Customizable Recorder of Animal Kinesis (CRoAK): A multi-axis instrumented enclosure for measuring animal movements

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A B S T R A C T

Accurately quantifying animal activity and movements is of fundamental importance in a broad range of disciplines, from biomedical research to behavioral ecology. In many instances, it is desirable to measure natural movements in controlled sensory environments in which the animals are not physically or chemically restrained, but their movements are nevertheless constrained to occur within a fixed volume. Here, we describe a novel device to quantify the movements of small animals in response to sensory stimulation. The device consists of an Arduino controlled inertial measurement unit that senses angular velocity (along three axes) of a suspended mesh enclosure that temporarily houses the animal subject. We validated the device by measuring the phonotaxis behavior of gravid female frogs in response to acoustic broadcasts of male mating calls. The system, as designed, proved effective at measuring natural movements made in response to acoustic stimulation.

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Specifications table

| Hardware name | Customizable Recorder of Animal Kinesis (CRoAK) |
|---------------|-----------------------------------------------|
| Subject area  | Biological Sciences                           |
| Hardware type | Measuring physical properties and in-lab sensors |
| Open Source License | CC BY 4.0                                   |
| Cost of Hardware | $270                                           |
| Source File Repository | https://doi.org/10.17632/6wyfr3ct5x.1 |

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1. Hardware in context

Movement is the most prominent output generated by animals in response to sensory input [1]. Therefore, biologists have been quantifying animal movements for a long time and for a wide variety of purposes, such as understanding migration and spatial navigation [2,3], energy expenditure [4,5], disease spread [6], pain assessment [7,8], and motor impairment [9,10], and also to study the preferences of animals in the contexts of habitat selection [11], foraging [12,13], and mating [14,15]. Given the breadth of contexts in which animal movements are quantified, numerous methods have emerged over preceding decades to measure movements. While early methods in the movement ecology of free-ranging animals mostly relied on labor-intensive observational, mark-recapture, and radio-tracking studies [16–18], technological advancements have now shifted the paradigm to the use of more data-driven methods, such as global positioning system (GPS), radiofrequency identification (RFID) chips and tags, and kinemeters (i.e., movement detecting sensors like accelerometers and gyroscopes) [19,20]. These methods have played an instrumental role in providing insights into animal movements across landscapes.

However, quantifying movements is not only desirable in an animal’s natural sensory landscape, but also in laboratory environments where sensory information can be precisely controlled and manipulated. Several behavioral and neurophysiological studies on perception and decision making in animals are in fact conducted in experimental arenas where the response to a stimulus is measured as some function of movement [14,21,22]. In such experiments, since the animals are not moving for large distances, it is convenient to track them either manually or by using automated video tracking software [23,24]. Although tracking freely moving animals as a behavioral assay has proven extremely useful, such studies can be logistically difficult to conduct and scientifically hard to interpret. For instance, many experiments with free moving animals require setting up test arenas or specialized mazes in large, isolated spaces where the sensory information available to animals can be precisely controlled [25,26]. Such requirements can be a significant obstacle for researchers with limited space and funding to perform rigorous experiments. Another major limitation concerning the scientific inference of such studies is that as the animals freely move in a given space, stimulus strength (for example, light intensity, sound amplitude, and odor concentration) changes with distance from the stimulus source, which in turn can influence the behavioral response of animals because of well-known principles of sensory coding [27].

To overcome these limitations, we present the Customizable Recorder of Animal Kinesis (CRoAK), a small, semi-automated, time- and space-efficient device to quantify the movements of small animals within a constrained volume in response to sensory stimulation. Although CRoAK, as described here, was designed to detect the movements of treefrogs in response to acoustic stimuli, the device can be easily customizable to also quantify the movements of other animals, such as laboratory mice and rats, and in response to stimuli in other sensory modalities.

