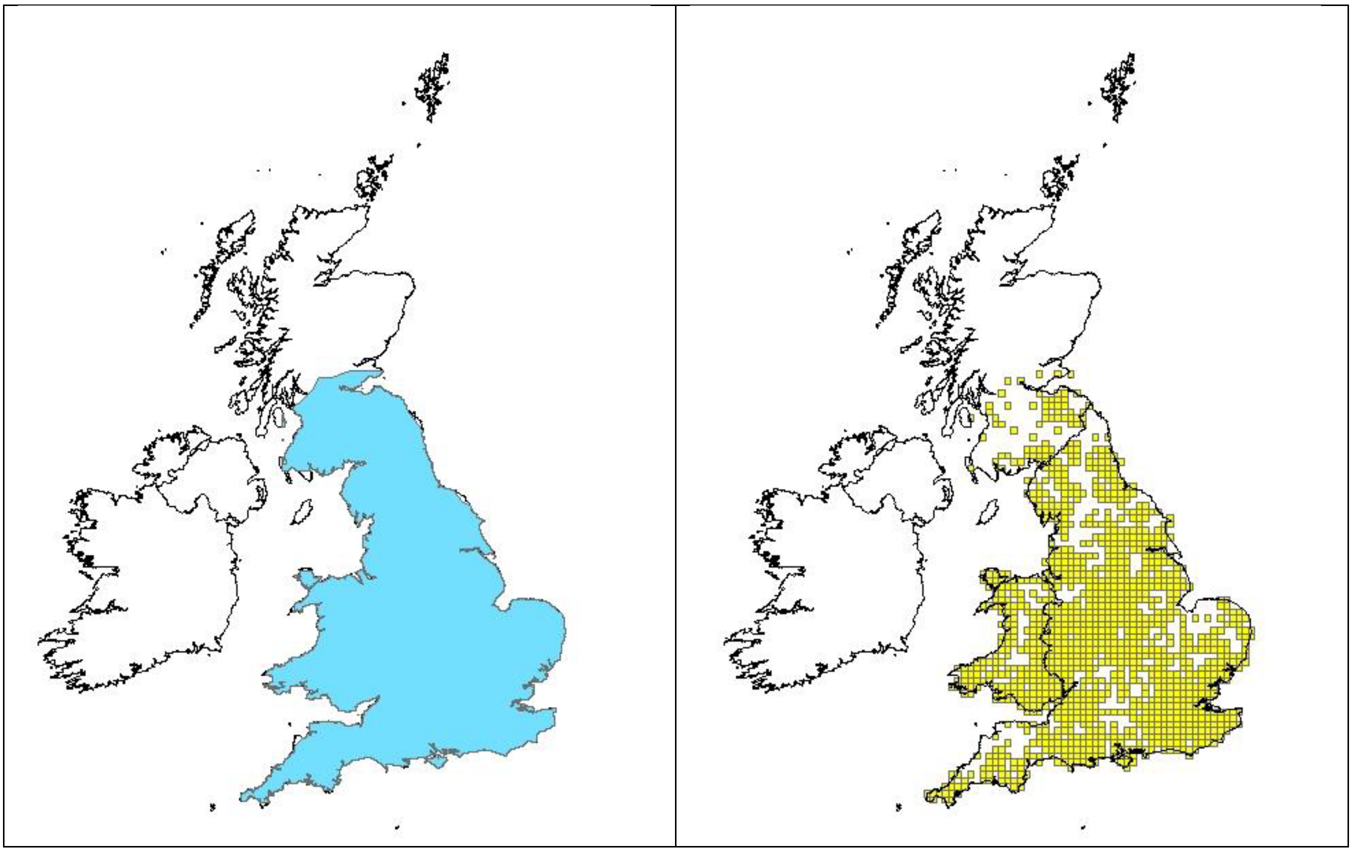
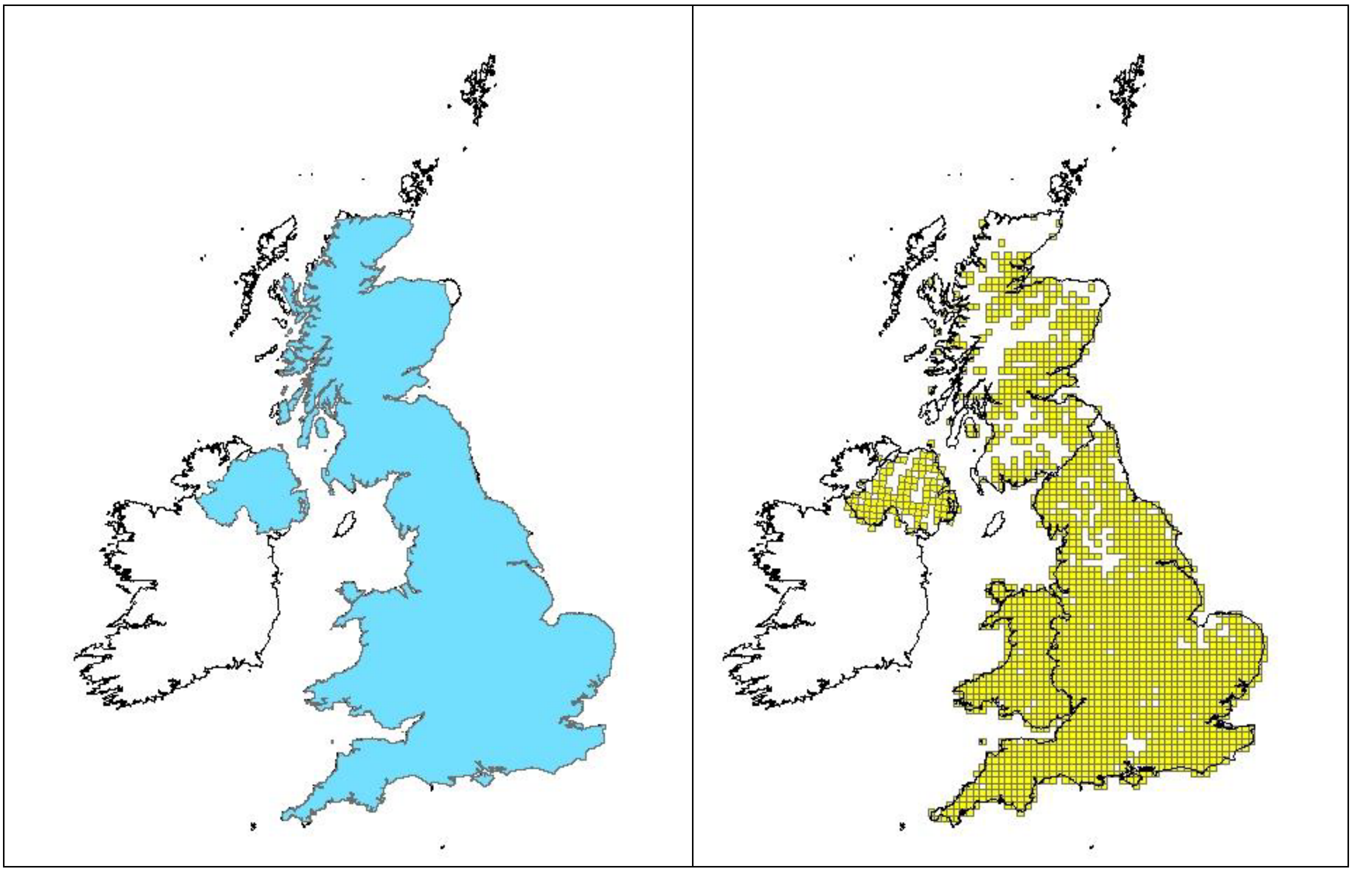


