Spatial and temporal variation in vibratory noise and its impact potential on a common urban arthropod

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Key words (up to 12):

Abstract (up to 300 words):

**INTRODUCTION**

* Animals use their senses to assess and interact with the world, but noise can interrupt or mask these abilities
  + Noise most prevalent in cities
  + Knowledge of the effects of acoustic, light, and chemical noise has impacted animal behavior is accumulating, but the role of vibratory noise lags behind
  + The use of vibratory information is widespread amongst animals, especially arthropods
    - Used especially in foraging, communication, and antipredator
  + Imperative to understand the impact of vibratory noise on an ecologically important group
* What we know about variation in vibratory noise comes from seismic stations looking to remove ambient vibrations, and is disconnected from animal responses
  + Bring in urban aspect
* In an environment where noise varies over space and time, animals may be able to assess the vibratory environment and control where and when they perform behaviors to decrease the impact of noise
  + This convalesces as microhabitat selection (where) and activity patterns (when)
  + If these are not modified, the animal may perform more fine scaled behaviors to cope with heightened noise
* Why spiders are a good model, especially Agelenopsis
* Aims/Questions
  + To what degree does vibratory noise vary across space?
  + How does vibratory noise vary over time?

**MATERIALS AND METHODS**

**Survey sites**

We recorded substrate-borne (vibratory) noise at 21 private properties in Lancaster County, Nebraska, United States in 2020 and added an additional private property (8A) and the University of Nebraska-Lincoln (UNL) city campus (8B) in 2022 (**Figure 1A**). To acquire access to the properties, we sent an email to listservs of the biological sciences and entomology departments at UNL in 2020 asking for volunteers to allow us to record ambient vibrations at their properties. We received permission to access private properties for the duration of the study from faculty, staff, and graduate students, as well as a few personal connections with properties randomly scattered across Lincoln, Nebraska, and into the surrounding rural area (**Figure 1A**). We added the two sites in 2022 (8A and 8B) because we collected some *Agelenopsis pennsylvanica* spiders from these sites for choice test experiments.

We sorted the 23 sites into two categories based on land cover class: rural and urban. Anthropogenic sources of vibratory noise are thought to travel up to 1 kilometer from the source (Lecocq et al., 2020). As such, we used QGIS (v. 3.16.3-Hannover, ESRI 102704) to calculate the area of each land cover class from the 2019 National Land Cover Database (Dewitz and U.S. Geological Survey, 2021; 30-meter resolution) within a 1-kilometer radius of each site. We combined designations of ‘Planted/Cultivated’ to describe agricultural areas and designations of ‘Developed’ to describe urban areas (<https://www.mrlc.gov/data/legends/national-land-cover-database-class-legend-and-description>). The remaining classes were combined as other area. We categorized sites as rural that had more agricultural than urban areas and sites as urban that had more urban than agricultural areas (**Figure S1A**).

**Recording ambient vibratory noise**

To record ambient vibrations, we deployed recording units (**Figure 1B**) at each of the sites. We recorded ambient vibrations by attaching a contact microphone (Kmise, Model KP-01, China) to substrates using XFasten double-sided tape. The microphone was connected to a Tascam DR-05X digital recorder (TEAC Corporation, Tokyo, Japan) where the files were stored on a Sandisk 32 GB microSD card. We used a waveform format with a 24-bit depth and a sampling rate of 48 kHz. The recorder was powered by a 10000 mAh portable battery (onn. Walmart, 3x charge, Bentonville, Arizona), which could power the recorder for approximately 24 hours. We stored the recorder and portable battery in Enther plastic 28 oz container (22 x 15.5 x 7 cm) covered in moisture- and UV-resistant Gorilla Tough and Wide White Duct Tape and applied caulk around the entrance of the audio cable leading to the microphone. Once a week, we coated the containers and microphones in Repels-All Animal Repellent, a mild nasal irritant, to safely reduce wildlife tampering with the equipment.