2. Hardware description

Historically, psychoacoustic studies have relied heavily on using conditioning techniques to examine auditory processing in animals [28–30]. However, conditioning has not been very successful in some taxa, including anurans (frogs and toads), which form a large taxonomic group among acoustically communicating animals [31]. In such cases, one common method to study auditory perception is by conducting phonotaxis experiments (phonotaxis: oriented movement) in which researchers exploit the natural tendency of animals to approach behaviorally relevant sounds [30]. In anurans, phonotaxis typically involves walking or hopping toward a sound source. A traditional laboratory setup to perform phonotaxis experiments in a controlled acoustic environment typically includes a large test arena (e.g., 2–3 m on a side or in diameter) built inside a heavy (and expensive) semi-anechoic sound chamber [32–35]. In such a setup, phonotaxis is measured by tracking the movements of an animal toward a speaker broadcasting the acoustic stimulus. Because this behavioral assay involves the active movement of animals inside an arena, in addition to being expensive and space-demanding, it is also a time-intensive method. This is a potential problem, especially when studying auditory perception in anuran species that may only be responsive to natural sounds for short time periods (measured in hours) during their annual breeding season, which itself may last just 1 to 2 days per year in some species [36].

With these factors in mind, we developed a new behavioral assay to measure the phonotaxis behavior of anurans and other small animals. In our new approach, instead of allowing free movement inside a large arena, we constrained the animals to a small enclosure and quantified the movements made in association with phonotaxis using an Inertial Measurement Unit (IMU). The inspiration for using such an approach came from our repeated observations made during traditional phonotaxis experiments that, when frogs are placed in a small cage (9 cm in diameter) before being released into a larger test arena, they move and reorient toward the sound source inside the cage in response to biologically relevant sounds, such as mating calls and chorus noise, but not during periods of silence or in response to biologically irrelevant sounds (e.g., tones or calls of different species). Therefore, in an attempt to measure such movements shown by animals in a restricted space, we
built a new setup in a mini acoustically insulated chamber in which we suspended a small, acoustically transparent animal enclosure. On the bottom of the enclosure, we attached an Arduino controlled 9 Degrees of Freedom (9DoF) IMU that combines three different 3-axis sensors, a gyroscope (angular velocity), an accelerometer (linear acceleration), and a magnetometer (direction of the magnetic field). In the implementation of CRoAK described here, we relied solely on the gyroscope to quantify the motion of the enclosure in response to the movements made in response to a stimulus. We did not use the accelerometer in this implementation because we anchored the suspended enclosure to minimize oscillations in the system after perturbation by an animal’s movement. This means that, by design, we eliminated the possibility of linear translation such that movements would result in angular displacement of the enclosure that could be captured by the gyroscope. Other users may find the accelerometer more useful in customized implementations of CRoAK more suitable to their purpose. We also did not use the magnetometer in our single-speaker setup, but it could be used in multi-speaker implementations of CRoAK to measure perturbations of the local magnetic field that occur when different speakers are being driven, for example to coordinate movements with sounds broadcast from different directions.

Our behavioral assay of measuring animal movements in response to sensory information provides a good alternative to the pre-existing behavioral assays for the following reasons:

- CRoAK is economical and easy to set up in a small space.
- CRoAK can be replicated in parallel to perform multiple experiments simultaneously without much additional cost.
- CRoAK provides control over the perceived amplitude of acoustic signals by constraining animal movement within a desired and specified sound field.
- CRoAK allows dissociation of the processes of stimulus recognition and localization because animals are not allowed to approach a stimulus.

### 3. Design files

#### 3.1. Design files summary

| Design file name | File type | Open source license | Description | Location of the file |
|------------------|-----------|---------------------|-------------|----------------------|
| Layout           | PDF       | CC BY 4.0           | The layout template for cutting the panel out of a piece of plywood. This assumes a 24”x24” (61 x 61 cm) project panel. | https://doi.org/10.17632/6wyfr3ct5x.1 |
| Top View         | PDF       | CC BY 4.0           | A general layout of how the parts on the modular enclosure plate are laid out in plan view. | https://doi.org/10.17632/6wyfr3ct5x.1 |
| Side View        | PDF       | CC BY 4.0           | A general layout of how the parts on the modular enclosure plate are laid out in elevation view. | https://doi.org/10.17632/6wyfr3ct5x.1 |
| Connections      | PDF       | CC BY 4.0           | A wiring diagram for the connections between the Sound Detector and 9DoF boards and the Arduino Nano microcontroller. | https://doi.org/10.17632/6wyfr3ct5x.1 |
| Code             | CPP       | CC BY 4.0           | The source code for the Arduino Nano microcontroller in this system to read sensor data from the 9DoF and Sound Detector | https://doi.org/10.17632/6wyfr3ct5x.1 |
4. Bill of materials

