

This is the citation of the final version of the article:

Oliveira, F.G., Tapisso, J.T., von Merten, S. *et al.* Behavioral responses of rural and urban greater white-toothed shrews (*Crocidura russula*) to sound disturbance. *Urban Ecosyst* (2021).

<https://doi.org/10.1007/s11252-020-01079-y>



---

Published: 03 January 2021

## Behavioral responses of rural and urban greater white-toothed shrews (*Crocidura russula*) to sound disturbance

Flávio G. Oliveira , Joaquim T. Tapisso, Sophie von Merten, Leszek Rychlik, Paulo J. Fonseca & Maria da Luz Mathias

*Urban Ecosystems* (2021) | [Cite this article](#)

87 Accesses | 5 Altmetric | [Metrics](#)

# Urban Ecosystems

## Sound disturbance does not explain behavioral differences between rural and urban greater white-toothed shrews (*Crocidura russula*)

--Manuscript Draft--

<b>Manuscript Number:</b>	
<b>Full Title:</b>	Sound disturbance does not explain behavioral differences between rural and urban greater white-toothed shrews ( <i>Crocidura russula</i> )
<b>Article Type:</b>	Manuscript
<b>Keywords:</b>	behavioral plasticity; heterothermy index; nocturnal activity; risk perception; Soricidae; urban noise
<b>Corresponding Author:</b>	Flávio Gomes Oliveira, MSc Universidade de Lisboa Centro de Estudos do Ambiente e do Mar Lisboa, Alvalade PORTUGAL
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Universidade de Lisboa Centro de Estudos do Ambiente e do Mar
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Flávio G. Oliveira
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Flávio G. Oliveira Joaquim T. Tapisso Sophie von Merten Leszek Rychlik Paulo J. Fonseca Maria L. Mathias
<b>Order of Authors Secondary Information:</b>	
<b>Funding Information:</b>	Fundação para a Ciência e a Tecnologia (PD/BD/109400/2015) Mr. Flávio G. Oliveira
<b>Abstract:</b>	The development of urban areas imposes challenges that wildlife must adapt to in order to persist in these new habitats. One of the greatest changes brought by urbanization was an increase in anthropogenic noise, which may impair communication and detection of acoustic cues required for survival. Small mammals have been neglected in the study of behavioral adaptations to urban environments and sound disturbance. In this study, we compared the behavioral responses of urban and rural greater white-toothed shrews, <i>Crocidura russula</i> , to different sound stimuli. A total of 32 shrews, 16 from each habitat, were exposed in captivity to four sound treatments: silence, tawny owl calls, traffic noise and white noise. Urban and rural shrews responded differently when exposed to the silence treatment. Urban animals were more active, fed more frequently, and used less torpor than rural counterparts. However, responses to the other three sound treatments were similar: both urban and rural shrews exhibited a slight decrease in activity and feeding behavior, as well as more fleeing responses, when exposed to traffic noise or white noise, but not to owl calls. These results confirm that adaptation to the urban environment leads to changes in the natural behavior of greater white-toothed shrews, but not to changes in their acoustic response. Factors besides soundscape are discussed in order to explain the behavioral differences between urban and rural shrews.
<b>Suggested Reviewers:</b>	Lisa Angeloni, Dr. Professor, Western State Colorado University Lisa.Angeloni@colostate.edu

	Effects of noise on wildlife
	<p>Mark Brigham  Professor, University of Regina  Mark.Brigham@uregina.ca  Energetics and daily torpor</p>
	<p>Hans W. Slabbekoorn, Dr.  Associate Professor, Universiteit Leiden  h.w.slabbekoorn@biology.leidenuniv.nl  Anthropogenic noise (in birds)</p>
	<p>Rafał Łopucki  Uniwersytet Medyczny w Lublinie  lopucki@kul.pl  Impacts on small mammals along an urbanization gradient</p>
	<p>Ernő Vincze  University of Pannonia  erno.vincze@gmail.com  Urban ecology. Developed method to calculate the "recovery rate"</p>

[Click here to view linked References](#)

1  
2  
3  
4 1 TITLE: Sound disturbance does not explain behavioral differences between rural and urban  
5  
6 2 greater white-toothed shrews (*Crocidura russula*)  
7  
8

9 3  
10  
11 4 Flávio G. Oliveira<sup>1,\*</sup>, Joaquim T. Tapisso<sup>1</sup>, Sophie von Merten<sup>1</sup>, Leszek Rychlik<sup>2</sup>, Paulo J.  
12  
13 5 Fonseca<sup>3</sup>, Maria L. Mathias<sup>1</sup>  
14  
15  
16 6

17  
18  
19 7 <sup>1</sup>Department of Animal Biology and CESAM – Centre for Environmental and Marine Studies,  
20  
21 8 Faculty of Sciences, University of Lisbon, Campo Grande, Lisbon, Portugal

22  
23 9 <sup>2</sup>Department of Systematic Zoology, Institute of Environmental Biology, Adam Mickiewicz  
24  
25 10 University, Poznań, Poland

26  
27  
28 11 <sup>3</sup>Department of Animal Biology and cE3c - Centre for Ecology, Evolution and Environmental  
29  
30 12 Changes, Faculty of Sciences, University of Lisbon, Campo Grande, Lisbon, Portugal  
31  
32

33 13  
34  
35 14 \*Corresponding author

36  
37  
38 15 F. G. Oliveira, Department of Animal Biology of Faculdade de Ciências da Universidade de  
39  
40 16 Lisboa & CESAM, building C2, 3<sup>o</sup> floor, room 2.3.03, Campo Grande, 1749-016 Lisboa,  
41  
42 17 Portugal. Phone number: 217500000. Email address: flavioagoliveira@gmail.com  
43  
44  
45 18

46  
47  
48 19 ORCID  
49  
50 20 Flávio G. Oliveira: <https://orcid.org/0000-0001-5510-7663>  
51  
52 21 Joaquim T. Tapisso: <https://orcid.org/0000-0002-8664-9956>  
53  
54 22 Sophie von Merten: <https://orcid.org/0000-0001-6010-1127>  
55  
56 23 Leszek Rychlik: <https://orcid.org/0000-0002-1479-1443>  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 24 Paulo J. Fonseca: <https://orcid.org/0000-0002-2663-9385>  
5

6  
7 25 Maria L. Mathias: <https://orcid.org/0000-0003-3876-958X>  
8

9 26  
10

## 11 27 **Acknowledgements** 12

13  
14 28 Shrews were trapped in Sintra and Lisbon under the license nº 37 / 2016 / CAPT, issued  
15  
16 29 by ICNF. We thank users “sean.townsend”, “YleArkisto” and “Benboncan” for allowing us to  
17  
18 30 freely download tawny owl calls and users “Robinhood76” and “C klankbeeld 2014” for  
19  
20 31 allowing us to freely download traffic noise from the freesound.org website. Tawny owl calls  
21  
22 32 were kindly provided by Dr. Tomi Trilar from the Slovenian Museum of Natural History. We  
23  
24 33 thank all the field assistants that helped us to trap and bring the shrews to the Faculty: Ana  
25  
26 34 Cerveira, Ana Filipa Durão, Daniel Mameri, Diogo Barros, Fernando Madeira, Inês Fontes, João  
27  
28 35 Gameiro, Pedro David, Rita Andrade, Sara Oliveira and Tiago Maçarico. Thanks are due to  
29  
30 36 FCT/MCTES for the financial support to (UIDP/50017/2020+UIDB/50017/2020), through  
31  
32 37 national funds. This work was supported through a PhD grant (ref: PD/BD/109400/2015) granted  
33  
34 38 to FGO and financed by the Portuguese Foundation for Science and Technology (FCT).  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 39 Abstract

40 The development of urban areas imposes challenges that wildlife must adapt to in order to persist  
41 in these new habitats. One of the greatest changes brought by urbanization was an increase in  
42 anthropogenic noise, which may impair communication and detection of acoustic cues required  
43 for survival. Small mammals have been neglected in the study of behavioral adaptations to urban  
44 environments and sound disturbance. In this study, we compared the behavioral responses of  
45 urban and rural greater white-toothed shrews, *Crocidura russula*, to different sound stimuli. A  
46 total of 32 shrews, 16 from each habitat, were exposed in captivity to four sound treatments:  
47 silence, tawny owl calls, traffic noise and white noise. Urban and rural shrews responded  
48 differently when exposed to the silence treatment. Urban animals were more active, fed more  
49 frequently, and used less torpor than rural counterparts. However, responses to the other three  
50 sound treatments were similar: both urban and rural shrews exhibited a slight decrease in activity  
51 and feeding behavior, as well as more fleeing responses, when exposed to traffic noise or white  
52 noise, but not to owl calls. These results confirm that adaptation to the urban environment leads  
53 to changes in the natural behavior of greater white-toothed shrews, but not to changes in their  
54 acoustic response. Factors besides soundscape are discussed in order to explain the behavioral  
55 differences between urban and rural shrews.

56 *Keywords:* behavioral plasticity, heterothermy index, nocturnal activity, risk perception,  
57 Soricidae, urban noise.

## Introduction

The increased concentration of human populations in urbanized areas is a global phenomenon severely affecting biodiversity (Seto et al. 2012). The resulting environment is extremely altered, with few patches of natural vegetation and high levels of anthropogenic disturbance such as chemical, light and noise pollution (Barber et al. 2010; Hölker et al. 2010). Animals persisting in these environments must overcome such disturbances through adaptation. These adaptations result from either a species' phenotypic plasticity of traits already present, or from genetic shifts in traits that increase the species' fitness under the new environmental conditions (Sol et al. 2013; McDonnell and Hahs 2015). Because the capacity to plastically adjust behaviors is limited (Snell-Rood 2013), not all species are able to adapt to these disturbances and colonize or persist in cities (Wong and Candolin 2015). In the case of small, ground-dwelling mammals, this is reflected in numerous studies showing a general decrease in abundance and richness of urban-sensitive species in cities when compared with adjacent areas (e.g. Cavia et al. 2009; Gomes et al. 2011; Klimant et al. 2017; Łopucki and Kitowski 2017). Without sufficient vegetation cover and high levels of habitat connectivity, many small mammals cannot persist in urban areas (Cavia et al. 2009; Gomes et al. 2011; Łopucki and Kitowski 2017).

