

AUTOMATED RECORDING OF DAILY VIBROACOUSTIC AND BEHAVIORAL ACTIVITY IN
XYLOCOPA VIRGINICA

An Honors Thesis Presented

By

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ABSTRACT

Only two studies offer detailed records of the behaviors performed by *Xylocopa virginica* (Hymenoptera: Apidae), the eastern carpenter bee. Vibroacoustic behaviors and circadian activity patterns remain unstudied. I built a custom, open-source data collection instrument and artificial nests to develop a more comprehensive ethological record of eastern carpenter bee behavior. A Raspberry Pi Zero WH managed instrument operation and data collection. A video camera and transducers were programmed to record activities at the artificial nest entrance and sounds produced within the artificial nest. Air temperatures were recorded daily with a digital thermometer. Photoperiod data (length of sunlight exposure) were recorded daily. The instrument was powered by mains power supply, with a supplemental lithium polymer battery in case of power outages. A PiJuice Uninterruptible Power Supply regulated electrical current flow through the instrument. With the video and audio recordings, I will identify circadian periods of high and low species activity (by the number of entries and exits to the nesting gallery) and I will count the daily frequencies of performed behaviors. Sound source localization will be performed on each audio recording to determine where in the nesting gallery vibroacoustic behaviors are performed. This custom data collection instrument can be used to automate naturalistic observations of behavior and modified to study other species.

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Table of Contents

Introduction.....	3
Methodology.....	13
Reflections.....	25
Conclusions.....	30
References.....	31
Appendix.....	34

Introduction

Historically, behavioral observations have been recorded with a pen and paper. Recently, researchers have begun to incorporate automated observation devices (such as trail cameras) into ecological research (Rhinehart *et al.*, 2020). Small, portable, automatic devices enable round the clock species monitoring, without mandating the constant presence of researchers to record species behaviors. Usage of these devices also reduces the amount of human disturbance to habitats and reduces human disturbance to behavioral activities.

Open-source software programs are used for a variety of tasks in scientific research. Open-source software creators permit users to view, modify, and distribute the software's source code. This permission enables users to customize the software's functioning to serve their own purpose. Source codes from one study are thus available for reuse in future studies.

Open-source codes can be combined to perform complex functions. When hardware and software are combined, custom instruments can be produced for use in research. Devices such as a Raspberry Pi (<https://www.raspberrypi.org/>) single board computer, make use of open-source software (e.g., Python Programming Language, <https://www.python.org/>) to operate connected hardware. With such devices, a user provides code programs that contain commands for the computer to execute.

Life-History of Xylocopa virginica

The eastern carpenter bee, *Xylocopa virginica*, (Hymenoptera: Apidae) is a large carpenter bee species native to North America. The species distribution extends from eastern Mexico to southern Ontario, Canada (Mawdsley, 2017; Prager, 2008; Richards, 2011; Skandalis *et al.*, 2009; Skandalis *et al.*, 2011; Vickruck & Richards, 2017A; Rau, 1933). Eastern carpenter bees are a generalist forager species (Barrows, 2016; Vickruck & Richards, 2017A; Tucker *et al.*,

2019A), though they may forage for food from specific plant species depending on local biodiversity and time of year (Gerling *et al.*, 1989; Rau, 1933). Like other bee species, females of *X. virginica* possess stingers and males lack stingers. Females and males can be distinguished by head morphology; males have a white, square-shaped spot in the center of their head, but females lack this spot (**Fig. 1**).

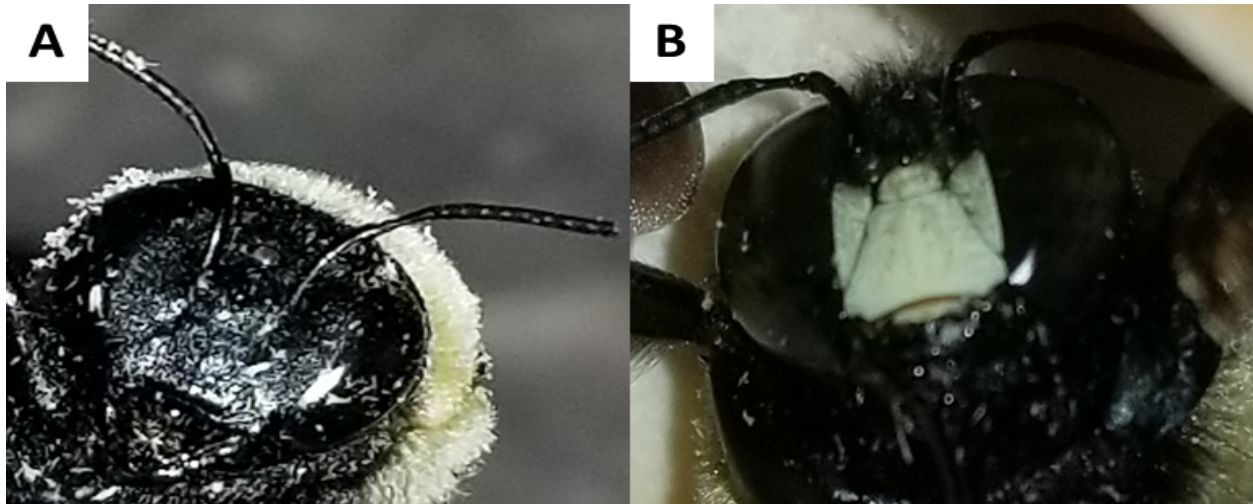


Figure 1. Head morphology of each *X. virginica* sex. A) Female of *X. virginica*. B) Male of *X. virginica*. Males are morphologically distinguished from females by the white square on a male's head.

Females of *X. virginica* maintain nests and perform all brood care in this species (Gerling *et al.*, 1989; Prager, 2008). Female bees bore nests into aged wood (such as fallen tree branches, fence boards, and barn joists) (**Fig. 2**), where they live and raise their brood (Gerling *et al.*, 1989; Prager & Hunter, 2011; Course, 2011). Nests are constructed with either a linear or a branched structure (Prager & Hunter, 2011). This is a univoltine species; females raise a single brood of eight to nine offspring per breeding season (Richards & Course, 2015; Skandalis *et al.*, 2009; Gerling & Hermann, 1978; Course, 2011). Within their nest, a female creates brood chambers (also referred to as brood cells) that eventually house their developing brood (Jenkins & Matthews, 2004; Prager & Hunter, 2011; Gerling *et al.* 1989; Rau, 1933; Course, 2011). A female will provision a pollen mass, lay a single egg on top of the pollen mass, and seal the

pollen mass and egg combination inside of a chamber with wood shavings (Gerling & Hermann, 1978; Prager & Hunter, 2011; Rau, 1933). The female repeats this process until several egg and pollen mass containing brood chambers are assembled adjacent to one another within the nest (Fig. 3). Females have been observed to fill a nest with animal hairs after sealing their newest (and consequently, last constructed) brood chamber (Gerling *et al.*, 1989). The hairs may serve as an additional defensive barrier against predators who enter the nest to prey on offspring (Fig. 4).

