Using Bioindicator Bat Activity to Compare Ex-Agricultural Habitats: A case study of Knepp Estate, West Sussex

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Using Bioindicator Bat Activity to Compare Ex-Agricultural Habitats: A case study of Knepp Estate, West Sussex

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Abstract

The use of bioindicators is an important tool in the monitoring of adaptive management conservation. Rewilding projects require effective monitoring of shifting baselines. Bat activity has been identified as a useful indicator for monitoring agricultural intensification and dissipation. The activity of bat species across Knepp Estate, a rewilding project in West Sussex, was monitored over a nine day period in late August/early September 2016. The estate was divided into three sections according to agricultural history prior to the rewilding project, and activity of bioindicator bat species in each area was compared. Through analysing the activity of species in each block, habitat characteristics could be inferred. The Northern block (ex-dairy) was found to have the highest over-all rate of bat activity, whereas activity in the Middle block (historic parkland) and Southern block (ex-arable) were found to be equal. Findings suggest that although large herbivores may increase levels of bat activity, they may prevent progress of natural habitat restoration. A patchwork of differing habitats results in greater diversity of bat species. These inferences are supported by scientific literature and estate records. Aspects for improvement in further studies are highlighted, as are areas for further research. This study concludes that bats are suitable bioindicators for monitoring habitat health in the Knepp rewilding project, and that they show varied progression of habitat regeneration across the estate.

Keywords: bats, rewilding, bioindicators, diversity, activity, agriculture.

Introduction

Use of Bioindicators: Ecosystems worldwide are facing major declines in biodiversity (de Groot *et al*., 1995; Jones *et al*., 2009). Accurate observations and reliable monitoring are essential to determine population trajectories, vulnerable species and areas of conservation priorities

(Jones *et al*., 2009). One of the most efficient and recently widely discussed methods of monitoring ecosystems is through the use of bioindicators. Many studies discuss the benefits of using bioindicators, as a method for saving time and resources (Park, 2015). Using insectivorous species as bioindicators saves a great amount of time and effort that may otherwise may have been spent on insect monitoring for the same results. Insect diversity is of vital importance in assessing ecosystem state (McGeogh, 1998). The use of insectivorous mammals as bioindicators has been discussed extensively in scientific literature (Pocock and Jennings, 2008). In particular, bats have been used to assess the impact of agricultural intensification (Pocock and Jennings, 2008). It is therefore hypothesised that UK bat species may be an efficient metric by which rewilding strategies can be compared (Park, 2015). The comparison of restoration progress for differing agricultural land is important, so that habitats with the most chance of regeneration can be identified and areas can be prioritised. Bats are viable bioindicators because they are taxonomically stable, yet vulnerable to habitat change. All UK bat species are insectivorous, and are easier to monitor than invertebrate diversity. It is believed that a global monitoring system for bats would be a valuable tool in monitoring the effects of large-scale threats such as climate change (Jones *et al.,* 2009). Bats as bioindicators have been utilized in varying ecosystem monitoring systems across the world, often successfully (Catto *et al*., 2003; Everard, 2008; Medellin *et al.,* 2000). There are now nationwide schemes in place for monitoring of UK bat species, which have shown an increase in some bat species numbers over recent years (JNCC, 2015). Monitoring activity of insectivorous bioindicators is easier, cheaper, and infers more information on insect diversity through identification of foraging preferences, than an abundance count. Abundance may be misleading as an indicator, as habitat fragmentation is not taken into consideration, and occupancy alone does not suggest which part of a bats extensive range they are most reliant on (Henry *et al.,* 2007). Behavioural monitoring shows habitat preferences, and may in conjunction with roost surveys, determine the most valuable habitat features for certain species (Siemers and Schnitzler, 2004).

Bioindicator bat species: This study will focus on bioindicator bat species, those identified as particularly informative of their environment, as listed by the JNCC (2015). For the purposes of this study the species identified as viable indicators for the study site were Common pipistrelle *(Pipistrellus pipistrellus),* Soprano pipistrelle (*Pipistrellus pygmaeus),* Daubentons bat *(Myotis daubentonii)* and Noctule bat (*Nyctalus noctula),* due to their abundance and consistent appearance across the estate (Greenaway, 2011). Each species indicates the state of a different aspect of its environment (JNCC, 2015). Pipistrelle bats are used as indicators of quality in riparian habitats (Scott *et al.,* 2010). High quality riparian habitat means more pipistrelle activity. *P. pygmaeus* activity is higher than *P. pipistrellus* in higher quality riparian habitats, but previous studies show there is little difference between the two species in low quality habitat, where both are relatively inactive (Scott *et al.,* 2010). Both Soprano and Common pipistrelle are very adaptable species, prevalent across the UK and increasing in numbers. They are the most adaptable British bat species to anthropogenic change, even using street lights to catch prey attracted by the light (Arlettaz *et al.,* 2000). There is some concern that the expansion of pipistrelle bat ranges due to climate change are increasing competition with other species such as the rarer *Rhinolophus hipposideros,* the Lesser Horseshoe bat (Lundy *et al.,* 2010; Arlettaz *et al.,* 2000). Pipistrelle diet consists mainly of moths and Diptera, meaning it is in direct competition with *R. hipposideros* (Arlettaz *et al.,* 2000). It should therefore be a primary concern to assess the degree of interspecific competition between UK bat species (Vaughan, 1997). Pipistrelle roosts tend to be close to trees or buildings, where bats will emerge earlier from a roost with more cover (Jenkins *et al.,* 1998). Pipistrelle bats emerge about 35 minutes after sunset in colder climates (Swift, 1980). They may emerge later during weaning season, whereas later in the season, toward the end of summer, activity will peak just after dusk and just before dawn (Avery, 1985). Males are more active in winter then females, and pipistrelles will leave the roost to feed on especially warm winter nights. Later in the season is therefore the easiest time to monitor pipistrelles (Avery, 1985).