Note: Fig. 1 contains images of the materials used for building CRoAK.

| Designator      | Component                  | Number | Cost per unit - currency | Total cost - currency | Source of materials | Material type |
|-----------------|----------------------------|--------|--------------------------|-----------------------|---------------------|---------------|
| Plastic Canvas  | 12pkx3                     | 1      | $24.95                   | $24.95                | Amazon              | Polymer       |
| 9DoF            | SEN-13944                  | 1      | $13.46                   | $13.46                | SparkFun            | Composite     |
| μC              | NANOX10                    | 1      | $35.99                   | $35.99                | Amazon              | Composite     |
| Sound Detector  | SEN-12642                  | 1      | $10.95                   | $10.95                | SparkFun            | Composite     |
| OpenLog         | DEV-13712                  | 1      | $13.46                   | $13.46                | Amazon              | Composite     |
| μSD Cards       | 8 GB                       | 2      | $7.30                    | $14.60                | Digi-Key            | Composite     |
| Chamber         | 10070158-00419XLF          | 1      | $18.95                   | $18.95                | Digi-Key            | Composite     |
| Connectors      |                            |        |                          |                       |                     |               |
| Plate Connectors| 10070165-00119ALF          | 1      | $1.70                    | $1.70                 | Menards             | Composite     |
| Plywood         | 2’x2’x1/2” (61x61x1.3 cm)  | 1      | $11.59                   | $11.59                | Amazon              | Wood          |
|                |                            |        |                          |                       |                     | (Biopolymer)  |
| Speaker         | TEBM65C20F-8 3–1/2”         | 1      | $14.39                   | $14.39                | Menards             | Composite     |
| Flathead Screws | 1/4”-20x6”                 | 1      | $3.98                    | $3.98                 | Amazon              | Metal         |
| 1/4”-20 Ball    | 2 pk                       | 1      | $8.99                    | $8.99                 | Amazon              | Metal         |
| Mount           |                            |        |                          |                       |                     |               |
| Speaker Wire    | 16 AWG Speaker             | 1      | $9.29                    | $9.29                 | Menards             | Composite     |
| Shelf Bracket   | 6’x8” (15.2x20.3 cm)       | 1      | $0.99                    | $0.99                 | Amazon              | Metal         |
| Thread          | 115 5–310                  | 1      | $1.79                    | $1.79                 | Amazon              | Cotton (Biopolymer) |
| Steel Rod       | 2x150 mm                   | 1      | $7.99                    | $7.99                 | Amazon              | Metal         |
| Spring          | 50456A                     | 1      | $11.96                   | $11.96                | Menards             | Metal         |
| Self-Tapping    | #10x1”                     | 1      | $5.19                    | $5.19                 | Menards             | Metal         |
| Screws          |                            |        |                          |                       |                     |               |
| Wood Screws     | #8x1/2”                    | 1      | $0.92                    | $0.92                 | Menards             | Metal         |
| Webcam          | v2                         | 1      | $19.99                   | $19.99                | Wyze                | Composite     |
| Sprout Lids     | 6pk                        | 1      | $7.99                    | $7.99                 | Amazon              | Polymer       |
| Jumper Wires    | EL-CP-004                  | 1      | $6.98                    | $6.98                 | Amazon              | Composite     |
| Header Pins     | 40pk                       | 1      | $7.99                    | $7.99                 | Amazon              | Composite     |
| Protoboard      | 10pk                       | 1      | $7.86                    | $7.86                 | Amazon              | Composite     |

5. Build instructions

5.1. Tools needed

- Drill or Screw Gun
- Phillips Screwdriver (PH #2)
- Soldering Iron
- Countersink Bit
- Table Saw, Jigsaw, or Circular Saw
- ¼” Drill Bit
- 3/16” Drill Bit
- 13/64” Drill Bit
- ¼” Drill Bit
- ¼”-20 Tap and Handle
- 7/16” Wrench
- Hot Glue Gun
- Superglue
- Needle Nose Pliers
- Wire Cutters
- Wire Stripper
5.2. Base plate construction

The base plate is a removable plywood board to which the enclosure is anchored. It is designed to be detachable so that multiple plates (potentially with different enclosure sizes based on the study system) can be replicated to fit in the system.