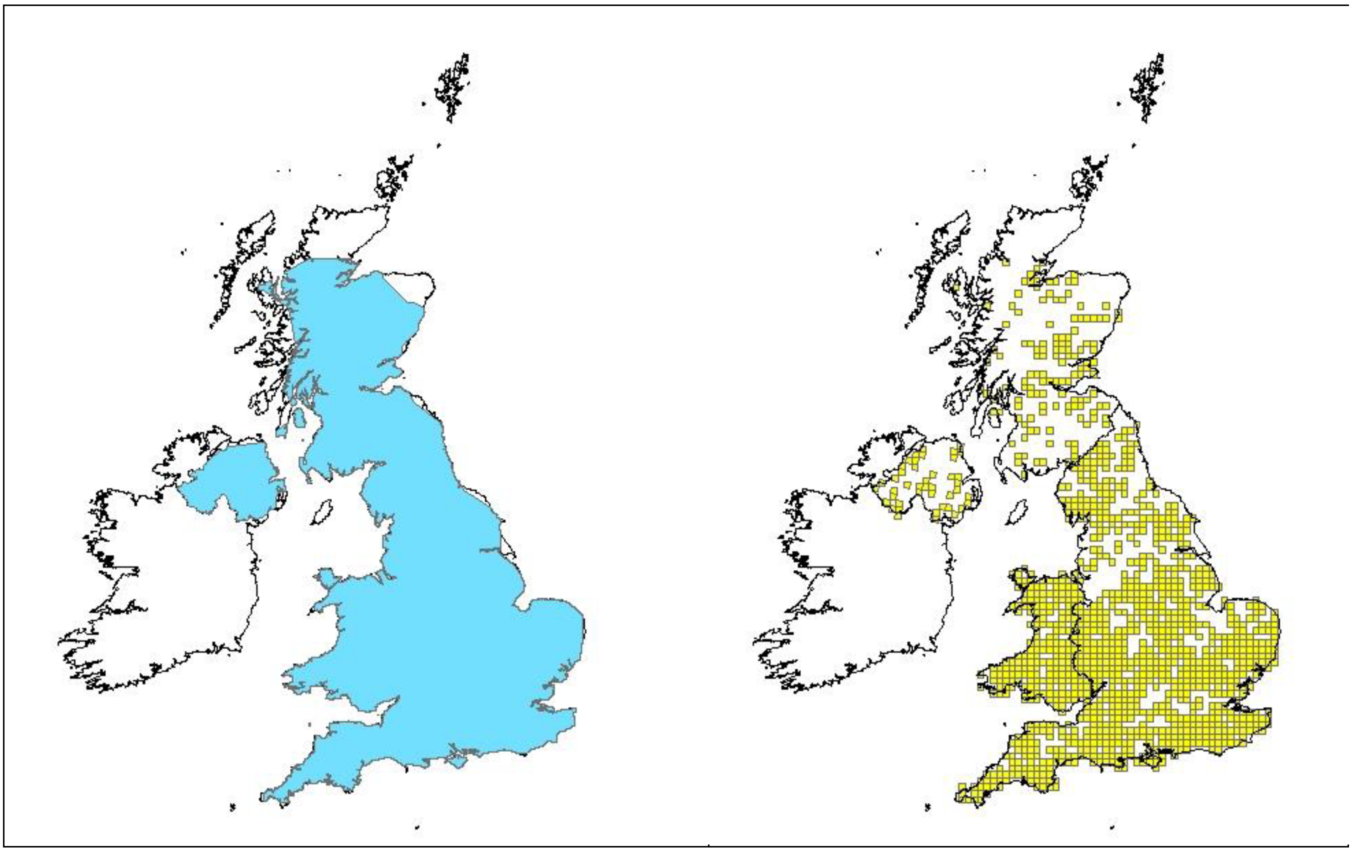
1c) Pipistrellus nathusii – Nathusius’ pipistrelle (JNCC species code: S1317)



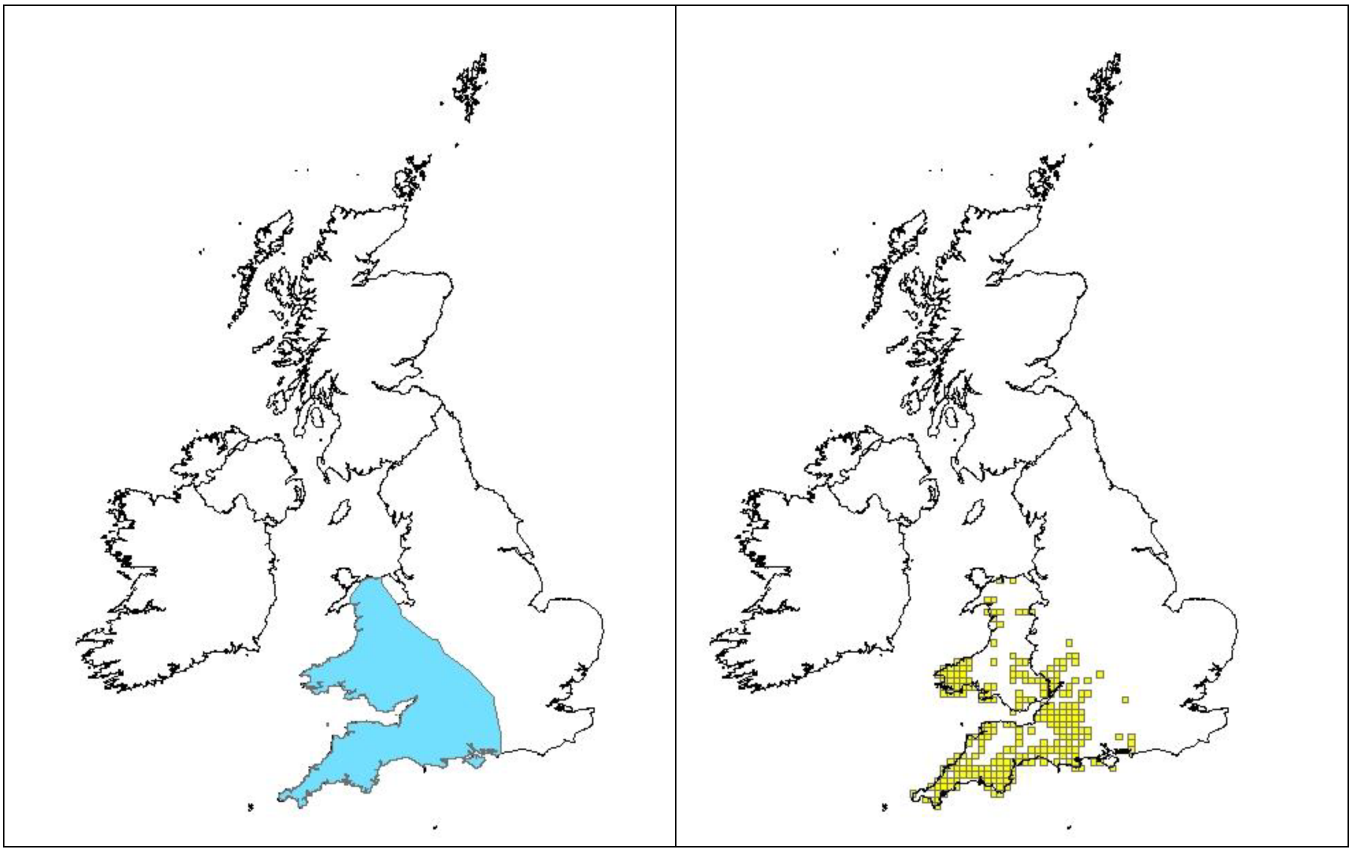
1d) *Nyctalus noctula* – Noctule (JNCC species code: S1312)