N. noctula stay close to wooded areas, and forage at the edges (Rachwald, 1992). They are important indicators of the quality of edge habitats, transitional areas known as ecotones, and habitat connectivity (Rachwald, 1992). *N. noctula* are widespread across Europe, though some populations contain themselves, such as Scandinavian populations, which are genetically isolated (Petit and Mayer, 2000). The monitoring of widespread species such as these is important to increase understanding of metapopulation dynamics across Europe, and identify whether migration is affected by threats such as global warming (Petit and Mayer, 2000). *N. noctula* emerge later than pipistrelles and Daubentons as is expected of larger, more conspicuous bats (Petit and Mayer, 2000; Jones and Rydell, 1994). *N. noctula* habitat use varies with reproductive state (Mackey and Racey, 2007). Non-lactating bats prefer marginal habitats, arable land/moorland areas, but typically lactating and non-lactating use broadleaved woodland and pasture. The end of summer is therefore a good time to monitor *N. noctula* as their foraging preferences are less affected by reproductive state.

M. daubentonii are used as indicators of water quality (Everard, 2007; Langton *et al*., 2010; Catto *et al*., 2003). High diversity and abundance of macro-invertebrates indicates high chemical water quality (Langton *et al.,* 2009). Daubentons activity is higher in areas with high macroinvertebrate diversity, therefore *M. daubentonii* are very vulnerable to fluctuations in water quality and pollution (Akasaka *et al.,* 2009). *M. daubentonii* activity is highest in large waterways with woodland nearby (Boonman *et al.,* 1998).

Study site background: Knepp estate is a 3500 hectare estate in West Sussex. It was intensively farmed from the 1930s to the turn of the 21st century (Rotherham and Handley, 2011). In 2001, the owners of the estate, the Burrell family, decided to establish a rewilding project with an adaptive management approach across the estate (Greenaway, 2007). The River Adur was restored, large herbivores were introduced as the ecological equivalents of Pleistocene mammals, and natural regeneration of plant species encouraged (Greenaway, 2006; Donlan *et al.,* 2006; Colson and Stone, 2000). The idea of rewilding in the south of England has long been theorised, but rarely practised in any way. Knepp Estate is a rare chance to put into practise various rewilding strategies (Rotherham and Handley, 2011); attempting to boost biodiversity in some of the most heavily populated and agriculturally exploited areas of the UK. Built on the research from Oostervasplassen, a large scale rewilding project in the Netherlands, it is hoped that the results of the Knepp project will contribute to future rewilding efforts (Rotherham and Handley, 2011). The estate is divided into three equally sized blocks by the roads intersecting it. Each of these blocks has a different history; the Northern block was dairy farmland, the Middle was (and is) an historic deer park, and the Southern was arable farmland. Each are now managed with the same approach and similar methods for the aim of rewilding. The agricultural intensification that occurred during the 20th century may have resulted in a varied restorative progress across these blocks of the estate.

Habitat variation and management: The sectioned blocks of Knepp are suitable units by which to compare the natural regeneration of natural habitats. Large herbivores are able to move between blocks by way of specialised gateways and are allowed to display their natural preferences (Rotherham and Handley, 2011). The blocks have the same clay based soil type, climate and management. However, the history of each block may affect biodiversity. The Northern block was once dairy farmland. Hedgerows were removed in the 1950s to allow more space for more cattle (Rotherham and Handley, 2011). This field system has been naturally maintained since dairy farming ceased, with grazing preventing the regeneration of hedgerows. However, the Northern block still contains the most woodland of the three blocks, with numerous plantations of various native and non-native species. Much of the headwater for Knepp Lake, which is in the Middle block, is within this area. The Middle block was (and is) a historic deer park. Though the majority of the block has always been parkland, some parts were used for arable farming in the 20th century. It has gone through the least transition over the last decade, with the habitat structure continuing as it has done for hundreds of years (Rotherham and Handley, 2011). The increased number of grazing herbivores, with the release of Exmoor ponies, red deer, and cattle, has meant that preservation of the parkland is now a natural process rather than through constant human maintenance. The Middle block has the largest single expanse of water, Knepp Lake. The Southern block was once arable farmland. Grazing herbivores were absent from the estate during most of the 20th century. The Southern block has undergone the most obvious change (Rotherham and Handley, 2011). Unlike the Northern block, hedgerows were never removed, and small fields between wide hedges show a vast amount of growth in shrubs and wildflowers since 2001 (Greenaway, 2011). Cattle and deer have always been present in the Middle and Northern block, but were absent from the Southern block during the first stage of the rewilding process. Mammal surveys throughout the years have shown the Southern block to have the highest abundance in small mammal species, many of which feed on insects (Greenaway, 2011). The majority of tourism is concentrated in the Southern block, as it shows the most obvious contrast with what it once was (Rotherham and Handley, 2011).

