The dark side of street lighting: impacts on moths and evidence for the disruption of nocturnal pollen transport

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Abstract

Among drivers of environmental change, artificial light at night is relatively poorly understood, yet is increasing on a global scale. The community-level effects of existing street lights on moths and their biotic interactions have not previously been studied. Using a combination of sampling methods at matched-pairs of lit and unlit sites, we found significant effects of street lighting: moth abundance at ground level was halved at lit sites, species richness was >25% lower, and flight activity at the level of the light was 70% greater. Furthermore, we found that 23% of moths carried pollen of at least 28 plant species and that there was a consequent overall reduction in pollen transport at lit sites. These findings support the disruptive impact of lights on moth activity, which is one proposed mechanism driving moth declines, and suggest that street lighting potentially impacts upon pollination by nocturnal invertebrates. We highlight the importance of considering both direct and cascading impacts of artificial light.

Keywords: ecosystem services, flowers, Lepidoptera, light pollution, population declines, sampling

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Introduction

Moths (Lepidoptera) are under threat, with long-term declines in populations and distributions of many species recorded in several European countries, including Great Britain (Conrad *et al.*, 2006) and the Netherlands (Groenendijk & Ellis, 2011). Whilst habitat degradation and climate change are likely to be the main drivers of these declines (Fox *et al.*, 2014), artificial night lighting is also a potentially important contributing factor (Fox, 2013). Light pollution from sources including industrial lighting and street lights (Luginbuhl *et al.*, 2009) is increasing internationally (but see Bennie *et al.*, 2014). It has the capacity to be a driver of environmental change (Gaston *et al.*, 2013, 2015) and affects individual moths through multiple mechanisms (reviewed in Macgregor *et al.*, 2015). Street lighting is one of the most widespread and important sources of light pollution (Luginbuhl *et al.*, 2009), yet surprisingly few studies have investigated the effect of artificial light on wild moth communities (but see Somers-Yeates *et al.*, 2013; Spoelstra *et al.*, 2015) and none have utilised existing light sources to do so.

In addition to direct effects upon moths, artificial light may also have secondary impacts upon other taxa, mediated through their effect on moths. Recent work has shown the potential for artificial light to affect interactions between species (Davies *et al.*, 2013), and so ecosystem functioning (Lewanzik & Voigt, 2014). Considerable evidence exists that moths are pollen vectors for a diverse range of plant taxa in ecosystems across the globe (Macgregor *et al.*, 2015), including in the UK (Devoto *et al.*, 2011), although no study has investigated the contribution of moths to pollination services in lowland agro-ecosystems. The attraction of moths to street lights could have functional consequences for nocturnal pollination, but the nature of these consequences would depend on the way in which moth communities respond to the presence of street lights (Macgregor *et al.*, 2015). For example, flower-visiting behaviour of moths in the immediate vicinity of lights could be disrupted if they are attracted upwards to fly around lights. However, if moths are attracted to lights from the surrounding area, the resultant increase in local abundance of moths could counteract such effects and lead to a local increase in pollen transport, whilst reducing pollen transport in the surroundings by acting as an ecological trap. These potential conflicting effects have not yet been investigated.

Many ecological census studies have demonstrated that moths are attracted to artificial light and, consequently, light traps are widely used for sampling

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nocturnal moths (Merckx & Slade, 2014). The number of moths caught by light traps is expected to decrease in the presence of other light sources, including moonlight and artificial lighting (Eisenbeis, 2006), and individual moth families may be affected to different degrees due to variation in the strength of response to light of different wavelengths (Van Langevelde *et al.*, 2011; Somers-Yeates *et al.*, 2013; Merckx & Slade, 2014). As a result, light-trapped samples may not be appropriate for direct comparison of moth communities between artificially lit and naturally dark sites. To evaluate this, we used an alternative sampling method, night-time transects, and compared the species composition of samples from each method.

We established a natural matched-pairs experiment to investigate the effects of street lighting at night on communities of wild moths and their contribution to pollen transport. Based on previous studies of the effects of artificial light on individual moths, we hypothesised four possible effects of street lights on moth communities (Macgregor *et al.*, 2015): (1) a concentration effect, whereby moths are attracted into the vicinity of street lights, but their behaviour is largely unaffected, leading to a greater density of moths below street lights; (2) an ecological trap effect, whereby moths are attracted into lit areas and up to street lights, leading to greater flight activity around street lights and, possibly, greater moth density below them; (3) a disruption effect, whereby moths in the vicinity of street lights are attracted upwards to the light source, but moths are not attracted into the vicinity from further away, leading to greater flight activity around street lights with correspondingly reduced moth density below them. Finally, as some moth families appear to be more strongly attracted to light than others, (4) a preferential disruption effect is plausible, whereby certain families are more strongly affected by the presence of street lights than others.

To find evidence for these effects, we used night-time transects to assess abundance and assemblage composition of moths in agricultural field margins lit by street lights and naturally dark field margins and compared the results to sampling with light traps. We used overhead flight activity surveys to quantify activity of moths high above ground level (at the street light level). Finally, we compared the abundance and diversity of pollen transported by moths at lit and unlit sites. Whilst pollen transport or flower visitation do not strictly prove the existence of a pollination interaction (King *et al.*, 2013), these measures are frequently used as a proxy for insect pollination; therefore, a reduction in pollen transport may indicate disruption of pollination services.