We used 12 recording units in total. At each of the sites in 2020, we deployed four recording units to record ambient vibrations for 24 hours next to webs of *A. pennsylvanica* (**Figure 1C**). Thus, we could survey three sites in a single day. Of the four recording units, we attached two to plant substrates and two to manmade substrates (e.g., cement, paneling, wood fences/porches, metal, etc.) to test whether substrates differ in vibratory noise levels (**Table S1**). We repeated recordings on the same substrates during three subsequent visits, for a total of four visits that occurred between August 3 and October 24, 2020. Visits to the same sites occurred approximately every three weeks (visit 1 = Aug 3-Aug 20; visit 2 = Aug 31-Sept 21; visit 3 = Sept 22-Oct 8, visit 4 = Oct 12-Oct 24) to investigate how vibratory noise varies across the penultimate instar and adult season of *A. pennsylvanica*. Recordings only took place on weekdays to avoid changes in anthropogenic activity patterns on weekends. We also only recorded on days when chances of rain were below 20% (both to protect the recording units and remove vibratory noise from rain) and the temperature was not forecasted to exceed 33 oC (to protect the recording units). We switched out the portable battery for a fully charged battery approximately 10 hours after deployment to ensure the recorders remained charged throughout data collection (see **Table S2** for data on start, check, and end times). We excluded recordings where the microphone fell from the substrate at any point during recording (see **Table S3** for final sample sizes).

In 2022, we added a private property (8A) and UNL city campus (8B, **Figure 1A**) to determine vibratory noise levels at sites where *A. pennsylvanica* spiders were collected for choice experiments. We deployed six recording devices (three on plants and three on manmade substrates) at the private property (8A) for three consecutive days (August 11-13, 2022). We deployed four recording devices (two on plants and two on manmade substrates) on UNL city campus (8B) for four consecutive days (August 17-20, 2022). We returned approximately every 24 hours to replace the portable batteries.

To measure vibratory noise levels, we used Raven Pro (v. 1.6.1) to divide each 24-hour recording into five-second time bins and calculate the equivalent continuous sound pressure level (Leq, units = dB) for each bin. We did this for the frequency range of 20 to 1000 Hz since anthropogenic noise occurs predominantly below 1000 Hz (Raboin and Elias, 2019). We removed the first five minutes of each recording to ensure that disturbance from setup was not included in the analysis. For the same reason, we removed about a minute of the recording where we replaced the battery.

**Vibratory noise across space**

First, to assess the range of vibratory noise that *A. pennsylvanica* is exposed to, we computed the site average for each of the 23 sites by averaging the Leq values for all five-second time bins recorded at a site. For further analysis, we calculated the daily average Leq by averaging the Leq of all five-second time bins for each 24-hour recording (unique by site, visit, and microphone, see **Table S3**).

We predicted that vibrations from nearby traffic likely contributed significantly to the recorded ambient vibrations. To test this, we first gathered four variables related to the traffic impact potential for each site: (i) impervious (i.e., building and pavement) cover in a 1 km radius, (ii) the average daily vehicles passing on the nearest road, (iii) the distance of the site to the nearest road, and (iv) the total length of roads in a 1 km radius.

(i) We used the 2019 Impervious Cover Data from the National Land Cover Database (Dewitz and U.S. Geological Survey, 2021; 30-meter resolution). In QGIS, we polygonized the file and used the intersection tool to calculate the percent cover for the entire 1 km buffer region for each site (as in Pessman et al., 2023?). (ii) We used data from the Lincoln Transportation and Utilities (<https://www.lincoln.ne.gov/City/Departments/LTU/Transportation/Traffic-Engineering/Average-Daily-Traffic-Volume>) and the Nebraska Department of Transportation (<https://gis.ne.gov/portal/apps/webappviewer/index.html?id=8ed4b009b0d546f19f0284e5bba0f972>) to determine the average number of vehicles per day that pass the nearest road for each site. (iii) We also calculated the shortest distance from the site to the road of its associated traffic data. (iv) We used the street centerlines layer (<https://www.nebraskamap.gov/datasets/nebraska::streetcenterlines/about>) in QGIS to sum the lengths of all of the roads within a 1 km radius of each site.