Application of bioindicator monitoring at Knepp: The adaptive management approach implemented at Knepp puts a great amount of pressure on effective monitoring strategies. Though the rewilding project began in 2001, a monitoring strategy was not established until 2007 (Greenaway, 2011). It is important for managers of passive management systems to have an idea of how the system is progressing in all aspects (Navarro and Pereira, 2012); for a rewilding project such as Knepp Estate, fast and efficient systems for monitoring habitat health would be extremely valuable. Insect diversity is a good indicator of ecosystem health (McGeogh, 1998), but it is not a simple process to evaluate landscape scale areas. Knepp currently has no comparative method for monitoring bat populations or activity, with sporadic surveys occasionally presenting progress every few years. The original management plan states that there are “no species of particular interest” at Knepp, with no mention at all of bat species (Colson and Stone, 2000). This is no longer the case, with numerous bat species now found at Knepp, as well as reintroduction of species such as red deer, and valuable invertebrate diversity (Greenaway, 2011). Bat biodiversity is now one of Knepp’s greatest achievements, as over the past 10 years, 16 out of the 17 resident UK bat species have been found foraging or resident at Knepp (Greenaway, 2006; Whitby, 2002; Greenaway, 2009; Love, 2005). Sussex Bat Group occasionally carries out trapping as part of wider surveys, but no comparative studies on bat populations have been carried out at Knepp (Greenaway, 2011). In Greenaway’s 2006 survey, eight species of bat were found, including Bechsteins bat, *Myotis bechsteinii*, which were then one of the UKs rarest mammals (Greenaway, 2006). Numbers of *Eptesicus serotinus* showed a significant increase in 2006 compared to an unpublished 2002 study (Greenaway, 2006; Whitby, 2002). The increase in numbers of *E. serotinus* was linked to natural grazing, which may increase dung beetle populations, an important source of prey for *E. serotinus* (Whitby, 2002). This trend seems to have continued throughout the rewilding project, with each bat survey showing slightly greater numbers and a slight increase in species richness (Greenaway, 2011). It should also be considered that over the years a significant increase in funding to the estate may have allowed for more rigorous surveying, and national trends in species increases may be reflected on a regional scale. However, there seems to be a clear increase in bat species, and it would appear the rewilding project has benefited bat biodiversity over the years.

The Southern block is expected to have the highest rate of bat activity, as denser vegetation should provide adequate foraging habitat for numerous species. Knepp is on a clay soil base which is typically considered unsuitable for intensive agriculture, but decades of work went in to making the Southern block suitable for growing plants (Rotherham and Handley, 2011). This may have benefited the restoration progress of natural plants. *Myotis nattereri* looks for prey close to dense vegetation and woodland edges, as do many larger UK bat species

(Siemers and Schnitzler, 2000). *P. pygmaeaus* relies on local woodland features, whereas more mobile species like *Myotis* bats and *P. pipistrellus* rely on larger scale variation. A patchwork of maintained pasture, trees and small woodland is ideal for the majority of microbat species (Lentini *et al.,* 2013). The mix of habitats in the Northern block should appeal to *P. pygmaeuas*, which relies on local differentiations in woodland, whereas the more mobile bats such as *P. pipistrellus* and *Mytois* species are more dependent on larger scale landscape variation (Feuntes-Montemayor *et al*., 2013). Although each blocks contains a range of microhabitats, the plentiful growth in the Southern block is mostly unimpeded by grazers. The denser vegetation here would suit the "gleaning" foraging strategy of *Myotis* bats, whereas the more open understory of woodland in the Middle and Northern blocks would appeal to the "aerial hawking" strategy of Pipistrelle bats (Fuentes-Montemayor *et al*., 2013*)*.

Hypotheses:

- Bat activity will vary across the sectioned blocks within Knepp Estate.
- Bioindicator bat activity will be highest in the area presenting the most natural regeneration since agricultural intensification ceased. This is expected to be the ex-arable farmland (Southern block).

Aims:

- To determine which species are most active.
- To determine in which area species are most active.
- To assess the viability of using bats as bioindicators at Knepp.

Methods

In order to compare differentiation of bat activity across the estate, Knepp estate was divided into three "blocks", according to historical records and geographical separation by roads (see Figure 1). Echolocation calls of bat species were monitored along approximate 6km transects through each block, with three repeats in each block. Location by block, time of occurrence and frequency (kHZ) of these calls, referred to as "passovers", were noted down, and a recording was made of each call.

Sampling: The estate was surveyed on nine occasions in late August to early September 2016. Days were not consecutive but all samples were taken within a 2 week period. Evenings were selected depending on suitable weather, humidity and wind speed. The Southern section of the estate (Southern block) was sampled on the 29th, 30th and 31st of August, the Middle section of the estate (Middle block) was sampled on the 2nd, 4th and 5th of September, and the Northern section of the estate (Northern block) was sampled on the 6th, 8th and 9th of September. An approximate transect of 6km was conducted for 100 to 120 minutes after sunset in each block, with three replicates of each (Figure 1). BCT citizen science recommendations were used to plan the methods of data collection where possible, though this was occasionally limited due to health and safety concerns over walkable habitat (BCT, 2016).

Equipment: Recordings were made on a Dodotromic Ultramic USB microphone using the Digital Biology bat detector app on a Nexus 7 tablet. A Bat Box II heterodyne bat detector was used as back up equipment.