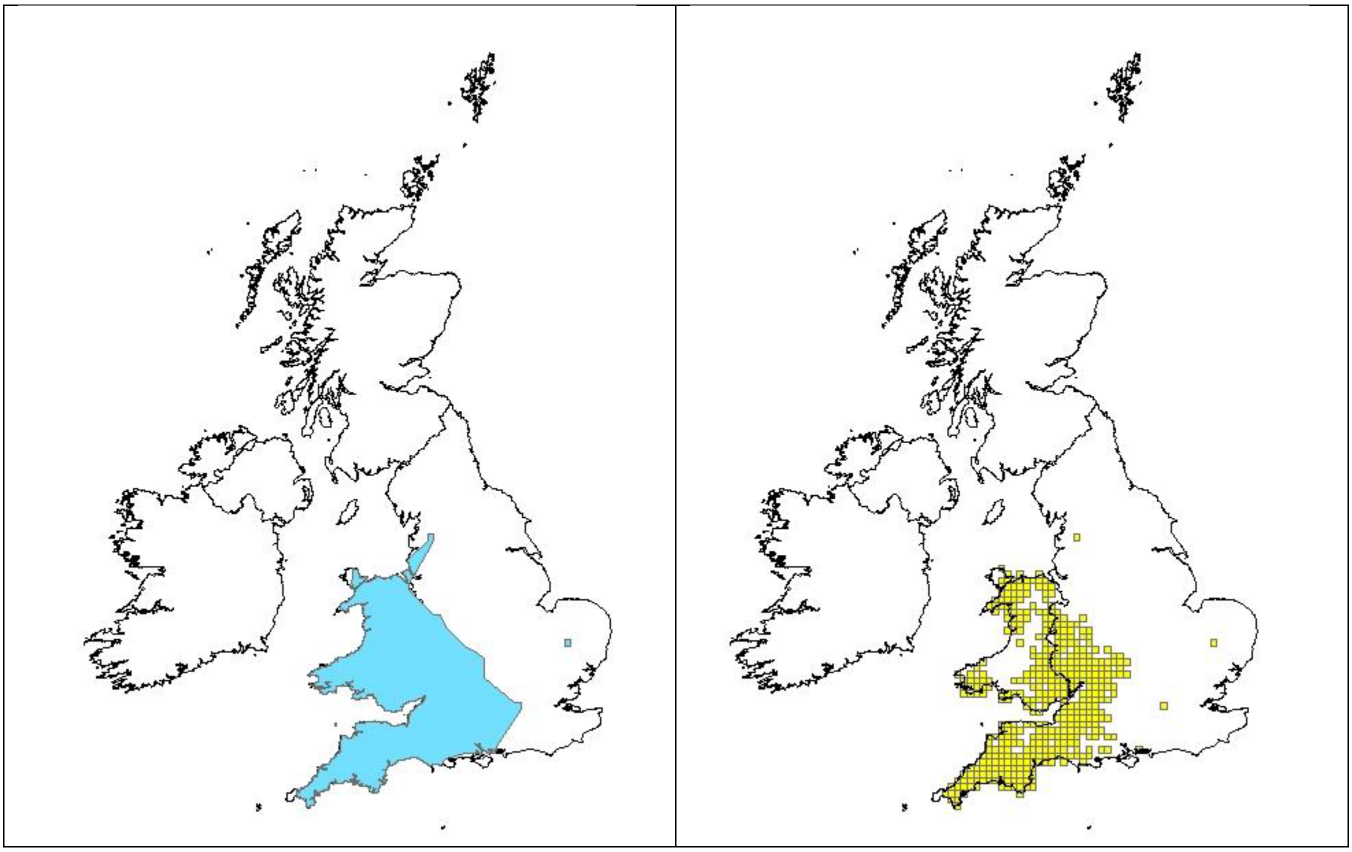
1e) *Plecotus auritus* – Brown long eared bat (JNCC species code: S1326)



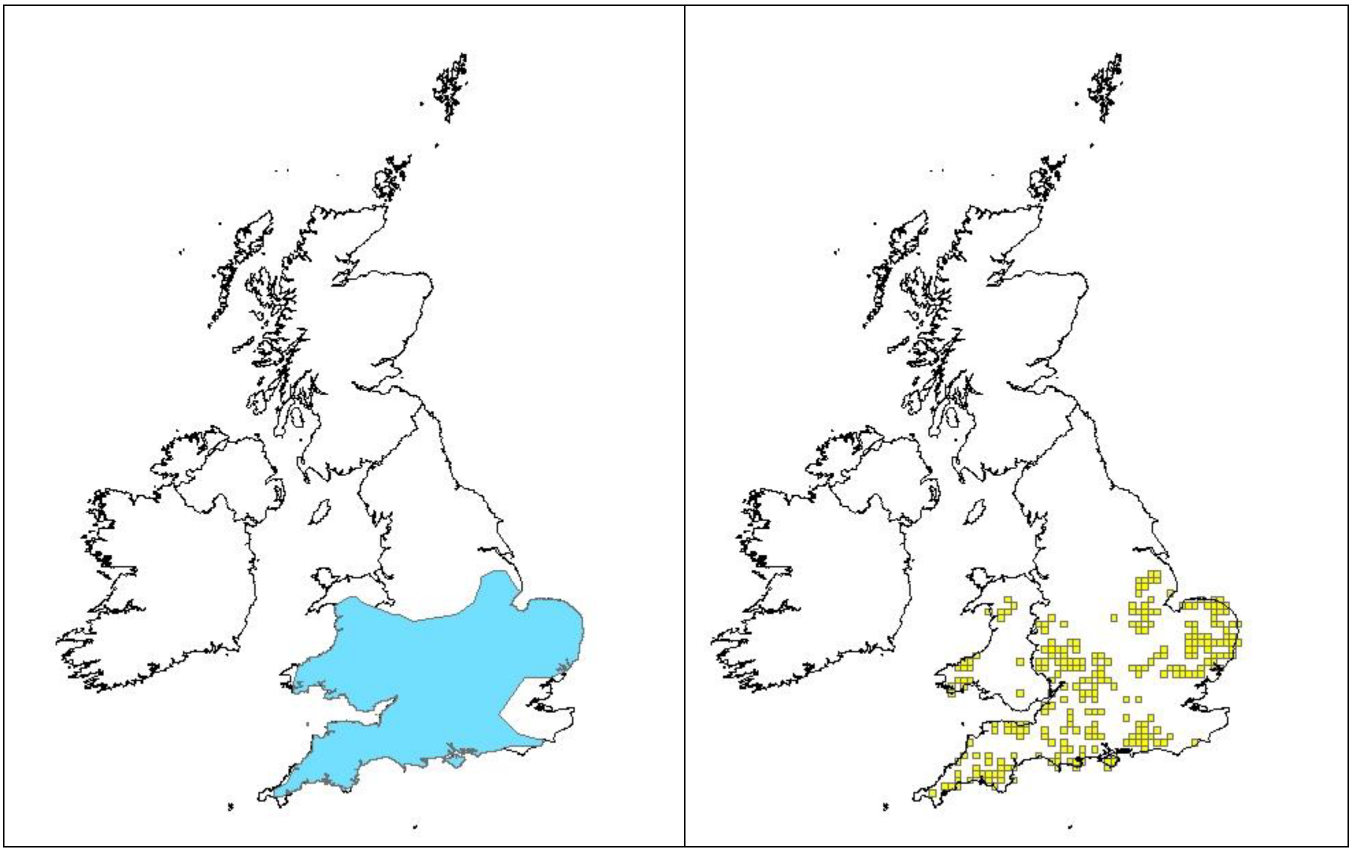
1f) *Myotis nattereri* – Natterer’s bat (JNCC species code: S1322)



