



UNIVERSITY OF MINNESOTA

Xu Lab

Department of Ecology, Evolution and Behavior
University of Minnesota, 2022
Laboratory Manual

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I. Introduction to This Manual (coming soon)

II. Lab Health & Safety

In the Xu lab, we are dedicated to ensuring the health and safety of all members. Here you will find information regarding a variety of steps all lab members are expected to take to reduce the risk of harm while working.

a) Protecting Yourself and Others From Mold and Spores

Many of the crickets studied in the Xu lab come from moist forest in Hawaii where humidity is high and mold is part of their natural habitat. In our lab, crickets are reared in containers with very high humidity inside. Such damp conditions lead to mold growth. We recommend the following safety precautions to protect you from inhaling spores or come in direct contact with mold on bare skin.

Personal Protective Equipment (PPE): To reduce the likelihood of inhalation and irritation when opening cups, jars, or bins, all lab members should:

1. Wear a disposable face mask to reduce the risk of inhalation (provided in lab).
2. Wear gloves to reduce the possibility of skin irritation (provided in lab).
3. Wear a lab coat to reduce the possibility of carrying spores with you when you leave the lab (provided in lab).

Used cups and lids: To reduce the likelihood of inhalation derived from used cups and lids awaiting washing, all lab members should:

1. Make certain that the tub containing the used plastic containers and lids is covered whenever it is not in use.

Trash can: To reduce the likelihood of inhalation derived from moldy food and paper in the trash awaiting disposal, all lab members should:

1. Make certain that the lid on the trash can in the cricket room stays closed.
2. When the trash can is nearing full, dispose of it according to section IV.c (waste disposal). If the trash is not yet full but the smell is irritating to you, feel free to dispose of it at any time. Make certain you replace it with a fresh bag.

HEPA air purifier: The cricket room has an anti-mold air purifier with HEPA filtration. The air purifier should run at all times. Do not turn it off. If you notice that it is not running, please notify lab manager Susan Balenger ASAP.

Lab floor: To reduce the likelihood of escaped mold spores persisting on the floor, all lab members should:

1. Sweep the floor of the cricket room before leaving if they have opened moldy containers.

Open communication: If you have concerns about the work environment or have health issues that may prevent you from working in the rearing room, Mingzi Xu (PI) and Susan Balenger (Lab Manager) are here to listen. We strive to do everything within our ability to offer you a safe work environment.

III. Incubators & Permits

Research crickets are housed in temperature and light controlled incubators. These look like large white refrigerators, but are maintained at a constant 20°C temperature with a 12:12 light dark cycle. Any time you are passing the incubators, it is always a good idea just to check that the temperature is $20 \pm 0.5^\circ\text{C}$ and that the light is on in the day and off in the night. (Temperature given on a digital display at the top of the incubator - see photo with yellow arrow.) If you are running an experiment, it is even more critical that someone check them each day.



Also of note, there should always be a current version of the lab's USDA permit posted to the front of the incubator. This permit must be displayed to prove that the crickets were imported from Hawaii legally.



Furthermore, every lab member must read and initial the last page of the USDA permit before they can work with Laupala crickets.

IV. Lab Hygiene & Maintenance

a) Setting Up the Workspace

Cricket collected from the field can carry a variety of pathogens. If not careful, it can easily wipe out a colony or the whole cricket stock. Therefore, it is important to ensure that the workspace is clean to prevent the spread of any pathogen from one cricket to another.

To clean the working surfaces, follow the steps below:

1. Spray the entire surface with 10% bleach solution (1 part bleach + 9 part tap water) spray bottle to disperse an even amount to disinfect the entire table. Wipe until dry with a paper towel.
 - A lab coat is recommended if bleaching your clothes is a concern.
2. Spray the surface with water and wipe dry to reduce the chance of creating dangerous fumes.
3. Spray the entire surface with 70% ethanol solution (7 part ethanol + 3 part tap water). Let the solution sit on the surface for 10 s, then wipe the surface dry with a paper towel.
 - Both bleach and ethanol can disinfect, but bleach itself is very harmful for crickets. Ethanol can help clean bleach away. So it is important to follow exactly this sequence.
4. As an added precaution, clean the surface with water to wipe any residual ethanol away.
 - As **bleach** and **ethanol** are harmful for crickets and **water** is safe, bleach and ethanol are stored in **RED** spray bottles and water is in **BLUE** bottles. Before you spray anything, always check the label on the bottle and the color of the bottle.



b) Cleaning Up the Workspace After You Are Done

Once the day's tasks are completed, disinfect the workspace once again with step 1-3 above.

c) Waste Disposal

Check the trash bag before you leave. If it is full or smells bad or you suspect it could contain any eggs, you must dispose of it. Proper disposal is simple:

1. Tie trash bag shut.
2. Label a piece of lab tape with the date and your initials. Stick tape to the bag in a visible location.
3. Put in a freezer (until the lab remodel is finished, we are sharing Dr. Zuk's freezer in 330) for at least 3 days.
4. After 3 days the bag can be thrown out with the regular garbage.

d) Sanitizing Cups

Cricket cups need to be switched out every few weeks as they get dirty. The cups from previous generations are recycled and need to be cleaned manually.