We wanted to see the degree of variation in vibratory noise levels across large-scale space (across 23 sites and by category – rural vs urban) and small-scale space (between substrates – manmade vs plant). We reduced the four traffic impact potential variables through principal component analysis (PCA) using the FactoMineR and factoextra packages in R (**Figure S1B-C**) and used principal components 1 and 2 for statistical analyses.

Statistical Analysis

For all analyses of vibratory noise, we used linear mixed-effect (LME) models using the lme4 package in R with site as a random factor since each site had multiple recordings. Since our data frequently had outliers (see **Figure S2**), we also tested identical robust LME models using the robustlmm package in R. We report the LME model results if the robust LME models produced similar results.

We first looked for differences in daily average Leq by category to assess large-scale differences in vibratory noise levels. To determine if vibratory noise levels are correlated with traffic impact potential and substrate type, we tested the daily average Leq against principal component 1 (PC1), substrate type, and the interaction as predictor variables. We also repeated this analysis while subsetting the data by each category to see if rural and urban areas experienced different relationships between daily average Leq and PC1. We used separate models to investigate PC1 because the categories were separated across PC1 (**Figure S1B**), making it difficult to interpret model predictions. We reported the results of the robust LME model for the rural subset.

We also assessed principal component 2 (PC2), this time including category as an interacting predictor variable because the categories were integrated across the PC2 axis (**Figure S1B**). Since the global model included a three-way interaction (PC2 x Substrate x Category) we used the drop1 function to perform backward selection. We performed a Type II Wald Chi-Squared test on the final LME model since the model included two multilevel variables (substrate and category).

**Vibratory noise across time**

For all temporal analyses of vibratory noise levels, we restrict our data to those collected in 2020 since 2022 data were collected across three or four consecutive days in August 2022. We first investigated whether vibratory noise varies temporally across the season throughout the penultimate instar and adulthood of *A. pennsylvanica* (August-October). We looked for relationships in the daily average Leq between each visit (1-4) and each category (rural vs urban) and the interaction between visit and category. We performed a Type II Wald Chi-Squared test using the car package in R on the LME model since visit has more than two levels. As a post hoc test, we performed a Tukey’s pairwise comparison using the emmeans package in R. We refrained from using date as a continuous variable as the data suggested a non-linear pattern across time.

We also predicted that vibratory noise from harvesting equipment was a significant source of vibratory noise in rural areas. To test this, we used the United States Department of Agriculture’s (USDA) National Agriculture Statistics Service data (<https://quickstats.nass.usda.gov/>) about the end-of-the-week percent harvested values for Nebraska in August-October 2020, specifically using crops that are most common in Lancaster County, Nebraska: corn and soybeans. We graphed these values across the season (Aug 3 – Oct 24, 2020), including the calculated mean of the percent harvested for corn and soybeans, to compare to the vibratory noise levels by visit. To directly assess if harvesting is related to rural vibratory noise levels, we tested the daily average Leq of rural sites against the percent harvested by matching the week from the harvest data to the week of the recording. We reported the results of the robust LME model.

Last, to determine whether vibratory noise exhibits a pattern by category across a 24-hour period, we averaged the Leq from the five-second time bins by each hour (i.e., daily average Leq broken down by the hour) to get the hourly average Leq. We then calculated and graphed the mean and standard error across 24 hours for each category. To see if 24-hour patterns change overtime, we also graphed this information by visit.

**Spider choice test**

In order to assess how spiders respond to variation in their vibratory environment, we collected 69 female *Agelenopsis pennsylvanica* in their penultimate instar across four different sites (Sites 5A, 6C, 8A, and 8B, **Figure 1A**). These sites varied in the recorded vibratory noise levels (5A and 6C [rural] = -69 dB, 8A [urban] = -64 dB, 8B [urban] = -55 dB). We collected spiders within a week’s time (August 4-10, 2022) and housed them in the lab under ambient vibratory conditions in 8.3 x 8.3 x 8.3 cm plastic containers until the choice test. The spiders received ad libitum water from a cotton wick inserted in the bottom of each container. We suspended the containers over a tub of water with metal chicken wire. We fed the spiders two crickets twice a week that were approximately half of their abdomen length. The spiders were maintained at 25oC and a 13:11 hr light:dark cycle that began at 07:30. We checked for molts every other day to determine the day of maturation.