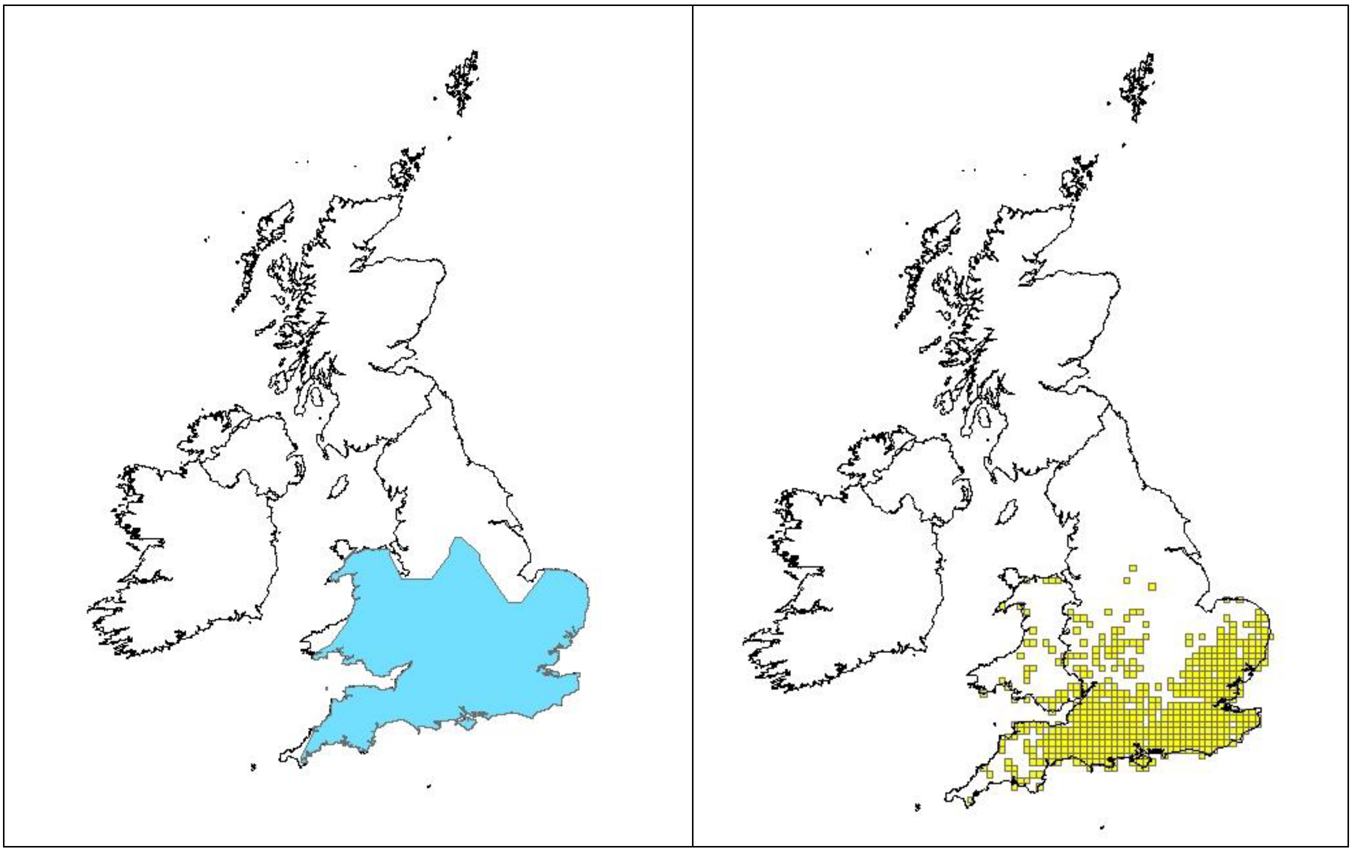
2a) *Rhinolophus ferrumequinum* – Greater horseshoe bat (JNCC species code: S1304)



2b) *Rhinolophus hipposideros* – Lesser horseshoe bat (JNCC species code: S1303)



2c) *Barbastella barbastellus*– Barbastelle (JNCC species code: S1308)



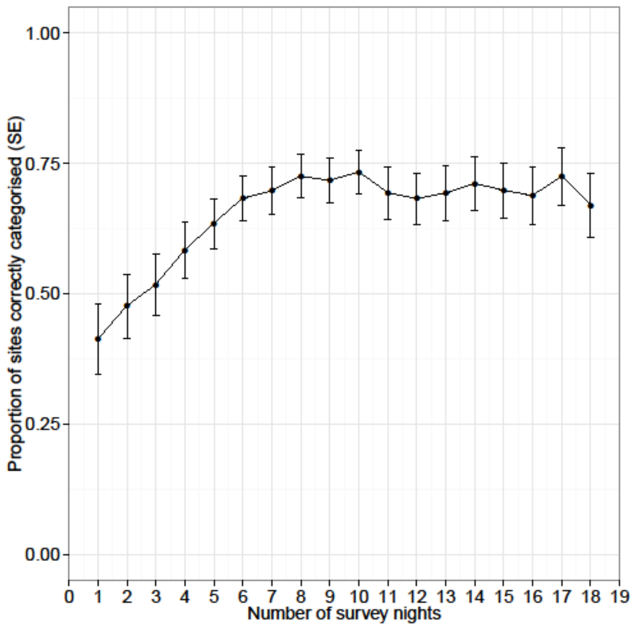
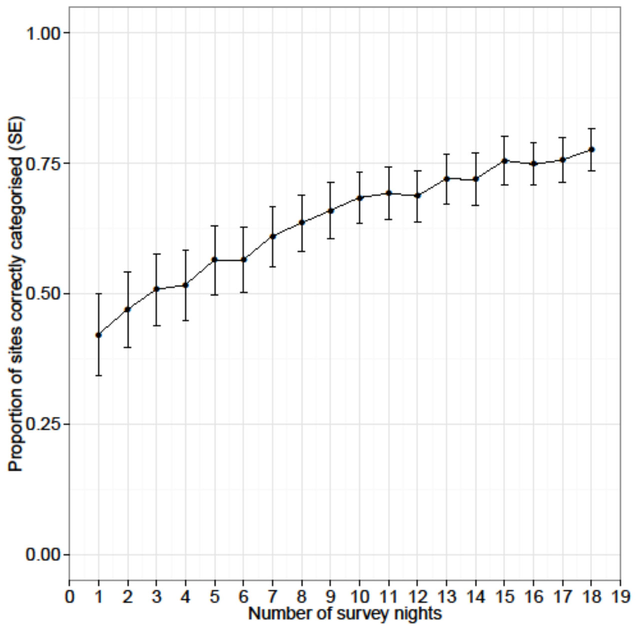
2d) *Eptesicus serotinus*– Serotine (JNCC species code: S1327)

APPENDIX 2: Bat activity recorded on transect routes at sites surveyed in 2013.

Site ID	Distance from turbine (m)	Total Passes	Count of 10m points	Proportion with bats
2	50	2	974	0.002
2	100	0	504	0.000
2	150	0	363	0.000
2	200	0	158	0.000
5	50	37	741	0.050
5	100	4	775	0.005
5	150	6	568	0.011
5	200	9	598	0.015
8	50	10	314	0.032
8	100	0	136	0.000
8	150	17	254	0.067
8	200	10	361	0.028
9	50	13	1068	0.012
9	100	3	1041	0.003
9	150	5	756	0.007
9	200	19	808	0.024
11	50	10	854	0.012
11	100	9	822	0.011
11	150	4	824	0.005
11	200	15	400	0.038
14	50	1	753	0.001
14	100	45	994	0.045
14	150	35	533	0.066
14	200	29	271	0.107
16	50	43	1131	0.038
16	100	13	711	0.018
16	150	22	627	0.035
16	200	3	294	0.010
20	50	10	546	0.018
20	100	24	862	0.028
20	150	63	1448	0.044
20	200	49	1214	0.040
21	50	8	544	0.015
21	100	38	364	0.104
21	150	11	521	0.021
21	200	1	652	0.002
26	50	4	454	0.009
26	100	21	410	0.051
26	150	37	323	0.115
26	200	31	239	0.130
30	50	8	385	0.021
30	100	12	216	0.056
30	150	48	169	0.284
30	200	58	175	0.331
31	50	10	42	0.238
31	100	8	37	0.216
31	150	14	82	0.171
31	200	11	100	0.110
35	50	8	1417	0.006