1. **Soak the cups.** Place dirty cups into the sink. Run hot water and add 3 small cups of bleach (need clarification on specific amount). Ensure that all cups are fully submerged. Let soak for 3-5 days to sanitize.
2. **Scrub the cups.** Once the cups have finished soaking, use a few drops of dish soap and the appropriate brush to scrub and rinse each cup and lid thoroughly. Place in a clean bin.
3. **Rinse the cups.** Once all of the cups have been scrubbed and rinsed, everything should be rinsed thoroughly one or two additional times to ensure that all of the bleach and dish soap has been rinsed thoroughly. Allow to dry completely before use.

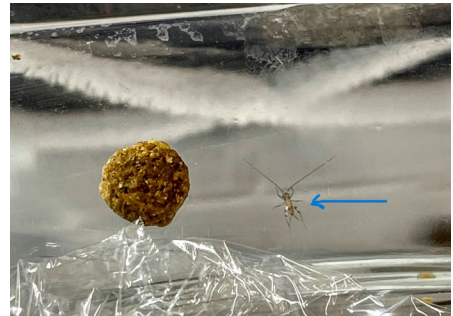


V. Cricket Husbandry & Care

a) Preparing Cups for Crickets

To prepare cups for cricket habitation, KimTech wipes, and clean cups with lids are needed.

1. **Burn holes in the cups.** Burn two holes in each cup alongside the translucent rectangle. The size of the hole is important. Too small and the cricket will not get enough fresh oxygen. Too large and the nymphs will be able to escape. For reference, the image to the right shows a piece of cat food compared to a cricket nymph.
2. **Prepare wipes.** Fold the KimTech wipe into quarters. Evenly moisturize the paper so that it is saturated with water but not dripping. Place on the opaque rectangle inside the wall of the cup. The paper should fully stick to the wall and be translucent when held up to the light.
3. **Assemble the cups.** Once the wipes have been folded and properly moisturized and the cups have appropriate hole sizes, it's time to put it all together. Place one wipe in each cup with the wipe covering the translucent rectangle. This allows for better visibility of the cup interior. Ensure that the wipe is not covering either of the two holes. Add a clean blue lid.



b) Preparing Jars for Crickets

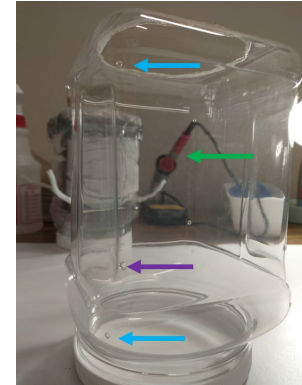
2-4 weeks after hatching, crickets can be moved from cups to medium-sized jars. To construct a jar for cricket habitation, you will need the following:

- ½ gal. jar (ULine part #S-15710), hereafter “medium jar”
- Burning tools (soldering iron & small hole burning tool)
- Kimwipes
- ⅔ of an egg carton torn into 4 squares
- 2 pipe cleaners
- Scissors
- 4” x 4” square of screen mesh
- Duct tape
- Saran wrap

1. **Burn large hole in bottom of medium jar.** Use the soldering iron to burn an opening in the bottom (soon to be the top) of the medium jar. So as not to cut too big an opening, keep in mind that the edges will shrink up a little from the heat.



2. **Burn tiny air holes at all 8 corners of the medium jar.** As shown in the photo to the right, using the tool for burning small holes (green arrow), burn 1-2 small air holes in each of the top and bottom corners of the medium jar (blue arrows). Ensure that they are small enough that juvenile crickets can not escape. Finally, burn 1 slightly larger hole in each corner, ~2.5-3" from the lid. This opening needs to accommodate a pipe cleaner, but still keep crickets from escaping.



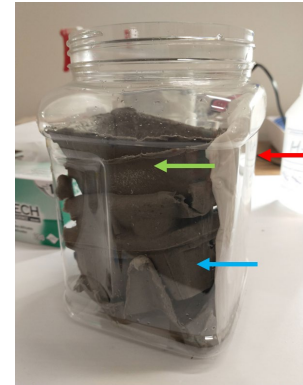
3. **Wipes 1.** Unfold 6-7 Kimwipes and stack on top of one another. Spray both sides liberally with water to saturate. Fold the stack of kimwipes into thirds, then insert it into the jar and stick it to one of the walls. Make sure not to cover any air holes.