One of the most prominent and well-studied urban disturbances is man-made noise as a byproduct of urbanization (Read et al. 2014; Shannon et al. 2016b). Exposure to anthropogenic noise has several consequences for wildlife, from interfering with the ability to forage and stay alert for predators (Klett-Mingo et al. 2016; Finch et al. 2020) to declines in reproductive success (Halfwerk et al. 2011) and altered trophic relationships (Francis et al. 2009). The diverse arrays of anthropogenic sound sources, which vary in frequency, intensity, and duration, are relevant to understand how organisms perceive, and react to, these sounds. Traffic noise is one of the most

1  
2  
3  
4 81 common and best studied sources of anthropogenic noise (Luo et al. 2014; Pieniżek et al. 2017).  
5  
6  
7 82 Depending on the location inside the urban matrix, exposure to traffic noise can either be  
8  
9 83 continuous and chronic or sudden and unpredictable. The later often elicits strong responses from  
10  
11 84 animals similar to antipredator behavior (Frid and Dill 2002; Shannon et al. 2014; Klett-Mingo et  
12  
13 85 al. 2016), and while the former may not be perceived as threatening due to habituation, it can  
14  
15  
16 86 interfere with the animals' ability to detect acoustic cues or signals such as calls from  
17  
18  
19 87 conspecifics or sounds made by predators or prey (Patricelli and Blickley 2006; Barber et al.  
20  
21 88 2010).

22  
23  
24 89 Small mammals partly depend on acoustic cues to communicate with conspecifics  
25  
26 90 (Simeonovska-Nikolova 2004; Musolf et al. 2010) and to assess the structure of their habitat  
27  
28 91 (Siemers et al. 2009). Several studies have shown the influence of noise on small mammal  
29  
30 92 behavior in both the field and the lab: traffic noise can impact the activity levels (Shannon et al.  
31  
32  
33 93 2014) and predator detection abilities (Shannon et al. 2016a) of black-tailed prairie dogs,  
34  
35  
36 94 interfere with communication signals (foot drumming) used by endangered Stephens' kangaroo  
37  
38 95 rat (Shier et al. 2012), and impact the locomotor activity of striped field mice (Pieniżek et al.  
39  
40 96 2017). Furthermore, the behavior and physiology of wild mice has been shown to be negatively  
41  
42  
43 97 impacted by mining machinery noise, with different effects based on its amplitude and frequency  
44  
45  
46 98 (Mancera et al. 2018). However, to the best of our knowledge, no laboratory studies have yet  
47  
48 99 compared the impacts of sound disturbance on the behavior of rural and urban populations of  
49  
50 100 small mammals.

51  
52  
53 101 The main aim of the present study was to compare the behavioral responses of small  
54  
55 102 mammals from rural and urban areas to sound disturbances, using as a model species the greater  
56  
57  
58 103 white-toothed shrew (*Crocidura russula*). Shrews are sensitive to urbanization, and decline in  
59  
60  
61  
62  
63  
64  
65



1  
2  
3  
4 104 abundance and richness in highly urbanized areas (Vergnes et al. 2013; Klimant et al. 2017;  
5  
6  
7 105 Łopucki and Kitowski 2017). Similar to other shrew species, *C. russula* is a vocal species, using  
8  
9 106 a range of different sounds for communication and allegedly a rudimentary form of echolocation,  
10  
11 107 termed echo-orientation (Siemers et al. 2009). As opposed to other small mammal species like  
12  
13  
14 108 mice, which communicate predominantly using ultrasonic calls over very short distances (Musolf  
15  
16 109 et al. 2010), shrews mainly use sonic frequencies (Zsebők et al. 2015), which travel further and  
17  
18  
19 110 might thus be more affected by noise. Consequently, noise might affect the vocal communication  
20  
21 111 and echo-orientation of shrews. They thus offer a good model species to test sound disturbance  
22  
23  
24 112 in a synurbic small mammal.

25  
26 113 To differentiate if shrews from different habitats (urban vs. rural) respond differently to  
27  
28 114 anthropogenic noise and natural sounds (the calls of predators), we exposed shrews at night to  
29  
30  
31 115 traffic noise and predator calls. We selected calls of the tawny owl (*Strix aluco*), a predator  
32  
33 116 relatively common in rural areas but mostly absent in urban areas (Capizzi 2000). Since the  
34  
35  
36 117 soundscape differs along the rural-urban gradient, we predict rural shrews will react more  
37  
38 118 strongly to owl calls and traffic noise. As owl calls are seldom heard in cities (personal  
39  
40  
41 119 observation), and urban shrews are likely habituated to traffic noise, we expect them to exhibit a  
42  
43 120 weaker response to these sounds.

44  
45 121 A recent study suggested daily torpor can be used not only as an energy-saving strategy  
46  
47  
48 122 but also as a strategy to avoid danger (Geiser and Brigham 2012; Nowack et al. 2017). Similarly  
49  
50  
51 123 to most shrew species, *C. russula* uses torpor to save energy when facing adverse environmental  
52  
53 124 conditions (Nagel 1977; Taylor 1998; Oliveira et al. 2016). We thus additionally studied the  
54  
55 125 effect of habitat and sound on torpor use. We predict that, if *C. russula* perceives traffic noise  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

126 and owl calls as a threat, they will use torpor more often when being exposed to either of the two  
127 disturbance sounds.

## Methods

### Capture Sites and Shrews

Thirty-two shrews were trapped with wooden-box live traps (“dziekanówka” type, size 16.5 × 8.0 × 9.5 cm; produced by PPUH A. Marcinkiewicz, Rajgród, Poland) during the night. Traps were opened before sunset and checked every three hours until 01h00. No bait was provided, and no mortality was observed inside the traps. Captured *C. russula* were brought to the animal facility of the Faculty of Sciences, University of Lisbon, while pregnant females and other trapped animals were released on site.

Shrews were captured in two distinct locations with different habitats. Rural shrews ( $N = 16$ ) were captured in Sintra-Cascais Natural Park in June and July 2016. Trapping sites were characterized by a wet climate, typical forest habitats with Mediterranean shrubby-type vegetation and *Quercus spp.*, and had minimal human disturbance. Urban shrews ( $N = 16$ ) were captured in the city of Lisbon in public gardens, parks and small patches of vegetation near buildings and roads in October and November 2016.

In the animal facility, shrews were housed in non-transparent plastic cages with a grid top (38×28×17 cm) enriched with soil, sand, and a cardboard eggbox, and maintained under room temperature ( $18 \pm 1$  °C). Photoperiod was 12L:12D with lights on from 08h00 until 20h00. They had access to a water bowl and were fed with 0.5 g of mealworms, 1.5 g of blowfly pupae and 4.0 g of minced beef (Oliveira et al. 2016). Water and food were replenished daily during the afternoon. After approximately three weeks, captive shrews were released at their original capture site.

## 149 **Experimental Procedure**

150 Each shrew was tested in four sequential trials, each lasting from 20h00 until 10h00 of  
151 the next morning. Subsequent trials were separated by three days to avoid habituation to the  
152 experiment itself. For each trial, shrews were taken out of their home cages at 19h00 and placed  
153 in the experimental arena (50×50×30 cm) inside another room with an ambient temperature of 18  
154 ± 1 °C. The experimental arena had white sand as substrate and a small (5×5×5 cm) cylindrical  
155 cardboard shelter (Fig. 1). To monitor shelter temperature, a pre-calibrated iButton datalogger  
156 (iButton DS1990A Thermocron, Maxim Integrated Products, Inc.), programmed to take  
157 temperature measurements every minute, was placed at the base of each shelter. In combination  
158 with video information (see below), we could deduct if a shrew inside the shelter was below  
159 normothermia or not (Willis et al. 2005). Food and water were provided to each shrew before the  
160 beginning of the experiment, at 20h00 (Fig. 1). Food consisted of 4.5 g of mealworms, which  
161 was shown in pilot studies to be enough to maintain their body mass throughout the experiment.  
162 At the end of the experiment, shrews were returned to their home cages.

163 The shrews' behavior was video-recorded during the entire experiment using a webcam  
164 (NGS Swift Cam-300). During the dark phase, the arena was illuminated with a dim red LED  
165 light (0.7 W) so we could observe shrews without disturbing them.

## 166 **Sound Playback Experiments**

167 Shrews were exposed individually to four different sound treatments, one per trial: tawny  
168 owl calls (as a predator sound), traffic noise (as an anthropogenic sound), silence and white noise  
169 (as control sounds). The order of sound treatments over each experimental night was randomized  
170 between individuals. During each night, the respective sound treatment was presented four times

1  
2  
3  
4 171 for five minutes, starting at 21h, 00h, 03h, and 06h (Fig. 1). To avoid pseudo-replication, four  
5  
6  
7 172 different playbacks were used for each sound treatment (see below).

8  
9 173 Silence was used as a baseline to analyse the behavioral differences between sound  
10  
11 174 treatments. Note that, when the playback chain was activated, the played file did not contain any  
12  
13  
14 175 sound. White noise (a continuous sound containing similar energy along a wide range of  
15  
16 176 frequencies) was used to assess the shrews' natural response to a disturbing and unfamiliar  
17  
18  
19 177 sound. Traffic noise was used to simulate anthropogenic disturbance and was obtained from  
20  
21 178 various sources on the website freesound.org. Tawny owl calls were used to simulate the sound  
22  
23  
24 179 of a natural predator, and were often heard at night at the capture sites in Sintra (the rural  
25  
26 180 habitat), but not in Lisbon (the urban habitat). Owl calls were also obtained from the  
27  
28  
29 181 freesound.org website and kindly provided by other sources (see Acknowledgements). Silence  
30  
31 182 and white noise were generated using Audacity 2.1.0. All playbacks were edited using the same  
32  
33 183 software so that playbacks for each sound treatment were similar in duration, intensity and  
34  
35  
36 184 frequency distribution (e.g. playbacks from different individuals but with similar numbers of  
37  
38 185 calls for the owl calls treatment) (exemplary spectrograms of the four playback sounds are  
39  
40  
41 186 available in the Online Resource 1, Figs. S1–S4).

42  
43 187 For playbacks, experimental stimuli generated by a laptop were converted to analog  
44  
45 188 format (Edirol UA25-EX, D/A rate 44.1 KHz, 16 bit), amplified (Technics V500 Mark II) and  
46  
47  
48 189 delivered through a Tamon LB-10 loudspeaker. The frequency response of this audio chain,  
49  
50  
51 190 which varied within  $\pm 7$  dB in the range 20–20,000 Hz (Online Resource 1, Fig. S5), was  
52  
53 191 measured by computing the transfer function between a constant amplitude sine sweep fed to the  
54  
55 192 chain and recorded with a measuring 1/2" microphone Bruel & Kjaer 4188 (50 cm in front of the  
56  
57  
58 193 speaker, i.e. the position later occupied by shrews) and the same sweep sound at the entrance of  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 194 the chain. Both signals (microphone and sweep) were simultaneously recorded (16 bit, 100 kHz)  
5  
6 195 in different channels of a multifunction I/O USB device (USB-6521, National Instruments,  
7  
8  
9 196 Austin, TX, USA) and the transfer function computed with a custom-made software (PF) written  
10  
11 197 in LabView. All experimental stimuli (except the silence treatment) were adjusted to 70 dB SPL  
12  
13  
14 198 (re 1  $\mu$ Pa) using the above-mentioned measuring microphone installed in a sound level meter  
15  
16 199 Bruel and Kjaer 2238 Mediator positioned in the experimental arena.