35	100	7	710	0.010
35	150	16	654	0.024
35	200	7	646	0.011
37	50	0	1010	0.000
37	100	1	667	0.001
37	150	3	665	0.005
37	200	2	639	0.003
38	50	5	920	0.005
38	100	9	364	0.025
38	150	11	294	0.037
38	200	3	376	0.008
39	50	2	184	0.011
39	100	12	443	0.027
39	150	24	471	0.051
39	200	22	570	0.039
40	50	3	1022	0.003
40	100	5	679	0.007
40	150	12	365	0.033
40	200	3	150	0.020
42	50	7	988	0.007
42	100	10	763	0.013
42	150	5	538	0.009
42	200	15	393	0.038
46	50	6	238	0.025
46	100	22	518	0.042
46	150	15	772	0.019
46	200	50	773	0.065

APPENDIX 3: Proportion of sites correctly categorised as low, medium or high activity, based on tertiles of sites, using the maximum nightly pass rate across 3 detectors. All nights and only sites where a species was detected at a site were included.

Ground	**Height**
Soprano pipistrelle	
	
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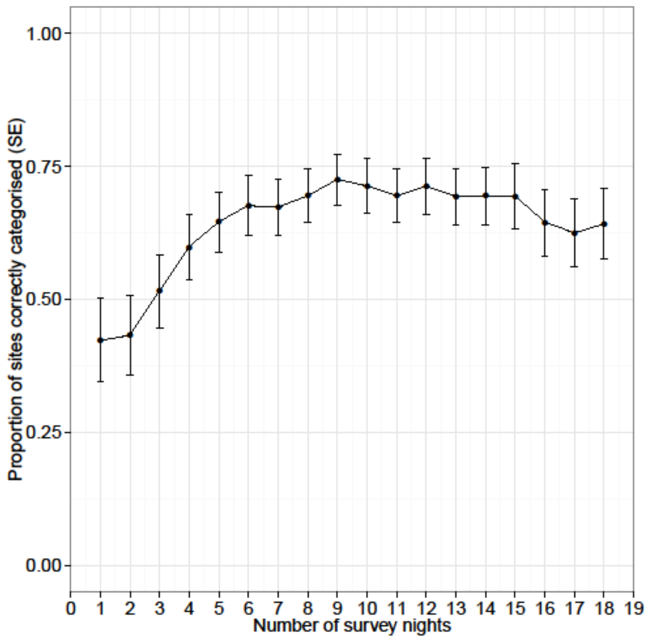	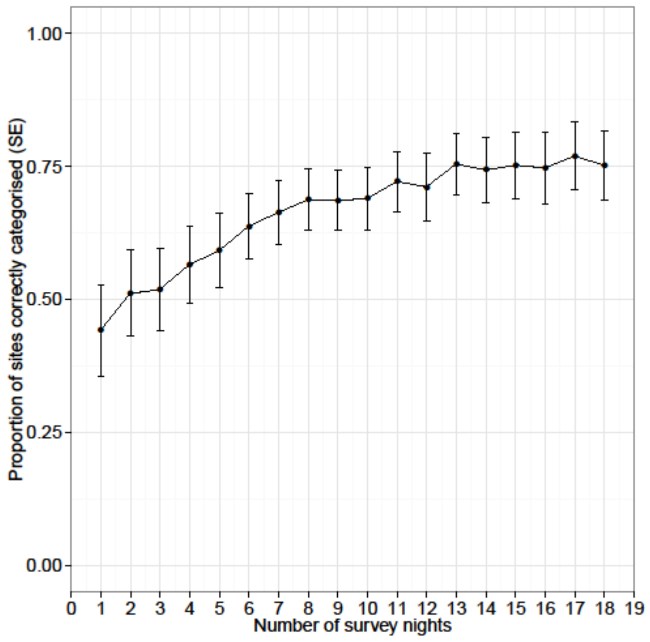

APPENDIX 4: Effectiveness of Search Dogs Compared With Human Observers in Locating Bat Carcasses at Wind-Turbine Sites: A Blinded Randomized Trial (see attached pdf for full text)

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Wind Energy and Wildlife Conservation

Effectiveness of Search Dogs Compared With Human Observers in Locating Bat Carcasses at Wind-Turbine Sites: A Blinded Randomized Trial

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ABSTRACT With the expansion of wind-energy generation, there is a growing need to develop accurate and efficient methods to detect bat casualties resulting from turbine collision and barotrauma. We conducted a formal blinded trial comparing the abilities of search dogs and human observers to locate bat carcasses. Dogs located 73% (46/63) of bats, whereas humans found 20% (12/60). We therefore recommend search dogs as an effective means of monitoring bat fatalities, particularly when a high degree of search accuracy is important. This includes surveys for rare species, or cases where searches are limited in extent or duration, because the application of correction factors is problematic where very few or no casualties are found. The dogs averaged 40 min to complete a survey, which was <25% of the time taken by humans. At large sites, the high initial set-up costs for search dogs can therefore be offset by the increased number of surveys that can be conducted within a given time. However, care must be taken with the selection and training of the dogs and handlers to produce consistent results. To allow fatality rates to be estimated from the number of casualties located, it is essential that assessments of the accuracy of the dog–handler team are made at each site. © 2013 The Wildlife Society.

KEY WORDS bat, carcass, casualty, conservation, scent, search dog, turbine, wind energy.

Wind-energy generation is undergoing a rapid global expansion and currently is the fastest growing sector of the renewable energy market. Unfortunately wind turbines, whilst being a relatively "clean" method of energy production, can present a threat to wildlife. Collisions by birds, particularly raptors, have been widely reported (Lucas et al. 2004, Drewitt and Langstone 2008). More recently, the risk to bats, both from direct impact and from barotrauma has been highlighted (Kunz et al. 2007, Rydell et al. 2010). However, evidence currently available for bats is limited. Most data are derived from anecdotal reports, usually obtained incidentally during walking transect surveys for bird fatalities. Studies are beginning to be conducted at wind-energy installations, particularly in the United States, which do search systematically for bat casualties, attempt to identify risk factors for collisions (Barclay et al. 2007, Smallwood and Karas 2010), and test potential mitigation techniques (Baerwald et al. 2009, Arnett et al. 2011). However, most work remains unpublished, and in many countries outside the United States, few, if any, bat surveys are conducted. Given the tremendous diversity in bat species composition and abundance, even at relatively small spatial scales, there is an urgent, international need for further research to establish local risks and develop appropriate mitigation.

A major barrier to such studies is the difficulty of conducting effective surveys for bat carcasses. Most species recorded as frequent casualties at wind-energy installations are extremely small. In Europe, for example, the common pipistrelle (*Pipistrellus pipistrellus*) weighs 3.5–8 g and has a head–body length of 36–51 mm; and in North America, the little brown bat (*Myotis lucifugus*) weighs 3.1–14.4 g and has a head–body length of 79–93 mm (Schober and Grimmberger 1997). This, coupled with their cryptic coloration, makes them difficult to spot. Yet estimates of true impacts on local populations depend on accurate quantification of casualty rates. Making these estimates within reasonable confidence limits becomes increasingly problematic the closer the underlying casualty rate is to zero. Even where kill rates for bats

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APPENDIX 5: A rapid PCR based test for identification of fifteen species of British bats

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Abstract

The development of a rapid method for identification of fifteen species of British bat is described. These are: *Barbastella barbastellus*, *Eptesicus serotinus*, *Myotis bechsteinii*, *Myotis daubentonii*, *Myotis mystacinus*, *Myotis nattereri*, *Nyctalus leisleri*, *Nyctalus noctula*, *Plecotus auritus*, *Pipistrellus nathusii*, *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus, Plecotus austriacus*, *Rhinolophus ferrumequinum* and *Rhinolophus hipposideros.* A multiplex of 5 fluorescently-tagged primers and 15 species-specific primers designed to regions of mitochondrial control region, cytochrome *b* and NADH dehydrogenase produces clear peaks when visualised on a DNA sequencer. The multiplex also includes primers to test for DNA quality and primers specific for the Y-chromosome as a test for sex. This method will aid high throughput species identification of British bats, and will particularly aid work involving partial or decomposing specimens, such as casualties from vehicle or wind turbine collision, or those submitted for rabies screening.