The choice chamber was custom-made from two 8.3 x 8.3 x 8.3 cm acrylic containers connected by a smaller (5.8 x 2.6 x 2.6 cm) acrylic container using acrylic plastic cement (Weld-On 16) to keep the material as consistent as possible for vibration transmission (**Figure 4**). The chamber was suspended in acoustic foam to reduce vibrations from traveling between containers. We attached Hylaea clear anti-slip tape (5 cm wide) to the sides of the larger containers to produce rough surfaces for silk attachment. We applied Vaseline above the anti-slip tape to discourage silk attachment to the lid of the containers. On the bottom inside of each large container, we placed black masking tape to better observe the silk. Centered on the bottom of each larger container, we attached a piezoelectric disc (Pagow 3-in-1 transducers) that played continuous vibrations from the same audio recorders used to collect ambient vibrations in the field (Methods: Recording ambient vibratory noise). This allowed us to present a different vibratory environment on each side of the chamber: one loud and one quiet. These treatments were produced from white noise, filtered to be concentrated in low frequencies (< 1000 Hz) with a 6 dB decrease per octave. The treatments differed only in amplitude similar to the variation in ambient vibrations from the noise survey (~ 15 dB, **Figure 4**). The treatments played continuously across the experiment, powered by the same power banks from field recordings (exchanged every 24 hours).

We had 18 chambers total and ran four iterations of trials for a total of 72 spiders. However, in three trials, we used spiders from an additional site where we do not have recorded noise levels, so we decided to remove these individuals. At the time of the trial, we weighed each spider and photographed it with a size standard to calculate body size. We added the spider to one side of the chamber and encouraged it to the other side with a paintbrush before trapping it in the smaller container connecting the two larger (hereafter, tunnel). After a ten-minute acclimation period, the barriers were removed and the spider was allowed to explore the chamber and build its web for four nights. In between each trial, we thoroughly cleaned the chamber with soap and water and randomly rotated each chamber. Spiders were fed on the third day with two crickets, one thrown on each side of the chamber. We conducted this experiment from September 13 through October 7, 2023 – the natural adult season of this species.

Each day, we recorded the position of the spider once between 10:00 and 15:00, which is the time of day when they show the least activity (Results: Spider activity patterns) and are often observed in the field either at the entrance of the retreat or deep into the retreat (personal observation, Riechert?). We also used CanaKit Raspberry Pi 4 4GB computers, Arducam wide angle, night vision cameras (Model: OV5647), and the libcamera-still application to take a photo every two minutes for the first 24 hours on a subset of spiders to assess where they spend their time during the first night of web-building. At the end of the four days, we used pre-dried, pre-weighed fishing lines (4 cm long) to collect the silk from each chamber part separately. We dried the silk at 50oC for 48 hours and recorded the dry mass as an estimate of where the spiders were laying their silk.

Statistical Analysis

We used spider age (days since maturation) and body condition at the time of trial as predictors in all of our models. We considered a spider to be one day old on the day when the final molt was found. We calculated body condition by obtaining the residuals of the linear model between body mass and cephalothorax width (both log-transformed, following Jacob…) at the beginning of the choice trial. We first used linear models to explore whether age and body condition of spiders differed by the site where the spider was collected. We also tested if age and body condition were correlated. Since age and condition are correlated (see Results: Spider choice test), we refrained from including their interaction in any model. For each final model where two or more continuous predictors remained in the model, we scaled and centered all continuous variables in the global model and ensured the variance inflation factor (VIF) remained below two for each of the variables in the final model. We used backward selection to select the best fit model.

Given the choice between loud and quiet vibratory environments, we wanted to test whether spiders collected from sites with different vibratory profiles would be more likely to (i) stay in the same chamber part all four days, (ii) be found on the loud or quiet side, (iii) lay more silk on the loud or quiet side, (iv) differ in the total amount of silk used, and (v) vary in the proportion of silk laid in each chamber part.