## 200 **Video Analysis**

201 Boris version 4.1.10 (Friard and Gamba 2016) was used to analyse the recorded videos.  
202 We analysed the behavior in three steps: instant reaction (behavior observed immediately after  
203 the respective playback started), recovery rate (see section Data Analysis for details) and long-  
204 term reaction (behavioral patterns during one hour, beginning with the start of the playback). For  
205 the instant reaction, we registered three groups of behaviors: “shelter” (behavior could not be  
206 observed), “no reaction” (no apparent changes in behavior were observed), and “reaction”  
207 (immediate changes in behavior were observed) (Table 1). For the recovery rate and long-term  
208 reaction, we registered activity and feeding whenever the shrew was outside the shelter for 60  
209 minutes after the start of the respective playback. The following criteria were used to ascertain  
210 activity and feeding behavior: activity was registered when the shrew was moving around, i.e.  
211 not being stationary for more than 30 seconds; feeding was registered when the shrew was eating  
212 mealworms for at least five seconds. When shrews were inside the shelter and their behavior  
213 could not be observed, we analysed the temporal variation in shelter temperature by calculating  
214 an heterothermy index (HI). The HI describes the temporal variation in body temperature of an  
215 individual during a given sampling period (see Boyles et al. 2011 for more details), and was  
216 calculated as follows:  $HI = \sqrt{\frac{\sum(T_{b-mod} - T_{b-i})^2}{n-1}}$ , where  $T_{b-mod}$  is the mode of the peak at the

1  
2  
3  
4 217 highest iButton temperature values,  $T_{b-i}$  is the iButton measurements at a given time, and  $n$  is the  
5  
6  
7 218 number of measurements sampled. Higher HI values indicate an individual's body temperature  
8  
9 219 was frequently below normothermia after a playback. Only data registered after every individual  
10  
11 220 had been 10 minutes inside the shelter permanently was analysed.

## 14 221 **Data Analysis**

16 222 Statistical analyses were performed using R version 3.4.2 (R Core Team 2018) and  
17  
18  
19 223 Microsoft Excel 2016.

21 224 To analyse if shrews from the same habitat display different behavior categories (“no  
22  
23  
24 225 reaction”, or “reaction”) during the instant reaction, regardless of sound treatment, a Pearson's  
25  
26 226 chi-squared test was performed. To analyse which sounds had significantly more reactions than  
27  
28  
29 227 silence, we followed with a post-hoc chi-square ( $X^2$ ) analysis using the “fifer” package (Fife  
30  
31 228 2017).

33 229 For the recovery rates, we calculated the cumulative durations of activity and feeding (in  
34  
35  
36 230 seconds) from the start of a playback until 60 minutes later. One was attributed when an  
37  
38  
39 231 individual was displaying the respective behavior at the respective second, and zero was  
40  
41 232 attributed otherwise. For each playback, we calculated a linear regression model in Excel with  
42  
43 233 the evolution of the respective cumulative behavior over time, and an intercept crossing zero.  
44  
45  
46 234 The slope of each model was used as an estimate of the defensive response's attenuation over  
47  
48 235 time, a modified version of the rate of recovery described in Vincze et al. (2016). A steeper  
49  
50  
51 236 positive slope indicates the respective individual performed a specific behavior earlier and/or for  
52  
53 237 a longer period of time, i.e. had a faster recovery rate for that behavior.

55 238 For the long-term reaction to each sound, we quantified the relative duration of activity  
56  
57  
58 239 and feeding (range 0–1) during the same period (60 minutes) for each individual. Together with  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 240 the recovery rate (range 0–1), these measurements allowed us to have a clearer picture about the  
5  
6  
7 241 shrews' reaction to specific sounds. For example, if the recovery rate of activity is smaller than  
8  
9 242 its relative duration, it means an individual's defensive response lasted longer, but the individual  
10  
11  
12 243 recovered quickly later.

13  
14 244 To analyse the difference in recovery rates and long-term reactions of shrews on activity  
15  
16 245 and feeding, linear mixed-effects models were used, accounting for repeated measurements from  
17  
18 246 the same individuals, using the lme function from the 'nlme' package (Pinheiro et al. 2019). We  
19  
20  
21 247 used as fixed terms habitat (rural vs. urban), sound treatment (silence, owl calls, traffic noise and  
22  
23  
24 248 white noise), playback time (21h, 00h, 03h, 06h), and all two-way and three-way interactions  
25  
26 249 between them. Homogeneity and independence assumptions were assessed graphically,  
27  
28  
29 250 visualizing the residuals versus fitted values (Zuur et al. 2010). Violations of these assumptions  
30  
31 251 were dealt with using appropriate random effects and variance structures, and testing these using  
32  
33  
34 252 the Akaike information criterion corrected for small sample sizes (AICc). Models with lower  
35  
36 253 AICc have less information loss, hence have higher quality. Using this procedure, the activity  
37  
38  
39 254 recovery model contained individual as a random slope, and time as the constant variance  
40  
41 255 covariate; the feeding recovery model contained individual as a random slope and as a constant  
42  
43  
44 256 variance covariate. For the models on relative duration of activity, relative duration of feeding,  
45  
46 257 and HI, we used the same fixed terms, and individual identity as a random intercept. Playback  
47  
48 258 time was used as a random slope for activity, and sound was used as a random slope for HI, as  
49  
50  
51 259 these random terms gave the lowest AICc to the model. As some models still showed residual  
52  
53 260 patterns, habitat and time were used as variance covariates in a constant variance function for  
54  
55  
56 261 activity. For feeding, we used individual identity as a variance covariate, and for HI, we used  
57  
58 262 habitat as a variance covariate.  
59  
60  
61  
62  
63  
64  
65



1  
2  
3  
4 263 We then performed model selection using maximum likelihood estimation. Candidate  
5  
6  
7 264 models were generated using the dredge function implemented in the ‘MuMIn’ package (Bartoń  
8  
9 265 2019). The models with the lowest AICc from all the generated models for a particular response  
10  
11 266 variable were selected as the best statistical models (Online Resource 1, Table S1), and the  
12  
13  
14 267 summary of their results are presented. Means  $\pm$  standard errors are reported.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Results

### Behavioral Differences Between Rural and Urban shrews

Urban shrews spent, on average, 26.0% more time in activity and 37.4% more time feeding than rural shrews (activity:  $1241.49 \pm 57.34$  s/h vs.  $926.87 \pm 52.84$  s/h; feeding:  $71.46 \pm 2.86$  s/h vs.  $45.12 \pm 2.34$  s/h; Fig. 2).

Shrews from both habitats increased their activity significantly over the night (Fig. 2, Table 2). Activity was the lowest in the first analysed hour (21–22h) in both shrew populations ( $470.71$  s/h), and the highest in the last analysed hour (06–07h:  $1683.70$  s/h). Regarding feeding behavior, while urban shrews increased feeding throughout the night, rural shrews decreased feeding near the morning (Table 2; Fig. 2). Specifically, urban shrews increased their feeding from  $77.05 \pm 5.05$  s/h to  $80.94 \pm 5.81$  s/h from 03–04h to 06–07h, while rural shrews decreased from  $49.57 \pm 4.10$  s/h to  $38.36 \pm 3.39$  s/h from 03–04h to 06–07h.

In 76.56% of the cases for rural shrews and 72.27% of the cases for urban shrews, individuals were inside the shelter long enough to allow a valid heterothermy analysis. HI was significantly lower (by 14–26%) in urban than in rural shrews at all measured hours (Fig. 3, Table 3). More HI values were obtained at 21h (rural: 64; urban: 59), when shrews were less active, and less at 06h (rural: 32; urban: 28), when shrews were more active. In both populations HI values were significantly lower at 21h ( $1.70 \pm 0.08$ ) and significantly higher at 06h ( $2.31 \pm 0.13$ ) (Fig. 3, Table 3).

### Influence of Sound on Behavior

The instant reaction to sound playbacks could not be observed in about two thirds of the shrews as they were inside the shelter when a sound was played (67.96% for rural shrews and 61.96% for urban shrews, Fig. 4). For shrews outside the shelter when a sound was played, the

1  
2  
3  
4 291 influence of sound treatment and habitat on the instant reaction was analysed. Shrews from both  
5  
6  
7 292 habitats showed significantly different instant reactions depending on the sound treatment (rural:  
8  
9 293  $X^2 = 22.164, p < 0.001$ ; urban:  $X^2 = 16.706, p < 0.001$ ). Compared with silence, both populations  
10  
11 294 reacted significantly to traffic noise (rural:  $p = 0.001$ , urban:  $p = 0.008$ ) and white noise (rural  
12  
13  
14 295 and urban:  $p < 0.001$ ), while no significant reaction was observed for owl calls (rural:  $p = 0.607$ ,  
15  
16 296 urban:  $p = 0.220$ ). Independently of the population, fleeing to the shelter was the most common  
17  
18 297 reaction to traffic noise and white noise (Fig. 4), with shrews taking  $8.2 \pm 2.6$  ( $n = 13$ ) minutes  
19  
20  
21 298 and  $12.8 \pm 2.1$  ( $n = 23$ ) minutes, respectively, to leave the shelter again. Urban shrews, however,  
22  
23  
24 299 showed more diverse behaviors when hearing traffic noise and white noise than rural shrews  
25  
26 300 (Fig. 4).

27  
28 301           There was no difference in the long-term reaction to sound between rural and urban  
29  
30  
31 302 shrews, as seen by the lack of an interaction between habitat and sound (Table 2, Online  
32  
33 303 Resource 1, Table S1). After owl calls and traffic noise playbacks, neither relative activity nor  
34  
35  
36 304 activity recovery were significantly different from silence (Fig. 5, Table 2). However, white noise  
37  
38 305 lowered both the relative activity (by 21.2%) and the activity recovery (by 29.0%) in comparison  
39  
40  
41 306 to silence (Fig. 5, Table 2). Both traffic noise and white noise had an influence on feeding  
42  
43 307 behavior, but not owl calls (Table 2). Shrews exposed to traffic or white noise spent less time  
44  
45  
46 308 feeding than shrews exposed to silence (decrease of 13.0% and 31.1% respectively; Fig. 5).  
47  
48 309 Furthermore, feeding recovery was significantly decreased by traffic noise by 14.9%, and by  
49  
50  
51 310 white noise by 40.8%, (Fig. 5).