1. Cut up the plywood project board using a saw according to the layout file (Layout.pdf).
2. Attach the shelf bracket to the largest panel on the centerline, offset so that the end of the leg on the panel is two inches shy of center. Use the #8 wood screws for this.
3. Drill $\frac{1}{4}$" holes in the places indicated on the top board of the layout.
4. Countersink the $\frac{1}{4}$" holes from underneath so that the $\frac{1}{4}$"-20 flathead screws are flush with the bottom of the board.
5. Enlarge the top hole of the shelf bracket so that one of the $\frac{1}{4}$"-20 screws can pass through it.
6. Attach one of the ¼”-20 screws through the shelf bracket top hole with a ¼”-20 nut, using the screwdriver and the 7/16” wrench.
7. Attach the spring to the center of the steel rod using glue.
8. Attach the opposite end of the spring to the end of the ¼”-20 screw by bending it around the end of the threads.
9. Solder four male-to-male jumper wires to the four contacts of the plate connector. The plate connector is used to connect the sensor to the Arduino Nano via a chamber connector on the docking strip.
10. Glue the plate connector to the edge of the board using superglue on the bottom and hot glue around the back.

5.3. Enclosure construction

The size of the animal enclosure can be customized to take into account the size of the animal to be enclosed. The enclosure described below is an acoustically transparent circular cage (86 × 60 mm, diameter × height). It was specifically designed for use with Cope’s gray treefrogs (Hyla chrysoscelis), which range between 26.15 mm and 47.5 mm in body length (snout-to-vent length) and between 2 and 12 g in body mass (LaBarbera and Bee, unpublished data). In addition, the enclosure was designed to closely match the dimensions of a release cage used successfully in several previous phonotaxis studies conducted with this frog species in our lab [37–41].

1. Cut a 60 mm wide strip off one of the sheets of plastic canvas.
2. Use superglue to glue the 9DoF onto the flat exterior of one of the sprout lids (86 mm in diameter), near its center. In contrast to hot glue, superglue is sufficiently rigid so as to not modify the response of the sensor.
3. Coil the plastic canvas strip inside this sprout lid.
4. Trim the plastic canvas strip so that its ends overlap by one or two columns of squares.
5. Glue the bottom of the plastic canvas strip to the inside of the sprout lid.
6. Solder the jumper wires from the plate connector to the four connections on the 9DoF, making certain to use the two smaller, middle connectors for the SDA and SCL pins, and the two larger, outer connectors for the VCC and GND pins on the 9DoF.
7. Tie and glue four pieces of thread each 15 cm long to the bottom plate of the enclosure, ~1 cm in from the edge at 90° positions to one another.
8. Tie and glue two pieces of thread each 15 cm long to the sides of the plastic canvas, each three rows down from its raw edge.
9. Place the other sprout lid on top of the raw end of the plastic canvas.
10. Wrap the two pieces of thread coming off of the enclosure around the suspended steel rod near its ends, tie them in place so the enclosure is balanced side to side, and glue them with superglue.
11. Pull the pieces of thread coming off of the bottom of the enclosure to one point on the project base plate beneath it so that they are just barely taut, and so that the enclosure can displace up to a maximum of 30°.
12. Tie the threads together and attach them to the plate using superglue (Fig. 2).