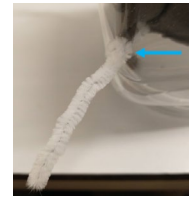
4. **Prepare the egg cartons.** Fill a tub with several inches of water. Tear (or cut with scissors) an egg carton into 6 equally sized pieces (3 top and 3 bottom pieces). You will need at least 4 pieces to put inside the medium jar. Submerge egg carton pieces in water, allowing them to completely saturate. Cartons are ready to come out of the water when they feel soft, but not fall apart.



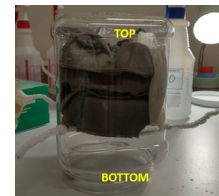
5. **Stack pieces of egg carton inside of jar.** Typically, I (SLB) use 4 pieces - 2 tops and 2 bottoms. Insert the tops first (blue arrow), then the bottoms (green arrow). Check that the kimwipes are still stuck to the wall (red arrow) and not covering the pre-drilled holes.



6. **Pipe cleaners.** Use the slightly larger holes you burn in #2 to string the pipe cleaners diagonally across the interior of the jar. The pipe cleaners are much longer than the diagonal length of the jar, and several inches of wire should extend through each hole to the outside. Tie these off by making a loop as close to the side of the jar as you can.



When you invert the jar - *what was originally the bottom is now the top!*



7. **Cover the opening at the (new) top with a square of screen mesh square.** Cut a square of mesh (approx. 4 x 4" will cover entire top) and place over opening. Use duct tape to tape the edges down, again being certain to avoid covering any air holes you created earlier.

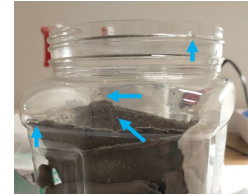


8. **Wipes 2.** Unfold 3 Kimwipes and stack on top of one another. Spray both sides with water to saturate. Fold stack in half, and lay over the screen mesh opening.



9. **Saran wrap 1.** Place saran wrap/clingfilm over damp kimwipes, ensuring a seal on all four sides. This layer is critical for keeping the kimwipes moist. Cut off excess to avoid covering any air holes while ensuring the water is sealed in.

- 10. Wipe droplets from bottom (former lid).** Turn the jar upside down (so that the screw top is facing upwards). Use kimwipes to soak up any water droplets that have accumulated inside the rim and shoulders. Crickets can get trapped in these and will drown/die, so they are critical to remove.



If you are ready to add crickets now, do the following:

- 11. Saran wrap 2.** Remove foam liner from lid and discard. Place a strip of saran wrap across the lid such that it completely covers the lid and some hangs over the edges after pressed into the lid to line it. Add 4-6 pieces of cat kibble.



- 12. Add crickets (See section on Transferring Crickets between containers for details), invert jar, and screw lid securely.** Before inverting the jar, check for crickets near the opening. In one smooth motion, turn the jar over and seat it into the lid. Gently turn the lid until it is snug.

- 13. Label jar.** Use lab tape of appropriate color (colors are matched to populations) to provide the following information:

Pop	Sex	Status
ID	Hatch dates	
n	Date of transfer	

Finally, transfer stickers from all cups to medium jar before returning to incubator.

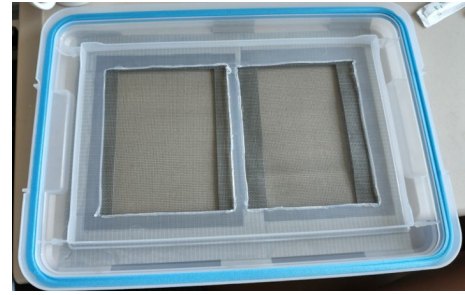
c) Preparing Bins for Cricket Breeding Colonies

Sometimes we will use large bins to house breeding colonies of crickets. To construct a bin for cricket habitation, you will need the following:

- Large bin with snap lid
- Soldering iron
- Scissors
- Duct tape
- 2 soft plastic trays
- 4 egg cartons
- Kimwipes
- Screen mesh
- Saran wrap
- Blue cap
- Plastic sandwich bag
- Pointed probe

- Lab tape

1. **Burn 2 large rectangular holes in the lid of a large bin.** Use the soldering iron to burn these 2 openings in the lid, leaving a ½-1” strip of plastic down the center.



2. **Cover each rectangular opening with screen mesh.** Cut 1 large or 2 smaller pieces of screen mesh such that they completely cover the holes you have just cut in the lid. Seal the sides down with duct tape, and run a piece down the center.



3. **Kimwipes 1.** Make 2 piles of 6-7 unfolded Kimwipes. Spray both sides liberally with water to saturate. Lay the piles flat across the lid so that they cover the screen mesh completely.



