Catching insects while recording bats: impacts of light trapping on acoustic sampling

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Abstract
Collecting information on bat prey availability usually involves the use of light traps to capture moths and flies that constitute the main prey items of most insectivorous bats. However, despite the recent awareness on the adverse effects of light on bats, little is known regarding the potential impacts of light trapping on the bat sampling outcomes when passive acoustic sampling and light trapping are implemented simultaneously. Using a before–after experimental design that involved the installation of a 6 W actinic light trap 1 m away from the bat detector, we tested the predictions that (1) slow-flying bat species will be less active when the light trap is present, while the opposite will be true for fast-flying species; and (2) bat species richness will be lower at lit conditions compared to dark ones. Our results suggest that the use of light traps in combination with bat detectors may considerably influence the outcomes of acoustic sampling. Although the activity of fast-flying bat species did not differ between the two treatments, we found that the activity of slow-flying ones such as Rhinolophus ferrumequinum and Rhinolophus hipposideros decreased significantly at lit conditions. Furthermore, we recorded fewer bat species when the light trap was deployed. To overcome this issue, we strongly recommend either (1) placing light traps at a considerable distance from bat detectors; or (2) using light traps during the night that follows the bat sampling if sampling needs to be at the same position; or (3) deploying non-attractant insect traps such as Malaise traps if Lepidoptera is not the main order targeted.

Introduction
In the face of ongoing biodiversity loss that is happening worldwide despite substantial recent conservation efforts (Butchart et al. 2010), it is essential that biodiversity surveys and monitoring are implemented in the most accurate, efficient and cost-effective ways. With the advent of ultrasonic bat detectors, Passive Acoustic Sampling (PAS) has become an increasingly popular non-invasive method for studying the ecology of echolocating bats (see review by Britzke et al. 2013). PAS may outperform trapping (MacSwinney et al. 2008) and active acoustic sampling such as transect surveys (Stahlschmidt and Bruhl 2012; de Torrez et al. 2017) in detecting elusive species and patterns in bat activity, although these different methods complement each other (Flaquer et al. 2007; Lintott et al. 2013). Obtaining reliable outcomes from PAS strongly depends, however, on its implementation in the field. This is particularly true for species with low detectability (e.g. short-range echolocators; Meyer et al. 2011), which moreover are generally of major conservation concern (Jones et al. 2003; Safi and Kerth 2004). While much attention has been given to the optimization of the acoustic sampling methods (Hayes 1997; Fischer et al. 2009;
Adams et al. 2012; Skalak et al. 2012; Froidevaux et al. 2014; Law et al. 2015), the potential biases arising from the use of PAS in concomitance with other field methods such as insect trapping have been poorly documented (but see Adams et al. 2005).

Information on prey availability are generally required to fully understand how bats utilize foraging habitats (Kusch et al. 2004; Fukui et al. 2006; Müller et al. 2012). As most insectivorous bat species feed on nocturnal moths and flies (e.g. Vaughan 1997), light trapping is commonly used simultaneously with PAS to quantify insect abundance/biomass alongside bat activity (e.g. Lumsden and Bennett 2005; Adams et al. 2009; Dodd et al. 2012; Müller et al. 2012; Wolbert et al. 2014; de Oliveira et al. 2015). However, the distances researchers are placing light traps relative to the bat detectors greatly vary between studies. It seems that caution is usually taken by installing light traps >20 m apart from the bat detectors (Lumsden and Bennett 2005; Adams et al. 2009), or even >50 m (Wolbert et al. 2014; de Oliveira et al. 2015), yet shorter distances may also be observed in the literature (e.g. 5 m; Müller et al. 2012).