5.4. Docking strip construction

1. Wire up a piece of protoboard according to the connections diagram (Fig. 3):
   a. Cut pieces of female header strip to fit each of the two sides of the Arduino Nano (cutting on the middle of the next pin after the one you want to be the last in a row ensures a proper length).
b. Put the pieces of female header strip onto the pins of the Arduino Nano and position it somewhere close to the center of the protoboard.

c. Invert the assembly on a flat surface and solder the female header pins in place in their holes (or vias).

d. Reinvert the board right side up. Cut a 5-pin section of female header and put it in place on the edge of the board, then invert the board to solder it in place.

e. Solder a wire between the GND pin on the Arduino Nano headers and the first pin of the 5-pin header.

f. Solder a wire between the 5 V pin on the Arduino Nano headers and the second pin of the 5-pin header.

g. Solder a wire between the A0 pin on the Arduino Nano headers and the fourth pin of the 5-pin header.

h. Cut a 4-pin piece of male header strip and insert it near the edge of the top of the board with the short side sticking through the vias.

i. Invert the board and solder the 4-pin header row in place.

j. Solder a wire between the GND pin on the Arduino Nano headers and the first pin of the 4-pin header.

k. Solder a wire between the 3.3 V pin on the Arduino Nano headers and the second pin of the 4-pin header.

l. Solder a wire between the A4 pin on the Arduino Nano headers and the third pin of the 4-pin header.

m. Solder a wire between the A5 pin on the Arduino Nano headers and the fourth pin of the 4-pin header.

2. Solder five male pins into the Sound Detector board so that the short side is coming through the top of the board.

3. Solder four male-to-female jumpers to the back of the chamber connector.

4. Attach the chamber connector to the plate connector and push it against the docking strip to mark out where it will be attached.

5. Drill two ½” holes about ¼” deep where the foot pins of the chamber connector will sit on the docking strip.

6. Glue the chamber connector in place with superglue on the bottom and in the pin holes, and with hot glue around and behind it.

7. Drill 3/16” holes in two opposite corners of the protoboard.

8. Attach the protoboard to the docking strip with two wood screws through these holes.

9. Place the Sound Detector board in its 5-pin header with the GND pins in the GND socket, so all pins are lined up (GND to GND, VCC to 3.3 V, and Envelope to A0) (Fig. 4).

10. Plug the female side of the jumpers into the 4-pin male header so that VCC and 5 V are connected, GND and GND are connected, SDA and A4 are connected, and SCL and A5 are connected.

5.5. Speaker attachment

1. Drill out the hole on the back of the speaker with the 13/64” drill bit.

2. Tap the hole in the back of the speaker with the ¼”-20 tap.

3. Screw the ball joint adapter into the hole on the back of the speaker.

4. Attach a length of speaker wire sufficient to reach your amplifier or sound source to the two tabs on the back of the speaker using solder.

5. Screw the ball joint adapter onto the ¼”-20 screw that you want to use as its support (Fig. 5).
5.6. Installation

This installation was performed in a portable acoustic chamber (51 × 61 × 60 cm, length × width × height; Eckel Noise Control Technologies, Cambridge, MA, USA) (Fig. 6).

1. Push the docking strip to the back interior of the chamber and screw it in place using at least two self-tapping screws.
2. Place the two alignment strips along the left and right floor of the chamber and screw them into place using at least two self-tapping screws.
3. Plug a USB A to miniB cord into the Arduino Nano and run it out through the chamber passthrough.
4. Glue acoustic foam on all the walls and on the roof. Also, place some foam pieces on the base plate.
5. Unpack and set up the Wyze Cam, putting in a µSD card before powering it up.
6. Run the power cable for the Wyze Cam out through the chamber passthrough.
7. Place the Wyze Cam on a foam piece such that you can get a good view of the center of the chamber (Fig. 7).
6. Operation instructions

1. Power Arduino Nano by plugging in the USB cable into a computer.
2. Connect the amplifier to the computer.
3. Load the stimulus for playback. We used Adobe Audition 3.0 (Adobe Systems Inc., San Jose, CA, USA) to broadcast sounds but other free software, such as Audacity can be easily used to present the desired stimuli.
4. Upload the .ino code onto the Arduino Nano.
5. Open SerialPlot (an open-source software from Hackaday developers) and set input to the USB channel that is connected to the Arduino Nano. Click on Data Format and set the number of channels. Click on Plot and select the measurements that you want to visualize on the monitor.
6. Take out the base plate and put the animal in the enclosure.
7. Connect the plate connector on the base plate to the chamber connector on the docking strip.
8. Open a new SerialPlot file and give it a desired name to start recording data into a file.
9. Start the experiment.
10. Click on Pause to stop recording data.
11. Monitor the experiment using the Wyze Cam.