Material and methods

Field sites

We used a matched-pairs experimental design to evaluate the effects of artificial lights upon communities of nocturnal moths (Lepidoptera). We selected twenty pairs of agricultural field margins, differing only by the presence of artificial lighting at one member of each pair. We made use of existing street lights for experimental treatments. The multiple types of street lighting in use in Britain are expected to affect moths in different ways, so we restricted our study to high-pressure sodium (HPS) lights, one of the most widely used forms of street lighting in the UK (Somers-Yeates *et al.*, 2013).

Twenty lit sites within 40 km of Wallingford, Oxfordshire, UK (51°35′ N, 1°8′ W; south-east England) were selected for sampling (Table S1). Each lit site was selected by the presence of at least one HPS street light adjacent to the hedgerow boundary of an agricultural field. At lit sites where more than one street light was present, the single light closest to the unlit site (see below) was selected as the mid-point of the lit site and used for sampling. Boundaries with fences or no delineation, rather than hedgerows, were not considered. Each lit site was paired with a nearby section of hedgerow (the 'unlit site'), controlling for a number of habitat variables (including hedgerow and field margin properties and crop type: see Supplementary Information for complete criteria). Unlit sites were >100 m from any street lights (so at least this distance from lit sites; Table S1); we considered this distance to be sufficient to ensure that moths at the unlit site would not be affected by lights at the lit site, as it was far in excess of the distance from which moths may be attracted to lights reported by several experimental studies (Truxa & Fiedler, 2012; Merckx & Slade, 2014; Van Grunsven *et al.*, 2014). Night-time light levels were measured at all sites using a CEM DT-1300 light meter (CEM, Shenzhen, China) held pointing directly upwards, 1 m above the ground. Measurements of 0 lux were made at all unlit sites (i.e. light intensity was <0.1 lux) compared to a median of 2.3 lux (range 0.2–12.1 lux) at lit sites (Table S1); the maximum expected intensity of natural light at night is <1 lux (Bünning & Moser, 1969). In most cases, the conditions for pairing were best satisfied by matching the lit site with a section further along the same hedgerow. In all cases, the distance between sites within pairs (mean = 207 m, range 120–330 m) was smaller than the distance between pairs (nearest neighbour distance: mean = 6000 m, range 2200–14000 m). Distance to the nearest human habitation was recorded, as domestic gardens can be floristically heterogeneous and highly biodiverse, and may act as refuges for insects in agro-ecosystems (Galluzzi *et al.*, 2010).

Sampling took place by three methods (night-time transects, overhead flight activity surveys and light traps) between May and September 2014. The sampling period was divided into three periods of 6 weeks each ('spring': May–mid-June, 'early summer': mid-June–July and 'late summer': August–mid-September). Sampling took place when weather conditions at the beginning of the sampling session were suitable for moths to be active: dry conditions, wind speed of force 3 or lower (Beaufort scale) and minimum temperature of 12 °C. Sites

within pairs were sampled on the same evening to further control for environmental conditions (including moon phase). Pairs of sites were sampled once in each season with each sampling method: night-time transects and overhead flight activity surveys on the same night and light trapping on a separate night. Due in part to unsuitable weather conditions, we sampled only six pairs for each method during the spring period; therefore, we obtained 46 sets of paired observations across the sites and the seasons for each of the sampling methods.

Captured moths were identified in the laboratory to species level using Waring & Townsend (2009) and Sterling & Parsons (2012), apart from *Mesapamea secalis* and *M. didyma* which would have required genitalia dissection for identification, and were recorded as *M. secalis* agg. Each individual was then sampled for pollen.

Night-time transects

We adapted the method for night-time transects set out by Birkinshaw & Thomas (1999), using a 25-m transect with a street light at the mid-point of the lit transect. Each 25-m transect was repeated three times on each sampling occasion, alternating between sampling the lit and unlit transect. The order of the lit and unlit transects at each pair was determined randomly ahead of the first sampling occasion and retained for subsequent visits. The first transect was begun exactly 15 min after the street light came on; this was after sunset, but shortly before full darkness. The second and third replicates of each transect followed immediately afterwards in full darkness.

Each 25-m transect consisted of five sections of 5 m each. This length of field margin was illuminated using a Cluson Clulite FL7 portable LED floodlight and a Petzl head torch. Birkinshaw & Thomas (1999) observed that moths appeared to fly rapidly away from the floodlight when it was turned on; therefore, we fitted the floodlight with a red filter to reduce its visibility to moths, which have low sensitivity to the red end of the colour spectrum (Eguchi *et al.*, 1982). This also reduced the contrast in brightness between the area lit by the floodlight and the surrounding area, making it easier to maintain focus on moths initially seen on-transect that flew off-transect before being captured.

Moths were sampled in each section for a net total of 1 min before moving forward 10 paces (approximately 5 m) and repeating. Moths were captured using a hand net and retained for later identification. This prevented the possibility of repeatedly recording the same moth. When a moth was first seen, it was pursued until either it was captured or its capture became impossible (either because it was lost from view, or because it escaped over the hedge or the crop or into dense vegetation). Any further moths seen during a pursuit were disregarded until observations were resumed. This method, combined with the relatively low frequency of recording moths (median 6 per site per sampling night; range = 0–26), gives us confidence that preferential sampling bias towards larger-bodied or more interesting individuals was excluded. The time taken to retain each moth after its capture was not included in the search time.

