**Supplementary Information for**

**Bats adjust echolocation and social call design as a response to urban environments**

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**Supporting methods**

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Figure S. Location of urban (n=3, u1-u3) and rural (n=3, r1-r3) study sites in Berlin and Brandenburg.

*Site characterization*

We measured different parameters that supported site selection and classification of sites as ‚urban‘ and ‚rural‘. We used a highly sensitive sensor (Sky Quality Meter SQM, Unihedron, Canada), to measure the brightness of the night sky at a solid angle of 42° around the central axis (full width at half maximum) resulting in magnitudes per arc second2 as values. Measurements were taken at five different nights of clear sky (not overcast) per site during the sampling period and averaged to mean±SD. Larger values in mag/arcsec2 indicate darker skies. During the same nights, we measured anthropogenic sound level using a commercial sound level meter (SLM, Model: Lutron, SL-4001), which makes calibrated measurements of sound pressure level (SPL; dB re 20 μPa). We measured the proportion of imperviousness land cover surrounding each site (landscape scale) at 500m radii with publicly available data on the surrounding landscape features from the Berlin Environmental Atlas (Senatsverwaltung für Stadtentwicklung und Umwelt, 2016; Landesamt für Umwelt at a 2 × 2-m resolution and using the Zonal statistics tool in QGIS software v. 2.18.0 (QGIS Development Team, 2018). To account for the influence of other influencing variables on echolocation behavior, all site locations of each category (urban/rural) were selected to have similar habitat features surrounding them. We calculated distances to nearest water source, trees, and buildings. Height of trees and buildings were measured using a laser range finder (LRF 600, Walther GmbH, Germany).

Table S. Measured parameters at study sites (n= 3 urban sites in Berlin city center and 3 rural sites in Brandenburg district)

|  |  |  |
| --- | --- | --- |
|  | **rural** | **urban** |
|  | **r1** | **r2** | **r3** | **u1** | **u2** | **u3** |
| mean sound pressure leveldB re 20 μPa | 24.05±3.08 | 26.02±3.01 | 29.6±2.06 | 56.0±7.42 | 47.2±4.54 | 57.8±4.58 |
| mean sky brightness (mag/arcsec2) | 18.96±0.21 | 18.9±0.6 | 18.08±0.43 | 16.06±0.77 | 16.0±0.71 | 15.4±0.56 |
| % impervious surface in 500m radius | 42.98 | 50.13 | 47.82 | 72.31 | 68.14 | 85.74 |
| distance to nearest building (m) | 50.5 | 43.7 | 63.8 | 82.5 | 59.4 | 49.3 |
| mean height of buildings in 100m radius (m) | 5.8 | 8.6 | 7.6 | 16.2 | 19.8 | 21.3 |
| distance to water (m) | 423 | 493 | 529 | 89 | 101 | 33 |
| distance to nearest tree (m) | 78.8 | 37.8 | 56.4 | 68.6 | 45.5 | 52.3 |
| mean height of vegetation in 100m radius (m) | 21.4 | 18.5 | 16.8 | 14.5 | 16.6 | 15.1 |

*Bat call analysis*

Recorded bat calls were identified using the software bcAdmin 2.0. followed by automatic species identification with the software batIdent 1.03 (ecoObs GmbH, Nuremberg, Germany). Because there is evidence, that automatic species identification is subject to a high rate of misidentification (Russo and Voigt, 2016; Rydell et al., 2017, Lopéz-Baucells et al., 2019), we postvalidated files with classification accuracy < 95 %. Using the software BatSound ver. 4.1.4 (Pettersson Elektronik AB, Uppsala, Sweden), we post-validated files comparing measured parameters on spectrograms (shape, peak frequency, duration, intervals) with those available from the literature (Ahlén, 1990; Vaughan et al., 1997; Barataud, 2015; Marckmann and Pfeiffer, 2020).

Manual analyses revealed that calls from *Pipistrellus pipistrellus* and *Pipistrellus* *pygmaeus* were assigned correctly with the software batIdent if automatically assigned ≥84% and for *Nyctalus noctula* ≥93% to this species. In contrast, calls assigned to *Eptesicus serotinus*, *Vespertilio murinus,* and *P. nathusii* were assigned correctly with batIdent only if automatically assigned ≥95% to this species. For *Myotis* spec. rates of accuracy ranged from 95 to 100%.

Overlap in call characteristics makes it difficult and sometimes impossible to distinguish species by their echolocation calls (Barataud, 2015). This is the case for the mid-frequency nyctaloid species *Eptesicus serotinus*, *Vespertilio murinus* and *Nyctalus leisleri.* Therefore*,* bats of the genera *Nyctalus*, *Vespertilio*, and *Eptesicus* are often grouped (pooled) as ‘nyctaloid’ or ‘NEV’. However, to our view, this has strong implications for conservation practice, especially in view of urban planning. Thus, we aimed at manual species identification on the basis of the most distinctive call types according to the following criteria of Marckmann and Pfeiffer (2020):