7. Validation and characterization

7.1. Characterization of the sound field

We characterized the sound field inside the enclosure by recording white noise from five different locations inside the enclosure. One recording was made from the center of the enclosure. Two recordings were made along the imaginary axis spanning the 9-cm distance between the speaker and enclosure, one from the edge of the enclosure closest to the speaker (i.e., the front) and the second from the edge furthest from the speaker (i.e., the back). Two additional recordings were made from the edges (i.e., the sides) of the enclosure along the imaginary perpendicular axis running through center of the enclosure. The noise was broadcast at a sound pressure level of 85 dB SPL (re 20 \text{mPa}, fast, C-weighted), and recordings were made using a Sennheiser ME66 microphone (Sennheiser USA, Old Lyme, CT, USA) and Marantz PMD 620 recorder (Marantz Professional, Cumberland, RI, USA) (44.1 kHz sampling rate, 16-bit resolution). Over the frequency range spanning the spectral components of our stimulus (1.2 to 1.3 kHz and 2.4 to 2.6 kHz) the frequency responses measured at the five locations inside the enclosure ranged between ± 0.7 dB and ± 2.4 dB. Relative to the center of the enclosure, SPL varied by ± 1.5 dB along the axis between the enclosure and the speaker and ± 0.5 dB along the perpendicular axis. This range of variation in sound pressure level is substantially lower than the variation that animals can experience in an open arena in more traditional phonotaxis tests [42]. Due to expected variation across different physical implementations of CRoAK, we encourage users to characterize their own sound fields to ensure the fidelity required for experimentation.

7.2. Sensitivity of the system

Our method of quantifying movements using CRoAK inherently depends on the sensitivity of the gyroscope to detect angular displacement of the enclosure. We characterized the sensitivity of our system by calculating the minimum deflection (in mm) of the edge of the enclosure that can be measured by the gyroscope when placed at the center of the bottom of a circular enclosure using the equation:

$$\text{deflection} = \left(\frac{d}{2}\right) \times \sin\theta$$

where $d$ is the diameter of the bottom of the enclosure in mm (86 mm in our case) and $\theta$ is the minimum angular displacement of the enclosure that can be measured by the gyroscope (which we calculated using the known sensitivity of the gyroscope). According to the manufacturer’s specifications, the sensitivity of the gyroscope is 0.07 dps/LSB (degrees per s/least significant bit), which means that a movement of 0.07 degrees per second is required to change the output of the gyroscope by 1 count. Hence, at a sampling rate of 50 samples per second, the minimum angular displacement measured in a single sample is 0.0014°. Using the equation given above, we calculated that our system was sensitive enough to measure deflections as small as 0.001 mm at each sampling point. The maximum deflection that could be measured in a single sample was 21.5 mm because we designed the system to have a maximum angular displacement of 30° from the enclosure’s resting position. Hence, the system proved to be quite sensitive for gray treefrogs, which were easily able to deflect the edge of the enclosure by at least 0.001 mm each time they moved.

7.3. Validation experiment

(a) Aim

We validated CRoAK by measuring the movements of six gravid, wild-caught females of Cope’s gray treefrog, a well-established model system in the field of animal communication [43,44], in response to broadcasts of their species specific mating call. Gravid female frogs – i.e., females in which egg laying is imminent – are highly motivated to respond with phonotaxis toward sources of mating signals because finding a mate is imperative. Failure to find a mate risks losing a clutch of eggs, which they only produce once or perhaps twice a year. In traditional phonotaxis experiments, gray treefrog females show a very well-defined motor pattern in response to repeated broadcasts of synthetic mating calls. They move towards the speaker by walking or hopping for 1–2 s immediately after playback of a call and remain mostly still for the rest of the silent interval between consecutive calls [45]. However, they do not necessarily move after playback of each call. Therefore, we
used this well-defined motor activity shown by gray treefrogs in traditional phonotaxis experiments to validate CRoAK. Our expectation was that females would exhibit measurable movements associated with phonotaxis inside the enclosure only in response to broadcasts of mating calls. We regarded these movements as indicative of phonotaxis behavior – and not a startle response or reflex [31] – due to the robust nature of the behavior in gravid females in response to mating signals presented during their mating season.