Overhead flight activity of moths

During night-time transects, moths could only be captured when flying ≤3 m above ground level, so to quantify the activity of moths at higher altitudes (approximately 3–12 m above ground), we conducted flight activity surveys at both lit and unlit sites, on the same nights as transects. A bright (70 lumen), narrow-beamed (beam diameter at 5 m was approximately 40 cm) Mactronic 9018 LED torch was held 2 m above the ground at the mid-point of each transect, pointing vertically upwards, for 1 min and the number of moth passes through the beam during this time was counted. All potential moths were counted, with individual insects that could be positively identified as Neuroptera, Diptera or other non-lepidopteran taxa excluded; however, the possibility remains that some non-moths were counted. These counts were repeated three times during each sampling session, immediately after each transect replicate.

Light traps

Light trapping is a standard method for sampling moths (Merckx & Slade, 2014), so in addition to night-time transects, each site was sampled using light traps. Sampling by light traps and night-time transects was carried out on different nights at each site, to preclude interference between the two methods. Heath-style light traps (Heath, 1965), operating 8-W actinic bulbs (electrics purchased from Entomological Livestock Group, UK) and powered by a 12-V, 7-Ah battery, were placed at the centre points of both the lit and the unlit site. Traps were turned on shortly before dusk and operated until the battery ran flat (c. 8 h). Traps were collected the following morning, and captured moths were retained for later identification and pollen sampling. The mean number of moths caught per trap was 11.8 (range = 0–47); this sampling intensity was sufficiently low that samples should not have been affected by removal of moths during previous sampling sessions at the same site.

Pollen transport

To assess the quantity and diversity of pollen transported by moths, all moths retained during sampling were examined for pollen. Euthanised moths were placed in a relaxing chamber for 24 h before being swabbed with fuchsin jelly (Beattie, 1972). Most moth species carry their proboscides tightly coiled when not actively feeding (Krenn, 1990), so it was considered unlikely that pollen held on the proboscis was present from a source other than flower visitation. Therefore, to avoid the risk of cross-contamination between specimens, pollen was swabbed only from the proboscides of moths. A 1 mm^3 cube of fuchsin jelly was used, smaller than in comparable studies (e.g. Devoto *et al.*, 2011), in order that the proboscis could be swabbed with precision. Moth species that do not have a functional proboscis (e.g. Hepialidae) were not swabbed.

Microscope slides were prepared with the fuchsin jelly swabs and examined at 400× magnification. Pollen present was morphotyped and identified using a combination of keys

(Moore *et al.*, 1994; Kapp *et al.*, 2000), pollen reference collections at the Department of Geography, Environment and Earth Sciences, University of Hull, and knowledge of locally observed plants and likely insect-pollinated plant taxa. Morphotypes represented groupings that could not be separated to a lower taxonomic level and might have contained pollen of more than one species; such approaches are common in studies of this nature (e.g. Jędrzejewska-Szmek & Zych, 2013; Tur *et al.*, 2014) due to the difficulty of unambiguously identifying pollen using morphological characteristics.

Statistical analysis

Statistical analyses were conducted using R version 3.1.1 (R Core Team, 2014). Generalised linear mixed-effects models (GLMMs) were constructed to test for differences in moth abundance, activity, diversity and pollen transport between lit and unlit sites, separately for each sampling method. Significance of fixed effects within the model was tested with likelihood ratio tests where appropriate, and type III (Wald) tests for quasi-Poisson error distributions. Distance to the nearest human habitation was included in GLMMs as a covariate and site pair was included as a random effect. GLMMs were built using the package lme4 (Bates *et al.*, 2014), except for quasi-Poisson error distributions, when MASS (Venables & Ripley, 2002) was used, and zero-inflated error distributions, when glmmADMB (Skaug *et al.*, 2015) was used.

We tested for effects of light on moth abundance separately for each of the three sampling periods. In addition to testing for differences in overall abundance of moths, we considered individual moth families, as well as macro- and micro-moths separately: although the latter division is largely arbitrary in taxonomic terms, among UK species it represents a well-recognised and practical division of moths by size at the family level, allowing us to examine the potential effects of the size bias in strength of attraction to artificial light (Van Langevelde *et al.*, 2011). As not all families were captured during each field visit, subanalyses were only carried out where moths of a given family were captured at a minimum of five site pairs in a sampling period.

Diversity measures were calculated for each site using the package Vegan (Oksanen *et al.*, 2015). Species accumulation curves were plotted for each site; as asymptotes were not reached in most cases, functional extrapolation was conducted using EstimateS (Colwell, 2013) to estimate the true value of species richness for each site. Measures used were species richness and Simpson's D index (deemed more appropriate than Shannon's H index as our data contained small sample sizes and intersecting species accumulation curves (Magurran, 2004)).

We tested for differences in community composition of sampled moths, comparing communities sampled at lit and unlit sites, and also comparing the communities sampled by night-time transects and light traps. Differences in community composition were assessed with Bray–Curtis dissimilarities and tested using the adonis function within Vegan (Oksanen *et al.*, 2015). Comparisons were made at both species and family level due to the high number of singletons in the species-level data, and randomisations were constrained to within-site pairs only.