(i) We used a binomial generalized linear model to determine if site Leq x age and Leq x body condition predicted the probability of a spider being observed in the same chamber part each day (1) or moving between parts (0). (ii) To investigate predictors of a spider being found on the loud (1) or quiet (0) side of the chamber, we used a similar test but included day as an interacting variable as we made daily observations of spider position. We took a mixed model approach by adding spider ID as a random effect. (iii) In addition to spider position, we assessed “choice” using dry silk mass. We built a model similar to (i) where spiders with higher silk mass on the loud side were coded (1) and more silk mass on the quiet side (0). (iv) We assessed total dry silk mass by each spider using a negative binomial generalized linear model with Leq x age and Leq x condition. As total silk mass varied by age (Results), (v) we tested variation in the proportion of silk mass by chamber part x Leq x age and chamber part x Leq x condition using a beta regression generalized additive model. These tests looked for differences between spiders collected from different vibratory profiles using Leq as a continuous predictor. We also performed similar tests for each response variable to assess variation by age and condition within each of the four sites.

We also graphically explored spider activity during the first night of web building from the raspberry pi images. We visualized the proportion of night-time photos where the spider is visible and the proportion of visible photos where the spider was on the loud versus quiet side. We also estimate activity levels of urban and rural spiders using the number of photos where the spider’s position changed from the previous photo for the first 24 hours of xxx spiders. These results are presented as supplemental material (Figure S).

**Spider activity patterns**

All analyses were completed in RStudio (v. 2023.03.0+386). We calculated model predictions from 1000 simulations of bootstrapping using the bootMer function in lme4 and broom.mixed package in R. We obtained p-values from LME models using the lmerTest package. To get model predictions for the robust LME models, we used the effects package. We used the insight package in R to calculate the R2 values for each LME and robust LME model. We used the tidyverse, ggrepel, ggpubr, viridis, ggExtra, and ggprism packages in R to build the graphs. For the maps, we used ggmap and ggsn packages. Tables were made using the flextable package in R or the Word document table function. All data and code are available at <https://github.com/brandipessman/Vibratory_Noise>.

**RESULTS**

**Vibratory noise across space**

Site average Leq varied by about 15 dB across the 23 sites – the loudest vibrations came from site 8B at -55 dB and the quietest vibrations came from site 6B at -70 dB (**Figure 2A**). Urban sites varied from -66 to -55 dB and rural sites varied from -70 to -66 dB (**Figure 2A**). Anecdotally, site average Leq appears to be higher at sites that are nearest to highways and interstates (**Figure 2A**).

When reducing our four traffic impact potential variables through PCA, the first principal component (PC1) explained 70.9% of the variation while PC2 explained 18.3% (**Figure S1B**). All variables contributed to PC1, but only traffic (average daily vehicles) had a substantial contribution to PC2 (**Figure S1C**). Daily average Leq significantly varied by category, with urban sites exhibiting higher Leq than rural sites (**Table 1**). Overall, daily average Leq showed a significant positive relationship with PC1, and manmade substrates carried significantly louder vibrations than plant substrates (**Figure 2B, Table 1**). There was not a significant interaction between PC1 and substrate, overall (**Table 1**). When we subset the data by category, daily average Leq for rural sites did not significantly correlate with PC1 or substrate, and there was not a significant interaction between PC1 and substrate (**Figure 2C, Table 1**). For the urban subset, we found similar results to that found overall (**Figure 2D, Table 1**).

We find similar results for PC2. Daily average Leq was positively correlated with PC2, with manmade substrates carrying louder vibrations than plant substrates and urban sites louder than rural sites (**Figure S3**). There was a trend in the interaction between PC2 and category where daily average Leq increased with PC2 in urban sites, but not rural sites (**Figure S3).**