(b) Procedure

Subjects were collected from local wetlands (Carver and Ramsey Counties, MN) and handled prior to testing following previously published protocols [40–44]. The acoustic stimulus consisted of a series of 10 synthetic mating calls broadcast at a rate of 6 calls/min, which approximates the call rate of a naturally calling male. Each call was modeled after the average call from local populations and had a pulsatile structure consisting of pulses produced at a rate of 50 pulses/s, with each pulse having a bimodal frequency spectrum with frequencies (and relative amplitudes) of 1.25 kHz (-11 dB) an 2.5 kHz (0 dB) (see [46] for additional details on the stimulus). Prior to commencing validation experiments, each subject’s behavioral responsiveness was confirmed in a standard phonotaxis test in which the stimulus was broadcast from a speaker and females were allowed to freely move in a 2-m diameter test arena [40–44]. The stimulus was delivered from a Dell OptiPlex 5060 (Dell Computer Corporation, Round Rock, TX, USA) using Adobe Audition 3.0 (Adobe Inc, San Jose, CA, USA) and amplified using Crown XLS 1502 amplifiers (Harman Professional, Northridge, CA, USA). The calls were calibrated before the start of each experiment to a sound pressure level of 85 dB (SPL re 20 μPa, C-weighted, LCF) using a Larson Davis System 824 sound level meter (Larson Davis, Depew, NY, USA). We chose this sound pressure level because it approximates the received level of a calling male located at a distance of 1–2 m away [47]. For calibration, the microphone of the sound level meter was pointed directly at the speaker at approximately the same height of subject at the center of the enclosure. All experiments were conducted in the dark and were monitored in real time using the night vision function of the Wyze camera. The camera allowed us to visualize the motion of the enclosure and to verify that the gyroscope was able to capture this motion. However, one thing to note is that we were not able to monitor every movement of the frogs because the mesh enclosure was not adequately visually transparent.

At the start of each experiment, a subject was placed in the enclosure and allowed to acclimate for at least a minute. During this time, a new file in SerialPlot was opened to record data from the 9DoF IMU and the sound detector, after which the acoustic stimulus was delivered. Each stimulus started with a 30-s silent interval that allowed us to measure the subject’s baseline activity and estimate the noise floor of the recording system. This 30-s silent interval was followed by a stimulus sequence consisting of 10 repetitions of the synthetic call. Overall, each experiment lasted for 130 s. Over the duration of an experiment, the IMU recorded (~50 samples per s) the angular velocity, linear acceleration, and direction of the magnetic field in three axes (x, y, and z) each, which created a total of nine measurements at each sampling point. Simultaneously, the sound detector recorded the magnitude of the sound envelope, allowing us to monitor the onsets and offsets of each stimulus call. From these raw measurements, movement at each sampling point was quantified by calculating the magnitude of angular velocity \( \omega = \sqrt{\omega_x^2 + \omega_y^2 + \omega_z^2} \). Recall that we used measurements obtained from just the gyroscope because only rotational motion was possible in the described implementation of CRoAK.

For each female (n = 6), time-series data of sound envelope and angular velocity magnitude were plotted to visualize movements in relation to the presentation of individual calls. Each frog’s average movement in response to mating calls was quantified by first computing the mean angular velocity over all samples constituting the first 1 s following each stimulus offset, and then averaging these mean values over all 10 stimulus presentations. For comparison, the movement of frogs in response to calls was then assessed in relation to the average movement of the frogs during the 1 s immediately preceding each stimulus onset and during the initial 30 s silent interval (i.e., baseline activity).