With the relatively recent growing awareness of the negative effects of light pollution on nocturnal biodiversity (Holker et al. 2010; Gaston et al. 2015) – including bats (Stone et al. 2009) – the use of light traps at close vicinity to bat detectors might need to be reconsidered. In fact, several studies have highlighted the pronounced effect of artificial lights on bat behaviours in relation to their eco-morphological traits such as echolocation call design and wing morphology (Rydell 1992; Stone et al. 2012). At the local scale, lights either attract fast-flying species (i.e. light-exploiting bats; medium- and long-range echolocation calls, high wing loading and high wing aspect ratio) that may benefit from insect aggregation around the light sources or deter slow-flying species (i.e. light-averse bats; short-range echolocation calls, low wing loading and low wing aspect ratio) due to high perceived predation risk (Jones and Rydell 1994; Stone et al. 2015; Rowse et al. 2016). It is therefore possible to envisage similar behaviours with the presence of light traps, which may lead to serious biases in the bat acoustic sampling outcomes and consequently wrong management decisions if both methods, PAS and light trapping, are implemented close to one another at the same time.

In this study, we aim to assess the potential effects of light trapping on bat acoustic sampling outcomes in terms of bat activity and species richness. We predicted that acoustic sampling under lit conditions would result in: (1) an increase in fast-flying species activity as we expected a greater abundance in insects at the sampling sites when the light trap is deployed; (2) a reduction in slow-flying species activity given that the costs of foraging at light would be higher than the benefits for these species that can be subjected to higher perceived predation risk; and (3) a decrease in species richness as slow-flying species may radically avoid lit areas.

Materials and Methods

Study design

We applied a before–after experimental design to investigate the effects of light trapping on bat activity and species richness. The study was carried out in 12 farms located in the south-west of England (Fig. 1) between June and August 2016. Within each farm, we selected between one and four hedgerows separated at least 200 m from each other to conduct the experiment (28 hedgerows in total; mean height: 3.34 m; height range: 1.45–7.13 m). We chose hedgerows as our sampling sites since they constitute important foraging and commuting habitats for a wide range of bat species present in the study area (Walsh and Harris 1996). We implemented a passive acoustic method to record bat echolocation calls using a Song Meter SM2BAT recorder (sampling rate: 384 kHz; Wildlife Acoustics, Concord) connected to a SMX-U1 ultrasonic microphone. Each site was acoustically sampled during 4 h starting 30 min before sunset and during two consecutive nights, weather permitting (i.e. no precipitation, temperature at dusk >10°C, wind speed <30 km/h). During the second sampling night, we installed a portable heath-type actinic light trap 1 m away from the bat detector. Temperature at dusk was registered during the two nights using a data logger RC-5 (accuracy: ±0.5°C; Elitech, London, UK).

Light trap characteristics

We measured the irradiance and illuminance of the portable heath-type actinic light trap (6 W 12 V actinic bulb) in a darkened room using a USB2000+ spectrometer and a QP400-2-UV-VIS fibre optic cable attached to a CC-3-UV-S cosine corrector (Ocean Optics, Dunedin). Irradiance measurements were taken at close vicinity (<50 cm) to the light trap, while the illuminance level was recorded 1 m away from the light trap at 1 m above ground with the device directed horizontally towards the light source. Using the OceanView software (Ocean Optics, Dunedin), spectra and illuminance level were collected for 1 min and 1 sec by triplicate respectively. This type of light trap emits ultraviolet light with peak intensity at 367 nm (Fig. 1) with an illuminance level of 3.36 lux.

Acoustic analyses

We defined bat activity as the total number of bat passes (i.e. series of minimum two echolocation calls lasting up
to 15 sec with inter-pulse duration <1 sec) recorded during a night. Bat echolocation calls were manually analysed using BatSound 4.1.4. (Pettersson Electronic, Sweden). Echolocation calls were assigned to the lowest taxonomic level possible. Our analyses focused on the main taxa recorded over sites, namely *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, *Nyctalus/Eptesicus* spp., *Myotis* spp., *Rhinolophus hipposideros* and *Rhinolophus ferrumequinum*. Foraging activity was assessed by counting the number of feeding buzzes present within the bat passes. Finally, we calculated the species richness considering some species groups where identification can be problematic as single taxa. This concerned the (1) *Myotis* group (*Myotis bechsteinii*, *M. brandtii*, *M. daubentonii*, *M. mystacinus* and *M. nattereri*); (2) *Nyctalus/Eptesicus* group (*Eptesicus serotinus*, *Nyctalus noctula* and *N. leisleri*); and (3) *Plecotus* group (*Plecotus auritus* and *P. austriacus*).