**Vibratory noise across time**

When looking across the season as *Agelenopsis pennsylvanica* mature and proceed through adulthood, we found significant variation in vibratory noise levels. There was a trend that daily average Leq varied by visit (**Figure 3A, Table 1**). A Tukey post hoc test revealed a significant increase in noise from visit 2 to visit 3 (*t* = -3.00, df = 241, *P* = 0.016). We still see a significant effect of category where rural sites are quieter than urban sites, and there is no interaction between visit and category (**Table 1**). Using the USDA data on harvest rates for corn and soybeans in Nebraska, we found that the majority of the corn harvest (51%) occurred during the fourth visit while the majority of the soybean harvest (70%) occurred during the third visit (**Figure 3B**). As a result, the mean harvest of corn and soybeans was at its peak during the third visit (**Figure 3B**), coinciding with the increase in daily average Leq at the third visit (**Figure 3A**). When we tested to see whether the mean percent harvest was correlated with the daily average Leq of rural sites, we found a positive trend from the robust LME (**Table 1**) that was significant in the LME (*t* = 2.12, df = 71, *P* = 0.037).

By graphing the trends in the hourly average Leq across 24 hours, we observed that vibratory noise in rural and urban areas goes through similar patterns (**Figure 3C**). Noise levels are highest in the morning, with an additional peak in the afternoon before decreasing and maintaining low levels during the night (**Figure 3C**). Rural noise levels stay consistently lower than urabn noise levels throughout the 24-hour span (**Figure 3C**). Peaks appeared to have occurred around rush hours (08:00 in urban, 09:00 in rural, 15:00 both). Across visits, rural sites showed variability in noise levels across 24 hours, while urban sites seemed to maintain relatively consistent patterns (**Figure S4**). During the third visit, we see high noise levels at night in rural areas that nearly match nightly recordings in urban areas (**Figure S4**). During the fourth visit, rural areas show heightened daily noise levels (**Figure S4**).

**Spider choice test**

We collected spiders from four different sites that varied in average Leq (5A: -69 dB, 6C: -69 dB, 8A: -64 dB, 8B: -55 dB). We first tested whether spiders differed in age or body condition by site. Spiders from different sites did not vary by age at the time of the trial (*F3,65*= 1.40, *P* = 0.250). Body condition showed marginally no difference between sites (*F3,65*= 2.62, *P* = 0.058). A Tukey post hoc test showed a trend that site 8A spiders were in better condition than spiders from sites 8B (*t* = -2.57, *P* = 0.059) and 6C (*t* = 0.246, *P* = 0.075) but not 5A (*t* = 2.18, *P* = 0.137). We also found that age and condition have a significant positive correlation (*t* = 3.80, *P* < 0.001, Adj. R2 = 0.165).

*Spider Position* – (i) Site Leq, age, and their interaction did not significantly predict whether a spider was found in the same chamber part (loud, quiet, or tunnel) all four days of observation (**Table 2**), and body condition was dropped from the model entirely. When we looked for variation within sites, only spiders from site 8B (the loudest site) were less likely to stay in the same chamber part as age increased (*z2,22*= -2.02, *P* = 0.043, *R2* = 0.174). We observed the spiders’ positions daily, but there were no trends associated with the day of observation as it was dropped from the model. (ii) Site Leq and age had no effect on a spider’s probability of being found on the loud or quiet side (**Table 2**). Yet, there was a non-significant trend exhibited in the interaction between Leq and age (**Table 2**) – i.e. spiders from loud environments decreased in the probability of being found on the loud side as age increased while age effects diminished for spiders from quieter sites (**Figure 5A**). We found further support for this finding when we looked for age effects within sites and found a trend for site 8B spiders (*z2,84* = -1.71, *P* = 0.088) but no effect of age for spiders from other sites (*P* > 0.360).

*Silk Mass* – (iii) The results for the side with more dry silk mass were similar to the findings from spiders’ positions (**Table 2, Figure 5B**). We also found that spiders from site 8A showed a trend of declining silk mass on the loud side with age (*z2,22* = -1.74, *P* = 0.083) but there were no age effects for the rest of the spiders (*P* > 0.140). (iv) When we looked at the total dry silk mass combined from each chamber part (loud, quiet, and tunnel), site Leq stayed in the model but showed no significant relationship (**Table 2**). However, there was a significant positive correlation between total silk mass and spider age (**Table 2**). When we look at age effects within sites, sites 8B (*z1,22* = 4.67, *P* < 0.001) and 6C (*z1,14* = 2.19, *P* = 0.029) indicated significant positive increases in silk mass with age, but sites 8A (*z1,9* = 1.08, *P* = 0.280) and 5A (*z1,16*  = 0.79, *P* = 0.430) did not.