(c) Results and Discussion

Five of the six subjects exhibited movements consistent with those expected for phonotaxis behavior in response to the species-specific mating call; the sixth subject largely showed indiscriminatory movements throughout the experiment but is not excluded from results of the validation experiment. In Fig. 8A, we depict that the overall mean (±95% CI) angular velocity (n = 6) immediately following a call (6.21 ± 2.26 deg/s) was higher than both the mean angular velocity measured immediately preceding a call (2.59 ± 0.39 deg/s) and during the initial 30-s silent interval (2.13 ± 0.89 deg/s). Results from paired t-test confirmed that there were significant differences between the angular velocity measurements taken 1-s immediately after each call and those taken 1-s preceding each call (t = -3.47, df = 5, P = 0.018) and also between measurements taken 1-s after each call and the during the 30-s preceding call sequence (t = -4.50, df = 5, P = 0.006). We also found that the angular velocity measured 1-s preceding each call was not statistically different than the angular velocity measured during the initial 30-s silent interval (t = -0.959, df = 5, P = 0.382), suggesting that the oscillations of the enclosure reached baseline level of motion before the presentation of each call. (Note that because measurements from each time point were used in two statistical comparisons, we use a significance criterion of \( \alpha = 0.025 \) to control for multiple comparisons.)

Visual inspection of the time-series data revealed a strong correspondence between the timing of the sound envelope and changes in angular velocity. In Fig. 8B, we present an example of time-series data from one subject, which shows that angular velocity increased rapidly immediately after a call and declined before the onset of the next call (see inset in Fig. 8B). It was often the case that several distinct movements were recorded shortly after one stimulus and before the next presentation of the stimulus (see multiple spikes in movement trace between stimuli in the inset of Fig. 8B). Such movements are indicative of attempted locomotion events (e.g., hops) and were unlikely due to the occurrence of multiple, repeated startle responses...
or reflex movements occurring over the course of 10 s between acoustic stimuli. In other experiments using CRoAK, gravid females do not exhibit similar movements in response to broadcasts of the mating calls of a different species (Gupta, Marchetto, and Bee unpublished data).

In general, the five frogs that showed phonotaxis behavior tended to move immediately after the playback of a mating call and to stop moving soon afterwards until they heard a subsequent call, although not necessarily the next call. This trend is illustrated in the schematic in Fig. 9, which compares the relative timing of calls (shaded bars) and movement (‘M’) measured using CRoAK (Fig. 9A) and in a more traditional phonotaxis experiment (Fig. 9B). Both types of assay capture the tendency of female frogs to exhibit phonotaxis shortly after perceiving a mating call, as previously described [45].
Results from this validation study confirm that movements associated with phonotaxis behavior were quantifiable using CRoAK and distinguishable from the baseline motion of the system. Overall, we believe CRoAK can provide a useful and potentially less expensive alternative to the predominant methods currently used to quantify behavioral movements made in response to sensory stimuli. We hope that this low-cost instrument will be more accessible to researchers and will prove to be especially useful in undergraduate teaching laboratories.

8. Capabilities and limitations

The hardware has the following capabilities and limitations:

- The system is highly customizable. The size of the acoustic chamber, distance of speaker from the enclosure, type of sensor, and the size of the enclosure are all customizable according to the needs of the researcher and suitability of the study system.
- While this study used movements associated with phonotaxis as a behavioral assay, other types of acoustic stimuli (e.g., loud tones or noise bursts) might also be used to elicit startle responses or reflex movements that could inform the generation of behavioral audiograms.
- Even though the sensitivity of our system is very high, detection thresholds of the measurements are limited by the background noise of the sensor as deployed in the entire system. Therefore, movement of the enclosure in response to a stimulus needs to be interpreted in relation to the baseline motion of the enclosure in the absence of a stimulus.
- The system as designed here should not be used for low-frequency sounds with long wavelengths because of the potential for impacts of near-field effects. The impacts could be mitigated by increasing the distance between the speaker and the enclosure.
- The enclosure was not adequately visually transparent to allow the webcam to be used to assess the correspondence between the actual movements of the animal and movements of the enclosure as measured by the sensor. A larger grid spacing in the materials used to construct the encloser could mitigate this limitation.

9. Human and animal rights

All work complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee (#1701-34456A, approved March 3, 2017).
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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