Keywords: Chiroptera, control region, cytochrome *b*, mtDNA, Cryptic species, *Myotis*,

Introduction

Bats are given strict legal protection across Europe and efforts are being made to reverse their historical population declines. Nevertheless, they face numerous anthropogenic challenges, ranging from habitat loss to direct mortality from vehicle and wind turbine collisions and domestic cat predation. Infectious disease poses additional threats: in North America, White Nose Syndrome (WNS) has decimated populations (Frick et al. 2010). The causal pathogen for WNS has also been isolated in a number of European species, though to date without any records of mass mortality (Wibbelt et al. 2010, Puechmaille et al. 2011). Human health may also be endangered by infectious agents of bats such as the lyssaviruses (rabies) (Lumio et al. 1986, Fooks et al. 2003, Mollgaard 1985)). Whether initiated by a concern for public health or for bat conservation, accurate species identification of specimens is crucial if research and management are to be meaningful: bats are a diverse group, and this is reflected in their ecology and epidemiology. For example, sympatric *Myotis* species have marked differences in their social structures (August et al. subm.) and prevalence of lyssaviruses (Schatz et al. 2013); and while Nathusius's pipistrelle (*Pipistrellus nathusii*) is known to be a long-distance migrant, common and soprano pipistrelles (*P. pipistrellus* and *P. pygmaeus*) are sedentary (Moussy et al. 2013).

Species identification is usually based on morphological characteristics. However, there are a number of similar and cryptic species, particularly in the genera *Pipistrellus* and *Myotis*, and identification frequently depends on simultaneous assessment of a range of characteristics, none of which is individually diagnostic. In addition, when samples are degraded or incomplete (for example dead specimens submitted for disease screening), morphological identification is not always possible. Therefore it has become commonplace to use genetic identification methods to complement morphological identification. One approach is to sequence regions of mitochondrial DNA followed by BLAST analysis or phylogenetic analysis of the sequence (Barratt et al. 1997, Mayer et al. 2007). However this can be time consuming when sample numbers are high. Another approach is to use the polymerase chain reaction (PCR) with species-specific primers; a technique that has been used, for example for differentiating *Myotis mystacinus*, *M. brandtii* and *M. alcathoe* based on the NADH dehydrogenase (*ND1*) gene (Boston et al. 2011), and *Pipistrellus pipistrellus* and *P. pygmaeus* based on the cytochrome *b* gene (Kanuch et al. 2007).

Our aim was to design a rapid PCR-based test to distinguish between all common species of British bats. To do this we designed and tested a range of species-specific primers for the mitochondrial control region, and also included primers previously designed for species of *Myotis* and *Pipistrellus* (Kanuch et al. 2007, Boston et al. 2011).

Methods

DNA extraction

Genomic DNA was extracted from the wing tissue of 64 bats belonging to 15 different British species. The number of individuals sampled for each species, and their geographical origins are given in Table 1. The HotSHOT method (Truett et al. 2000) was used, but was modified by the addition of an initial digestion step with 10 µl 20 mg/ml proteinase K at 56 °C for 1 hour in the lysis buffer.

Control region sequencing

Primers were designed for PCR amplification mitochondrial control region from an alignment of complete mitochondrial genomes from five diverse bat species (*Artibeus jamaicensis*, accession no. AF061340; *Pipistrellus abramus*, AB061528; *Plecotus auritus*, HM164052; *Mystacina tuberculata*, AY960981; *Rhinolophus formosae*, EU166918). All alignments in this study were made using Clustal W (Thompson et al. 1997) in BioEdit, version 7.2.3 (Hall 1999). The control region of several species was amplified and sequenced (Beckman Genomics UK) in both directions using various combinations of the forward primers CR3f, CYRCCHTCARCACCCAAAGCTG; CR9f, GGTCTTGTAAACCRRMRAAGGG and reverse primers CR2r, ATGGGCTGATTAGTCATKAAYCCATCG; CR3r, ATGYSKCGGCATGGGCTGATTAGTC and CR9r, CCTGAAGHAAGAACCAGATGYCGTTTAC. The PCR conditions for the reaction consisted of 10 µl multiplex Type-it Microsatellite PCR Kit (Qiagen) with 0.15 µM of each primers and 1 µl DNA, made up with water to give a total volume of 20 µl per reaction. PCR was conducted using a 'Touchdown' protocol with a successively reducing annealing temperature, consisting of 5 mins at 95 °C followed by 35 cycles of 30s at 95 °C, 30s at the annealing temperature (5 cycles each at 62, 58, 55, 53, 51, 49 °C) and 1 min at 72 °C, then 1 min at 72 °C and 35 mins at 60 °C (modified from Hamilton and Tyler 2008). Products from five independent reactions were pooled for sequencing. Consensus sequences for each species were assembled from electropherogram alignments using DNABASER (http://www.dnabaser.com/home.html). Accession numbers for new sequences obtained in this study are *Plecotus auritus* (KJ150736), *Myotis bechsteinii* (KJ150730), *Myotis nattereri* (KJ150733), *Nyctalus noctula* (KJ150734) and *Rhinolophus hipposideros* (KJ150732).

Species-specific primer design

Alignments of DNA sequences obtained in this study and those from other published studies obtained from National Center for Biotechnology Information (NCBI) (see Table 2) were used to design a suite of species specific primers (Table 3). Other species-specific primers used in this study have been published previously (Kanuch et al. 2007, Boston et al. 2011) or were modified from them (Table 3). For each species, the PCR product(s) were produced using one specific primer and another fluorescently labelled primer, which in some cases was common to several species. To determine the exact size of the PCR product, PCR products were run on a DNA sequencer (CEQ 8000 Genetic Analysis System, Beckman Coulter) and fragment sizes were determined using CEQ 8000 Genetic Analysis System, Version 9.0 (Beckman Coulter). We aimed for PCR products to be less than 400bp, to aid identification in cases where DNA is degraded.

Species-specific primers were designed from DNA sequence alignments to regions that are conserved within the species, but differ from closely related species. Sequences from up to five individuals (if available) from each species were included, originating from different geographic locations and representing different haplotypes (see Table 2). Primer specificity was confirmed by Blastn. Primers for *M. bechsteinii, M. nattereri, N. noctula, P. auritus, P. austriacus,* and *R. ferrumequinum* were designed from control region alignments. For the control region, each species-specific primer produced two products with two fluorescently labelled universal reverse primers (CR2r, CR9r) resulting in two distinct (green and black in Figure 1) fragments differing in size by approximately 75 bp. The exception was *R. hipposideros* for which a single primer pair was used.

For *Pipistrellus* species, the published species-specific primers for amplification of the *cytb* gene in *P. pipistrellus* and *P. pygmaeus* (Kanuch et al. 2007) were used. A new specific primer for *P. nathusii* and a new universal primer were designed to reduce the sizes of the amplification products. The remaining primers were designed to the *ND1* region, using a combination of published primers (Boston et al. 2011) and primers designed in this study. PCR conditions were as described above except 10µl reaction volumes were used with 0.15 µM (0.015 µl) of each species-specific primer, 1.2 µM (0.12 µl) of the *Zfx* primers, 1.8 µM (0.18 µl) of the *Zfy* primers. The multiplex was then tested with multiple individuals for each species that had been visually identified (see Table 1). To test for the presence of PCR-amplifiable bat DNA, primers for amplification of a zinc finger gene present on the X chromosome (*Zfx*) was used by (Bryja and Konecny 2003), were also included in the multiplex mix.