52  
53 311           Sound treatment did not influence HI in both populations as this variable was not featured  
54  
55  
56 312 in the top two models for HI (Table 3, Online Resource 1, Table S1).  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Discussion

### Rural vs. Urban Shrews

Our results suggest *C. russula* inhabiting urban environments has modified its natural behaviour, with individuals captured in urban areas spending more time in activity and feeding than individuals captured in rural areas. One explanation might be an increased level of boldness typical of urban individuals (Sol et al. 2013). Higher activity levels have already been linked in other species with high levels of boldness (Herde and Eccard 2013; Rangel-Patiño et al. 2018) while an increase in time spent feeding has been correlated with a decrease in vigilant behaviors (Shannon et al. 2014; Klett-Mingo et al. 2016; Uchida et al. 2019). Such behaviors were likely selected in shrews to explore more territory and access more resources in the complex urban matrix, increasing their fitness. Another explanation for the increased activity and feeding observed in urban *C. russula* during our night-time tests might be that individuals adjusted their foraging behavior to periods of lower human activity, a behavior already described for other mammals (Dowding et al. 2010; Gaynor et al. 2018). Crocidurines are predominantly nocturnal, but still show some bouts of activity during the day (Baxter et al. 1979; Genoud and Vogel 1981; Oliveira et al. 2016). Possibly, urban individuals of *C. russula* changed their diel activity to peak at night to avoid exposure to human activities, such as traffic. However, as we did not test shrews during daylight, we cannot confirm urban individuals indeed showed lower levels of day-time activity and feeding than rural ones.

Food intake (feeding behavior) was constant throughout the night in rural shrews, with a slight drop at the last hour (06h). In contrast, feeding behavior kept increasing throughout the night in urban shrews. This provides some support to our previous interpretation: if urban shrews shift their activity peak to the night period when human disturbance is lower, feeding behavior

1  
2  
3  
4 336 will occur mainly during this period. Additionally, activity increased along the night for both  
5  
6  
7 337 populations. Activity results obtained at 06h match the mean nocturnal hourly rate of activity  
8  
9 338 registered for wild and captive adult *C. russula* in summer by Genoud and Vogel (1981). Likely,  
10  
11 339 individuals were more hesitant at the beginning of the experiment, then resumed their regular  
12  
13  
14 340 activity and exploration as the territory became more familiar.

15  
16 341 The use of heterothermy was different between rural and urban shrews, with urban  
17  
18  
19 342 shrews making generally less use of daily torpor than rural shrews. The higher use of  
20  
21 343 heterothermy in rural shrews may serve as an energetic compensatory mechanism. Such a  
22  
23  
24 344 mechanism has already been suggested in bats, in which populations roosting in buildings appear  
25  
26 345 to use less torpor than rock-roosting populations (Lausen and Barclay 2006). For urban shrews,  
27  
28  
29 346 in contrast, a higher food intake may be more important than torpor as a compensatory behavior  
30  
31 347 for their higher energy expenditure caused by higher levels of activity. Torpor use and depth is  
32  
33 348 heavily influenced by food availability (Bozinovic et al. 2007; Doucette et al. 2012; Leslie et al.  
34  
35  
36 349 2015), but also predation risk (Bieber and Ruf 2009; Nowack et al. 2017; Turbill and Stojanovski  
37  
38 350 2018) and competitor pressure (Geiser and Brigham 2012; Kronfeld-Schor and Dayan 2013;  
39  
40  
41 351 Vuarin and Henry 2014). Predation pressure is generally lower in urban environments (Eötvös et  
42  
43 352 al. 2018) and shrews can forage with low mortality risk. But in Sintra, like many other natural  
44  
45  
46 353 areas, predators and competitors are more diverse and abundant (ICNF 2018). Therefore, we can  
47  
48 354 assume rural *C. russula* struggle more to acquire resources, and consequently spend more time in  
49  
50  
51 355 torpor to save energy (Levy et al. 2011; Geiser and Brigham 2012; Kronfeld-Schor and Dayan  
52  
53 356 2013). Furthermore, shrews from both populations increased torpor use over the night. This  
54  
55  
56 357 matches findings from other studies that show small mammals enter torpor more frequently in  
57  
58 358 the morning, when they start becoming less active (Nagel 1977; Oliveira et al. 2016).  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 359 Although our results were based on an adapted HI, rather than direct body measurements,  
5  
6  
7 360 they suggest an interesting possible influence of urban environments on torpor patterns in daily  
8  
9 361 heterotherms such as *C. russula*. Our results gain additional importance, as torpor is perceived as  
10  
11 362 a possible relevant ability for endotherms facing global change (Nowack et al. 2017).  
12

### 13 363 **Influence of Sound on Behavior**

14  
15  
16 364 Contrary to what we initially predicted, shrews from rural and urban habitats had similar  
17  
18  
19 365 reactions to the same sounds. Unlike other small mammals (Hendrie et al. 1998), shrews outside  
20  
21 366 the shelter showed no changes in behavior when hearing tawny owl calls. Shrews exposed to  
22  
23  
24 367 traffic noise fled to the shelter initially, but returned quickly to their normal activity. However,  
25  
26 368 time spent feeding was significantly reduced after hearing traffic noise. Other studies have  
27  
28  
29 369 shown significant negative impacts of traffic noise, e.g. in the locomotor activity of striped field  
30  
31 370 mice *Apodemus agrarius* (Pieniżek et al. 2017) and the activity and feeding behavior of *Myotis*  
32  
33 371 *spp.* (Finch et al. 2020). White noise had an even stronger effect on the shrews' behavior: they  
34  
35  
36 372 fled to the shelter more frequently and took longer to recover their regular behavior, as evidenced  
37  
38 373 by reduced activity and feeding behaviors and recovery rates.  
39

40  
41 374 A possible explanation why shrews in our study showed no behavioral reaction to the  
42  
43 375 playback of owl calls might be related with their hearing abilities. Branis & Burda (1994)  
44  
45  
46 376 suggested the hearing capabilities of all shrews should be tuned to higher frequencies and their  
47  
48 377 ability to localise sound below 15 kHz should be limited. We only know of one audiogram  
49  
50  
51 378 conducted on a similar sized shrew species, namely *Sorex araneus*, which have their best range  
52  
53 379 of hearing at 5–20 kHz (Konstantinov and Movchan 1985). These findings fit to the frequency  
54  
55 380 spectrum of typical call types used by *C. russula*, like courtship (~10 kHz; Hutterer 1977) and  
56  
57  
58 381 twittering calls (4.4–7.0 kHz; Zsebök et al. 2015). Owl calls have their highest energy at  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 382 frequencies below 1 kHz and do not reach higher than 5 kHz (Figure S7). The lack of a reaction  
5  
6  
7 383 to owl calls in our study might thus confirm the hearing range of *C. russula* to be above 5 kHz  
8  
9 384 like in *S. araneus*. Both traffic noise and white noise are broadband sounds, with higher energies  
10  
11 385 within the alleged range of hearing of shrews (especially white noise; Online Resource 1, Figure  
12  
13 386 S6), which can explain the shrews' reaction. It appears the higher the sound frequency, the more  
14  
15  
16 387 pronounced the defensive response of *C. russula* is. Comparable results have been observed in  
17  
18 388 house mice, which show higher stress responses to anthropogenic noise emitted at higher  
19  
20  
21 389 frequencies than at low frequencies (Mancera et al. 2018).

## 23 390 **Conclusion**

26 391 Our results suggest cities have imposed new selective pressures on *Crocidura russula*  
27  
28 392 that forced individuals inhabiting urban environments to change their natural behavior. Urban  
29  
30 393 shrews were active for longer periods of time, spent more time feeding, and were heterothermic  
31  
32  
33 394 less often than rural shrews. Despite these behavioral differences and the fact that rural and urban  
34  
35 395 environments have a vastly different soundscape, shrews from both habitats responded to  
36  
37  
38 396 different sound disturbances in the same way. Furthermore, behavioral differences observed  
39  
40 397 when hearing sound disturbances were never as strong as behavioral differences observed  
41  
42  
43 398 between rural and urban populations. This means factors other than noise disturbance are  
44  
45 399 responsible for this species' synurbic adaptations and should be investigated. Food availability  
46  
47  
48 400 (invertebrates, Ishitani et al. 2003), predator abundance (Eötvös et al. 2018), climatic differences  
49  
50 401 (Oke 1995), or inter- and intraspecific competition (Francis et al. 2009) have all been shown  
51  
52  
53 402 previously to be dramatically different between rural and urban environments. It would also be  
54  
55 403 interesting to compare our overall results with other small mammals living in cities, particularly  
56  
57  
58 404 rodents, to better understand what factors drive species' different responses to noise.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

405 Understanding how these evolutionary processes work would allow city managers and  
406 politicians to better plan green infrastructures and allow rural biodiversity a better chance to  
407 adapt to an ever-increasing urbanized world.