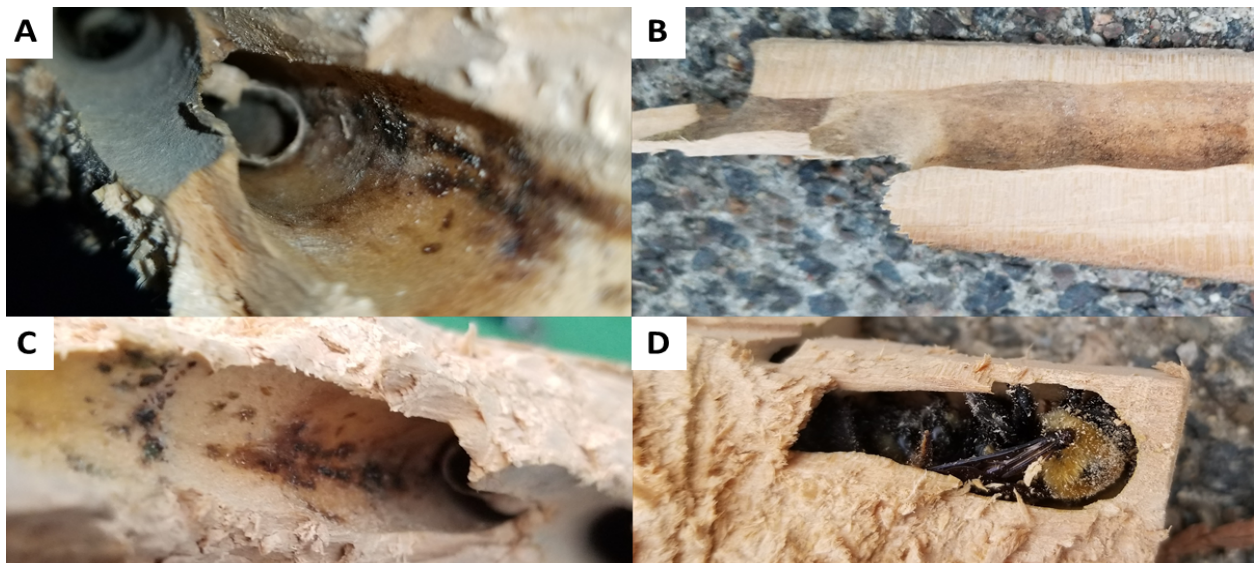


Figure 2. Examples of nests bored by females of *X. virginica*. A) - C): Vacant nest tunnels. D) Eastern carpenter bees overwintering within a nest tunnel. All depicted nests were parts of the same nesting gallery.

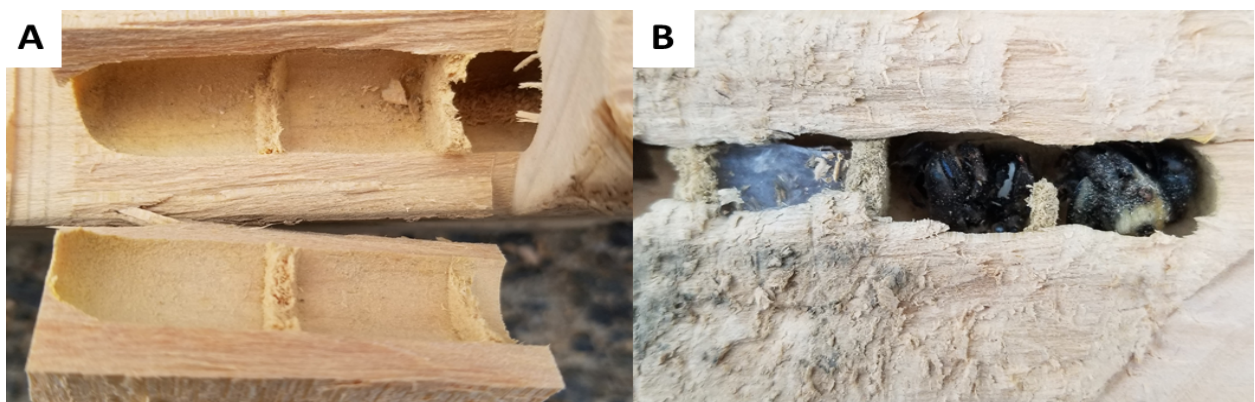


Figure 3. Brood chambers constructed by a female eastern carpenter bee. A) Empty brood chambers within an unoccupied nest. B) Decaying eastern carpenter bees within their brood chambers. These bees died inside of their brood chambers during development.



Figure 4. Animal hairs stuffed inside of an *X. virginica* nest.

A developing larva consumes its associated pollen mass while inside of the brood chamber. Where the first egg laid is the oldest, this is usually the most matured offspring at any given time within the nest. This matured bee will break through the wood shaving partitions that separate its siblings from each other, and begin to move about the nest (Rau, 1933). Maturing offspring of *X. virginica* are entirely dependent upon their mother to provide food resources during development. After maturing, offspring appear to remain in the nest for several more weeks. During this time, the maternal female continues provisioning food for the offspring (Gerling *et al.*, 1989). Where offspring develop rapidly from the larval stage to the imago stage, matured bees may require additional nutrients before they are ready to emerge from the nest (Gerling *et al.*, 1989).

Behavior of X. virginica

Female carpenter bees are facultatively social; they may nest in a solitary setting or they may nest in a semi-social setting (Peso & Richards, 2010; Richards & Course, 2015; Vickruck & Richards, 2018; Gerling *et al.*, 1989; Course, 2011). Facultative sociality is a plastic behavioral phenotype; a female may nest in solitary during one breeding season and nest in a semi-social gallery during the next breeding season, and vice versa. The decision to mate in solitary or in a semi-social nesting gallery may depend on space availability and food resource availability (Gerling *et al.*, 1989; Course, 2011). Course (2011) suggested that individuals of *X. virginica*

reduce competition for previously constructed nests by living in shared semi-social nesting galleries. Some studies have shown no difference in fitness between reproductive females that nest in solitary versus females that nest in a nesting gallery (Prager, 2008; Prager, 2014).

Within a nesting gallery, there is a hierarchy of dominant females and subordinate females. The dominant female breeds, bores new nest tunnels, and forages for the entire nest (Richards & Course, 2015; Vickruck & Richards, 2018; Gerling *et al.* 1989). Subordinate females may serve as helpers females. Previously, it was concluded that helper females guard nests while dominant females forage and reproduce (Course, 2011; Prager, 2014; Gerling *et al.*, 1989; Vicruck & Richards, 2018). However, more recent data suggest that subordinate, helper females may perform some foraging activities and may produce a brood later in a breeding season than dominant females (Course, 2011). Course (2011) suggested that reproducing by order of dominance during a breeding season may account for why some females (likely the most dominant) commence foraging early in the breeding season, while other females commence foraging late in the breeding season.

Helper females gain no immediate fitness benefit by supporting the dominant female's nesting activities, unless the helper female is genetically related to the dominant female. If the helper female and the dominant female are related, then the helper female receives an indirect fitness benefit because it will share some of its DNA with the dominant female's offspring (Course, 2011). Subordinate females may benefit by taking over a previously constructed nest within the gallery during their second year, after the prior dominant female has left the nesting gallery or has died (Vickruck & Richards, 2018; Richards & Course, 2015). In this situation, the former helper female acquires a previously constructed nest and expends less energy than would otherwise be the case if an entire new nest were constructed.

Two types of subordinate females have been identified in *X. virginica*. A secondary female assumes dominance once the dominant female is gone (Vickruck & Richards, 2018; Richards & Course, 2015). Secondary females guard nests while dominant females are foraging. Tertiary females perform essentially no work during a breeding season (Course, 2011). Tertiary females are often smaller than primary and secondary females (Vickruck & Richards, 2018). Tertiary females generally delay reproduction until their second breeding season, whereas primary females will reproduce during their first breeding season. Secondary females may attempt to reproduce during their first breeding season or they may delay reproduction until their second breeding season (Vickruck & Richards, 2018).