Finally, we used GLMMs (as described above) to test for differences between lit and unlit sites for three metrics of pollen transport: probability of a moth carrying pollen, total number of pollen grains recorded (pollen load) and total number of pollen types (pollen richness). The probability of a moth carrying pollen was analysed both with all moths included, and with only 'likely pollen carriers' included. 'Likely pollen carriers' were selected by excluding known nonfeeding macro-moth families and some smaller-bodied micro-moth taxa, and therefore comprised moths in the families Erebidae, Geometridae, Noctuidae, Pterophoridae, Sphingidae and Tortricidae, as well as larger-bodied genera of Crambidae (*Anania, Donacaula, Pleuroptya* and *Udea*). Pollen load and pollen richness were analysed at both per-moth and per-sample levels, to assess whether changes in moth abundance and diversity could affect overall pollen transport in the system. Noncarriers of pollen were excluded from the per-moth analyses.

Data collected in the course of this experiment have been archived in the Environmental Information Data Centre (EIDC), and can be accessed at http://doi.org/10.5285/31c c5cec-d33b-4dd6-a932-061ff947e708.

Results

Abundance

A total of 609 moths of 124 species in 17 families were caught on night-time transects, and 990 moths of 139 species in 19 families were caught in light traps (total 1599 moths of 203 species in 22 families; Table S2). A total of 434 overhead moth passes were recorded in flight activity surveys.

The presence of street lights had a significant effect on overall moth abundance. For the night-time transects, moths were significantly less abundant at lit sites (Fig. 1b) during both early summer ($P < 0.001$) and late summer ($P = 0.001$), although not in spring ($P = 0.588$). The overall effect (combining seasons) was that moths at ground level were 0.5 times (=$e^{-0.648}$; Table 1) as abundant at lit sites compared to unlit sites. Abundance at lit sites was significantly lower in at least one season for both macro- and micromoths, and for every individually analysed family (Table 1). For the light traps, abundance of moths at lit sites was significantly lower in late summer ($P = 0.042$) only. The overall effect (combining seasons) was that the abundance of moths in light traps at ground level was not significantly different between lit and unlit sites (Table 1). Abundance at lit sites was significantly lower in at least one season for macromoths and Noctuidae, but not micromoths or any other individual family (Table 1). In contrast, overhead flight activity at lit sites was significantly higher in both early ($P = 0.015$) and late summer ($P < 0.001$;
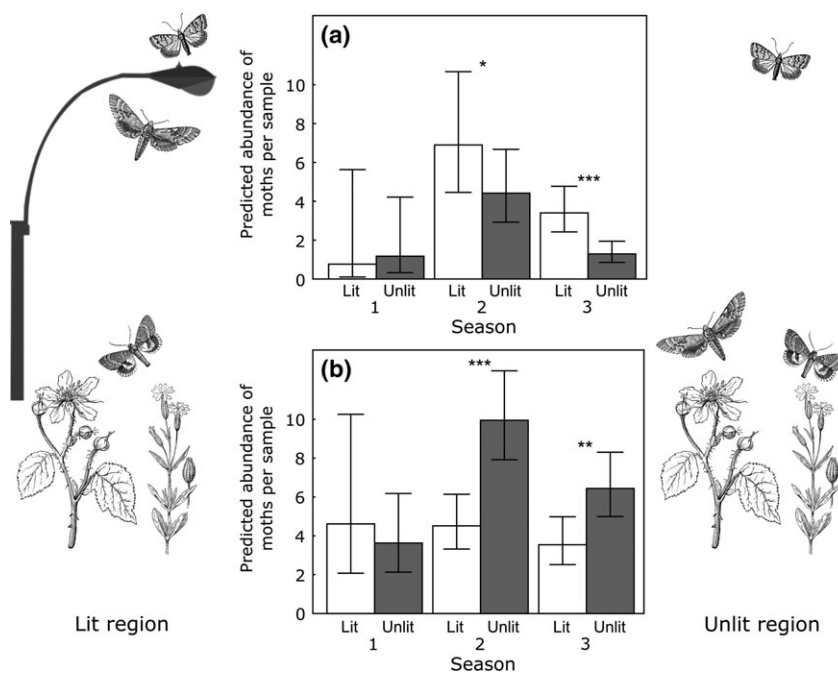
Fig. 1 The predicted abundance of moths at (a) street light level, measured by overhead flight activity surveys, and (b) vegetation level, measured by night-time transect walks, in the presence and absence of a street light (Table 1). Seasons are numbered as follows: 1 = spring; 2 = early summer; 3 = late summer. Significance indicates likelihood ratio tests on street light presence/absence in GLMMs (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Error bars show 95% confidence intervals. Drawings are used under licence from ClipArt ETC (see Supplementary Information for full acknowledgements).

Fig. 1a, Table 1). The overall effect (combining seasons) was that moths at light level were 1.7 times ($=e^{0.557}$; Table 1) as abundant at lit sites compared to unlit sites. These results were broadly similar when considering either the number of street lights within 100 m or the measured lighting intensity, instead of the presence/absence of street lights (Table S3).