(v) Since total silk mass increased with age, we used the proportion of silk mass between chamber parts to test whether spiders differentially apportioned silk between chamber parts. Site Leq, age, and the interaction between chamber part and age did not significantly predict the proportion of silk (**Table 2**). There was a significant effect of the chamber part on the proportion of silk (**Table 2**) where spiders invested more silk in the larger containers than in the tunnel (following a Tukey post hoc using the emmeans function). We also found a significant interaction between chamber part and Leq (**Table 2**). Exploring this interaction post hoc (using the lstrends function) revealed significant interactions between the loud-quiet parts (*t195* = 3.973, *P* < 0.001) and quiet-tunnel (*t195* = -3.011, *P* = 0.008) but not loud-tunnel (*t195* = 0.636, *P* = 0.801) parts at the 25th percentile age. In other words, spiders were using more silk in the tunnel and loud parts and less in the quiet as their site Leq increased (**Figure 5C**). At the median age, there was only a trend for the interaction between quiet and tunnel silk proportions (*t195* = -2.184, *P* = 0.076). At the 75th percentile age, there were no significant interactions between age and Leq. There was also a significant three-way interaction between chamber part, Leq, and age (**Table 2, Figure 5C**). When we compared parts pairwise for interacting age trends for each site Leq (using the lstrends function), the only significant finding was that spiders from the loud site decreased silk in the loud part and increased in the quiet part as age increased (*t195* = -3.109, *P* = 0.006). We tested for chamber part x age within sites, and spiders from each site had a significantly higher proportion of silk in the loud and quiet parts compared to the tunnel (8B: *P* < 0.001, 8A: *P* = 0.014, 6C: *P* = 0.052, 5A: *P* = 0.040). The loudest site also had a higher proportion of silk in the loud than quiet side (*P* = 0.004).

When we analyzed the raspberry pi images taken every two minutes during the first night for a subset of spiders, we find that spiders from each site explored both sides of the chamber (**Figure S5A**). Further, the side that the spider was most frequently observed on in the nighttime photos was not always the side that the spider was initially observed on during the first daily position observation nor the side that had the higher proportion of dry silk mass (**Figure S5B**). From the number of photos where the spider was observed changing positions, we anecdotally observed higher activity from urban spiders (sites 8B and 8A) than rural spiders (Site 5A and 6C, **Figure S5C**).

**Spider activity patterns**

**DISCUSSION**

Main findings

* Vibratory noise is variable across space in urban areas (especially due to proximity to high-traffic areas)
* Vibratory noise is variable across time in rural areas (especially due to harvest)

Spatial noise evidence

* Urban area ranges -55 to -66 dB; rural area ranges -66 to -70 dB
* Anecdotally, high-noise sites seem to be near highways/interstates from Figure 2A
* Urban site noise levels are positively related to traffic impact potential.
* Manmade substrate carries louder vibrations than plants in urban, not rural areas

Temporal noise evidence

* Increase from visit 2 to 3 more dramatic in rural areas
* Peak in harvest in visit 3 matches increase in noise in rural
* Percent harvest with positive relationship to noise levels
* Changes in 24-hour patterns in rural in third/fourth visit match harvest. Harvest occurs at night (visit three high noise in the middle of the night). Cleaning equipment in day in visit four?

Limitations

* Traffic impact potential is approximation. Traffic levels are annual averages, so they will fluctuate daily
* Harvest data is used for entire state of Nebraska. Did not record when harvest took place at each site individually.
* Manmade substrates may be louder due to proximity to human activity (garages with cars in and out, porches with dogs going in and out)
* Some outliers can be due to activities like unexplained or explained reasons - mowing (6A v2, 7A v1), trees being cut down (3A v1), pop-up storm cell at 3 AM (2 v1), yard work (1B v3) (we told property owners to carry out their normal activities)

**CONCLUSIONS**

**ACKNOWLEDGEMENTS**

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**FIGURE LEGENDS**

**TABLES**