Individuals of *X. virginica* use nestmate recognition, not kin recognition, to identify familiar conspecifics (Peso & Richards, 2010; Vickruck & Richards, 2017B). Male and female eastern carpenter bees aggress more against non-nestmate conspecifics than against nestmate conspecifics who they overwintered with, regardless of whether the non-nestmate conspecific is a sibling or not (Vickruck & Richards, 2017B). A foraging female that returns with little or no pollen after a foraging trip also receives more aggression from nestmates than a foraging female that returns with a large pollen provision (Vickruck & Richards, 2017B).

When foraging, eastern carpenter bees use two strategies to extract pollen and nectar from flowers. Like other pollinating species, carpenter bees will enter the flower's corolla to extract nectar and pollen. When they are unable to reach nectar at the base of flowers with elongated corollas, carpenter bees engage in nectar robbery (Gerling *et al.*, 1989; Rogers *et al.*, 2013; Sampson *et al.*, 2004; Tucker *et al.*, 2019A; Tucker *et al.*, 2019B). The eastern carpenter bee slits the corolla near its base and extracts pollen and nectar through the slit. Engaging in nectar robbery decreases pollen transfer efficiency for some plant species visited by *X. virginica*

(Tucker *et al.*, 2019A). Many *Xylocopa* species use buzz pollination (sonication) to free pollen from a flower's anthers (Gerling *et al.*, 1989; Tucker *et al.*, 2019A). After freeing the pollen, carpenter bees carry pollen to their nest with their hind legs (Gerling *et al.*, 1989).

Males of *X. virginica* are territorial. Larger, more aggressive males claim territories that contain active nests; smaller, less aggressive males claim territories around the periphery of active nests or farther away (Barthell & Baird, 2004; Prager, 2008; Skandalis *et al.*, 2009; Course, 2011). Smaller males hover near nest entrances within territories held by larger males if the larger males are removed or fly away. Upon returning, the larger males reclaim their territories and expel the smaller males back to the periphery of the active nests or farther away (Prager, 2008; Prager & Richardson, 2012). Males pursue intruders if intruders are actively flying within their territory but they do not pursue motionless intruders (Gerling & Hermann, 1978).

Males of *X. virginica* demonstrate female defense polygyny (Barthell & Baird, 2004, Prager, 2008; Prager & Richardson, 2012). In this mating system, a male defends a nest of female carpenter bees. The male mates with reproductively active females and prevents other males from mating with females in the nest. Most intrasexual interactions occur while flying around a nest entrance. Reproduction is skewed toward larger, more aggressive males. These males mate disproportionately more frequently than smaller, less aggressive males.

Each sex demonstrates different nest inhabiting activities. Males drift from nest to nest, and will remain inside of a nest that is near or within their territory overnight (Peso & Richards, 2011). Females also demonstrate nest drifting behaviors, but do not relocate as frequently as males do (Peso & Richards, 2011). Not all female eastern carpenter bees nest drift; some females remain in their natal nest after maturing (Course, 2011). Other females drift to solitary nests or

nesting galleries, possibly in search of a space to construct their own nests (Richards, 2011). Despite nest drifting, eastern carpenter bees demonstrate regional fidelity; Ballare & Jha (2020) found high regional philopatry and high regional genetic relatedness among conspecifics at the 1 km level.

Present Study

Two comprehensive ethograms detailing behaviors performed by eastern carpenter bees have been published to date (Vickruck & Richards, 2017B; Duff, 2018). However, neither of these records account for the species vibroacoustic behaviors, nor do they account for the circadian activity pattern in this species (**Fig. 5**, **Fig. 6**). Vibroacoustic behaviors are those consisting of vibration productions and sound productions (Hunt & Richard, 2013). Circadian activity patterns are those behavioral performances occurring on a twenty-four hour cycle. Arbitrarily, if a species forages intensely during the same two-hour time period daily, then this is a period of high circadian activity. If the intense foraging activity period were followed by, for example, a one hour period of inactivity daily, then this is a period of low circadian activity for the species. The sum of these active and inactive periods form a circadian activity pattern.

Table 1

Ethogram of all behaviours recorded from observation nests of *X. virginica*. All behaviours were counted as events (E), although two behaviours (marked S) sometimes occurred for long enough to be measured as state variables. The frequency of each behaviour was calculated relative to a total of 667 behavioural events from the perspective of the focal bee. The relative number of nests in which each behaviour occurred was divided by 40. (The total number of nests in the aggregation).

Behaviour by focal bee	Definition	Event or state	Relative frequencies in females and males	Relative number of nests in which behaviour occurred
<i>Feeding behaviours</i>				
Trophallaxis	Second bee rotates horizontally 180° to the focal female. Both bees extend their tongues. Focal female regurgitates nectar and second female consumes it.	E	5.1%, 0%	37.5%
Scrape pollen off	Focal female rubs two hind legs together to remove pollen from her legs.	E	5.5%, 0%	55%
Eat pollen	Focal bee consumes pollen from a slant at one end of the nest.	E	2.5%, 0%	30%
Receive beg	Second bee extends her front pair of legs and repeatedly 'taps' the focal female on the head or abdomen, depending on her orientation.	E	1.8%, 0.14%	20%
Eat pollen off focal female	Second bee consumes pollen directly off focal bee. This may occur while the focal bee is engaged in an interaction with a third bee.	E	1.20%, 0%	7.5%
<i>Aggressive behaviours</i>				
Bite	Focal bee opens mandibles and closes them forcefully on the second bee.	E	0.14%, 0%	2.5%
C- posture	Focal bee curves abdomen under her thorax while facing second bee.	E	0.28%, 0%	2.5%
Ejected	Focal bee is forcibly pushed out of the nest entrance by second bee.	E	2.85%, 0.28%	27.5%
Push	Focal and second bee face each other and attempt to drive each other backwards through contact with their heads.	E or S	3.14%, 0%	37.5%
Receive bite	Second female bites focal female.	E	0.75%, 0.28%	17.5%
Receive C-posture	Second female displays C-posture to focal female.	E	0.45%, 0%	7.5%
Receive push	Second female pushes while focal female backs up.	E	0.14%, 0%	2.5%
<i>Other interactions</i>				
Pass	Focal bee meets second bee. One of the two bees turns over and they pass each other venter to venter.	E	9.1%, 0.14%	57.5%
Attempted pass	Second bee blocks focal bee from passing by pushing head and abdomen to fully block tunnel.	E	0.75%, 0%	10%
Head to head touch	Focal bee touches the head of the second bee with her head and remains motionless.	E	2.25%	25%
<i>Individual behaviours</i>				
Back	Focal bee moves backwards without turning.	E	0.14%, 0%	2.5%
Exit	Focal bee leaves the nest of her own accord.	E	13.64%, 0.28%	82.5%
Groom	Focal bee passes her legs or antennae through her mouthparts.	E	13.49%, 3.15%	87.5%
Still	Focal bee remains motionless.	S	2.85%, 1.65%	47.5%
Turn	Focal bee changes direction.	E	25.49%, 2.10%	92.5%

Figure 5. Ethogram describing the behaviors performed by *X. virginica* bees. This ethogram was published in Vickruck & Richards (2017B). Vibroacoustic behaviors and the circadian activity pattern of behavioral performances are unaccounted for in this ethogram.

Table 1: An ethogram of male *Xylocopa virginica* behaviours observed in 2016. The total number of male behaviours and frequency for each behaviour relative to the number of ordered scan samples (1487 ordered scan-sampling points). Hyperlinks and timestamps of videos are provided to show examples of behaviours when possible.