Transects: All transects were walked in an anti-clockwise direction, as shown in Figure 1. Recordings were continuous throughout the transect. The Southern block transect was dependent on footpaths and public bridleways, as vegetation is too thick to allow a route off track. The Middle block transect was dependent on footpaths and open ground sections. A large proportion of the southern section of the Middle block was water logged, and so this was avoided in accordance with Knepp Estate health and safety protocol. A large proportion of the Northern block is open fields, and so the transect was not restricted by footpaths.

Analysis: Spectrograms of bat calls were generated using WaveSurfer software, and analysed for frequency, duration and call pattern in order to identify species (see Figure 2). Literature was referred to for species identification (Russ, 2012; Preatoni *et al*., 2005; Szewczak *et. al*, 2004; Ahlen *et al*., 2004). The timing, location by block, and species ID of each call was recorded in an Excel spreadsheet. Repeated measures ANOVA was selected for appropriate statistical analysis but could not be performed due to insufficient replicates. Species activity in comparison to location by block was found not to be normally distributed, and data transformations could not remedy the distribution. SPSS, Excel and GraphPad were used for statistical tests. A Chi Square goodness of fit test was performed individually for four bioindicator bat species, and for species richness by block, to determine whether activity significantly differed across the blocks. The total number of passovers of all bat species across the estate was analysed using a Chi Square test of independence to examine the relationship between activity (measured in numbers of passovers) and location by block.

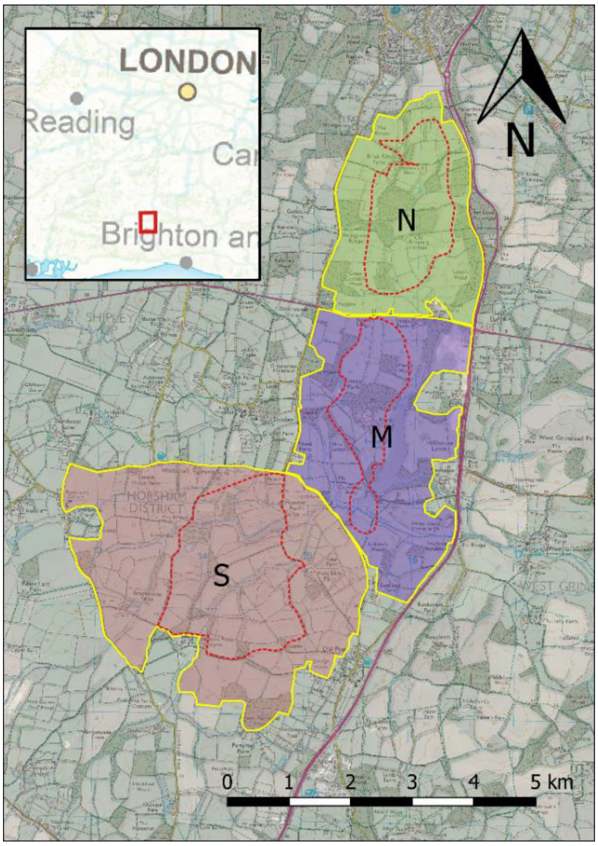

Figure 1. The total area of the estate, with transects shown as the red dotted line. Blocks are labelled S= Southern Block (ex-arable), M= Middle Block (historical deer park), N= Northern Block (ex-dairy).The positioning of the estate in the South of England is also shown.

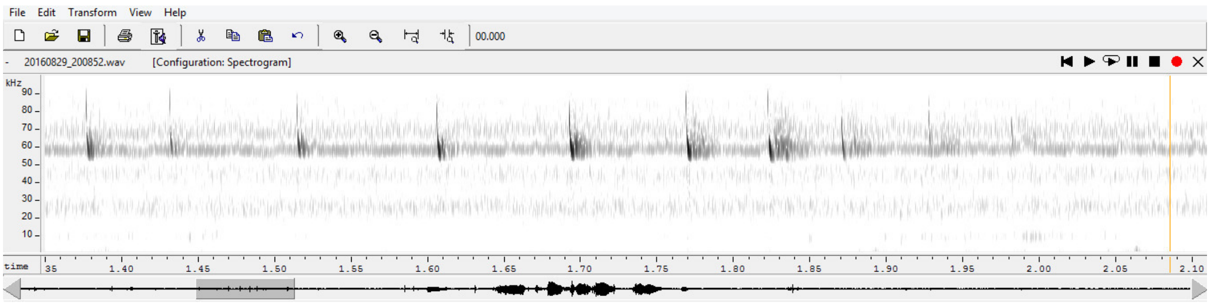

Figure 2. WaveSurfer spectrogram of Soprano pipistrelle passover, recorded at 20:08 pm on the 30th August 2016 in the Southern block of Knepp Estate.

Results

Species Richness and Composition: Number of species recorded varied for each block (see Figure 3). A Chi Square goodness of fit test was performed, showing that the observed species richness was not significantly different from the equal distribution of species that was expected, X^2=0.438, (df=2), *p*=0.8033. The percentage species composition was calculated but could not be effectively statistically analysed as some data points were consistently below the minimum required for Chi Square (see Figure 4). *P. pygmaeus* made up 34.6% of activity in the Southern block, and *P. pipistrellus* made up 15.38 % (Figure 4). In comparison, *P. pygmaeus* made up 25.38% of activity in the Middle block and *P. pipistrellus* made up 16.15% of activity, but DB activity was highest at 26.15%. *P. pipistrellus* was the highest in the Northern block with 29.22% of activity, *M. daubentonii* at 22.72% and *P. pygmaeus* at 16.88% (Figure 4). Species composition naturally follows the same pattern as individual species activity, but species richness does not follow the same pattern as activity (Figures 5 and 6).