In contrast to other nyctaloid species, calls of ***E*. *serotinus*** reveal no changes in call structure within sequences. On average, *E*. *serotinus* shows shorter calls and call intervals than other nyctaloid species. Call intervals between qcf calls are relatively constant at around 300 ms. In contrast, *V*. *murinus* and *N*. *leisleri* have longer and more irregular call intervals. In addition, the calls of *V*. *murinus* and *N*. *leisleri* are almost constant (without frequency changes). Qcf calls are characterized by a downwards frequency change of 5 to 10 kHz over the entire course of the call. Thus, call sequences with fm-qcf and qcf calls have been identified if there were no call changes and the calls were uniform in shape, frequency and call interval. For species assignment, we only used sequences with call intervals of approximately 300 ms and lowest frequencies between 21 and 25 kHz for qcf calls. For fm-qcf calls we used only sequences with call intervals between 100 and 300 ms and sequences with calls which did not exceed 26 kHz and which showed a pronounced uppercut at the end. Fm-qcf and fm calls for ***N*. *leisleri*** are not characteristic, since they show strong overlaps in the frequency range with all other "nyctaloid" calling types. Therefore we only used qcf calls with a characteristic frequency of 23 kHz for reliable species determination. We used at least three sequences with regular call changes (typical „plip-plops“) and unmistakable qcf calls (together > 10 calls) and excluded sequences with confusion species in temporal proximity (+/- 2 min.). As ***V*. *murinus*** shows an extreme overlap in the call repertoire with other "nyctaloid" species, echolocation calls cannot be reliably determined. Our species identifications are solely based on courtship calls during the mating season. We used at least one typical sequence of courtship calls with intermediate quiet fm calls.

Calls, which could not be identified to species level according to these criteria were grouped to sonotype Nycmi (containing *Nyctalus leisleri*, *Eptesicus serotinus* and *Vespertilio murinus*).

**Supporting results**

Median and range values for echolocation and social calls

Table S2. Median and range values for time and frequency variables of echolocation calls in urban and rural Pipistrellus pipistrellus; FStart = start frequency; Fend = end frequency; FmaxE = frequency of maximum; freq=bandwith; dur = duration; IPI= Inter pulse interval; Frequencies are given in kHz, time variables in ms. n=number of calls used to calculate mean; N= number of recorded sequences

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Urban (n=1,810/N=486) |  | FStart (kHz) | FmaxE (kHz) | FEnd (kHz) | freq (kHz) | dur (ms) | IPI (ms) |
| Median | 70.2 | 49.0 | 47 | 23.5 | 3.9 | 88.3 |
| Range | 65-85.3 | 47.5-52.2 | 47-51.5 | 15.5-36.5 | 2-4.7 | 81.6-92.6 |
| Rural(n=1,659/N=486) | Median | 64.5 | 48.1 | 44.7 | 20.5 | 5.9. | 101 |
| Range | 50.8-95.2 | 41.1-50.6 | 40.7-49.9 | 13.1 | 3.2-8.6 | 58.7-210.6 |

Table S3. Median and range values for time and frequency variables of social calls in urban and rural Pipistrellus pipistrellus; FStart = start frequency; Fend = end frequency; FmaxE = frequency of maximum; freq=bandwith; dur = duration; IPI= Inter pulse interval; Frequencies are given in kHz, time variables in ms. n=number of calls used to calculate mean; N= number of recorded sequences

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Urban (n=343/N=108) |  | FStart (kHz) | FmaxE (kHz) | FEnd (kHz) | freq (kHz) | dur (ms) |
| Median | 35.5 | 20.5 | 16.2 | 19.5 | 32 |
| Range | 33.1-37.0 | 17.5-24.0 | 15.5-17.1 | 17.5-22.2 | 27.8-40.0 |
| Rural(n=163/N=108) | Median | 34.5 | 19.0 | 15.3 | 19.1 | 27.5 |
| Range | 33.0-38.0 | 14.9-20.5 | 14.9-20.5 | 13.6-26.7 | 18.0-35.5 |

Median values for overall activity, foraging activity and social call rate on urban and rural study sites

Table S4: Median values for ecological parameters for each of the urban sites (a) and rural sites (b)

|  |  |  |  |
| --- | --- | --- | --- |
| **(a) urban sites** | ***P. pipistrellus*** | ***E. serotinus*** | ***N. noctula*** |
|  | u1 | u2 | u3 | u1 | u2 | u3 | u1 | u2 | u3 |
| Overall activity (recordings/h) | 4.9 | 6.6 | 5.3 | 2.9 | 4.9 | 3.2 | 0.4 | 0.6 | 1.1 |
| Foraging activity(final buzzes/night) | 0.9 | 1.9 | 2.1 | 0.7 | 1.3 | 2.3 | 0.01 | 0.07 | 0.09 |
| Social call rate (calls/night) | 1.1 | 1.4 | 2.1 | 0.03 | 0.07 | 0.04 | 0.0 | 0.0 | 0.01 |

|  |  |  |  |
| --- | --- | --- | --- |
| **(b) rural sites** | ***P. pipistrellus*** | ***E. serotinus*** | ***N. noctula*** |
|  | u1 | u2 | u3 | u1 | u2 | u3 | u1 | u2 | u3 |
| Overall activity (recordings/h) | 2.8 | 1.5 | 0.9 | 1.9 | 0.5 | 1.1 | 3.2 | 2.7 | 1.8 |
| Foraging activity(final buzzes/night) | 1.61 | ´2.1 | 3.2 | 0.9 | 1.8 | 3.9 | 3.9 | 4.2 | 2.7 |
| Social call rate (calls/night) | 0.26 | 0.5 | 0.3 | 0.0 | 0.0 | 0.01 | 0.07 | 0.05 | 0.03 |

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