Behaviour by focal male	Definition	Frequency of behaviour (percentage of sampling points)	Hyperlink & timestamp
<i>Territorial behaviours</i>			
Hovering	Stationary flight 10-20cm above the ground; usually facing nesting substrate sometimes pivoting in place. Flight sometimes drifts side to side.	938 (63.1)	youtube.com/watch?v=-0EWk0LGzps 0:00-0:05, 0:10-0:15, 0:45-0:53, 1:04-1:40
Loop flights	Quick circular or figure-8 flights that start and finish at a hovering position. The size of loops appeared to be quite variable.	118 (7.9)	youtube.com/watch?v=-0EWk0LGzps 0:54, 0:58-1:00
<i>Male-male interactions</i>			
Chase	A rapid darting flight in pursuit of a nearby male. Contact was sometimes made between males.	108 (7.3)	youtube.com/watch?v=-0EWk0LGzps 0:16-0:19
Flee	A quick flight to escape the aerial pursuit of another male.	108 (7.3)	youtube.com/watch?v=-0EWk0LGzps 0:16-0:19
Fight	Mid-air grappling of legs with another male.	4 (0.3)	N/A
<i>Male-female interactions</i>			
Chase (female)	The male pursuit of a female (see the description of Chase).	1 (0.0)	See male-male chase video
Copulation	Pouncing on a female followed by abdominal thrusting lasting longer than ~2s. Two types: 1. Females carried high into the air during intercourse, and 2. a mating pair landed, and intercourse continued.	16 (0.1)	youtube.com/watch?v=6LmkKX3FMtl 0:00-0:14
Mating attempt	Pouncing on a female that was quickly dislodged by the female. Females sometimes land after rejecting copulation.	15 (0.1)	N/A
Female guarding	Hovering facing a landed female at a very close distance ~5-10cm. The female was a mate when the behaviour followed copulation.	Video evidence only	youtube.com/watch?v=iH2o76ndPlc 0:00 – 0:41. youtube.com/watch?v=4TBJOBfwjuA 0:00 – 1:04.
<i>Non-territorial behaviours</i>			
Searching	Side-to-side flights very close to nest entrances.	67 (4.5)	N/A
Rest	Stationary and on the ground, sometimes with abdominal pumps.	12 (0.1)	youtube.com/watch?v=Zou5P5WV2Rk 0:00 - 0:18.

Figure 6. Ethogram describing behaviors performed by males of *X. virginica*. This ethogram was published in Duff (2018). Vibroacoustic behaviors and circadian activity patterns are also unaccounted for in this ethogram.

I constructed a custom, open-source data collection instrument to elicit the behaviors performed by *X. virginica* bees. With my data collection instrument, I will produce a more complete record of this species ethology. This instrument automates interval sampling of behaviors performed by eastern carpenter bees. Three questions about behaviors performed by *X. virginica* were asked: 1) What are the daily frequencies of performed behaviors in *X. virginica*?

2) what is the species circadian activity pattern?; and 3) What vibroacoustic behaviors are performed inside of the nesting gallery?

Due to the unusually cold spring season in New England in 2021, the male eastern carpenter bees in my study have emerged from their nests only four times (20 April 2021, 24 April 2021, 2 May 2021, and 3 May 2021). Female bees have yet to emerge from their nests. Therefore, intended data analyses will be presented, without results or discussion.

Methodology

Artificial Nesting Galleries

Artificial nests were built based on Vickruck & Richards (2017B) design. With an electric router, I cut a tunnel 12 mm deep and 200 mm long into a nominal 2x6 non-pressure treated pine board cut to 600 mm in length. The tunnel is positioned 12 mm inward from the lower edge of the board. To create a nest entrance, I then drilled a 12 mm wide x 12 mm deep hole into the board at the 100 mm tunnel length. I cut a sheet of 457 mm x 610 mm x 2 mm plexiglass (Optix® Acrylic Sheet) with a RotoZip to match the exterior dimensions of the pine board. I placed this plexiglass over the tunnel. The plexiglass served as a barrier between the eastern carpenter bees and myself, were I to open an artificial nest to observe bee activity. I then cut a piece of plywood with the RotoZip to match the exterior dimensions of the pine board and the plexiglass. I placed this plywood over the plexiglass to darken the tunnel for the eastern carpenter bees.

All artificial nest layers were held together with four screws drilled into the periphery of the pine board, to minimize future interference with nest boring. I then attached a piece of plywood measuring 152.4 mm x 600 mm to the top of the artificial nest in perpendicular fashion with four screws. This plywood shaded the entrance tunnel of the artificial nest and provided

some weather protection to the artificial nest. Parts were disassembled and later reassembled after I translocated the eastern carpenter bees into their artificial nests.

On 19 March 2021, I picked up a cedar board infested with individuals of *X. virginica* outside of The Durfee Conservatory at UMass Amherst, Amherst, Massachusetts, United States. The board had been part of one of several wooden planters that eastern carpenter bees have inhabited for numerous years outside of The Durfee Conservatory. To minimize any risk of bee stings, the cedar board was sealed inside of a black trash bag and placed into a car trunk for transportation. I exposed the nesting gallery tunnels with an electric miter saw, a miter hand saw, a tooth hand saw, a hack saw, and wood chisels.

Carpenter bees were present in four nests within the cedar board; the remaining nesting tunnels were vacant. Of the four nests, three contained live carpenter bees (the fourth nest contained three decaying bees that never emerged from their brood chambers). The live *X. virginica* containing nests contained eight bees, five bees, and five bees, respectively. In total, I extracted 22 bees; four of these bees were deceased, and one bee was injured during extraction (from a nest of 5 bees), yielding $n=17$ live eastern carpenter bees from the cedar board nesting gallery. (Only one nest of eastern carpenter bees was used for data collection. See **Appendix A.**) A single individual of the genus *Vespula* and a single individual of *Halyomorpha halys* emerged from the nesting gallery during extraction.

In each instance when I found an occupied nest in the nesting gallery, I proceeded with extraction. I tapped the cedar board with my hand until a single bee slid out of its nest and into the exposed part of the tunnel. I then picked up the carpenter bee with tweezers and placed the carpenter inside of a mesh bag. Once all carpenter bees were extracted from a nest, I zipped the

mesh bag shut and placed the bag inside of a residential refrigerator with an air temperature of about 3.3°C (Fig. 7).

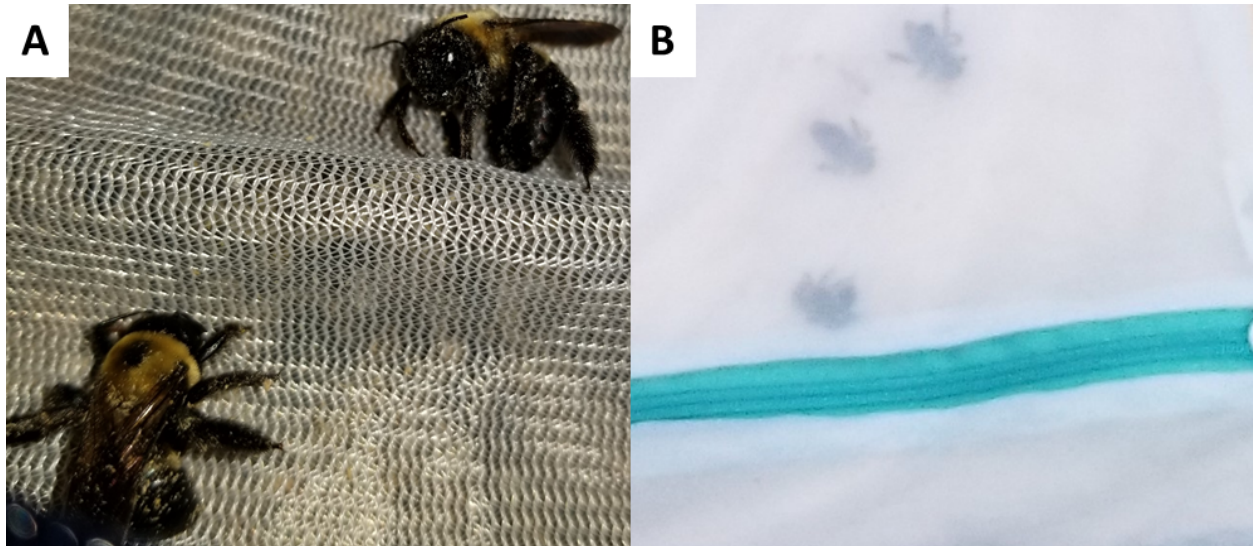


Figure 7. Eastern carpenter bees inside of a mesh bag. Bees were stored in mesh bags (one nest of bees per bag) until translocation into the artificial nests. A) Eastern carpenter bees within a mesh bag. B) View of eastern carpenter bees within a closed mesh bag.