Individual Species Level: The bioindicator bat species that were observed in sufficient numbers for individual statistical analysis to be performed were *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, *Myotis daubentonii* and *Nyctalus noctula* (Figures 4 and 5). With only three replicates, advanced parametric statistical analysis could not be performed. A total of all passovers over the three days was calculated for each species and a Chi goodness of fit test was performed. *P. pipistrellus* was shown to significantly vary across the three blocks, X^2= 14.966 (df=2), *p*=0.0006. The highest count for *P. pipistrellus* was in the Northern block. *M. daubentonii* activity was shown to significantly vary across the blocks, X^2=7.140 (df=2), *p*=0.0282, and the Southern block having the lowest count (see Figure 5). *P. pygmaeus* X^2= 5.327 (df=2), *p*= 0.0697, which suggests the difference may be significant if there were more replicates/greater sampling effort. *N. noctula* activity was shown not to vary significantly X^2= 1.750 (df=2), *p*= 0.4169. *P. pipistrellus* and *P. pygmaeus* were the most active species in all blocks. *N. noctula* activity was consistently the lowest, and *M. daubentonii* activity varied for each block.

Total Bat Activity: A Chi Square test of independence was performed, and a relationship between block and total bat activity over 3 days was found, X^2= 13.2247 (df=4), *p*=0.010229. Total activity of all species was shown to significantly vary across blocks, the Northern block having the greatest count of activity, and the Middle and Southern being equal (see Figure 6).

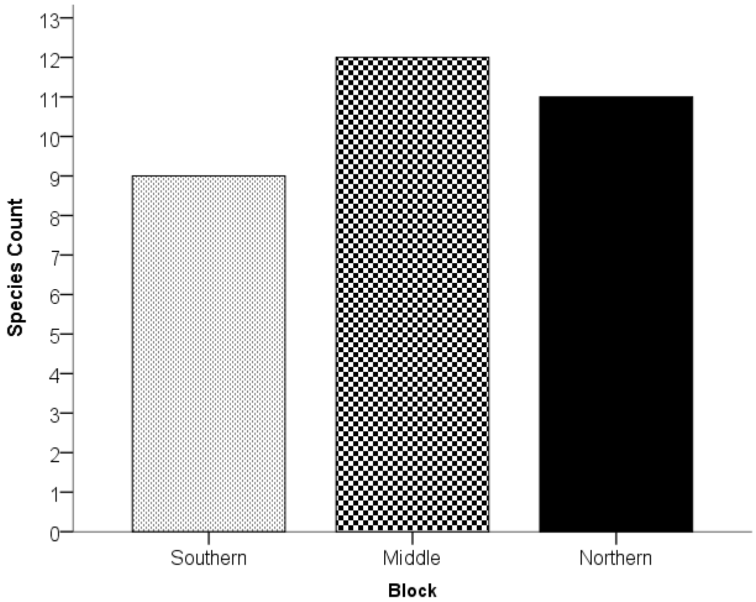

Figure 3. The number of bat species found in each block of Knepp estate, after 3 days of sampling in each, August/September 2016.

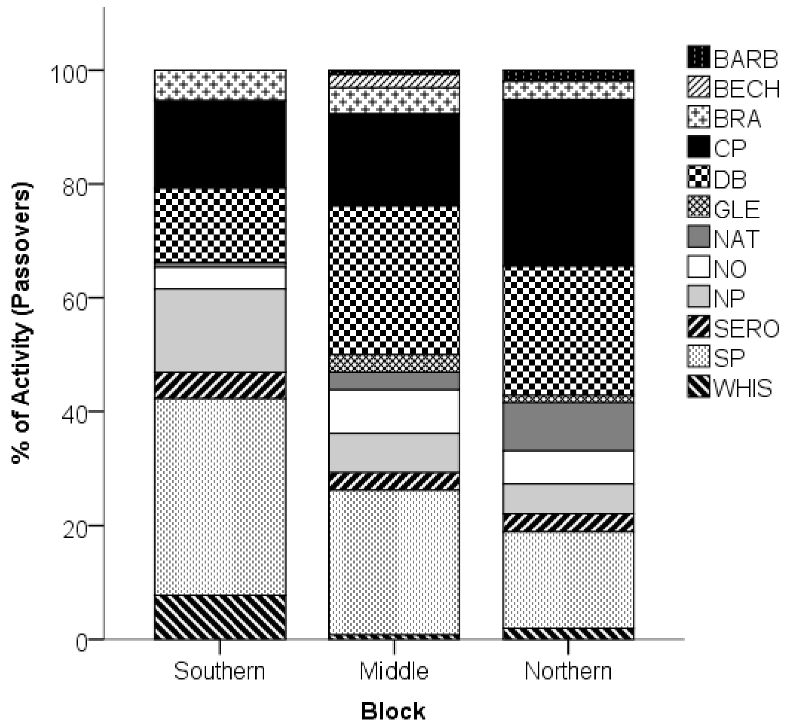

Figure 4. The species composition of activity recorded in each block of Knepp Estate, after 3 days of sampling in each, August/September 2016. BARB= *Barbastella barbastellus*, BECH= *Myotis bechsteinii*, BRA= *Myotis brandti*, CP= *Pipistrellus pipistrellus*, DB= *Myotis daubentonii*, GLE= *Plecotus austriacus*, NAT= *Myotis nattereri*, NO= *Nyctalus noctula*, NP= *Pipistrellus nathusii*, SERO= *Eptesicus serotinus*, SP= *Pipistrellus pygmaeus*, WHIS= *Myotis mystacinus.*