**Statistical analyses**

We tested the effects of light trapping on (1) bat activity of each species and species group; (2) total bat activity; (3) overall foraging activity; and (4) species richness by fitting a series of (generalized) linear mixed-effect models (functions *lmer* and *glmer* in ‘lme4’ package; Bates et al. 2015) with the appropriate distribution (Gaussian for models on species richness and Poisson or negative binomial when overdispersion was detected otherwise). Data on species richness were beforehand squared to meet normality assumptions. Treatment (unlit vs. lit) and temperature at dusk were included as fixed effects in the models while hedgerows nested within farms were considered as random effects to account for the before–after experimental sampling design that took place within different farms. We used an information theoretic approach to assess the importance of temperature as covariate in our models (Burnham and Anderson 2002). For all of them, the inclusion of temperature (as well as its quadratic term) did not lead to lower AICc (i.e. ΔAICc ≥ 2) compared to models incorporating treatment only; temperature was therefore disregarded for the analysis. Model validation was performed using the ‘DHARMa’ package (Hartig 2017). Statistical analyses were undertaken using R 3.4.0 (R Development Core Team, 2017).
Results

We recorded a total of 7176 bat passes and 3027 feeding buzzes along 28 hedgerows located in 12 farms. Pipistrellus pipistrellus was the most frequent species with 4460 bat passes (62% of the total bat activity), followed by P. pygmaeus (12%), Nyctalus/Eptesicus spp. (11%) and Myotis spp. (9%). Although relatively few passes from R. ferrumequinum (115 passes) and R. hipposideros (135 passes) were recorded, these species were detected in 20 and 23 sites out 28 respectively. We also recorded the presence of Barbastella barbastellus (59 passes), Pipistrellus nathusii (43 passes) and Plecotus spp. (10 passes). We assigned 40 bat passes to Pipistrellus pipistrellus-pygmaeus given that we could not confidently identify to species level these series of calls recorded (i.e. calls with end frequency around 50 kHz).

We found that the presence of the light trap had a significant negative effect on the activity of R. ferrumequinum and R. hipposideros (Table 1; Fig. 2). The same trend was observed for Myotis spp. and total bat activity, although not significant ($P = 0.07$ for each model). Our results suggested, however, that the activity of P. pipistrellus, P. pygmaeus and Nyctalus/Eptesicus spp. as well as the overall foraging activity level (i.e. no. of feeding buzzes) were not significantly different between the two treatments. When looking at bat species richness, significantly less species were recorded the second night when the light trap was deployed (Table 1; Fig. 2).

Table 1. Estimates with associated standard errors and lower and upper 95% confidence intervals (CI) of the (G)LMMs relating to the effect of light trapping (unlit vs. lit hedgerow) on taxon-specific and total bat activity, overall foraging activity and bat species richness.