Diversity and community dissimilarity

Across both night-time transect and light-trap methods, and using both raw data and functional extrapolation, lit sites were consistently found to have significantly lower species richness than unlit sites (Fig. 2). Simpson's D index was significantly higher (and therefore diversity lower) at lit sites for the light traps ($P = 0.007$), but not the night-time transects ($P = 0.785$; Table S4).

At the family level, the communities sampled at lit and unlit sites were significantly different to each other, and this was found for both sampling methods (transect: $P = 0.017$; trap: $P = 0.023$; Table 2). Significant effects were not found at the species level (transect: $P = 0.070$; trap: $P = 0.246$), despite the family-level approach being more conservative; this may be due to the effect of large numbers of singletons being recorded at the species level.

The subsets of the moth community sampled by light traps and on transects were significantly different ($P < 0.001$; Table 2).

Pollen transport

Nearly a quarter of sampled moths carried pollen: 23.5% (143 moths) of moths sampled on transects and 22.2% of moths sampled by light traps (220 moths), in total representing 83 species of 10 families (Table S5). Of the pollen-carrying moths, 72.2% carried only a single morphotype. Twenty-eight pollen morphotypes were distinguished (Table S6), of which seventeen were identified to genus or species level. A total of 94% of total pollen came from five morphotypes, identified as *Buddleja davidii* Franch. (Scrophulariaceae), *Lamium* spp. (Lamiaceae), *Rosa* spp. (Rosaceae), unknown Apiaceae spp. and *Tilia* spp (Malvaceae). Several known moth-pollinated taxa were also identified, including *Hedera helix* L. (Araliaceae), *Jacobaea vulgaris* Gaertn. (Asteraceae), *Lonicera periclymenum* L. (Caprifoliaceae), *Silene* spp. (Caryophyllaceae) and *Convolvulus* spp. (Convolvulaceae).

The probability of a moth carrying pollen was significantly lower at lit sites than unlit sites for light-trapped moths ($P = 0.031$) but not for transect-collected moths ($P = 0.875$), and not when the analysis was restricted to

Table 1 Summary of analyses testing for a change in moth abundance at different levels of taxonomic organisation attributable to presence/absence of a street light. Sampling seasons are numbered as follows: 1 = spring; 2 = early summer; 3 = late summer. No *P*-value is given for taxa with too few observations to analyse. Lit site abundance = unlit site abundance $\times$ e^{ES}, where ES = lighting effect size from the statistical model, so e^{ES} is the multiplicative effect due to lighting. Lighting test statistic is for likelihood ratio test unless indicated by *, in which case a Type III ANOVA was used. Significant *P*-values (<0.05) are italicized.

| Method	Moth subset	Sampling season	N (site pairs)	N (moths)	Lighting effect size	Standard error	Lighting test statistic	P
Transect	All families	All	46	605	−0.648	0.116	31.04	*<0.001*
		1	6	52	0.240	0.432	0.29	0.588
		2	20	332	−0.790	0.154	26.15	*<0.001*
		3	20	221	−0.598	0.184	10.22	*0.001*
	Macromoths	1	6	42	−0.017	0.006	<0.01	0.967
		2	20	200	−0.786	0.195	16.21	*<0.001*
		3	20	184	−0.740	0.208	12.28	*<0.001*
	Micromoths	1	6	10	1.504	0.931	2.97	0.085
		2	20	135	−0.701	0.217	10.40	*0.001*
		3	17	38	−0.044	0.382	0.01	0.908
	Noctuidae	1	5	11	−0.437	0.906	0.24	0.625
		2	14	40	−0.100	0.357	0.08	0.780
		3	19	120	−0.832	0.295	8.63*	*0.003*
	Geometridae	1	5	19	0.660	0.895	0.51	0.474
		2	19	97	−1.007	0.286	12.41	*<0.001*
		3	16	34	−0.157	0.419	0.14	0.708
	Crambidae	1	4	4	–	–	–	–
		2	19	82	−0.841	0.279	9.17	*0.002*
		3	11	17	0.322	0.560	0.33	0.564
	Erebidae	1	5	9	−2.133	1.127	4.84	*0.028*
		2	14	50	−0.867	0.429	4.13	*0.042*
		3	7	18	−1.920	0.846	6.36	*0.012*
Trap	All families	All	42	988	−0.170	0.099	2.90	0.088
		1	6	76	−0.240	0.406	0.35	0.551
		2	19	694	−0.069	0.127	0.29	0.589
		3	17	218	−0.443	0.215	4.13	*0.042*
	Macromoths	1	6	70	0.027	0.335	0.01	0.936
		2	19	301	−0.477	0.180	7.01	*0.008*
		3	17	160	−0.429	0.255	2.72	0.099
	Micromoths	1	3	7	–	–	–	–
		2	18	393	0.244	0.176	1.87	0.172
		3	12	58	−0.323	0.380	0.73	0.394
	Noctuidae	1	5	33	−0.615	0.364	4.08*	*0.043*
		2	19	183	−0.595	0.216	7.45	*0.006*
		3	16	118	−0.505	0.291	2.90	0.089
	Crambidae	1	1	3	–	–	–	–
		2	17	327	0.139	0.187	0.54	0.465
		3	10	41	0.058	0.471	0.02	0.901
	Erebidae	1	4	5	–	–	–	–
		2	14	83	−0.065	0.003	0.04	0.843
		3	1	1	–	–	–	–
	Hepialidae	1	6	27	0.096	0.533	0.03	0.857
		2	3	3	–	–	–	–
		3	11	30	0.040	0.491	0.01	0.936
	Tortricidae	1	1	2	–	–	–	–
		2	12	49	0.790	0.458	3.23	0.072
		3	3	3	–	–	–	–
Overhead	All families	All	43	434	0.557	0.151	13.63	*<0.001*
		1	4	30	−0.426	0.756	0.31	0.577
		2	20	313	0.446	0.182	5.91	*0.015*
		3	19	91	0.970	0.269	13.84	*<0.001*
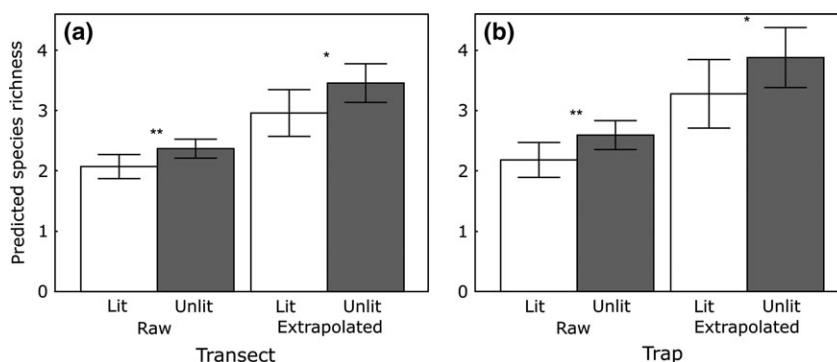
Fig 2 The predicted species richness of moths at vegetation level in the presence and absence of a street light, measured by (a) night-time transect walks and (b) light trapping (Table S5). Both raw and functionally extrapolated values for species richness are shown. Significance indicates likelihood ratio tests on street light presence/absence in GLMMs (*$P < 0.05$; **$P < 0.01$). Error bars show 95% confidence intervals.