## References

- 1  
2  
3  
4 408  
5  
6  
7 409 Barber JR, Crooks KR, Fristrup KM (2010) The costs of chronic noise exposure for terrestrial  
8  
9 410 organisms. *Trends Ecol Evol* 25:180–189. <https://doi.org/10.1016/j.tree.2009.08.002>  
10  
11 411 Bartoń K (2019) MuMIn: Multi-model inference. R Packag. version 1.43.6 75  
12  
13  
14 412 Baxter RM, Goulden EA, Meester J (1979) The activity patterns of some southern African  
15  
16 413 *Crocidura* in captivity. *Acta Theriol (Warsz)* 24:61–68. <https://doi.org/10.4098/AT.arch.79-7>  
17  
18  
19 414 Bieber C, Ruf T (2009) Summer dormancy in edible dormice (*Glis glis*) without energetic  
20  
21 415 constraints. *Naturwissenschaften* 96:165–171. <https://doi.org/10.1007/s00114-008-0471-z>  
22  
23  
24 416 Boyles JG, Smit B, McKechnie AE (2011) A new comparative metric for estimating  
25  
26 417 heterothermy in endotherms. *Physiol Biochem Zool* 84:115–123.  
27  
28 418 <https://doi.org/10.1086/656724>  
29  
30  
31 419 Bozinovic F, Muñoz JLP, Naya DE, Cruz-Neto AP (2007) Adjusting energy expenditures to  
32  
33 420 energy supply: food availability regulates torpor use and organ size in the Chilean mouse-  
34  
35 421 opossum *Thylamys elegans*. *J Comp Physiol B* 177:393–400.  
36  
37 422 <https://doi.org/10.1007/s00360-006-0137-0>  
38  
39  
40 423 Branis M, Burda H (1994) Visual and hearing biology of shrews. *Carnegie Museum Nat Hist*  
41  
42 424 18:189–200  
43  
44  
45 425 Capizzi D (2000) Diet shifts of the tawny owl *Strix aluco* in central and northern Italy. *Ital J Zool*  
46  
47 426 67:73–79. <https://doi.org/10.1080/11250000009356298>  
48  
49  
50 427 Cavia R, Cueto GR, Suárez OV (2009) Changes in rodent communities according to the  
51  
52 428 landscape structure in an urban ecosystem. *Landsc Urban Plan* 90:11–19.  
53  
54 429 <https://doi.org/10.1016/j.landurbplan.2008.10.017>  
55  
56  
57 430 Doucette LI, Brigham RM, Pavey CR, Geiser F (2012) Prey availability affects daily torpor by  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 431 free-ranging Australian owl-nightjars (*Aegotheles cristatus*). *Oecologia* 169:361–372.  
5  
6  
7 432 <https://doi.org/10.1007/s00442-011-2214-7>  
8  
9 433 Dowding C V., Harris S, Poulton S, Baker PJ (2010) Nocturnal ranging behaviour of urban  
10  
11 434 hedgehogs, *Erinaceus europaeus*, in relation to risk and reward. *Anim Behav* 80:13–21.  
12  
13  
14 435 <https://doi.org/10.1016/j.anbehav.2010.04.007>  
15  
16 436 Eötvös CB, Magura T, Lövei GL (2018) A meta-analysis indicates reduced predation pressure  
17  
18 437 with increasing urbanization. *Landsc Urban Plan* 180:54–59.  
19  
20  
21 438 <https://doi.org/10.1016/j.landurbplan.2018.08.010>  
22  
23  
24 439 Fife D (2017) *fifer*: a biostatisticians toolbox for various activities, including plotting, data  
25  
26 440 cleanup, and data analysis  
27  
28 441 Finch D, Schofield H, Mathews F (2020) Traffic noise playback reduces the activity and feeding  
29  
30 442 behaviour of free-living bats. *Environ Pollut* 263:114405.  
31  
32 443 <https://doi.org/10.1016/j.envpol.2020.114405>  
33  
34 444 Francis CD, Ortega CP, Cruz A (2009) Noise pollution changes avian communities and species  
35  
36 445 interactions. *Curr Biol* 19:1415–1419. <https://doi.org/10.1016/j.cub.2009.06.052>  
37  
38 446 Friard O, Gamba M (2016) BORIS: a free, versatile open-source event-logging software for  
39  
40 447 video/audio coding and live observations. *Methods Ecol Evol* 7:1325–1330.  
41  
42 448 <https://doi.org/10.1111/2041-210X.12584>  
43  
44 449 Frid A, Dill LM (2002) Human-caused disturbance stimuli as a form of predation risk. *Conserv*  
45  
46 450 *Ecol* 6:art11. <https://doi.org/10.5751/ES-00404-060111>  
47  
48 451 Gaynor KM, Hojnowski CE, Carter NH, Brashares JS (2018) The influence of human  
49  
50 452 disturbance on wildlife nocturnality. *Science* (80- ) 360:1232–1235.  
51  
52 453 <https://doi.org/10.1126/science.aar7121>  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 454 Geiser F, Brigham RM (2012) The other functions of torpor. In: Living in a Seasonal World.  
5  
6  
7 455 Springer Berlin Heidelberg, Berlin, Heidelberg, pp 109–121  
8  
9 456 Genoud M, Vogel P (1981) The activity of *Crocidura russula* (Insectivora, Soricidae) in the field  
10  
11  
12 457 and in captivity. *Zeitschrift für Säugetierkd* 46:222–232  
13  
14 458 Gomes V, Ribeiro R, Carretero MA (2011) Effects of urban habitat fragmentation on common  
15  
16 459 small mammals: species versus communities. *Biodivers Conserv* 20:3577–3590.  
17  
18  
19 460 <https://doi.org/10.1007/s10531-011-0149-2>  
20  
21 461 Halfwerk W, Holleman LJM, Lessells CkM, Slabbekoorn H (2011) Negative impact of traffic  
22  
23  
24 462 noise on avian reproductive success. *J Appl Ecol* 48:210–219.  
25  
26 463 <https://doi.org/10.1111/j.1365-2664.2010.01914.x>  
27  
28  
29 464 Hendrie CA, Weiss SM, Eilam D (1998) Behavioural response of wild rodents to the calls of an  
30  
31 465 owl: a comparative study. *J Zool* 245:439–446. <https://doi.org/10.1111/j.1469->  
32  
33 466 [7998.1998.tb00118.x](https://doi.org/10.1111/j.1469-7998.1998.tb00118.x)  
34  
35  
36 467 Herde A, Eccard JA (2013) Consistency in boldness, activity and exploration at different stages  
37  
38 468 of life. *BMC Ecol* 13:49. <https://doi.org/10.1186/1472-6785-13-49>  
39  
40  
41 469 Hölker F, Wolter C, Perkin EK, Tockner K (2010) Light pollution as a biodiversity threat. *Trends*  
42  
43 470 *Ecol Evol* 25:681–682. <https://doi.org/10.1016/j.tree.2010.09.007>  
44  
45  
46 471 Hutterer R (1977) Paarungsrufe der Wasserspitzmaus (*Neomys fodiens*) und verwandte Laute  
47  
48 472 weiterer Soricidae. *Zeitschrift für Säugetierkd* 43:330–336  
49  
50  
51 473 ICNF (2018) Fauna do Parque Natural de Sintra-Cascais. <http://www2.icnf.pt/portal/ap/p->  
52  
53 474 [nat/pnsc/fauna](http://www2.icnf.pt/portal/ap/p-nat/pnsc/fauna). Accessed 25 Apr 2020  
54  
55  
56 475 Ishitani M, Kotze DJ, Niemelä J (2003) Changes in carabid beetle assemblages across an urban-  
57  
58 476 rural gradient in Japan. *Ecography (Cop)* 26:481–489. <https://doi.org/10.1034/j.1600->  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 477 0587.2003.03436.x  
5  
6  
7 478 Klett-Mingo JI, Pavón I, Gil D (2016) Great tits, *Parus major*, increase vigilance time and  
8  
9 479 reduce feeding effort during peaks of aircraft noise. *Anim Behav* 115:29–34.  
10  
11 480 <https://doi.org/10.1016/j.anbehav.2016.02.021>  
12  
13  
14 481 Klimant P, Klimantová A, Baláž I, et al (2017) Small mammals in an urban area: habitat  
15  
16 482 preferences and urban-rural gradient in Nitra city, Slovakia. *Polish J Ecol* 65:144–157.  
17  
18 483 <https://doi.org/10.3161/15052249PJE2017.65.1.013>  
19  
20  
21 484 Konstantinov AI, Movchan VN (1985) Acoustic communication in different groups of mammals.  
22  
23 In: *Sounds in the life of animals* (in Russian). Leningrad, pp 63–81  
24 485  
25  
26 486 Kronfeld-Schor N, Dayan T (2013) Thermal ecology, environments, communities, and global  
27  
28 487 change: energy intake and expenditure in endotherms. *Annu Rev Ecol Evol Syst* 44:461–  
29  
30 488 480. <https://doi.org/10.1146/annurev-ecolsys-110512-135917>  
31  
32  
33 489 Lausen CL, Barclay RMR (2006) Benefits of living in a building: big brown bats (*Eptesicus*  
34  
35 *fuscus*) in rocks versus buildings. *J Mammal* 87:362–370. <https://doi.org/10.1644/05->  
36 490  
37 MAMM-A-127R1.1  
38 491  
39  
40 492 Leslie AM, Stewart M, Price E, Munn AJ (2015) Daily changes in food availability, but not long-  
41  
42 493 term unpredictability, determine daily torpor-bout occurrences and frequency in stripe-faced  
43  
44 494 dunnarts (*Sminthopsis macroura*). *Aust J Zool* 63:12. <https://doi.org/10.1071/ZO14058>  
45  
46  
47 495 Levy O, Dayan T, Kronfeld-Schor N (2011) Interspecific competition and torpor in golden spiny  
48  
49 496 mice: two sides of the energy-acquisition coin. *Integr Comp Biol* 51:441–448.  
50  
51 497 <https://doi.org/10.1093/icb/icr071>  
52  
53  
54 498 Łopucki R, Kitowski I (2017) How small cities affect the biodiversity of ground-dwelling  
55  
56 499 mammals and the relevance of this knowledge in planning urban land expansion in terms of  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 500 urban wildlife. *Urban Ecosyst* 20:933–943. <https://doi.org/10.1007/s11252-016-0637-y>  
5  
6  
7 501 Luo J, Clarin B-M, Borissov IM, Siemers BM (2014) Are torpid bats immune to anthropogenic  
8  
9 502 noise? *J Exp Biol* 217:1072–1078. <https://doi.org/10.1242/jeb.092890>  
10  
11 503 Mancera KF, Besson M, Lisle A, et al (2018) The effects of mining machinery noise of different  
12  
13 504 amplitudes on the behaviour, faecal corticosterone and tissue morphology of wild mice  
14  
15 505 (*Mus musculus*). *Appl Anim Behav Sci* 203:81–91.  
16  
17  
18 506 <https://doi.org/10.1016/j.applanim.2018.02.010>  
19  
20  
21 507 McDonnell MJ, Hahs AK (2015) Adaptation and adaptedness of organisms to urban  
22  
23 508 environments. *Annu Rev Ecol Evol Syst* 46:261–280. [https://doi.org/10.1146/annurev-](https://doi.org/10.1146/annurev-ecolsys-112414-054258)  
24  
25 509 [ecolsys-112414-054258](https://doi.org/10.1146/annurev-ecolsys-112414-054258)  
26  
27  
28 510 Musolf K, Hoffmann F, Penn DJ (2010) Ultrasonic courtship vocalizations in wild house mice,  
29  
30 511 *Mus musculus musculus*. *Anim Behav* 79:757–764.  
31  
32 512 <https://doi.org/10.1016/j.anbehav.2009.12.034>  
33  
34  
35 513 Nagel A (1977) Torpor in the European white-toothed shrews. *Experientia* 33:1455–1456.  
36  
37 514 <https://doi.org/10.1007/BF01918804>  
38  
39  
40 515 Nowack J, Stawski C, Geiser F (2017) More functions of torpor and their roles in a changing  
41  
42 516 world. *J Comp Physiol B* 187:889–897. <https://doi.org/10.1007/s00360-017-1100-y>  
43  
44  
45 517 Oke TR (1995) The heat island of the urban boundary layer: characteristics, causes and effects.  
46  
47 In: Cermak JE, Davenport AG, Plate EJ, Viegas D. (eds) *Wind Climate in Cities*, 277th edn.  
48 518 Springer Netherlands, Dordrecht, pp 81–107  
49  
50 519  
51  
52 520 Oliveira FG, Tapisso JT, Monarca RI, et al (2016) Phenotypic flexibility in the energetic strategy  
53  
54 521 of the greater white-toothed shrew, *Crocidura russula*. *J Therm Biol* 56:10–17.  
55  
56 522 <https://doi.org/10.1016/j.jtherbio.2015.12.002>  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 523 Patricelli GL, Blickley JL (2006) Avian communication in urban noise: causes and consequences  
5  
6 of vocal adjustment. *Auk* 123:639–649. <https://doi.org/10.1642/0004->  
7 524  
8  
9 525 8038(2006)123[639:ACIUNC]2.0.CO;2  
10  
11 526 Pieniążek A, Boguszewski PM, Meronka RA (2017) The impact of urban noise on the behavior  
12  
13 of two mouse species belonging to the genus *Apodemus*. *Nat Resour* 08:55–68.  
14 527  
15  
16 528 <https://doi.org/10.4236/nr.2017.82004>  
17  
18  
19 529 Pinheiro J, Bates D, DebRoy S, et al (2019) Package “nlme”: linear and nonlinear mixed effects  
20  
21 530 models  
22  
23  
24 531 R Core Team (2018) R: A language and environment for statistical computing. In: *R Found. Stat.*  
25  
26 532 *Comput.* <https://www.r-project.org/>  
27  
28  
29 533 Rangel-Patiño C, García-Morales C, Mastachi-Loza C, et al (2018) Personality and its variation  
30  
31 534 between sexes in the black-bellied bunchgrass lizard *Sceloporus aeneus* during the breeding  
32  
33 535 season. *Ethology* 124:796–803. <https://doi.org/10.1111/eth.12814>  
34  
35  
36 536 Read J, Jones G, Radford AN (2014) Fitness costs as well as benefits are important when  
37  
38 537 considering responses to anthropogenic noise. *Behav Ecol* 25:4–7.  
39  
40  
41 538 <https://doi.org/10.1093/beheco/art102>  
42  
43  
44 539 Seto KC, Guneralp B, Hutyra LR (2012) Global forecasts of urban expansion to 2030 and direct  
45  
46 540 impacts on biodiversity and carbon pools. *Proc Natl Acad Sci* 109:16083–16088.  
47  
48 541 <https://doi.org/10.1073/pnas.1211658109>  
49  
50  
51 542 Shannon G, Angeloni LM, Wittemyer G, et al (2014) Road traffic noise modifies behaviour of a  
52  
53 543 keystone species. *Anim Behav* 94:135–141. <https://doi.org/10.1016/j.anbehav.2014.06.004>  
54  
55  
56 544 Shannon G, Crooks KR, Wittemyer G, et al (2016a) Road noise causes earlier predator detection  
57  
58 545 and flight response in a free-ranging mammal. *Behav Ecol* 27:1370–1375.  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 546 <https://doi.org/10.1093/beheco/arw058>