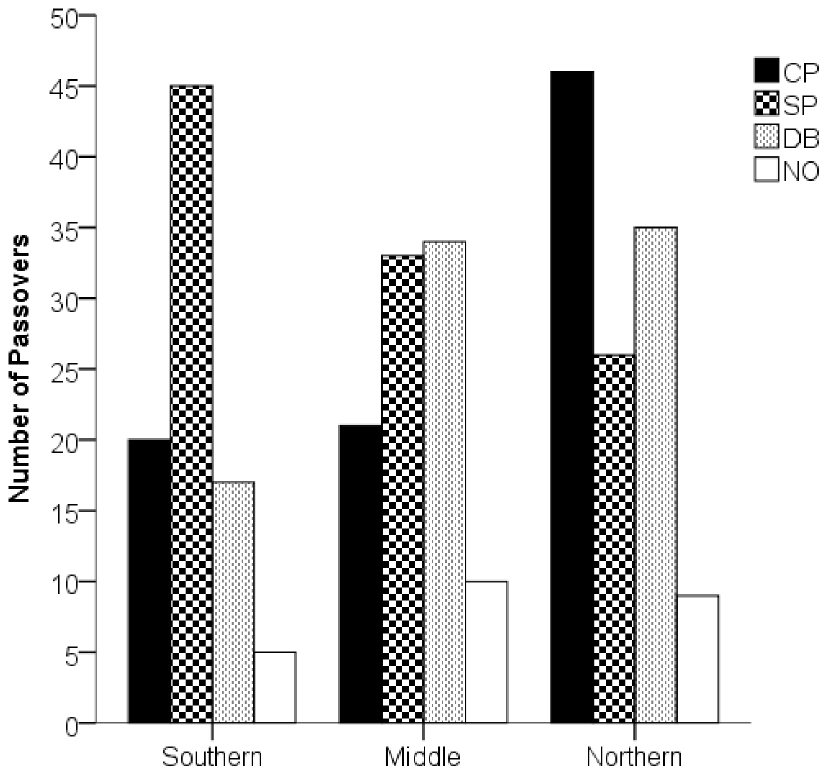

Figure 5. The total number of "passovers", or counts of activity by call, for selected bioindicator bat species in each block of Knepp Estate. CP= *Pipistrellus pipistrellus,* SP= *Pipistrellus pygmaeus,* DB= *Myotis daubentonii*, NO= *Nyctalus noctula.*

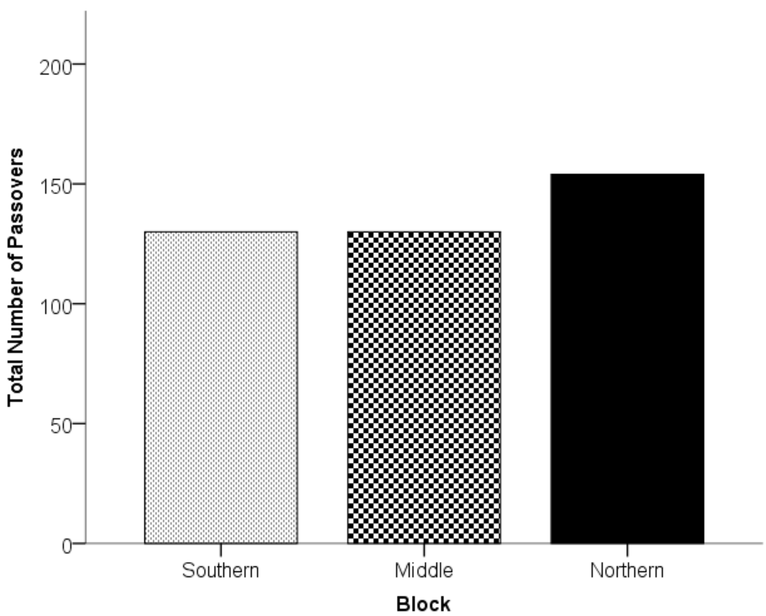

Figure 6. The total number of "passovers", or counts of activity by call, for all bat species, in each block of Knepp estate after 3 days of sampling in each, August/September 2016.