Refrigerating the bees prevented them from reanimating from hibernation. To avoid dehydration in the refrigerator, I placed wet paper towels over and underneath each mesh bag. I continued exposing carpenter bee tunnels, extracting bees from nests, and refrigerating them until all tunnels were exposed. Once all bees were extracted, I placed one nest of bees into each artificial nesting gallery. Carpenter bees were translocated into the artificial nests in the same order of bee extraction (Fig. 8).

After the artificial nest was occupied with eastern carpenter bees, I moved the artificial nest outside. I sealed the exterior edge of the artificial nest with a clear kitchen and bath Advanced Silicone (General Electric Company). The silicone prevents water from entering the artificial nest between the pine board, plexiglass, and plywood layers. I hammered two L-shaped metal brackets flat and attached them to the perpendicular plywood on the artificial nest. I screwed these brackets to the top of a wooden post (Fig. 9). Once suspended upon the post, the

artificial nest was approximately 1.1 m above the ground (measured from the ground to the entry tunnel). I covered the entry tunnel with a piece of mesh cloth for a one week. This served as an acclimation period for the carpenter bees after moving the artificial nest outside. After the week elapsed, I uncovered the entry tunnel and the eastern carpenter bees were free to leave the artificial nest.



Figure 8. Translocation of *X. virginica* bees from their natural nesting gallery to an artificial nest. A) Nominal 2x6 cedar board from which eastern carpenter bees were extracted. B) Artificial nest tunnel. This artificial nest design follows that used by Vickruck & Richards (2017B). C) One nest of *X. virginica* bees were placed into the artificial nest. Bees were placed into the nest in the same order from which they were extracted (From right to left - first extracted to last extracted).



Figure 9. Assembled artificial nest. A) Side view of the artificial nest. B) Artificial nest from below. The hole drilled into the nominal 2x6 board serves as the entry and exit tunnel for the artificial nest. C) View of the three artificial nests constructed for this research.

Data Collection Instrument Assembly

Device operation, data collection, and data storage were managed with a Raspberry Pi Zero WH (Raspberry Pi Foundation). The Raspberry Pi Zero WH (referred to hereafter as Raspberry Pi) is the pre-soldered version of the Zero W model, which can make wireless local area network (LAN) and Bluetooth connections. This Raspberry Pi includes a camera serial interface (CSI), a mini HDMI output port, a mini-USB power port, one additional micro-USB port for connecting other electronics to the Raspberry Pi, one microSD card port, and a forty pin GPIO header. The entire board itself measures 65 mm x 30 mm x 5 mm, which makes the Raspberry Pi convenient for transportation and easily implementable into larger electronic apparatuses. I used the Raspbian Lite operating system for the Raspberry Pi. Programming for device operations is contained within the Raspbian Lite operating system configuration files, and is contained within Python Programming Language Script (.py) files.

The Raspbian Lite operating system, plus all programming for instrument operation, and data were stored on a SanDisk 1 TB microSDXC card

(<https://shop.westerndigital.com/products/memory-cards/sandisk-extreme-uhs-i-microsd#SDSQXA1-1T00-AN6MA>). The Raspberry Pi's data storage capacity is limited to available microSDXC card memory. I stored data locally on the microSDXC card and remotely in a shared folder via a computer network (See *Data Collection* below).

For vibroacoustic data collection, I made use of the Raspberry Pi's micro-USB port. Two transducers were attached to the exterior of an artificial nest. With an interconnecting 0.25 inch to Radio Corporation of America (RCA) male jack adapter cable, two audio adapters, and a USB to microUSB adapter, I connected the transducers to the Raspberry Pi. The RCA adaptor cable has a 0.25 inch male plug and an RCA male plug on either end. The Sound eXchange (SoX) open source software (<http://sox.sourceforge.net/sox.html>) was used to manage the vibroacoustic data. I downloaded the SoX software from GitHub (<https://github.com/>).

For visual data collection, an Arducam OV5647 5MP 1080P Day-Night Vision camera module (referred to hereafter as camera module) was connected to the Raspberry Pi. The Arducam Day-Night Vision camera module (<https://www.arducam.com/product/arducam-m12-night-vision-ir-cut-raspberry-pi-camera/>) consists of one camera lens, plus three infrared photoresistors. The photoresistors automatically switch the camera to record infrared light at night. The camera module was connected to the Raspberry Pi CSI port with a mini ribbon camera cable. I attached the camera module to the lid of the data collection instrument enclosure with nylon screws. Attaching the camera module to the lid eliminates video distortion from reflective glare on the enclosure lid.

The open-source software motion was used as the basis of the Raspberry Pi's camera module programming (<https://motion-project.github.io/>). I changed the motion configuration file to detect changes in pixels as movement. I also permitted pre-capture and post-capture recording functions (See *Data Collection* below).

A PiJuice HAT Portable Power uninterruptible power supply (UPS), a 1,800 mAh lithium polymer battery, and the mains power supply powered the data collection instrument. With the mains power supply, data collection continued indefinitely, unless a power outage occurred. The UPS (<https://uk.pi-supply.com/products/pijuice-standard>) regulated current flow throughout the data collection instrument. I mounted the battery on top of the UPS board. The UPS board sent and received operational signals via the Raspberry Pi's GPIO pins. In the event of a mains power outage, the UPS board switches the device to battery power. Data collection would therefore continue, even in the event of a power outage.

The daily air temperature was recorded with a DS18B20 thermometer (<https://www.adafruit.com/product/381>). I attached the thermometer to the GPIO header on the UPS. The thermometer makes use of four GPIO pins: two 5 V power pins, one ground pin, and one GPCLK0 data pin. I connected the data wire to 5 V power with a 10,000 ohm resistor, which regulates electrical current flow through the data wire¹.

The assembled instrument was housed inside of a NEMA 4X rated enclosure (**Fig. 10**). This enclosure (https://www.budind.com/product/nema-ip-rated-boxes/pts-series-fiberglass-box/pts-25328-c/#group=series-products&external_dimensions_group=0&internal_dimensions=0&cover_style_group=0) protected the instrument from dust and water damage, which increases its field usability

¹ The thermometer does not require two 5 V pins. I directed the data pin's resistor to a second 5 V pin because this arrangement simplified the thermometer's connections to the GPIO pins. The thermometer will also work with both the data wire (via the resistor) and the red power wire soldered to the same 5 V power pin.

and longevity. The cover of the enclosure is transparent, to allow for video recording. The walls and base of the enclosure are opaque. I cut holes into one side of the enclosure with a Rotozip saw for the audio cable, the power cable, and the thermometer. I created a water and dust tight seal around the cables and the thermometer with hot glue.