Primers for identification of sex (Korstian et al. 2013) were incorporated within multiplex, designed to amplify the *Zfy* gene present on the Y chromosome (details on species) . The presence/absence of Y chromosome-related fragments was analysed for 8 known male and female individuals. The Y chromosome test was also conducted, using the PCR conditions specified by (Korstian et al. 2013), and analysed using agarose gel electrophoresis.

Results

Species identification

Our new test was able to identify all 15 species of British bat we aimed to identify (Tables 1 and 3). All species-specific primer sets produced peaks which were unique to each species in the range of 129-382 bp. Examples of pictures are shown in Figure 1 and size ranges are provided in Table 3. The primer pair for *Zfx* gave a band peak at 234-235 bp for all species, serving as a positive control for amplifiable DNA.

Sex identification

The primers for identification of sex (Korstian et al. 2013) gave bands of the expected size when included within the multiplex, but also introduced a blue peak at 167 bp. There was a good correlation between the strength of bands visualised on the gel and the peak intensity of sequence fragments obtained from the multiplex mix for 50 individuals of undetermined sex for a range of species. However, small sequence fragments were present in the size range expected for the Y chromosome test in females as well as males. Small peaks also occurred in water controls so are likely a PCR artefact, but contamination of the tissue samples can also not be ruled out. Therefore while some individuals were clearly identified some males due to the intensity of the peak there were also cases where it was difficult to clearly identify sex. The bleaching protocol prior to DNA extraction of may aid removal of surface contamination (Gilbert et al. 2006), but also new primers outside the range of the background peaks would also be required for unequivocal sex determination.

Discussion

This method will provide a fast and accurate method for identification of British bats including cryptic species and some that are difficult to differentiate morphologically. It will be particularly useful with dead specimens, which are commonly difficult to identify at post-mortem, either because the preservation method obscures key features, or because the carcasses are incomplete or degraded. As the PCR products are relatively small, the method has potential for identification of droppings, and was able to identified 3 *Myotis nattereri* using DNA isolated from faeces, but will need to be tested using a wider range of species. The test does not include primers for *M. brandtii and M. alcathoe*. However species specific *ND1* primers have been designed previously for these species (Boston et al. 2011) and these are potentially compatible with this multiplex as they are predicted to give peaks within the detectable range that do not overlap with the other species. There is also potential to expand the method to include to all European species. The primer sets could also be used individually using standard PCR and agarose gel electrophoresis.

Table 1. Details of the origin of individual bats used in this study to test the sensitivity and specificity of primers for each species.

Species	Common name	No. verified (matching visual id)	Origin
Barbastella barbastellus	Barbastelle	1	Devon
Eptesicus serotinus	Serotine	4	Wiltshire, Devon, Cornwall
Myotis bechsteinii	Bechstein’s	4	Devon
Myotis daubentonii	Daubenton’s	3	Devon
Myotis mystacinus	Whiskered	2	Devon
Myotis nattereri	Natterer’s	4	Devon
Nyctalus leisleri	Leisler’s	1	Bristol
Nyctalus noctula	Noctule	5	Devon, Cornwall
Plecotus auritus	Brown long eared	2	Devon

Pipistrellus nathusii	Nathusius's pipistrelle	2	Devon
Pipistrellus pipistrellus	Common pipistrelle	15	Devon, Cornwall
Pipistrellus pygmaeus	Soprano pipistrelle	9	Devon, Cornwall
Plecotus austriacus	Grey long eared	3	Devon
Rhinolophus ferrumequinum	Greater horseshoe	4	Devon
Rhinolophus hipposideros	Lesser horseshoe	5	Devon

Table 2. Details of DNA sequence alignments used in the design of species-specific primers.

	Species	Region	Accession numbers and geographic origin
Alignment 1	*Plecotus* spp.	control region	*Plecotus auritus*: AY030077 - Austria (Spitzenberger et al. 2001); HM164052 - Korea; KJ150736 – UK (this study); *Plecotus austriacus*: AF498260, AY030054 - Austria (Spitzenberger et al. 2001, Spitzenberger et al. 2002); AF515182, AF515184 - Mediterranean region (Juste et al. 2004)
Alignment 2	*Rhinolophus* spp.	control region	*Rhinolophus hipposideros*: KC978716, KC978513 (Spain, Dool et al 2013); KC978639 - Middle East, (Dool et al. 2013); DQ297610 - East Asia (Li et al. 2006); KJ150732 - UK (this study); *Rhinolophus ferrumequinum*: AY923063 – Turkey (Bilgin et al. 2009); RFU95335 - Switzerland or Luxemburg (Wilkinson et al. 1997); xxxxx UK (this study)
Alignment 3	*Myotis* spp.	control region	*Myotis bechsteinii*: EU531424, EU531434, EU531435 - Balkan region (Kerth et al. 2008); AY030078 -Austria {Spitzenberger, 2001 #3042); KJ150730 UK (this study) *Myotis nattereri*: JX852950, JX852949, JX852948 - Mediterranean, {Salicini, 2013 #3047}; KJ150733 – UK (this study)
Alignment 4	*Nyctalus noctula*	control region	*Nyctalus noctula*: U95353 - Germany (Wilkinson et al. 1997); KJ150734 - UK (this study)
Alignment 5	*Pipistrellus pipistrellus*	*cytb*	KF218401, KF218401 - Turkey (Coraman et al. 2013) Coraman et al 2013); EU360676, EU360671, EU360662, DQ120853 - Spain (Ibanez et al. 2006, Garcia-Mudarra et al. 2009)

"	*Pipistrellus pygmaeus*	*cytb*	JX566938, JX566929 - Portugal (Medinas et al. 2013); EU084884 - Cyprus (Hulva et al. 2007); EU084882 - Iran (Hulva et al. 2007)
"	*Pipistrellus nathusii*	*cytb*	EU084883 - Cyprus (Hulva et al. 2007); DQ120850, DQ120849 - Spain (Ibanez et al. 2006); U95510, U95509 - UK (Barratt et al. 1997); AJ504446 - Switzerland (Stadelmann et al. 2004)
Alignment 6	*Barbastella barbastellus*	*ND1*	KF218432, KF218431 - Turkey (Coraman et al. 2013); DQ915032 - Greece (Mayer et al. 2007); DQ915031 - Hungary (Mayer et al. 2007)
"	*Myotis mystacinus*	*ND1*	AF401376 - Germany (Kiefer et al. 2002)), AB106583 - Japan (Kawai et al. 2004); KF218482 - Turkey, (Coraman et al. 2013); JX645318, JX645316 - Poland (Bogdanowicz et al. 2012))
"	*Myotis daubentonii*	*ND1*	KF218481 - Turkey (Coraman et al. 2013); HQ657356, HQ657354, HQ657328 - UK; AF401452 - Germany (Mayer et al. 2007)
"	*Nyctalus leisleri*	*ND1*	DQ915046 - Spain, (Mayer et al. 2007), KF218487 - Turkey (Coraman et al. 2013); DQ915013, DQ915012 - Greece, (Mayer et al. 2007); DQ887582 - Switzerland (Salgueiro et al. 2007))
"	*Eptesicus serotinus*	*ND1*	DQ887580 - Morocco, (Salgueiro et al. 2007); KF218441 - Turkey (Coraman et al. 2013); DQ915024 - Greece, (Mayer et al. 2007); AF401472 - Germany, (Mayer and Von Helversen 2001b, a); EU786984 - Spain (Juste et al. 2004)

