

SUPPLEMENTARY INFORMATION to:

Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light

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Collection of echolocation signals:

We used Pettersson D500x detectors, with firmware 2.1.1 in 2012, 2.1.5 in 2013, and 2.D.6 in 2014, 2015 and 2016. All detectors were powered by external 6V Panasonic dry-fit lead acid batteries, and placed in Explorer Cases (GT Line srl, Bologna, Italy) permanently mounted in a tree close (max distance 6 meters) to the middle light post in each transect, see figure S2. Detectors were fitted with three 32GB Compact Flash data cards (Adata or Transcend). Detectors were set to Profile 3 (sampling frequency 500 kHz, pretrigger off, 5 seconds file duration, high-pass filter on, auto recording on, trigger sensitivity 1), and with the input gain at level 40, and the trigger at level 40. Detectors were set to start recording from 10 min after sunset until 30 min before sunrise. Sound files were only included as long as each detector at all transects within a site had logged wave files all night; e.g. until one hour before sunset or the next night. We collected in total 1,554,723 files; with in total 518,832 bat passes.

Sound analysis

Wave files were analysed with the SonoChiro software package (Biotope Research & Development, Mèze, France). The Northern Temperate region was selected to exclude most species not present at the experimental sites. We used the default program settings, e.g. the sensitivity of the program was set to 7 and the minimum call duration was set to 0.5 ms. We only included bats of which the calls were assigned to one of the species groups with a confidence index of six or above, and we did not use the program to identify calls to the species level. With these conditions, manual inspection of the groups assigned to the bat calls revealed these were correct.

Species grouping: species which are most likely present in the three groups, based on the distribution data for the Netherlands according to [1]:

Group 1: *Myotis daubentonii*, *Myotis mystacinus*, *Myotis brandtii*, *Myotis nattereri*, *Plecotus auritus*;

Group 2: *Pipistrellus pipistrelles*, *Pipistrellus nathusii*

Group 3: *Nyctalus nyctalus*, *Nyctalus leisleri*, *Eptesicus serotinus*.

Calculation of the number of passes per night per group:

If pulses of a bat species group were present in a wave file, and the confidence index for the group identification was six or above, we added one pass to the total passes of that group for the current night.

Insect data collection and processing

Sticky sheets: Thick A4 sheets of white paper were cut down to approximately 17.5 cm x 29.6 cm and laminated with clear, transparent A4 laminate sheets. The sheets were cut down to approximately 18.8 cm x 30.2 cm and then coated in tangle trap glue on both sides (Andermatt Biocontrol). The area that was coated was approximately 17.5 cm x 28 cm per side (± 1 cm in length), with an uncoated area at the top of the sheet to allow for handling in the field. Sticky sheet cages: PVC pipe (20 cm diameter) and aluminum strips attached with rivets, these cages were constructed to be light and durable, and stood 30 cm high. The sticky sheets were placed within the two pipe sections and held by the aluminium strips (see also figure S3).

Sticky sheets were consequently scanned using a flatbed scanner, set the file format to TIFF with 600 dpi and 24 bits colour quality. Files were processed using ImageJ program [2]. Non-insect material (leaves, dirt, pollen) and wings from *Lepidoptera* were removed using the clear tool (due to their small biomass compared to the area they occupy). For each image, a fixed area was selected (B4 size). The threshold for selecting dark pixels was set to a saturation level of 175, and, in order to avoid including dust particles, dark pixels were only included if they were part of a cluster of dark pixels exceeding a surface of 0.5 mm².

Light treatment:	Mean (Lux)	1 s.e.m.
Dark	0.004	0.002
White	10.061	1.139
Green	7.030	0.771
Red	5.717	0.974
White, green and red together	7.630	1.222

Table S1. Average light levels for the different treatments. Light intensity was measured at ground level underneath each light post with an LMT B36 Lux meter (LMT lichtmesstechnik GMBH, Berlin, Germany).

site	2012		2013		2014		2015		2016		2012-2016 total nights	passes/night (lights on)		
	1	2	1	2	1	2	1	2	1	2		group 1	group 2	group 3
1	2	4 (1)	6	2	7 (1)	6	9	12	9	3	62 (2)	2.90	677	47.1
2				2		6	9	11	7 (1)	3	39 (1)	7.55	562	12.4
3	5	4 (1)	5	6	5 (2)	5		10	6 (1)	9	59 (4)	1.18	347	164.5
4	1	2	5	1	6 (1)	6	9	4	8 (1)	4 (1)	48 (3)	1.26	968	95.7
5	5	8 (2)	4	3	5 (1)	8	9	12	7 (1)	4 (1)	69 (5)	1.15	725	36.1
6	4	4	5	3	12 (1)	13	9	7		1	59 (1)	1.38	219	15.2
7	3		4	2	4	6	9	5	8	3	44	1.59	222	6.7
8	2	5 (1)	5	4	8 (3)		8 (1)	3	6 (1)	8 (1)	55 (7)	2.41	1148	174.2