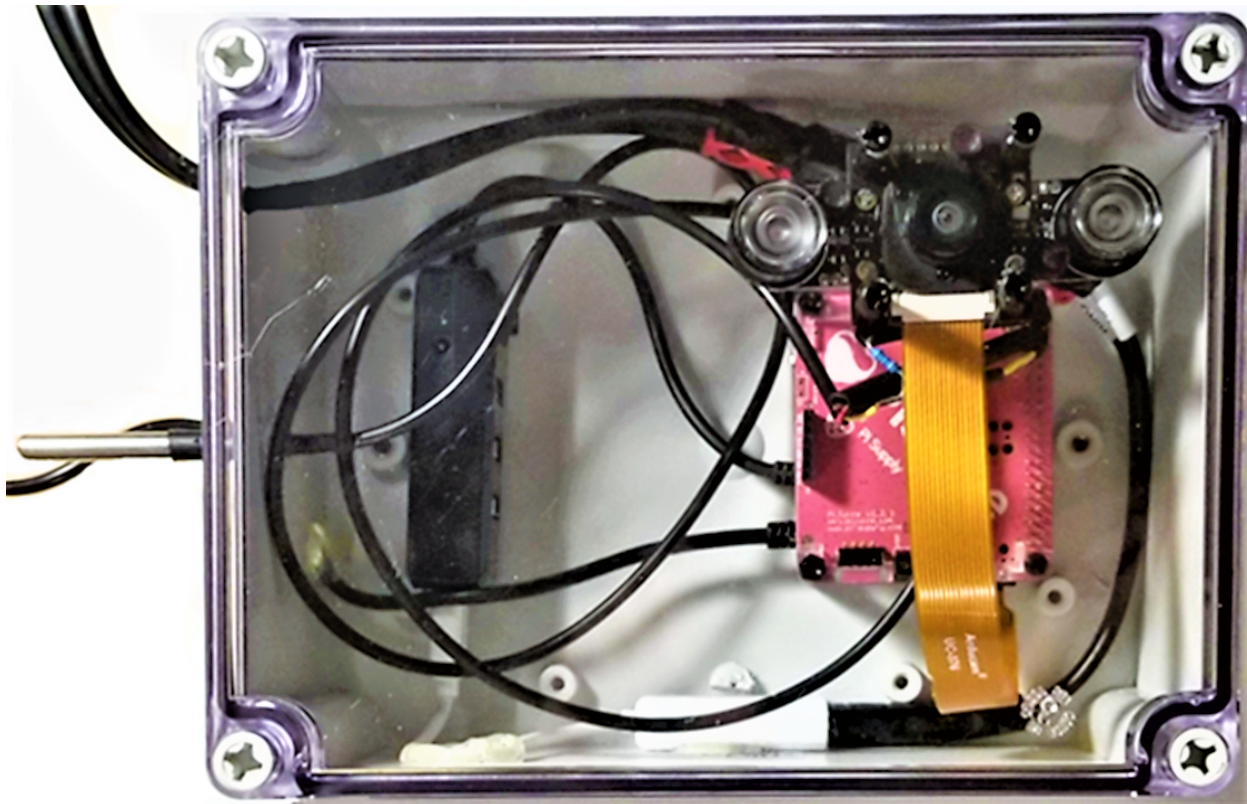


Figure 10. Fully assembled custom data collection instrument. From left to right: the thermometer (metal rod) extends out of the side of the data collection instrument, as well as the power cord and RCA cable (The transducers (not pictured) are attached to the RCA cable); the audio cable and adapters wrap around the interior perimeter of the enclosure; the thermometer wire is coiled inside of the enclosure and attached to the GPIO header on the UPS. The UPS is attached to the Raspberry Pi (underneath the UPS) via the Raspberry Pi's GPIO header. The camera module is connected to the ribbon camera port on the Raspberry Pi and attached to the enclosure cover with nylon screws.

Data Collection

Currently, data collection is ongoing and will continue past my date of graduation. As aforementioned, eastern carpenter bees have only emerged four times from the artificial nests. The data collection instrument was positioned diagonally below the nest entrance, in an orientation where the camera module faced the gallery entrance. The interconnecting cable used

for connecting the transducers to the Raspberry Pi is 2 m long, therefore, the orientation of the data collection instrument was not limited by the transducers being attached to the wood surface outside of the artificial nest.

Bee behaviors are recorded using automated interval sampling methods. All activities performed by the bees (entering and exiting an artificial nest and the sounds they produce within the nest) are recorded by the data collection instrument.

Sticky pads secured the two transducers to the wood outside of the gallery entrance. All sounds produced within the gallery were recorded by the transducers. The Raspberry Pi receives all audio data recordings and saves the audio data in the Wave file (.wav) format.

Every movement detected outside of the gallery entrance is recorded with the camera module. Live footage is temporarily stored in a five second data buffer. Whenever motion is detected via a change in the camera pixels, the buffered footage becomes the beginning five seconds of a saved video clip (the pre-capture footage). The camera module continues recording the movement event. After movement ceases, the subsequent sixty seconds are also recorded (the post-capture footage). The pre-capture, movement event, and post-capture footage are appended together into one video clip. The Raspberry Pi saves each video clip as a single visual datum in the Matroska video file (.mkv) format. The camera records footage constantly, but only these movement-containing video clips are saved by the Raspberry Pi. Recording without saving footage then resumes, until movement is detected again.

Consequently, *X. virginica* activity, nest parasite activity, and nest predator activity will all be recorded by the camera module. I will therefore be able to identify any intruders that the eastern carpenter bees encounter during this study. Although Prager (2008) reported that *X. virginica* bees remain inside of their nests overnight, the camera will be operated overnight to

detect activity from any parasites and predators that may be present (e.g., *Megachile sculpturalis* (Laport & Minckley, 2012; Roulston & Malfi, 2012), or *Xenox tigrinus* (Prager, 2008; Yeates & Greathead, 1997).

For environmental parameters, air temperature and photoperiod data are being collected. The ambient air temperature at which eastern carpenter bees emerge varies by geographic location (Gerling & Hermann, 1978; Skandalis *et al.*, 2011; Rau, 1933). Where temperature constrains activity in *X. virginica*, foraging trips and the number of broods produced annually are also constrained by temperature (Gerling *et al.*, 1989; Course, 2011). Temperature therefore influences behavioral performances in *X. virginica*. The thermometer records the air temperature every thirty minutes. All recordings (in degrees Celsius), plus their date and time stamps, were outputted in a csv file.

To measure the daily photoperiod, I recorded the daily visible light period for Salem, Massachusetts. (Here, photoperiod refers to the time period during which incident sunlight reaches the nesting galleries. This is not the period of time between sunrise and sunset.) Prager (2008) did not observe eastern carpenter bee activity overnight. Photoperiod may therefore influence behavioral performances and circadian activity patterns. Daily visible light data were recorded from Weather Underground (www.wunderground.com).

The data collection instrument stores data in two places. Data are stored locally in a folder dedicated to this research in the Raspberry Pi's directory. Data are also stored remotely in a shared Google Drive network folder. Upon being sent to the shared folder, the data are saved in Google Drive. Storing data in two places reduces the risk of data loss in case of an unexpected data collection instrument failure.

Data Analyses

Once data collection concludes for this breeding season, the following analyses will be undertaken.

Circadian activity patterns will be identified by comparing the date and time stamps on each datum. I will manually search for timestamps that are similar to one another on consecutive days. A daily time period is considered to be a species' typical high activity period whenever many movements or sounds are recorded at approximately the same time across consecutive days. A daily time period is considered to be a species' typical low activity period whenever there are few to no movement or sound recordings at approximately the same time across consecutive days.