likely pollen carriers only (transect: $P = 0.751$; trap: $P = 0.267$). Neither pollen load per moth nor pollen richness per moth was significantly associated with presence/absence of street lights for either sampling method (Table 3). Total pollen count per sampling session was not significantly different between lit and unlit sites for either method (Fig. 3); however, pollen richness per sampling session was significantly lower at lit sites for night-time transects ($P = 0.013$), although not for light traps ($P = 0.773$).

Discussion

Our results show clearly that moths are affected by artificial light from street lights (specifically, high-pressure sodium street lights). Overall, ground-level moth abundance on night-time transects at lit sites was 0.5 times that of unlit sites, whereas overhead flight activity at lit sites was 1.7 times that of unlit sites, although there was some seasonal variation (Table 1). Species richness at ground level at lit sites was between 0.5 and 0.75 times that of unlit sites (depending on sampling and analytical methods; Table S4), and community composition at the family level was significantly changed. This pattern best corresponds to our hypothesis of a disruption effect: moths in the vicinity of street lights are attracted upwards, away from field margins, to fly at higher level around the light. However, it is not clear from our data whether this observed behavioural effect is local (and temporary) disruption of moths moving upwards to lights or whether moths are attracted from further afield (potentially an ecological trap effect); sampling across a longer, continuous transect moving away from lights may clarify this. We did not find any clear evidence of a preferential effect; the effects of light were similar across macro- and micromoths and all of the abundant moth families, with a significant, negative

Table 2 Summary of analyses testing for differences in community composition attributable to sampling method (full dataset) and presence/absence of a street light (within sampling methods). Significant P-values (<0.05) are italicized.

Comparison	Taxonomic level	Sampling method	Model F	P
Treatment: lit vs. unlit	Family	Transect	2.00	*0.017*
		Trap	0.95	*0.023*
Treatment: lit vs. unlit	Species	Transect	1.21	0.070
		Trap	0.66	0.244
Sampling method: transect vs. trap	Family	–	15.41	*<0.001*
	Species	–	11.23	*<0.001*

effect of light on abundance in at least one sampling season for all subanalyses using the night-time transect data (Table 1). Nevertheless, the existence of a significant effect of light in the spring on only Erebidae corresponds with previous work that this family is most strongly attracted to light (Merckx & Slade, 2014). The lack of detectable effects of light on other groups in this sampling season may be due to smaller sample sizes and could be clarified by further spatial replication.

Our data do not show whether there is any long-term impact on moth populations. Recent studies have provided evidence that behavioural responses to artificial light might disrupt reproduction in moths (Van Geffen *et al.*, 2015a,b), thereby suggesting one potential causal pathway to population-level effects, but increased predation and disrupted nocturnal activity are also plausible causes (Macgregor *et al.*, 2015).