Table S2. Number of nights sampled for each of the 8 sites during early (1) and late (2) summer in the years 2012 – 2016. The numbers only include nights at which detectors were functional all night at all four transects within a site. The number of nights sampled with the lights off (because of the sampling of moth species) is indicated between brackets.

	est.	SE	z-ratio	p-value	
Group 1: <i>Myotis sp.</i> + <i>Plecotus sp.</i> (with lights on)					
dark - green	1.0951	0.2717	4.031	< 0.0005	*
dark - red	0.0153	0.2581	0.059	1	
dark - white	0.7533	0.2658	2.833	< 0.05	*
green - red	-1.0799	0.2718	-3.973	< 0.0005	*
green - white	-0.3419	0.2791	-1.225	1	
red - white	0.7380	0.2660	2.775	< 0.05	*
Group 2: <i>Pipistrellus sp.</i> (with lights on)					
dark - green	-0.9796	0.2812	-3.484	< 0.005	*
dark - red	-0.4985	0.2812	-1.773	0.46	
dark - white	-1.5545	0.2812	-5.529	< 0.0001	*
green - red	0.4811	0.2812	1.711	0.52	
green - white	-0.5749	0.2811	-2.045	0.25	
red - white	-1.0560	0.2811	-3.756	0.001	*
Group 2: <i>Pipistrellus sp.</i> (during insect sampling with lights on)					
dark - green	-0.7186	0.3545	-2.027	0.26	
dark - red	0.3450	0.3589	0.961	1	
dark - white	-0.6356	0.3547	-1.792	0.44	
green - red	1.0636	0.3564	2.984	< 0.05	*
green - white	0.0830	0.3522	0.236	1	
red - white	-0.9807	0.3566	-2.75	< 0.05	*
Insects (with lights on)					
dark - green	-0.7646	0.1002	-7.631	< 0.0001	*
dark - red	-0.4796	0.1002	-4.787	< 0.0001	*
dark - white	-0.7676	0.1002	-7.662	< 0.0001	*
green - red	0.2850	0.1002	2.844	< 0.05	*
green - white	-0.0031	0.1002	-0.031	1	
red - white	-0.2881	0.1002	-2.875	< 0.05	*

Table S3. Results of the (Bonferroni corrected) post-hoc pairwise comparisons of the best fitted models with a significant effect of the main fixed term (table 2). Significance values are Bonferroni corrected; significant differences between pairs are indicated with an asterisk.

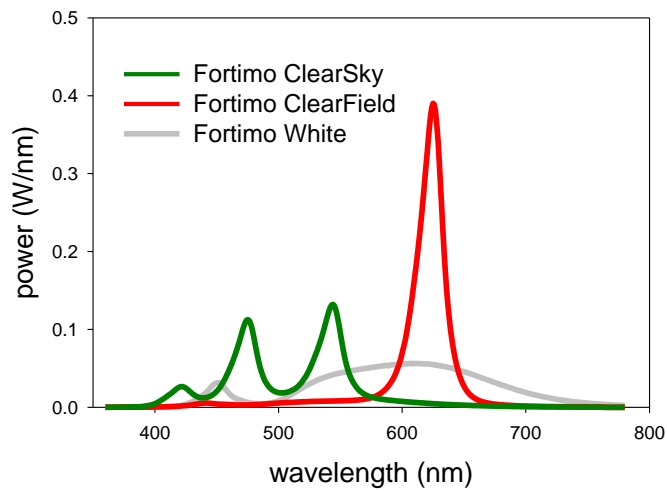


Figure S1. Spectral composition of the light installed at the experimental field sites. The total output of each spectrum is normalized to Lux.



Figure S2. D500x detectors were placed in Explorer Cases permanently mounted in the tree closest to the light post in the forest edge.



Figure S3: position of custom-made white sticky traps below the light post.

References

1. Broekhuizen S, Spoelstra K, Thissen JBM, Canters KJ, Buys JC, editors. 2016 Atlas van de Nederlandse Zoogdieren. Leiden: Naturalis.
2. Rasband W. 2012 ImageJ: Image processing and analysis in Java. *Astrophys. Source Code Libr.* 1, 06013.