The visual and vibroacoustic data together provide insight into the frequencies of behaviors performed at the nest. To determine the frequency of performances for a given behavior, the cumulative performances of each behavior will be manually counted while viewing or listening to each recording. Behaviors will be identified based on the aforementioned published ethograms (see **Fig. 5** and **Fig. 6**), plus published observations in other literature describing eastern carpenter bee behavior. Predator and parasite presence will also be noted.

The approximate sound source location for each vibroacoustic datum will be determined. Sound source localization is a method for identifying the location of a sound's origin, using only the sound itself (Rascon & Meza, 2017). Sound localization requires three dimensions: the azimuth, which is the horizontal plane angle from which the sound is received; the elevation, which indicates the vertical origins of the received sound; and the depth, which is the distance from which the sound originated (Risoud *et al.*, 2018) (**Fig. 11**). Where the transducers were

placed on the exterior wood surface outside of an artificial nest, I will assume the elevation to be zero. (If the female bees bore new nest tunnels within the artificial nest, then I will account for the elevation .)

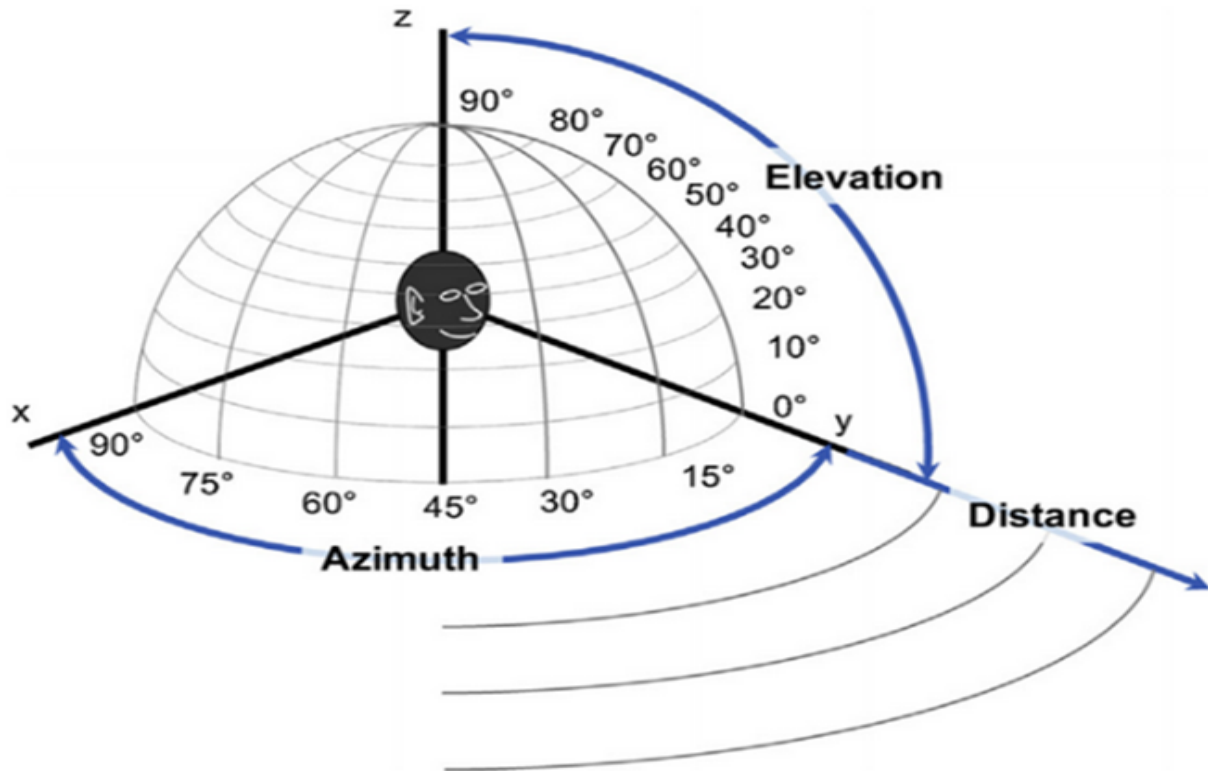


Figure 11. Demonstration of the azimuth, elevation, and distance axes used when localizing sound sources. This figure was published in Risoud *et al.* (2018).

With a minimum array of two audio receivers, the time difference of arrival (TDOA; also referred to as interaural time difference, or ITD) method will be operationalized to estimate the location of a sound's origin (Crocco *et al.*, 2016; Rascon & Meza, 2017). Each transducer has a distinct position in a three-dimensional Cartesian coordinate system (such as the ears on the person do in **Fig. 11**). After a carpenter bee emits a sound, the corresponding sound wave will arrive at each transducer at slightly different times because of their differing positions. This phenomenon is due the sound wave traveling farther to reach the second transducer (Crocco *et al.*, 2016; Rascon & Meza, 2017). Determining this time difference enables sound source

localization. The emitted sound will be captured by the transducer closest to the sound source's location first. A large TDOA suggests that the first transducer to receive the sound is much closer to the sound source's location than the second transducer. A TDOA equal to 0 s suggests that the sound source's location is exactly intermediate to the positions of each transducer. I will determine the TDOA of each vibroacoustic datum by comparing the timestamps on each pair of recordings from the two transducers.

Reflections

Possible Improvements to The Data Collection Instrument

Several hardware changes would improve user friendliness by simplifying the data collection instrument further, without changing the instrument's functionality. Once I attach the camera module to the enclosure cover, there is little range of motion for the enclosure cover. This limits my ability to manipulate hardware inside of the enclosure. The mini ribbon camera cable is 15 cm long, leaving little room for reorienting the enclosure cover during assembly. Due to this, it is easy to damage the ribbon cable ports on both the Raspberry Pi or the camera module.

Longer mini ribbon camera cables are not available for the Raspberry Pi Zero. The cable length could be extended by connecting the mini ribbon camera cable to the Raspberry Pi Zero and attaching it to a standard size ribbon camera cable with an adapter. The Arducam camera module requires a standard size ribbon cable, so an additional adapter and ribbon cable will not affect the choice of a camera module.

A second solution for avoiding instrument damage and increasing cover mobility during assembly is to make use of a smaller enclosure. The current enclosure has an external height of 100 mm (internal height of 80 mm), which provides excess internal volume to house the instrument hardware. I chose this enclosure with the expectation that I would use an audio board

that connected to the Raspberry Pi's GPIO header for vibroacoustic data input. However, after multiple tests, the audio board failed to work. I then changed the instrument's design to receive vibroacoustic data via the mini-USB port on the Raspberry Pi. This arrangement requires less vertical volume than the original design. Unless larger, or more hardware is added to change to the instrument's functionality, then the instrument would be unaffected by switching to a smaller enclosure.

One additional benefit to operationalizing a smaller enclosure is improved portability. I designed the data collection instrument to be tiny and portable, for ease of transport. A small data collection instrument would be more readily deployed at remote sites than a large data collection instrument. Multiple small units are also more easily packed for transport than multiple large units.

During the initial design phase, I planned to power the data collection instrument with a 12 W solar panel and a 10,000 mAh lithium polymer battery. Unfortunately, shipping issues and a lack of communication from the supplier prevented me from obtaining the solar panel. I selected a panel intended for use with the PiJuice UPS to minimize the need for cable adapters in the power hardware design. For the current data collection period, I am powering the instrument primarily with a mains power supply and, in the event of a power outage, there is an 1,800 mAh lithium polymer battery attached to the UPS, which will power the device for several hours and continue data collection.