Although our results do not conclusively demonstrate an effect of artificial light on pollen transport per moth, there are some indications that nocturnal pollen transport was disrupted. Moths were significantly less likely to carry pollen at lit sites than at unlit sites in the light-trap samples (although when analysis was

Pollen transport metric	Method	N (moths or site pairs)	Effect size	Standard error	Lighting test statistic	P
Probability of a moth carrying pollen	Transect	608	−0.044	0.277	0.02	0.875
	Trap	991	−0.476	0.219	4.67	*0.031*
'Likely' pollen carriers only	Transect	460	0.101	0.316	0.10	0.751
	Trap	463	−0.281	0.253	1.23	0.267
Total pollen load per moth	Transect	142	0.397	0.361	1.21*	0.272
	Trap	221	0.411	0.418	0.97*	0.326
No. pollen types per moth	Transect	142	0.067	0.183	0.13	0.716
	Trap	221	0.120	0.148	0.65	0.419
Total pollen count per sample	Transect	43	−0.686	0.489	1.84*	0.175
	Trap	37	−0.725	0.639	1.34	0.247
No. pollen types per sample	Transect	43	−0.525	0.211	6.21	*0.013*
	Trap	37	−0.055	0.191	0.08	0.773

Table 3 Summary of analyses testing for differences in pollen transport metrics between lit and unlit sites. Lit site metric = unlit site metric × e^{ES}, where ES = lighting effect size from the statistical model, so e^{ES} is the multiplicative effect due to lighting. Lighting test statistic is given for likelihood ratio test unless indicated by *, in which case a Type III ANOVA was used. Significant *P*-values (<0.05) are italicized.

restricted to 'likely pollen carriers', there was no significant difference between lit and unlit sites), whilst in the night-time transect samples, the number of pollen types transported was significantly reduced at lit sites, probably as a result of reduced moth abundance at vegetation level. Additionally, several of the pollen metrics showed nonsignificant trends towards a reduction in overall pollen transport at lit sites (Fig. 3). Any conclusions must be treated with caution, as the majority of analyses conducted on pollen-transport metrics were nonsignificant. However, this result is consistent with our predictions for a behavioural effect on moths in the immediate vicinity of lights; such an effect could lead to reduced flower-visiting activity, causing the reduction we found in per-moth pollen transport and decreasing the contribution made by moths to pollination. Therefore, our results suggest that artificial light might have negative effects not only upon moths but also upon other taxa with which moths interact. Further study is necessary to confirm the existence of an effect of artificial light on pollen transport, and to determine whether this reduction in pollen transport leads to a reduction in pollination.

The majority of previous whole-ecosystem studies of pollination by moths are from tropical ecosystems (Macgregor *et al.*, 2015). This study is the first to measure pollen transport by moths in a temperate agro-ecosystem and found clear evidence that a diverse range of moth species transport pollen of a diverse range of plants in lowland England. Of 1599 moths captured across both night-time transects and light trapping, 363 (22.7%) were found to be carrying pollen on the proboscis. Measures of flower visitation, such as pollen transport, do not prove effective pollination (King *et al.*, 2013), but we sampled pollen only from the proboscis, the most likely location for pollen transport to result in pollen deposition, and an unlikely location for pollen transport to be recorded as the result of contamination.
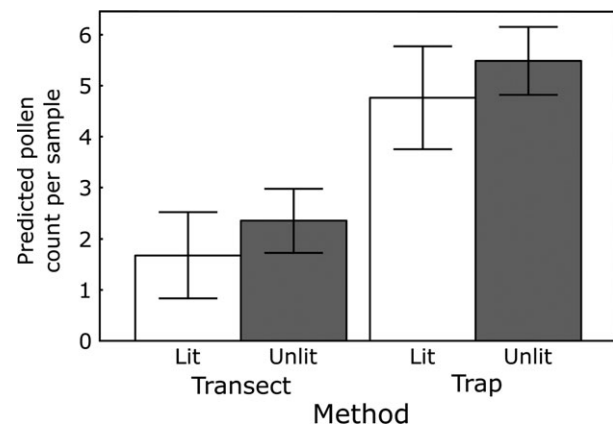

Fig 3 Pollen count per sample of moths at vegetation level in the presence and absence of a street light, predicted by Poisson GLMM and measured by night-time transects and light trapping (Table 3). Error bars show 95% confidence intervals; comparisons between lit and unlit treatments were nonsignificant.

Moth pollination is frequently associated with Sphingidae; however, less than 1% of moths in our study came from this family (Fig. S1). This finding adds to a growing body of evidence suggesting that the contribution of moths, including nonsphingids, to pollination in temperate ecosystems has been previously underappreciated (Macgregor *et al.*, 2015).

Compared to two previous studies of pollen transport by moths, in a Scottish pine forest (Devoto *et al.*, 2011) and a Portuguese meadow (Banza *et al.*, 2015), our findings were broadly intermediate in terms of the proportion of moths carrying pollen and the number of pollen types per moth. We found 28% of moths to be carrying ≥2 pollen types, and a total of 28 pollen types (0.08 per pollen-carrying moth), compared to 3% of moths carrying ≥2 pollen types and 12 plant taxa (0.04 per pollen-carrying moth) in Devoto *et al.* (2011), and 27 plant taxa (0.28 per pollen-carrying

moth) in Banza *et al.* (2015). As our study system, agricultural field margins in lowland central England, can also be considered to be intermediate between these two studies in floral abundance and diversity, this pattern indicates that the contribution of moths to pollen transport may be chiefly determined by the availability of flowers to visit. It is important to note that both previous studies only recorded a pollen-transport interaction where five or more pollen grains of any particular taxon were counted on a single moth; this conservative approach could have led to a lower rate of detection of pollen-transport interactions in these studies compared to ours. In contrast, by collecting pollen solely from the proboscis, our methods were more precise, but could have led to a lower detection rate in our study.