4. **Burn a large circular opening in the front of the box** (diameter approx. 3.5”) using the soldering iron. This opening will be your primary access when feeding crickets.



5. **Cut 2 of the soft (peach colored) plastic trays in half.** These trays are a bit longer than the interior width of the box, so they must be cut in half and stacked. One will overlap the other by ~1”. To facilitate movement and airflow, cut out 1-2” square openings from each half.



6. **Invert the trays and stack the 2 halves over one another.** Place them into the bin so they extend across its full width, while still exposing the small square openings you cut towards the interior of the bin. Place one of these stacks at each end of the bin.



The bins have rounded, recessed bottoms. To keep crickets from getting trapped or squashed in water droplets, we fill these spaces with damp kimwipes.

7. **Kimwipes 2.**

- Make 4 crumpled balls of 2 kimwipes each. Spray with tap water so they are fully damp without dripping.
- Make 4 piles of 4 unfolded kimwipes each. Spray with tap water so they are fully damp without dripping. Fold each pile in half and roll each into a tube shape.
- Place the kimwipe tubes and balls into the bin as shown in the photo (note: the balls are in the corners while the tubes will rest of the spaces along the edges), making certain that the sides of the bin are covered with kimwipes or the soft plastic trays.



8. **Prepare the egg cartons.** Fill a tub with several inches of water. You will need 4 complete (top and bottom) egg cartons to put inside a large bin. Submerge egg cartons in the water, allowing them to completely saturate. Cartons are ready to come out of the water when they feel soft, but do not fall apart.



9. **Stack the egg cartons in the bin.** Prior to putting cartons in the bin, open one and gently place it on top of the soft plastic trays already inside. The interior of the egg carton should be facing upwards. The next one should be opened and placed on top of the first, with its exterior side facing up. Repeat with the remaining 2 egg cartons.



10. **Seal the lid.** Place the lid you prepared in steps #1-3 on top of the bin. Do not clamp the latches yet. First, stretch a piece of saran wrap over the top. This will seal in the moisture from the kimwipes. The piece of Saran wrap should be a little longer than the lid, so when you close the latches down, the saran wrap will be tucked under them.

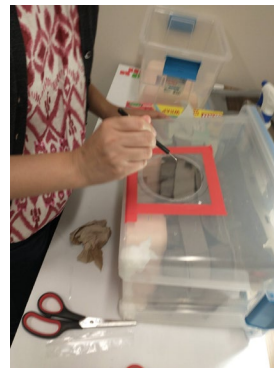
11. **Kimwipes 3.** Reach in through the hole in the front of the bin and wipe any water droplets from the bottom. Young crickets in particular can get stuck/drown in small droplets, so make sure you do this check before adding any crickets to the bin.



12. **Cover the front opening with a sandwich bag and lab tape.** Cut the 4 sides off of a sandwich bag so that it becomes 2 clear plastic squares. Using lab tape, tape 1 plastic square over the front of the access hole.



13. **Air holes.** Create air holes by repeatedly stabbing the plastic square with a pointed dissecting probe.



d) Feeding the Cups

1. **Open the cup.** Firmly tap the bottom of the cup to encourage the cricket to jump off of the bottom of the lid. Carefully unscrew the lid and dispose of the old food.
2. **Feed the cup.** Place two fresh pieces in the lid. Inspect the paper for moisture. If the paper is getting dry, add more water. If it is too wet, wait until the next feeding to reevaluate.
3. **Close the cup.** Nymphs will sometimes hide in the grooves of the cup, leading to their death if sealed with them inside. Ensuring that the cricket is in a safe location inside the cup, screw the lid back on.

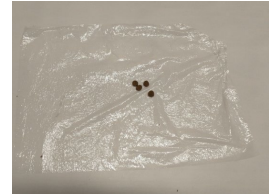


Note: As the cups are cleaned, the crickets should be inspected for any obvious health issues (sitting on the bottom of the cup, not jumping when prompted, lethargic), make sure to note it by sticking a piece of colored tape by the label. Additionally, if

there are any cups that don't have holes burned into them, make sure to switch it out with one that has holes as soon as possible.

e) Feeding the Medium Jars

1. **Prepare the new food.** Before you unscrew the lid of a container with crickets in it, make sure you have the new food ready to go. To do so, place a clean piece of saran wrap flat on the table. Place 3-5 pieces of food onto the plastic.



2. **Opening the jar.** Check the floor (lid) of the medium jar for crickets. If you see any on the floor or near the opening, try to tap them up into the jar. When it is safe, you can unscrew the lid (with lid still facing down), gently pulling on the old saran wrap as you do so. Keep your eyes peeled for crickets while you do this!



3. **Exchange the old and new plastic wrap.** Discard the old saran wrap & food. Quickly slide the new saran wrap + food (from step #1) into the lid, ensuring it covers the bottom and the sides of the lid.