Table 3. Primers used in this study

Species	Common name	Region amplified	Forward primers (primer code)	Reverse primers (primer code)	Product size 2	Reverse primer	Product size 1
Plecotus auritus	Brown long eared	Mt. control region	TAGATCTAAGAATTCAGCT	†D2CCTGAAGHAAGAACCAGATGYCGTTTAC (CR9r)	129-132	†D3ATGGGCTGATTAGTCATKAAYCCATCG (CR2r)	204-207
Myotis bechsteinii	Bechstein’s	Mt. control region	CGACCAATGGAAATCCCATGATCTA	“	138-141	“	213-216‖
Myotis nattereri	Natterer’s	Mt. control region	TAAGTCAAACAATATCCAGTCAAC	“	176-179‡	“	251-254‡
Nyctalus noctula	Noctule	Mt. control region	CATAATACATCTAATGCGTA	“	213-216	“	289-292
Plecotus austriacus	Grey long eared	Mt. control region	TGGAATGTTTATATTACATAGT	“	225-228	“	299-302
Rhinolophus ferrumequinum	Greater horseshoe	Mt. control region	GAATCAAGAAATTACATAAGAC	“	305-308	“	380-382
Rhinolophus hipposideros	Lesser horseshoe	Mt. control region	†D4: GGTCTTGTAAACCRRMRAAGGG (CR9f)	ATTACATAGGGGCGGAGGGTTG	173-186*		
Pipistrellus pipistrellus	Common pipistrelle	Mt. CytB	TCGAGATGTGAATTACGGATG[1] (Ppip-F2)	†D3CRGCRAATAGTARRATAACYCC	156-158#		
Pipistrellus nathusii	Nathusius's pipistrelle	Mt. CytB	TCCTCGCTATACATTATACATC	“	214-218		
Pipistrellus pygmaeus	Soprano pipistrelle	Mt. CytB	CTGTCTAGGGCTGCAAAT[1] (Ppyg-F)	“	250-253		
Myotis daubentonii	Daubenton’s	Mt. ND1	ACGCTTTAATTGGGGCCCTACG	†D2GTWGAGATRAATCATATTAT	189-190		
Eptesicus	Serotine	Mt. ND1	CGCCATTATATGAT	“	225-		

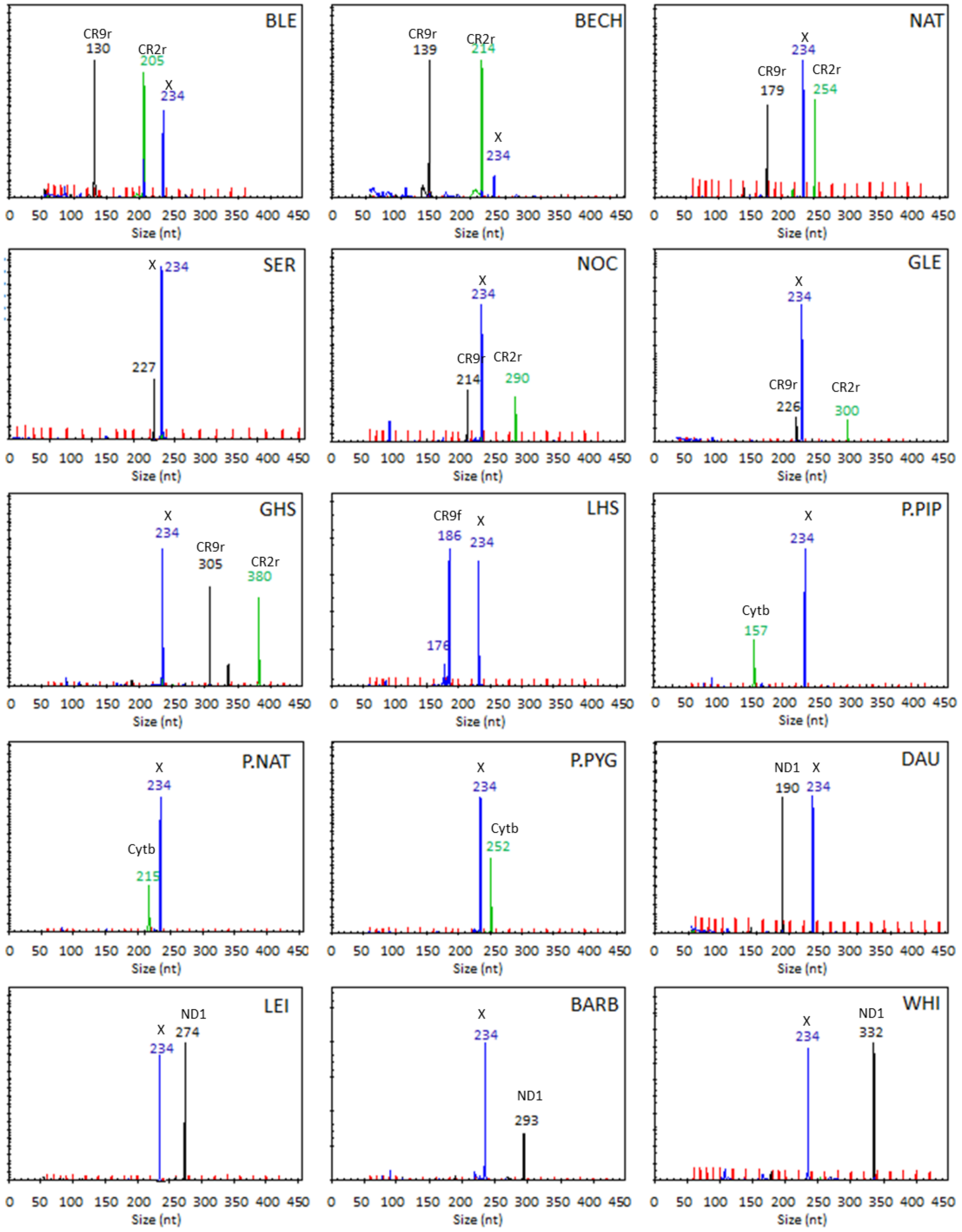
serotinus			CAGGATGAGCC		228
Nyctalus leisleri	Leisler's	Mt. ND1	GAATTTAAGTGTTTTGTTTATA	"	273-275
Barbastella barbastellus	Barbastelle	Mt. ND1	CGCACCCACTGGTAAACATAA	"	293-295
Myotis mystacinus	Whiskered	Mt. ND1	CCCTAACYCTAGCCCTAACAA[2] (ND1Mys04_f)	"	332-336
All species (positive control)		*Zfx gene*	†D4AGTCAAGGGRTGTCCATCR[3]	GTTTGYASACCAGGTTCCTC	234-235
All species (sex test)		*Zfy gene*	GGTRAGDGCACAYRAGTTCCACA	†D3TGCYATTACAAAACCTTTRTAGATAC	85-90*

† universal fluorescent primer colours as follows: D2=black, D3= green, D4=blue; * multiple peaks in this range of size fragments; ǂ minor peaks often present at b143 and g 218; # minor peaks sometimes present at b214 and g290; ‖ green peak not always present

[1] (Kanuch et al. 2007), [2] (Boston et al. 2011). [3] (Bryja and Konecny 2003). The published species specific *ND1* primers (Boston et al. 2011) are predicted to give peaks with the labelled reverse primer, but were not tested in this study: *M. brandtii* (ND1Bra02_F: ACGAAGTAACCCTGGCCATC) predicted size = 143 bp and and *M. alcathoe* (ND1Alc01f, GCCCTAACCTTGGCTCTCAT and ND1Alc06_f: AGCCGTATACGCTATCTTATGGTC) predicted sizes = 336 and 234 bp respectively. The other primer from this study for *M.mystacinus* is included and the predicted size of 335 bp and therefore matches the sequencer-determined size.

Figure Legends

Figure 1. Example electropherograms from each species of bat used in this study. Small red peaks are size standard. Numbers above peaks are sizes (bp). Letters above peaks indicate the gene primer set giving rise to the peak. X = peak given by primers for a region to the X chromosome, that serves as a positive control for PCR-amplifiable DNA.



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