Although the level of certainty that we ascribe to some of our identifications of pollen types (Table S6) was low, there nevertheless appeared to be pollen from several flowers that have not traditionally been associated with moths (e.g. Apiaceae spp., *Lamium* spp. – see Macgregor *et al.*, 2015 for a review of previously documented moth-pollination interactions), in addition to some flowers that are already known to be moth-pollinated, especially those with white flowers with a long corolla (e.g. *Lonicera* spp., *Silene* spp.). The high abundance of *Buddleja davidii* pollen in the data is also notable, as it suggests this nonnative species may compete strongly with native flowers for nocturnal pollination services.

As predicted, our results indicate a difference between moth collection by night-time transects and by 8-W actinic light traps. We knew in advance that it is not sensible to study the effects of artificial lighting with light traps, even though light trapping is a very popular way of sampling moths, as light traps are expected to be less efficient at lit sites than unlit sites (Eisenbeis, 2006). Light-trap samples did show reduced abundances of moths at lit sites in some sub-analyses, but with lower frequency than night-time transect samples, and it is uncertain whether these reductions are driven by the reduced efficiency of trapping at lit sites, the reduced abundances of moths (as recorded by the night-time transects) or a combination of the two.

We have shown the great utility of night-time transects for sampling moths, and we suggest that in most cases, night-time transects provide a less biased sample of the moth community than light traps. The two methods sample a significantly different subset of the moth community present (Fig. S1); light-trap samples were dominated by Noctuidae and Crambidae, with Geometridae in particular under-represented when compared to night-time transects. Transect samples appear to contain a more even spread among families, although strong-flying families such as Hepialidae and Sphingidae are relatively under-represented. We suspect that this is because light traps are more a measure of activity than abundance (Devoto *et al.*, 2011) and these families, being highly active, are over-represented in light-trap samples. However, it could also be because these families, being relatively harder to catch with a hand net, are under-represented in transect samples. The biased nature of light trapping should be considered when using light-trap data for ecological research.

We captured 1.6 times more moths using light traps than on night-time transects across the same number of sampling sessions. However, it may be argued that night-time transects captured moths more efficiently than light traps: whilst a typical night-time transect sampling session lasted for less than 1.5 h (mean = 75.1 min, range 51–92 min) and required one return trip to the field site, light traps were run unattended for c. 8 h per night but required two trips for set-up and collection. We therefore estimate a rate of capture of 10.6 moths per hour on night-time transects, compared to 2.7 moths per hour using light traps. Capture rates for high-powered light traps (e.g. 125-W mercury-vapour Robinson traps) may be considerably higher than our 8-W actinic Heath traps, but light-trap designs with low attraction radii (and therefore lower capture rates) are preferable if they facilitate the linking of samples to local conditions (Van Grunsven *et al.*, 2014). Night-time transects allow such links to be made, but at a higher rate of capture. Nevertheless, although we successfully used night-time transects to sample pairs of sites on the same night, light traps may be preferable for the sampling of larger numbers of sites simultaneously, especially if they are geographically dispersed.

This study has demonstrated that artificial light from street lights affects the abundance and community composition of moths at vegetation level in field margins; a population-level effect that can be explained by individual moths being attracted to lights. Such behavioural effects could have impacts for both moth populations and their interactions with other organisms, but our data do not reveal whether there are underlying negative impacts. There is a need for further study into the links between light pollution and observed moth population declines.

Our study focused on the current most-used type of street light in the UK, high-pressure sodium (HPS). Other lighting types, such as light-emitting diodes (LEDs), may be more, or less, attractive to moths (Huemer *et al.*, 2010; Pawson & Bader, 2014) and therefore have correspondingly greater or lesser impacts than those reported in this study. Recent studies have shown

that such changes in street lighting technology, and similarly the uptake of part-night lighting, can affect nocturnal organisms (Azam *et al.*, 2015; Stone *et al.*, 2015); it is important to address what the effects of such changes are on moths and their interactions.

We demonstrated that light trapping and night-time transects differ in the assemblages of moths they sample. Although light trapping is an important sampling method for nocturnal moths, we demonstrate that night-time transects are a valuable tool for sampling moths that may be free from some of the biases associated with light trapping. In addition, night-time transects offer an opportunity to standardise sampling of Lepidoptera and other insect taxa across both day- and night-time. Although such studies would require careful experimental design to account for factors such as different detectability of insects, these direct comparisons are otherwise not possible when using light traps to sample the nocturnal component of the community.

In conclusion, this study demonstrates that artificial light from street lights attracts moths upwards to fly at higher levels above the field margin. We found for the first time that a high proportion of moths within an agro-ecosystem (representing many species from several families) transported pollen of a substantial range of plant species. The wider importance of moths within ecological networks demands attention, particularly as we found some evidence that this pollen-transport service might be disrupted by artificial lights, potentially leading to impacts on pollination. Future research should extend beyond the direct effects of light upon moths, to understand the cascading effects of lighting on ecosystem functioning.

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Author contributions

All authors contributed to designing the experiment, analysing the data and preparing the manuscript. Field and laboratory work was conducted by C.J.M.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Criteria for matched-pairs.
Appendix S2. Image references for Fig. 1.
Figure S1. Composition by family of samples from night-time transects and light traps.
Tables S1-6. Summaries of data and additional analyses.