<table>
<thead>
<tr>
<th>Model</th>
<th>Estimate ($\pm$SE)</th>
<th>Lower 95% CI</th>
<th>Higher 95% CI</th>
<th>Test statistic$^d$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow-flying taxa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. ferrumequinum activity$^1$</td>
<td>$-1.04 \ (\pm 0.21)$</td>
<td>$-1.45$</td>
<td>$-0.63$</td>
<td>$-4.90$</td>
<td>***</td>
</tr>
<tr>
<td>R. hipposideros activity$^1$</td>
<td>$-1.21 \ (\pm 0.20)$</td>
<td>$-1.60$</td>
<td>$-0.82$</td>
<td>$-5.92$</td>
<td>***</td>
</tr>
<tr>
<td>Myotis spp. activity$^2$</td>
<td>$-0.57 \ (\pm 0.31)$</td>
<td>$-1.18$</td>
<td>$0.04$</td>
<td>$-1.84$</td>
<td></td>
</tr>
<tr>
<td>Fast-flying taxa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. pipistrellus activity$^2$</td>
<td>$-0.38 \ (\pm 0.24)$</td>
<td>$-0.85$</td>
<td>$0.09$</td>
<td>$-1.61$</td>
<td>NS</td>
</tr>
<tr>
<td>P. pygmaeus activity$^2$</td>
<td>$-0.31 \ (\pm 0.33)$</td>
<td>$-0.96$</td>
<td>$0.34$</td>
<td>$-0.93$</td>
<td>NS</td>
</tr>
<tr>
<td>Nyctalus/Eptesicus spp. activity$^2$</td>
<td>$-0.13 \ (\pm 0.31)$</td>
<td>$-0.74$</td>
<td>$0.48$</td>
<td>$-0.42$</td>
<td>NS</td>
</tr>
<tr>
<td>Global</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bat activity$^4$</td>
<td>$-0.42 \ (\pm 0.23)$</td>
<td>$-0.87$</td>
<td>$0.03$</td>
<td>$-1.81$</td>
<td></td>
</tr>
<tr>
<td>Foraging activity$^5$</td>
<td>$-0.48 \ (\pm 0.47)$</td>
<td>$-1.40$</td>
<td>$0.44$</td>
<td>$-1.03$</td>
<td>NS</td>
</tr>
<tr>
<td>Species richness$^3$</td>
<td>$-7.64 \ (\pm 2.42)$</td>
<td>$-12.38$</td>
<td>$-3.16$</td>
<td>$-3.14$</td>
<td>**</td>
</tr>
</tbody>
</table>

$^1$GLMMs with a Poisson distribution.

$^2$GLMMs with a negative binomial distribution.

$^3$LMM (Gaussian distribution). Data were squared to meet normality assumptions.

$^d$z value for GLMMs and t value for LMM.

NS $P \geq 0.1$; $P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Discussion

Our results demonstrate that the bat acoustic sampling outcomes might be significantly biased when light traps are used in conjunction with bat detectors. As hypothesized, the activity of slow-flying species such as Rhinolophus spp. and Myotis spp. (though not significant) drastically decreased when the light trap was present. These findings corroborate previous studies that found that artificial light at night adversely affects the foraging, commuting and drinking behaviours of slow-flying bats (Stone et al. 2009, 2012; Azam et al. 2015; Russo et al. 2017) as they might be subject to higher perceived predation risks (Jones and Rydell 1994). Moreover, although R. ferrumequinum and R. hipposideros were recorded flying along most of the unlit hedgerows, they were often found absent from the inventory when the hedgerow was lighted by the trap, resulting in lower species richness when sampling bats around lights. As we used light traps of relatively low intensity, we can reasonably assume stronger impacts when using high-intensity light traps that are available on the market.

Contrary to our expectations, the activity of fast-flying species was not significantly affected by the presence of the light trap. In fact, we hypothesized that due to their eco-morphological traits (medium- and long-range echolocation calls, high wing loading and high wing aspect ratio), fast-flying species would be able to fully exploit the abundance of their insect prey that aggregate around the light source (Rydell 1992). Although this

Statement
might be true at sites where the light trap has been
installed for a relatively long time (i.e. several nights), it
seems that – as suggested by Stone et al. (2012) – bats
need some time to discover these new foraging opportu-
nities within the landscape. Strong foraging site fidelity
observed in some bat species (Rydell 1989; Hillen et al.
2009) might explain this time gap.

Our results on total bat activity, overall foraging
activity and species richness contradict those of Adams
et al. (2005) who demonstrated that (1) forest bats in
Australia were significantly more active (higher number
of bat passes and feeding buzzes per pass) at sites where
the light traps were present; and (2) more species were
identified at lit conditions. The authors argued that the
use of light traps in combination with PAS enhance
species identification of bats at faster rates, as a signifi-
cantly higher number of bat passes with long duration
were recorded around lights. Although these results may
indicate that Australian bats are overall attracted by
lights, more recent studies have highlighted the negative
effect of artificial light at night on bats in Australia
(Threlfall et al. 2013; Straka et al. 2016). We therefore
recommend extreme prudence when implementing both
methods in the field, especially when known light-averse
species may occur in the study area. In fact, our results
suggest that when sampling simultaneously bats and
their insect prey, we may miss from the inventory slow-
flies bat species that are already difficult to detect due
to their short-range echolocation calls (Barclay and
Brigham 1991) and that are of major conservation con-
cern (Jones et al. 2003; Safi and Kerth 2004). As
emphasized in other studies, less mobile species are
more sensitive to habitat loss and fragmentation (Duch-
amp and Swihart 2008; Meyer et al. 2008; Bader et al.
2015; Farneda et al. 2015). Furthermore, biased acoustic
outcomes in which species richness is underestimated
and threatened taxa are not detected may undoubtedly
lead to wrong management decisions and alter