4. **Screw the lid on.** Slide the grooves of the lid into place, and gently turn the lid until it is snug. If you screw too fast or too tightly, you will likely tear the saran wrap which will make changing the food more difficult next time.

5. **Rewet the kimwipe on the top of the inverted container.** The kimwipes lying across the top of the container can dry out with time (even under saran wrap). Thus, it is important to check them regularly. If you find that the kimwipes are dry, rewet them by lifting the saran wrap and spraying them with tap water. Finally, don't forget to re-cover them with saran wrap when you are done.



f) Feeding the Large Bins

1. **Prepare the new food.** Before opening the container, prepare a clean blue lid with ~10 pieces of food.
2. **Open the bin.** Firmly tap the covered opening on the side of the bin to clear any crickets from the area for ease of opening. Peel the cover away from the bin, making sure to keep an eye out for any crickets that may be on the cover.
3. **Feed the bin.** Remove lid, tap to remove any crickets. Dispose of the old food. Place the newly prepared food dish in the center of the bin.
4. **Seal the bin.** Place the covering back on the side of the bin, firmly pressing the tape back onto the surface to ensure that no crickets can escape.
5. **Rewet the kimwipes on top of the bin.** Remove the plastic wrap from the top of the container. Peel back kimwipes, fan to oxygenate and spray with water for humidity as needed. Place the plastic wrap back on top, ensuring that the kimwipes are fully covered to retain moisture.



g) Anesthetizing Crickets

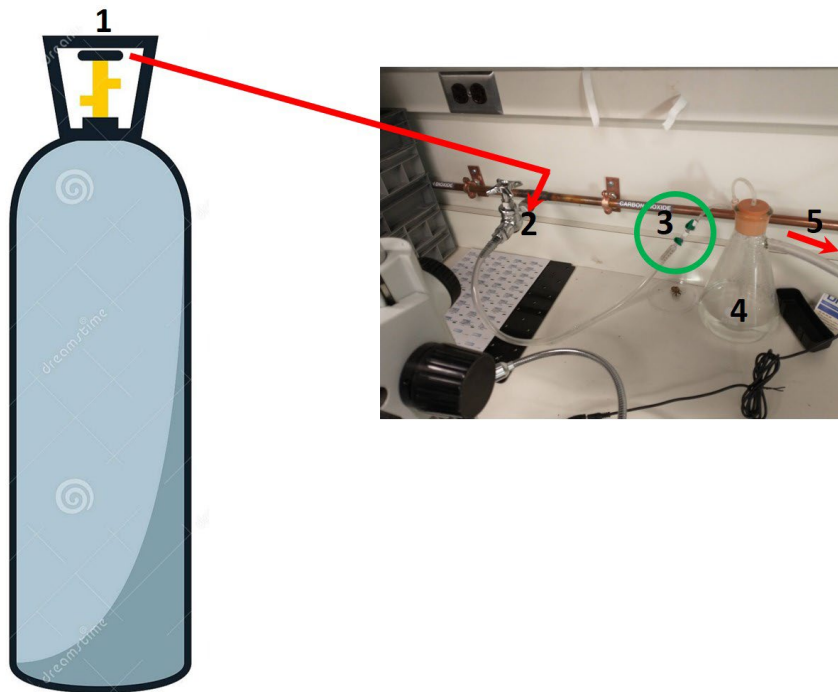
Laupala crickets are somewhat small and very jumpy. This can make it very difficult to handle them when they are awake. Fortunately, we can briefly anesthetize them using CO₂ gas. As of 9/29/2022 we are using gas in the Wardell lab (Ecology 240) because the Xu lab is still under renovation. Basic protocol will likely be very similar to what is described below once the lab is finished.

1. **Get a plastic bag.** Obtain a plastic bag that is large enough to easily fit over the container you currently have the crickets living in. Clear bags are preferred so you can easily see when the crickets are “knocked out”.
2. **Cover the bin with the open plastic bag.** Carefully remove the lid to the plastic jar/bin containing crickets while completely covering the bin with the bag opening.
3. **Transfer bin contents to the plastic bag.** Invert the bin so that the opening is facing the ground, and tap out all of its contents.
4. **Lift bin out of plastic bag.** Once you have ensured that all of the bin contents (including crickets) are in the bag, grasp it tightly below the bin. Slowly lift the bin out of the bag, double checking contents.
5. **Tie bag.** Knot or tie the bag securely. Check the bin to ensure all trash is removed and no crickets remain. Set the dirty bin aside for washing.