5  
6  
7 547 Shannon G, McKenna MF, Angeloni LM, et al (2016b) A synthesis of two decades of research  
8  
9 548 documenting the effects of noise on wildlife. *Biol Rev* 91:982–1005.

10  
11 549 <https://doi.org/10.1111/brv.12207>

12  
13  
14 550 Shier DM, Lea AJ, Owen MA (2012) Beyond masking: endangered Stephen's kangaroo rats  
15  
16 551 respond to traffic noise with footdrumming. *Biol Conserv* 150:53–58.

17  
18 552 <https://doi.org/10.1016/j.biocon.2012.03.007>

19  
20  
21 553 Siemers BM, Schauermann G, Turni H, von Merten S (2009) Why do shrews twitter?

22  
23 554 Communication or simple echo-based orientation. *Biol Lett* 5:593–596.

24  
25 555 <https://doi.org/10.1098/rsbl.2009.0378>

26  
27  
28 556 Simeonovska-Nikolova DM (2004) Vocal communication in the bicoloured white-toothed shrew  
29  
30 557 *Crocidura leucodon*. *Acta Theriol (Warsz)* 49:157–165.

31  
32 558 <https://doi.org/10.1007/BF03192517>

33  
34  
35 559 Snell-Rood EC (2013) An overview of the evolutionary causes and consequences of behavioural  
36  
37 560 plasticity. *Anim Behav* 85:1004–1011. <https://doi.org/10.1016/j.anbehav.2012.12.031>

38  
39  
40 561 Sol D, Lapiedra O, González-Lagos C (2013) Behavioural adjustments for a life in the city. *Anim*  
41  
42 562 *Behav* 85:1101–1112. <https://doi.org/10.1016/j.anbehav.2013.01.023>

43  
44  
45 563 Taylor JRE (1998) Evolution of energetic strategies in shrews. In: Wojcik JM, Wolsan M (eds)

46  
47 564 Evolution of shrews. Mammal Research Institute, Polish Academy of Sciences, Bialowieza,  
48  
49 565 Poland, pp 309–346

50  
51  
52 566 Turbill C, Stojanovski L (2018) Torpor reduces predation risk by compensating for the energetic  
53  
54 567 cost of antipredator foraging behaviours. *Proc R Soc B Biol Sci* 285:20182370.

55  
56  
57 568 <https://doi.org/10.1098/rspb.2018.2370>  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 569 Uchida K, Suzuki KK, Shimamoto T, et al (2019) Decreased vigilance or habituation to humans?  
5  
6  
7 570 Mechanisms on increased boldness in urban animals. *Behav Ecol* 30:1583–1590.  
8  
9 571 <https://doi.org/10.1093/beheco/arz117>  
10  
11 572 Vergnes A, Kerbiriou C, Clergeau P (2013) Ecological corridors also operate in an urban matrix:  
12  
13  
14 573 A test case with garden shrews. *Urban Ecosyst* 16:511–525. <https://doi.org/10.1007/s11252->  
15  
16 574 013-0289-0  
17  
18 575 Vincze E, Papp S, Preiszner B, et al (2016) Habituation to human disturbance is faster in urban  
19  
20  
21 576 than rural house sparrows. *Behav Ecol* 27:1304–1313.  
22  
23  
24 577 <https://doi.org/10.1093/beheco/arw047>  
25  
26 578 Willis CKR, Goldzieher A, Geiser F (2005) A non-invasive method for quantifying patterns of  
27  
28  
29 579 torpor and activity under semi-natural conditions. *J Therm Biol* 30:551–556.  
30  
31 580 <https://doi.org/10.1016/j.jtherbio.2005.07.001>  
32  
33 581 Wong BBM, Candolin U (2015) Behavioral responses to changing environments. *Behav Ecol*  
34  
35  
36 582 26:665–673. <https://doi.org/10.1093/beheco/aru183>  
37  
38 583 Zsebök S, Czabán D, Farkas J, et al (2015) Acoustic species identification of shrews: Twittering  
39  
40  
41 584 calls for monitoring. *Ecol Inform* 27:1–10. <https://doi.org/10.1016/j.ecoinf.2015.02.002>  
42  
43 585 Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common  
44  
45  
46 586 statistical problems. *Methods Ecol Evol* 1:3–14. <https://doi.org/10.1111/j.2041->  
47  
48 587 210X.2009.00001.x

588



## Figure captions

1  
2  
3  
4 589  
5  
6  
7  
8 590 **Fig. 1** Experimental design. On the left, the schematic representation of the experimental arena  
9  
10 591 (images not to scale). On the right, the schematic representation of the timing of each playback,  
11  
12 592 as well as the beginning and end of the experiment  
13  
14

15 593  
16  
17 594 **Fig. 2** Duration of activity and feeding during one hour after the start of each playback (21–22h,  
18  
19 595 00–01h, 03–04h, 06–07h) in greater white-toothed shrews trapped in rural and urban habitats.  
20  
21 596 Boxplots show the median and the upper and lower quartiles, with the whiskers representing data  
22  
23 597 within the  $1.5\times$  interquartile range. Points represent outliers.  $n = 16$  for each boxplot  
24  
25  
26

27 598  
28  
29  
30  
31 599 **Fig. 3** Heterothermy index (HI) calculated every three hours for shrews captured in rural and  
32  
33 600 urban habitats. Boxplots show the median and the upper and lower quartiles, with the whiskers  
34  
35 601 representing data within the  $1.5\times$  interquartile range  
36  
37

38 602  
39  
40  
41 603 **Fig. 4** Number of behavioral reactions observed in rural and urban shrews during a sound  
42  
43 604 playback. See Table 1 for the definition of each behavior  
44

45 605  
46  
47  
48 606 **Fig. 5** Relative activity (proportion of time shrews were active in an hour after each playback  
49  
50 607 started) and activity recovery (estimate of the speed by which shrews recovered their normal  
51  
52 608 behavior), as well as relative feeding and feeding recovery of shrews from two habitats exposed  
53  
54 609 to silence, tawny owl calls, traffic noise and white noise. See Fig. 2 for explanations of boxplots  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**Table 1**

Behavioral reactions observed in shrews during a playback

Category	Behavior	Description
Shelter	Shelter	Shrew inside the shelter during playback
No reaction	Ignore	No apparent reaction to noise
	Rest	Shrew resting outside the shelter, with no apparent reaction
Reaction	Flee	Shrew fled to the shelter
	Wake	Shrew left the shelter during playback
	Freeze	Shrew stopped moving after hearing playback
	Freeze and flee	Shrew stopped moving after hearing playback, then fled to shelter

**Table 2**

Summary results of the top models regarding relative duration of activity, recovery rate of activity, relative duration of feeding and recovery rate of feeding