Discussion

It is unsurprising that the species richness does not significantly vary across the estate, as each block caters for the same types of species in differing amounts. The habitat type across Knepp is much the same across the estate; a patchwork of open fields, woodlands, lakes and shrubs (Rotherham and Handley, 2011; Greenaway, 2011). However, though these environmental "ingredients" vary, the amount of each type varies block to block (Rotherham and Handley, 2011). The fact that *M. daubentonii* and *P.pipistrellus* activity does significantly vary suggests that there may be variation in riparian habitats and water quality (Everard *et al*., 2007; Scott *el al*., 2010). The fact that *P. pygmaeus* activity does not significantly vary, but is approaching statistical significance at p=0.0697, may represent a significant difference and therefore differentiation in riparian habitats with further sampling. However, it is a possibility that it is not the quality but the amount of riparian and aquatic habitat available. If this is the case, habitat structure rather than habitat health makes the difference in activity across the estate. The fact that species richness does not significantly vary supports this structure over quality hypothesis, as higher quality habitats would have a significantly different number of species present, rather than just differing activity rates. Alternatively, it may be an example of optimal foraging theory, with some bats pushed out onto the lower quality habitat of the less popular Southern block (Charnov, 1976).

Greenaway (2009) suggested that during the arable farming at Knepp, there was a lack of foraging areas for bats. Now that intensive farming has ceased, bat diversity is increasing, especially in the Southern block (Greenaway, 2009; Rotherham and Handley, 2011). This is in contrast to the Northern and Middle blocks, which have stayed more constant in their species composition (Rotherham and Handley, 2011). The earlier stage of succession may mean greater biodiversity than a habitat further along in development, where certain species have become dominant (Townsend *et al*., 1997). However, another possibility is the bovine influence. Cows attract insects, which attract bats. Insect species richness is higher in grazed areas (Kruess and Tscharntke, 2001). Therefore bat activity will be far higher over areas that cows occupy (Downs and Sanderson, 2010). The cows at Knepp are able to move between the separate blocks of the estate, but the Northern block landscape has for years been specifically maintained for cows (Rotherham and Handley, 2011). This study therefore represents a case where bovine habitat preference has impacted bats habitat preference.

The four species that were observed in sufficient numbers for analysis qualify as sensitive, easy to monitor species, representative of the health of the habitat as a whole (Pocock and Jennings, 2008). *P. pygmaeus* activity is higher than *P. pipistrellus* in the Southern and Middle blocks, but not in the Northern. This may suggest that riparian habitats are lower quality in the ex-dairy farmland (Scott *et al.,* 2010). Ungulate herbivory may impact restoration of dredged riparian corridors (Opperman and Merenlender, 2000) and species richness is shown to be lower in grazed areas (Humphrey and Patterson, 2000). However, the highest activity of all bats is still in Northern block. This may throw the use of bats as valid bioindicators into question, because they appear to show a preference for an area that may be regenerating more slowly. However, *M. daubentonii* are not significantly less active in the Northern block, as they may be expected to be if herbivores were impeding the restoration of aquatic habitats. Grazing may alternatively benefit *M. daubentonii*, as herbivores may prevent obstructive growth of plants (Steinman *et al.,* 2003*;* Kauuffman and Krueger, 1984). *M. daubentonii* activity significantly varies across the estate, potentially indicating variation in wetland quality (Everard, 2007). Recordings of Daubentons have greatly increased since 2001 (Rotherham and Handley, 2011; Greenaway, 2011; Love, 2005). Removing intensive agriculture benefits aquatic ecosystems (Gagliardi and Pettigrove, 2013). Therefore this increase in *M. daubentonii* can be explained by the cessation of agricultural processes. It may have been expected that the Middle block would provide the highest *M. daubentonii* activity, but there was no significant difference found between the Middle and the Northern. The Southern block had significantly less *M. daubentonii* activity. This may be due to the proximity of human inhabitants affecting water quality, or perhaps the amount of water plants. Pond plants affect foraging success for Daubentons, duckweed especially (Akasaka *et al.,* 2009). However, this variation is likely explained by habitat structure, as the Southern block has less open expanses of water than the Middle, and tributaries than the Northern. Greenaway (2011) and previous surveys found little to no variation in water quality across the estate, and so habitat structure is the most likely cause of *M. daubentonii* activity distribution. Habitat structure is also likely to have had the largest influence on *N. noctula.* The ecotone between woodland and pasture is very important for *N. noctula* foraging. The fact that the Southern block has significantly less *N. noctula* activity suggests that the transition areas between woodland and pasture are not suitable for *N. noctula* foraging. This is unsurprising, considering the length of time that the Southern block has not had effective grazing.

Therefore, shrubs have grown up, and field structures have not been conserved (Greenaway, 2011). There is likely to be little prime habitat for *N. noctula* in the Southern block.

It is clear from bat surveys over the years that Knepp provides foraging habitat for greater numbers of bats than those who roost there (Greenaway, 2011). This study highlights the uses of monitoring bat activity, and suggests that a later study should look in to how far bats have travelled to reach Knepp's resources. Tagging should be utilized in the future to determine this. It has been found that some species travel from reserves on the other side of the county in one night to forage at Knepp (Rotherham and Handley, 2011). There have been fluctuations in bat populations over the years as well as seasonally. Using abundance as a predictor of ecosystem health is an outdated approach which may have limited previous surveys at Knepp. Simple presence and number is not sufficient, and further studies should take greater account of behavioural findings rather than focusing on population. Continuing monitoring of activity patterns is required for accurate comparison over the years. It may also be useful to compare with nearby agricultural land, in the absence of historical data on bat activity across Knepp when it was farmed. Progression of natural restoration may have occurred at differing rates for each ex-agricultural system, and so the differences found in this study may be more pronounced than have been represented here. This study provides further support for the use of bats as bioindicators, at a regional scale as well as in a global context.