6. **Go to Wardell lab.** Take the bag to the Wardell bench along the far wall of Ecology 240. Make sure your ID card is activated for entry to this lab space.
7. **CO2 tanks.** Walk to the farthest end of the lab. You will see a couple of gas tanks attached to the wall.
8. **Pressure gauges.** Check the pressure gauges on the tank closest to the wall to make sure there is pressure (i.e., check that the tank is not empty). You can leave the tanks alone from here on. *If the pressure is very low or even zero, contact Susan Balenger or Mingzi Xu so they can notify the Wardell lab.*



The following is an overview of how the Wardell CO2 system is set up:



Explanation of figure above: The gas cylinder (1) contains pressurized CO₂. The gas is piped to a valve (2) that should be closed unless you are using it. When (2) is opened, Co₂ gas will flow into the plastic tubing. There is a junction (3) between the tubing and a glass flask where 2 green valves are located. The one to the left should be closed unless you are using it. It is closed

when perpendicular to the hose line and open when parallel to the line. After passing through this set of valves, the gas enters the flask containing water (4). This flask acts as a bubbler, which is often used when anesthetizing fruit flies. The crickets do not need this, but it does not negatively impact our ability to anesthetize them. The gas then exits out the flask into the final plastic tube (5), which will be inserted into the plastic bag containing crickets.

- 9. Check hoses and open green valves.** Check to make sure that the plastic hose lines are properly connected and that both of the green valves are open.



- 10. Insert the hose line into the bag,** making sure it is closed tightly around the hose. The picture on the right shows how long the hose coming out of the bubbler will be. As you can see, it is easily long enough to insert several inches into the plastic bag.



- 11. Open the metal CO2 valve attached to the wall.** Now that the hose is safely inserted into the plastic bag, you can slowly open the metal gas valve to allow the CO2 to begin flowing through the line. It will take a moment or 2 for the gas to reach the crickets. First you will notice the water in the flask bubbling. Wait ~2 min with the gas on, all the while checking to see if the crickets have stopped moving. You will know they are anesthetized because they will fall to the bottom of the bag and not attempt to climb up.

- 12.** When you are done, **close the metal CO2 valve attached to the wall *before* removing the hose from the plastic bag.**

- 13. Tie the plastic bag** so no crickets can accidentally escape.

- 14. Close the green valve.** Turn the green plastic valve(s) to close.

- 15. Leave everything exactly as you found it!**

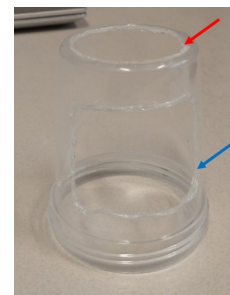
VI. Recording a cricket

a) Preparing Individual Recording Cups

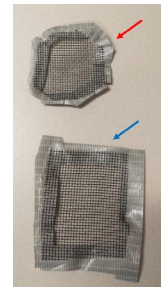
When collecting acoustic recordings of individual captive crickets, cups must be modified to minimize sound resistance. To prepare cups for this purpose, you will need the following:

- Individual cricket cup
- Soldering iron
- Screen mesh
- Duct tape

1. **Burn 2 large holes in the cup.** Remove the blue lid from the cup. Using the soldering iron, burn out a circular hole in the bottom of the cup (red arrow; this will be the top of the inverted cup!), as well as a rectangular opening on the side (blue arrow; approximately 1 x 2" h x w). It is perhaps easiest to follow along the edge of the labeling rectangle on the side of the cup.



2. **Cut screen mesh.** Cut 2 pieces of screen mesh, ensuring they are each just slightly larger than the openings you have cut.



3. **Pre-tape the mesh.** Attach duct tape along the edges of the mesh pieces before inserting them into the cup.

4. **Insert mesh pieces into the cup.** Insert the circular piece of mesh into the cup first. Once you are certain the tape has sealed along the inside of the cup, insert the rectangular piece and tape it to the interior walls of the cup.