The solar panel was intended to make the instrument more useful in remote locations. By recharging a 10,000 mAh battery regularly with the solar panel, depending on the battery to operate the device overnight, and programming the UPS to reduce energy consumption whenever data are not actively being collected (e.g., reduce instrument functioning during the night, when

less activity is expected), the instrument would operate without requiring maintenance until the microSDXC card's storage capacity is depleted. This design would make the instrument useful in remote locations that are difficult or costly to reach. This design would also reduce human disturbance to the site because a researcher's presence would not be required to perform regular maintenance on the instrument, such as replacing batteries.

The data collection instrument audio design uses three adapters to connect the interconnecting RCA cables to the Raspberry Pi. The interconnecting RCA cables first connect an RCA male jack to a single 3.5 mm stereo jack adapter. The single 3.5 mm stereo jack connects to an audio to USB male adapter. This adapter is plugged into a USB Mini Hub, which I was able to connect to the Raspberry Pi's micro-USB port. A single RCA to micro-USB male adapter would eliminate the three adapters currently used by the instrument. Simplifying the instrument improves user friendliness, reduces the number of components that can malfunction or break within the instrument, and would allow me to use a smaller enclosure (in terms of length and width), which improves portability (as previously discussed).

Possible Sources of Error in and Possible Improvements to the Vibroacoustic Analysis

Numerous algorithms for calculating a sound source's location have been developed. I selected TDOA based on the method's simplicity, with respect to other methods. There is a tradeoff between the simplicity of TDOA and its limitations. TDOA is more user friendly than other algorithmic methods because it is simpler to understand and apply. Consequently, this method accounts for fewer errors and is more susceptible to localization inaccuracies. To solve these analytical weaknesses, in a future iteration, I may program the instrument to automatically localize sounds using a more complex algorithm from the literature. A discussion of some potential errors follows below.

Background noise may be captured by the transducers (e.g., wind shaking the artificial nest). These noises may interfere with the vibroacoustic data collection. Where I intend to manually identify vibroacoustic sounds by listening to the recordings, decreased audio quality may prevent me from identifying sounds. A potential solution to this problem is training the Raspberry Pi to separate the vibroacoustic data from background noise automatically. Audio surveillance methods have been developed to distinguish between sounds in a noisy environment. Crocco *et al.* (2016) described a four-step workflow for audio analysis. First, background subtraction is performed to extract data containing recordings from non-data containing recordings. I could make use of background subtraction by training the Raspberry Pi to drop any silent periods in recordings (e.g., retain recording segments with audio but drop intermediate segments without audio). With enough practice in identifying background noise versus vibroacoustic data (e.g., identify wind versus buzzing), I could expand the background subtraction to drop silent periods and background noise from recordings.

The second and third steps in Crocco *et al.*'s (2016) workflow are event classification and sound source localization. The goal of event classification is to identify what event corresponds to the audio data. (For example, “does a buzzing sound come from a carpenter bee or from a bumble bee”?) After the source of the sound is known, the source is tracked and its location is identified. With the audio array, a multi-dimensional view of where sound waves originate from can be ascertained.

The final step in Crocco *et al.*'s (2016) workflow is situational analysis. After all sounds in an audio datum have been separated, identified, and localized, the sounds are considered together in an attempt to understand the overall environmental activity being recorded (e.g., a series of buzzes from two distinct but close origins could be an aggressive interaction among

carpenter bees.). Situational analysis therefore provides the greatest insight into the recorded environment. By identifying and localizing vibroacoustic sound sources within a nest, I will gain insight into what behaviors are performed during interactions inside of the nest. Automating background subtraction, event classification, sound source localization, and situational analysis would streamline the vibroacoustic data analyses and eliminate possible errors from background noise. The data collection instrument's user friendliness would also improve because a user would not have to depend on listening to each recording themselves for accurate data analyses.

Environmental parameters influence the speed of a sound wave's travel (Dowling & Sabra, 2015; Rhinehart *et al.*, 2020). For example, the speed of sound differs when air temperature is high versus when air temperature is low because of changes in air compressibility and air pressure (Dowling & Sabra, 2015). Changes in the speed of sound influence the amount of time it takes for a sound wave to travel from its source location to an audio receiver (Rhinehart *et al.*, 2020). Weather conditions will therefore influence the time required for sounds to reach the transducers. If I erroneously conclude that a TDOA is greater because of a bee's location than because of, for example, a temperature change, then I may indicate that a sound producing bee was much farther away from one transducer than was actually the case. Where the artificial nest and transducers are a small study system, I assume the change in speed of sound will be minimal as temperature varies. For this reason, I do not think weather conditions will be a major source of error in this study.

Other characteristics of sound travel and the artificial nest may introduce error into the vibroacoustic analyses. Dowling & Sabra (2015) identified three challenges in sound detection: background noise, reverberation, and unknowns about the sound wave's travel (propagation path and environmental parameters). I expect background noise to be minimal because the transducers

are attached directly to the exterior of the artificial nest. Reverberation is perhaps the largest potential source of error in the vibroacoustic analysis. When sound travels, some of it is reflected off of physical objects (creating an echoic sound effect) and some of it is absorbed by physical objects. The distance over which a sound is propagated increases as the sound reflects off of more physical barriers and travels in a different direction. Assuming the eastern carpenter bees will bore their own nest tunnels inside of the artificial nest, then sound will reflect and reverberate throughout the nest tunnels before reaching the transducers. Sounds will also reflect as they pass through the pine board and into the plexiglass, through the plexiglass to the plywood, and through the plywood and into the transducers. Consequently, the transducers may record sound events equal to the original sound plus reverbs, which would yield much longer, less clear vibroacoustic recordings.

Conclusions

With my custom, open-source data collection instrument, I will elicit behaviors performed by *Xylocopa virginica*. Once data collection and data analyses are complete, I will use this research to produce a more complete record of *X. virginica* ethology. Specifically, I will identify vibroacoustic behaviors produced by this species, I will identify the frequencies of these vibroacoustic behavioral performances, and I will identify the species circadian activity pattern. My data collection instrument is a demonstration of what open-source software and hardware can be used for in behavioral studies. By modifying the software and the hardware, it is possible to repurpose this data collection instrument for studying behaviors performed by other species.

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Appendix

A. Effect of the SARS-CoV-2 on this Research

This research was conducted remotely in Salem, Massachusetts. I transported the eastern carpenter bee infested board from The Durfee Conservatory at UMass Amherst, Amherst, Massachusetts, United States, to Salem, Massachusetts, United States.

Of the four occupied nests within the nesting gallery, three contained live individuals of *X. virginica*. I translocated each nest of eastern carpenter bees into a separate artificial nest. The data collection instrument is designed to collect data on one nest (or nesting gallery) of eastern carpenter bees at a time. Therefore, only one of three artificial nests that I built were included in visual and vibroacoustic data collection.

Due to pandemic-related hardware shortages, one artificial nest was assembled following a different design protocol from that described in the Methodology. Instead of using a single nominal 2x6 pine board, this nest was built with two nominal 1x6 boards that I nailed together and cut to a length of 600 mm. I cut the nest tunnel and the plexiglass to the same dimensions that I cut the other artificial nests (See Methodology). Instead of using plywood to cover the plexiglass, I used a third length of nominal 1x6 board to cover the plexiglass in this artificial nest. Consistent with the other artificial nests, I attached plywood to the top of the artificial nest to shade the nest entrance.

Another consequence of pandemic-related hardware shortages are two different wooden post designs. I created one post by erecting a scrap piece of nominal 2x6 pine board. I created two more posts using a nominal 4x4x8 pine post. I cut the pine post into two lengths that matched the height of the nominal 2x6 post. As described in the Methodology, each nest was mounted approximately 1.1 m above the ground on these posts. For stability, I attached scrap

wood to the bottom of each post. The scrap wood balances the post to prevent the whole structure from tipping over.