Response	Parameter	Relative duration		Recovery rate	
		Effect $\pm$ SE	<i>P</i>	Effect $\pm$ SE	<i>P</i>
<b>Activity<sup>a</sup></b>	(Intercept)	0.090 $\pm$ 0.015	<0.001	0.094 $\pm$ 0.030	0.002
	Urban	0.111 $\pm$ 0.026	<0.001	0.084 $\pm$ 0.040	0.044
	Owl calls	0.005 $\pm$ 0.017	0.783	-0.002 $\pm$ 0.019	0.928
	Traffic noise	-0.001 $\pm$ 0.017	0.942	-0.005 $\pm$ 0.019	0.788
	White noise	-0.044 $\pm$ 0.017	0.010	-0.056 $\pm$ 0.019	0.004
	00h	0.179 $\pm$ 0.033	<0.001	0.185 $\pm$ 0.022	<0.001
	03h	0.213 $\pm$ 0.033	<0.001	0.204 $\pm$ 0.020	<0.001
	06h	0.362 $\pm$ 0.041	<0.001	0.365 $\pm$ 0.024	<0.001
<b>Feeding<sup>b</sup></b>	(Intercept)	0.015 $\pm$ 0.002	<0.001	0.015 $\pm$ 0.002	<0.001
	Urban	0.007 $\pm$ 0.002	0.006	0.008 $\pm$ 0.002	<0.001
	Owl calls	-0.001 $\pm$ 0.001	0.157	-0.002 $\pm$ 0.001	0.075
	Traffic noise	-0.003 $\pm$ 0.001	0.001	-0.003 $\pm$ 0.001	0.010
	White noise	-0.007 $\pm$ 0.001	<0.001	-0.008 $\pm$ 0.001	<0.001
	00h	0.002 $\pm$ 0.001	0.111	NA	NA
	03h	0.002 $\pm$ 0.001	0.203	NA	NA
	06h	-0.000 $\pm$ 0.001	0.677	NA	NA
	Urban:00h	-0.001 $\pm$ 0.002	0.667	NA	NA
	Urban:03h	0.002 $\pm$ 0.002	0.434	NA	NA
Urban:06h	0.006 $\pm$ 0.002	0.007	NA	NA	

Baseline levels: rural habitat, silence sound and 21h playback

<sup>a</sup>Individual identity was used as a random intercept. Different variances were allowed for each habitat and playback

<sup>b</sup>Individual identity was used as a random intercept and as a variance covariate (different variances were allowed for each individual

**Table 3**

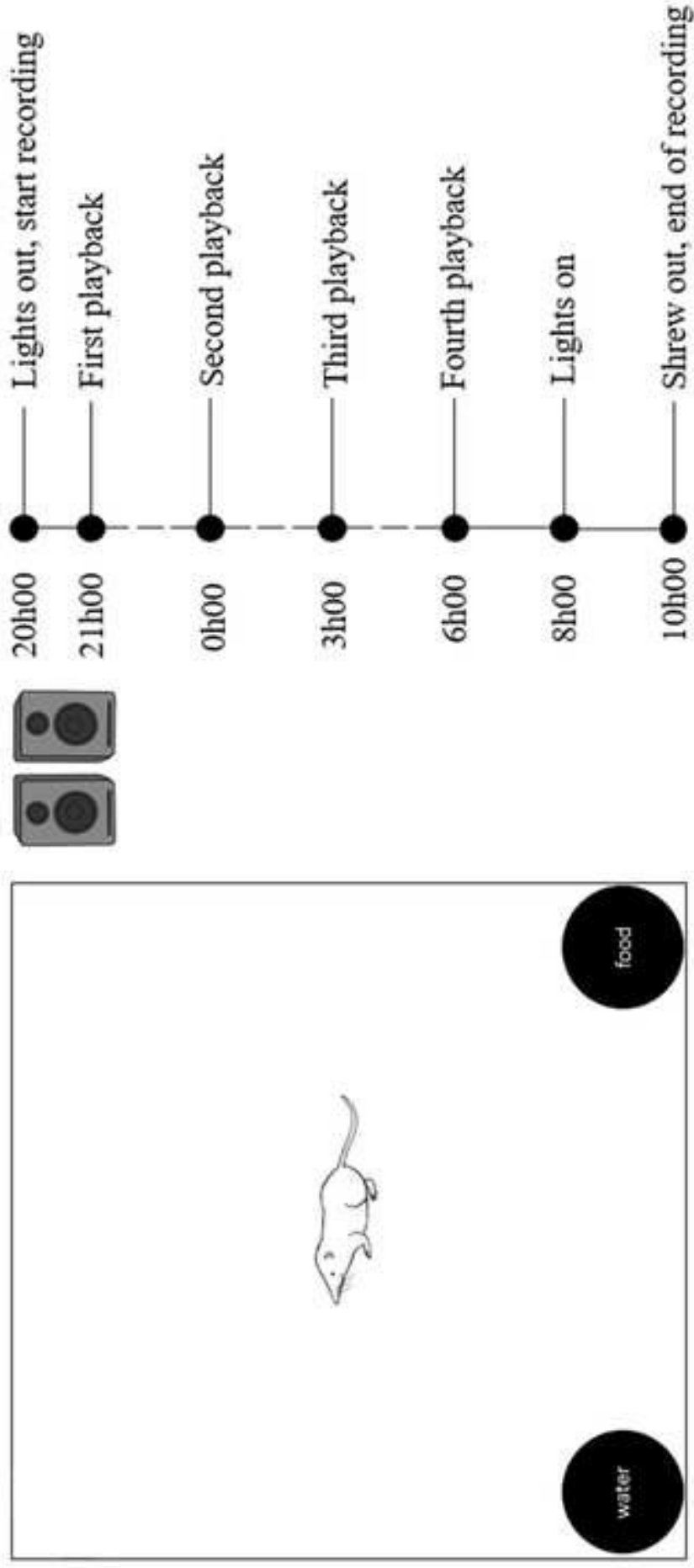
Summary results of the top model for the heterothermy index

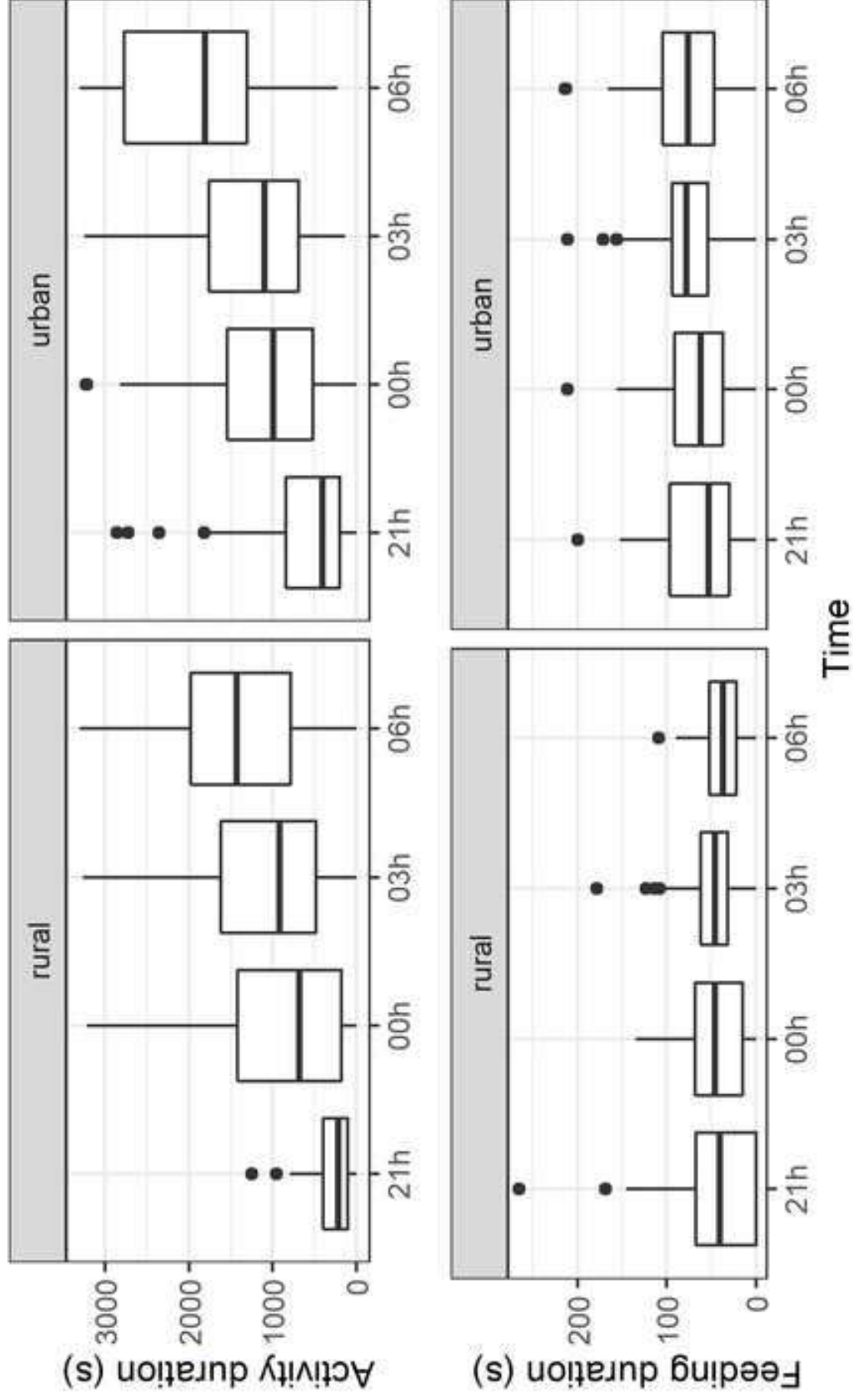
	Parameter	Effect $\pm$ SE	<i>P</i>
<b>Heterothermy Index (HI)</b>	(Intercept)	-0.004 $\pm$ 0.083	0.958
	Urban	-0.238 $\pm$ 0.082	0.007
	00h	0.101 $\pm$ 0.047	0.016
	03h	0.105 $\pm$ 0.042	0.023
	06h	0.252 $\pm$ 0.048	<0.001