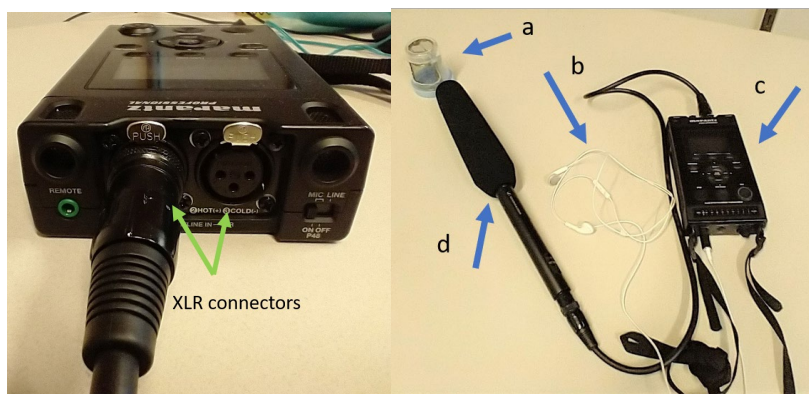
5. **Replace the blue lid and invert.**

b) Preparing audio equipment for recording

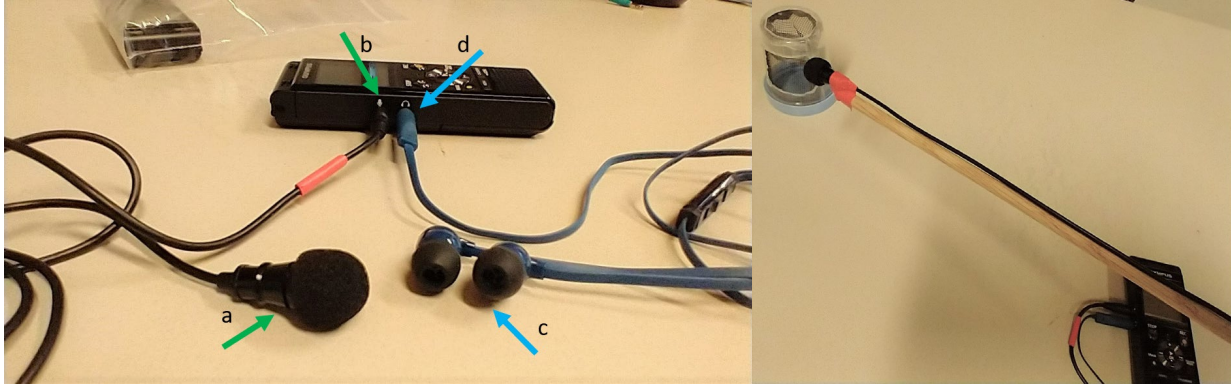
- Sennheiser directional microphone or EnerSound microphone
- Digital audio recorder
- SD card (depending on the audio recorder)
- Ear buds (may need adapter for these depending on audio recorder)

Before actually recording any crickets, make certain that you understand how to use the recording equipment and have all the necessary pieces assembled for recording and data storage. Generally this will simply entail assembling the equipment listed above and making certain you have the settings on the recorder selected correctly. Currently (October 2022) we can use 2 setups - one belongs to the Xu lab (EnerSound microphone + Olympus digital recorder) and one belongs to the Zuk lab (Sennheiser microphone + Marantz recorder). In the future, the Xu lab will likely have a Sennheiser microphone and some other, professional grade, recorder.

The Marantz recorder has a slot for an SD card. This is where you must save your recordings. The Olympus recorder has a slot for a microSD card, but it also has 8BG of internal storage and its own USB plug that you can use to directly download files to your computer. Regardless of how/where you save your files, you must immediately back them up by downloading them to a computer (and uploading them to the cloud if possible) each time you take a break from recording.



Photos above: Marantz digital audio recorder with Sennheiser directional microphone. *Left:* XLR connectors are 3+ prong electrical connector used for professional audio equipment. The microphone plugs into this recorder with an XLR connector. *Right:* Photo of basic recording setup. a) recording cup containing cricket; b) ear buds plugged into the audio recorder (you will typically listen through these as you record); c) Marantz digital audio recorder; and d) Sennheiser directional microphone. The foam sleeve that covers the end of the microphone reduces any noise from wind. In the anechoic room it isn't necessary, but it in the field you will want it!



Photos above: Olympus digital audio recorder with EnerSound microphone. *Left:* The Olympus recorder uses basic audio jacks for input from the microphone (a & b) and output to the earbuds (c & d). *Right:* The EnerSound microphone is very small and connects to the recorder with a thin wire. Therefore, it should be taped (use lab tape for ease of removal) to a thin dowel rod which will allow you to extend the microphone near to the recording cup without having to be so physically close that you disturb the cricket.

Saving audio files and adjusting recorder settings

Eventually the Xu lab will want all recordings to be collected as .wav files. Unfortunately, both of the recording devices we currently use will save exclusively as .mp3. It is fine for now, but is a lower quality recording. As a result, though, you should not have to adjust the output file type because you have no options!

The primary setting you want to double check before you collect any recordings is the folder location specified for saving your files.

Olympus folders:

1. When looking down at the screen, tilt the body of the recorder so you are looking at the right side of it. Here you will find the OFF/ON(power) tab. Switch to ON.
NOTE: because of the location of this tab, it can be easy to forget to switch it OFF when you are done. This piece of equipment runs on AAA batteries, so it is particularly important that you do turn it OFF when you are done. It's not a bad idea to always have 2 extra AAA batteries on hand when you start a recording session.
2. The screen will turn on. It is not backlit, so you will not be able to read it if you are recording in a dark room. It should contain similar information to that shown on the left.
3. Press the MENU button and scroll through the various options to see what you have control over. For example, if the Time & Date are not correct, scroll down to the Device Menu using the “-” button (the device menu is the wrench). To enter the menu, press the forward arrow button once, then scroll down using the “-” button until you reach “Date & Time.” Select by pushing



the center OK button. Check to make certain all of the information is correct, or correct it yourself using the +/- buttons. To leave the menu, push the STOP button.