Figure 2. Mean bat activity (number of bat passes), feeding buzzes and bat species richness between the two treatments (unlit vs. lit hedgerow). Error bars represent the standard errors of the mean. (A) Rhinolophus ferrumequinum activity; (B) Rhinolophus hipposideros activity; (C) Myotis spp. activity; (D) Pipistrellus pipistrellus activity; (E) Pipistrellus pygmaeus activity; (F) Eptesicus/Nyctalus spp. activity; (G) total bat activity; (H) overall foraging activity; and (I) bat species richness. NS $P \geq 0.1$; $* P < 0.1$; $** P < 0.05$; $*** P < 0.01$; $**** P < 0.001$. 

conservation actions. It is therefore important to implement alternative sampling strategies to overcome this major issue.

To avoid possible interference between light trapping and PAS, three main alternatives might be considered. The first one consists of trapping insects during the consecutive night that follows the bat sampling (e.g. Lentini et al. 2012). As temperature may influence nightly catches of insects (Jonason et al. 2014), it is recommended that light trapping takes place during similar weather conditions to those during bat sampling to get a realistic picture of prey availability. The second option is to install the light trap at a certain distance away from the bat detector, generally >20 m (e.g. Lumsden and Bennett 2005; Adams et al. 2009; Wolbert et al. 2014; de Oliveira et al. 2015), but to the best of our knowledge, its effectiveness remains to be tested. The effect of distance to the light source will depend on its intensity (inverse square law) as well as on its spectrum. Thus, this sampling strategy leads to a certain trade-off between (1) maximizing the distance between the traps and the detectors to limit the adverse effects of the lights on bats; and (2) minimizing it to capture the effect of the habitat structure. Furthermore, setting up light traps away from bat detectors but along linear elements such as hedgerows and tree lines is very likely to affect the acoustic outcomes as the light may also act as a barrier to movement and may induce insect depletion in the area where bat are sampled, thus reducing the level of foraging activity. Considering these potential limitations, the first option seems to be more relevant. Finally, a third alternative is to use passive, non-attractant insect trapping instead of light trapping. The Malaise trap is one of the most popular and effective static, non-attractant traps that can be used to collect large number of insect individuals (Häuser and Riede 2015; Muirhead-Thomson, 1991), and has been used to assess insect biomass/abundance alongside bat activity (e.g. Morris et al. 2010; Brooks et al. 2017). Nevertheless, when comparing the use of light traps and Malaise traps, Scanlon and Petit (2008) found that the former (using an 8 W fluorescent black tube in combination with an 8 W white fluorescent tube) attracted higher number of individuals, insect orders and biomass. Similar results were obtained by Dodd et al. (2012) when using a 10 W black light trap. Although these findings may also raise potential issues regarding the use of light traps when relating bat activity to insect abundance/biomass as some insects might be attracted from a longer distance than the bat detector detection range (5–100 m depending on the species), several studies have emphasized the local sampling ranges (<30 m) of low-wattage black light and actinic traps (Muirhead-Thomson 1991; Truxa and Fiedler 2012; van Grunsven et al. 2014; Merckx and Slade 2014).

Considering the advantages of light trapping, the use of Malaise traps as an alternative of light traps will mainly depend on the targeted insect orders. Malaise traps are more efficient for catching dipterans than lepidopterans, while the opposite is true with light traps (Dodd et al. 2012).

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