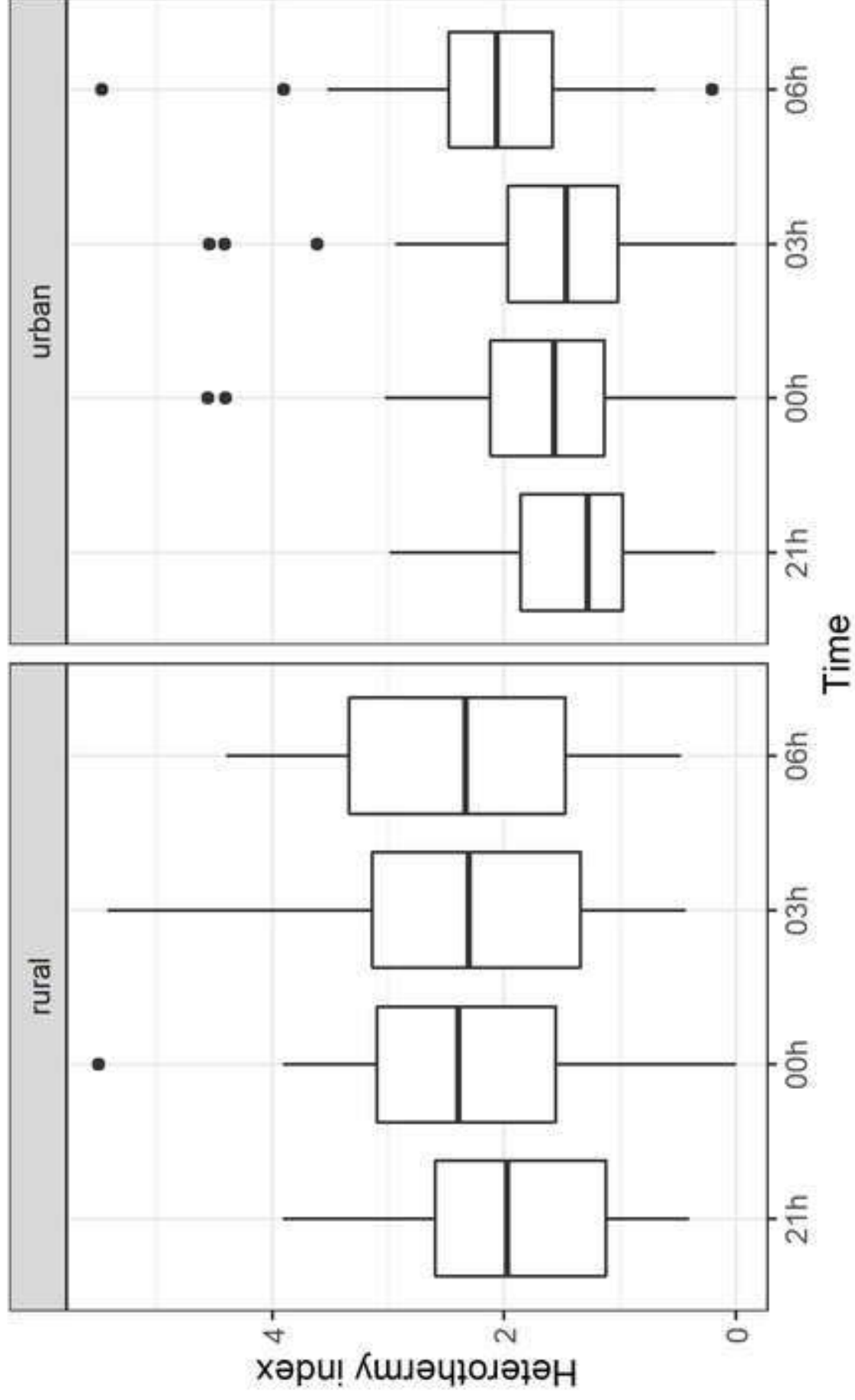
Baseline levels: rural habitat, silence sound and 21h playback

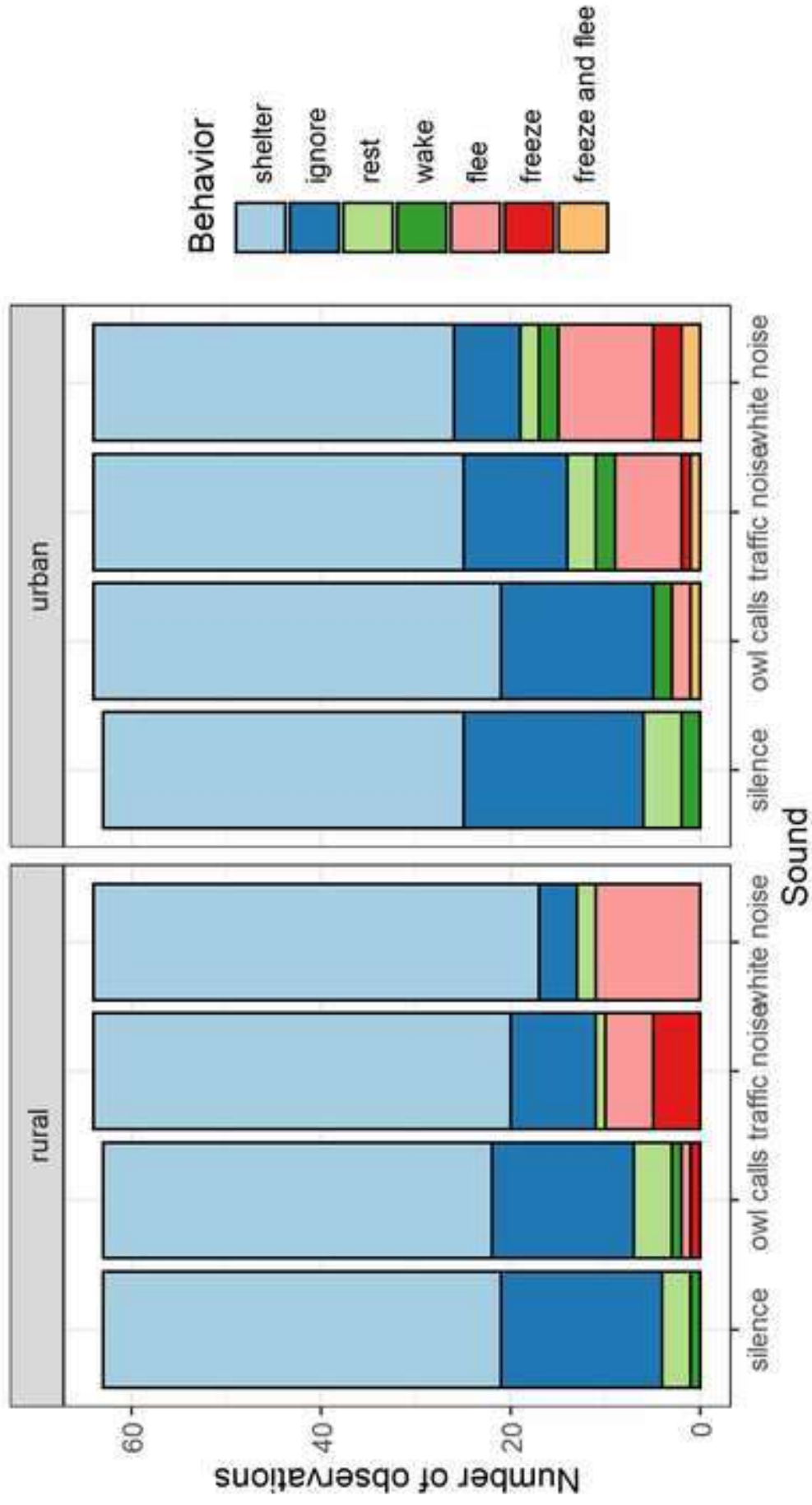
Individual identity was used as a random intercept, and sound was used as a random slope

Different variances were allowed for each habitat

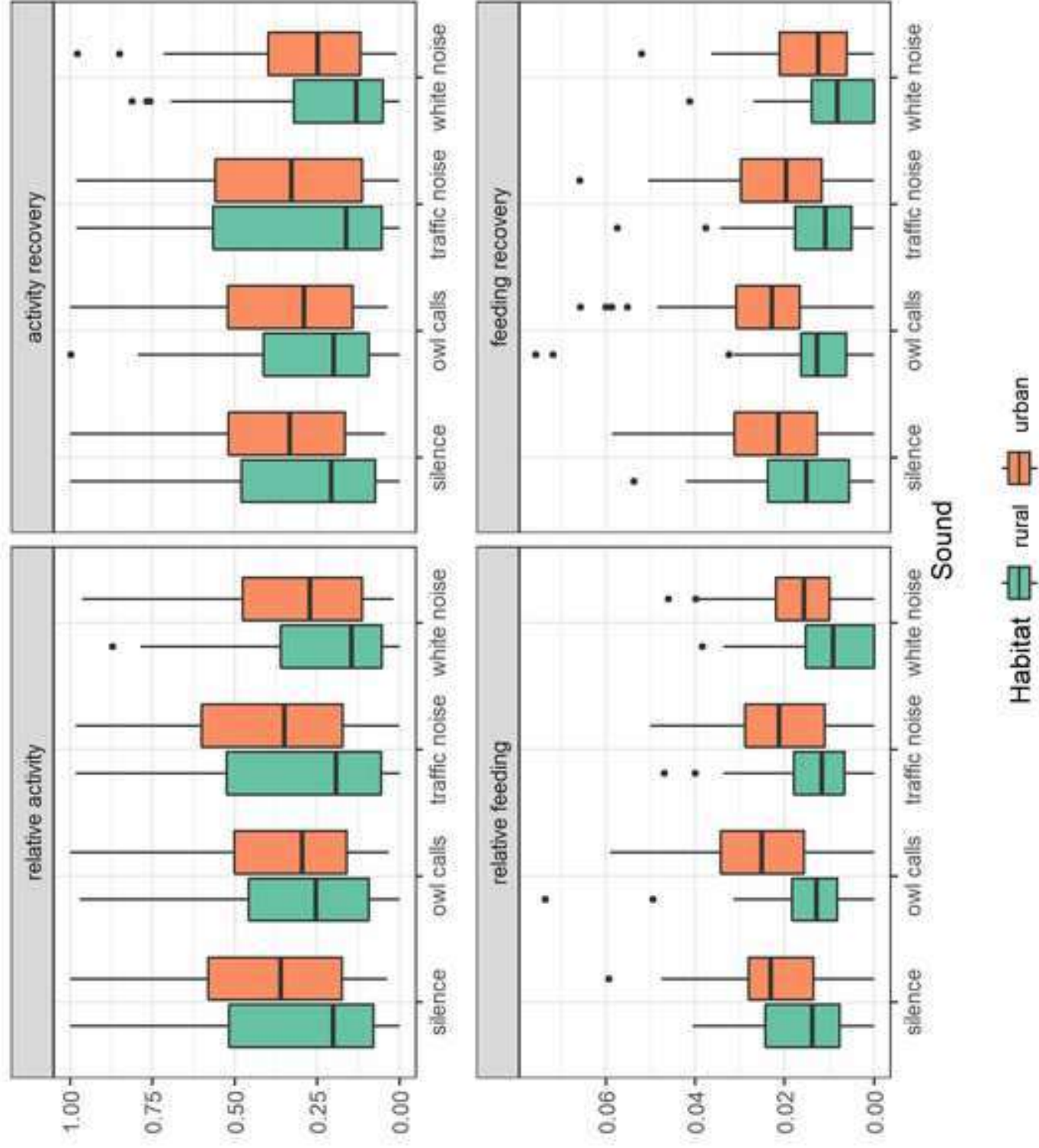


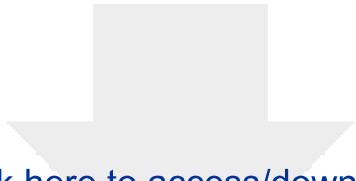












Click here to access/download  
**Supplementary Material**  
ESM1.docx