4. If no microSD card is inserted, you can save to 1 of 5 folders (A-E). You can scroll through them from the default screen by pushing the FOLDER/INDEX button. Make sure you make a note of which folder you are using so you do not lose track of your files.
5. If you wish to save to a microSD card, go to the DEVICE MENU and select "Memory Select." You will only be allowed to select this drive if a card is already inserted.

Marantz folders:

1. TBD

c) Recording a single cricket in an anechoic chamber

You will likely be recording crickets under two very different conditions. The best/cleanest recorder will come from inside of the anechoic room, which is specifically designed to absorb sound. Cricket songs will be clean and crisp because they will not be bouncing off of the walls.

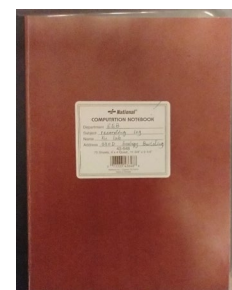
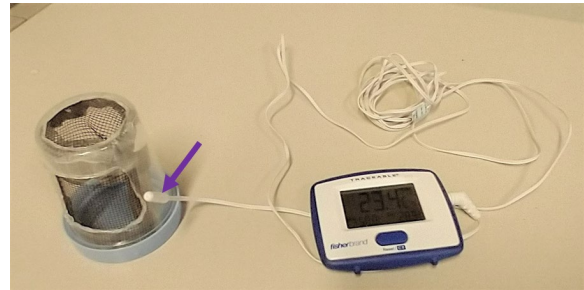
To record a cricket in the anechoic room you will need:

- An adult male cricket
- A recording cup (see section V.a)
- Sennheiser directional microphone
- Digital audio recorder
- SD card
- Headphones/earbuds
- Digital thermometer
- Recording notebook
- Clock - Mark Bee does not allow cell phones in his anechoic room, so you will need another way to tell time. If set correctly, the audio recorder can be used to tell time, but the Xu lab will also be purchasing a clock that can be carried in and out or simply remain in the chamber.

NOTE: it is good to have a female or 2 available just in case the male of interest will not sing for you. If you have to wait more than 30 min, it is likely that you will need to put a female in the recording cup with him or give up for the day and try again tomorrow.

1. Transfer male to recording cup.
2. Set cup on the floor.

3. Wait motionless and silently for cricket to begin chirping.
NOTE: if he does not start singing after 20-30 min, add a female to the cup. Often this will stimulate a male into singing. Fortunately it take hours before courtship/copulation ends, so he will sing for a long time.
4. Once he begins to chirp, wait at least 10-15 seconds to make sure he is going to continue to sing. Sometimes if you startle them at the beginning they will stop and you will have to go back to step V.c.3.
5. When you are confident he is producing a continuous string of regular chirps, slowly and calmly switch on the audio recorder.
6. Slowly extend the microphone towards the cup, getting it as close as possible without distracting the cricket (a few cms if possible). Assess the directionality of the chirps and place the microphone so you are recording at maximum amplitude. The best angle will be to point the microphone at his rear end - the chirps will be emanating from his raised wings and most of the sound is being pushed rearward.
7. Record for at least 30 seconds of continuous calling. If the cricket pauses during this time, you can pause the recording and then restart it when he resumes.
8. Temperature is always collected during every recording because it influences the rate at which crickets stridulate. The Xu lab has a highly sensitive digital thermometer that you should place near the cricket either while he is singing (if it does not disturb him), or at the end of the 30 sec recording.
9. When you have collected the desired recording, speak directly (but softly) into the microphone and record the following information:
 - a. Your name
 - b. Location
 - c. Date
 - d. Time of day (use 24 hour clock or be certain to say am/pm)
 - e. Temperature
 - f. Cricket ID
 - g. Project name if relevant
 - h. Any notes regarding irregularities in the recording
10. Stop the recording.
11. Fill all relevant information into the Xu lab Recording notebook.
12. Download any recordings to a computer and the cloud right away to ensure you have a copies!



VII. Cricket colony bookkeeping

a) Keeping track of family lines

Research in the Xu lab is heavily reliant on the careful tracking of inbred lines (typically full-sib crosses) which facilitate the generation of homozygous crickets utilized in quantitative genetic studies. Therefore it is of utmost importance that crickets be tracked with great care, and any movement of individuals or families to new containers be given your full attention.

There is a master spreadsheet which will be shared with all lab members

VIII. Requesting Lab Supplies

IV. Filling in Timesheets