Future Priorities: Future bat studies at Knepp should monitor throughout the summer months. Due to the short sampling period, few replicates were gained for this study, but valuable observations were still possible with low sampling effort. A limit of three replicates meant the study lacked parametric statistical analysis. The study therefore has low sample size with weak statistical power, which could be improved in future studies with a longer sampling period. The transects walked in each block were not uniform, with length, direction and speed varying slightly for each. Although this is to be expected with any field study, further steps should be taken in future studies to monitor speed, and geotagging of activity points should be recorded. Expertise in terms of equipment use and species identification was well controlled for in this study, with back up equipment in terms of a heterodyne bat detector and recordings taken for later analysis where literature could be referred to when needed. However it is possible that some human error may have resulted in inaccurate identification. In future studies, the use of several researchers to check over passover analysis would benefit more robust results. For example, accurate identification of pipistrelle species is hard for an amateur but very important, as pipistrelle, species may have different conservation needs, and monitoring is important to ensure they are not categorized as having the same preferences (Davidson-Watts *et al.,* 2006). The timing of the study was relatively late for bat monitoring, the prime time for monitoring being spring to late summer (Ahlen *et al.,* 2004). Roost selection varies depending on season; insectivorous bats select colder roosting locations and limit energy expenditure in late autumn, whereas summer roosts are preferred to be warmer (Speakman and Rowland, 1999). Differing habitats may be preferred at different times; *N. noctula*s have been shown to prefer different areas when breeding than they do outside of breeding season (Mackey and Racey, 2007). Therefore, the timing of this study may have affected results, but this is to be expected with a brief pilot study, and these findings still serve purpose in directing further research. In order to gain more representative results the study should begin earlier in August and continue for at least another 9 days, or ideally throughout summer. However, late summer is the easiest time to monitor pipistrelles, and after the breeding season ensures little reproductive behavioural variation in noctules (Ahlen *et al.,* 2004; Mackey and Racey, 2007). Proximity to housing may also affect bat monitoring. The transects may have passed a roost during emergence, which would result in a spike in activity. Alternatively, the amount of human activity may disrupt or increase bat foraging, particularly if it is in an area with less human interference, such as the Northern block. This is something that should be addressed in future studies by prolonging the sampling time, and monitoring for habituation indicated by declining activity rates as the weeks go on. Further analysis of the spectrograms collected from Knepp during this study may allow us to judge which type of activity may be occurring where. Identifying the difference between foraging, social and navigating calls may rule out the possibility of chance, such as passing by a roost site during emergence of *P. pygmaeus* resulting in a burst of activity. Spikes in activity may also relate to bats investigating observers, over a short period or consistently flying over them for a time. Low but constant counts of one bat species over time may suggest the bat could be using the observer to hunt. Especially in the Northern block, where there are fewer footpaths, more vegetation would be disturbed by the observer, bats may use this to prey on disrupted insects.

Agricultural abandonment is suggested as a major threat to insect abundance, particularly butterflies, by some studies. Most Lepidoptera are forest dependent, and species composition should be considered at regional rather than local scale (Merckx *et al.,* 2015; Schmitt and Rákosy, 2007). Goal driven conservation management is better than passive management for rewilding impacts on butterflies. Bats benefit from butterfly targeted conservation, which may mean an increase in their prey, as does eco-tourism (Howorth, 2009; Rotherham and Handley, 2011). Higher invertebrate diversity results in a more resilient ecosystem. Lack of agricultural intensification seems to be working well for invertebrates, particularly butterflies and moths at Knepp, with the planting of native species such as sallow (Howorth, 2009; Greenaway, 2011). "Small organisms provide the bulk of biodiversity" and so this is a positive sign for rewilding (Merckx *et al.,* 2015). Bat species should be used to manage the Lepidoptera focused ecotourism at Knepp.

Using bats as bioindicators may be non-representative (Cunto and Bernard, 2012). They forage in large areas and are robust to site specific declines in habitat health. All bioindicators should be species specific and over generalizing is a problem in many studies (Cunto and Bernard, 2012). Using behaviour as an indicator combats the robustness of bat species failing to reflect fluctuation in the environment, as concerns Cunto and Bernard (2012). Activity shows changes that may not be represented in the abundance of an adaptable species (Fenton, 1970). Terrestrial insects can be used effectively as bioindicators but sampling needs to occur large scale and with consistent effort (McGeogh, 1998). It is necessary to take a more grounded approach as has been done in this study by selecting four representative species, and decided based on area, not based on a priori theories (Nunes *et al.,* 2010; McGeogh, 1998).

Conclusion

Knepp is not representative of all other places in the UK or all other rewilding strategies; it's a small place and unique in many factors (Rotherham and Handley, 2011). However, it is the only rewilding project currently ongoing in the south of England, and though many factors present difficulties and inconsistencies, it is a valuable research opportunity. It is concluded that the presence of cattle and other large herbivores affect bat activity. The question of grazers presence preventing riparian habitat restoration should be further examined. Despite the small sample size and limitations of study design, bat activity has been shown to vary across the estate, presenting habitat differences. Findings are supported by previous literature and surveys. A more rigorous, continuous monitoring strategy may be able to further identify these variations. As a preliminary study, this research has been successful; variations have been identified with minimal sampling effort. The use of bioindicators at Knepp may not be successful in terms of within-estate-habitat-comparisons, but are viable in terms of change over time. The study should be repeated in following years.

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