Standard Operating Protocol

For the Marlene Zuk “Cricket Lab”

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Welcome to the Zuk Lab

Paychecks are administrated by CBS. Upon being hired you will need to make a welcome appointment with Kirsten Domer. She can be reached at cbshr@umn.edu. At this first appointment you will need your offer letter, your student ID, and a copy of your birth certificate or social security card.

Time sheets are available in 124 Snyder Hall, or online. Completed timecards need to be signed by Susan, or Marlene, and submitted to room 124 Snyder Hall by Tuesday at 4:30 pm. Timesheets that are received late may be subject to additional withholding tax.

We are excited to have you explore the wonderful world of Cricket Care with us! At the Zuk lab we are studying the patterns of evolution for a particular species of cricket found in the Pacific Rim. The crickets are found on isolated islands of the Pacific Ocean and we name them according to the island that they are native to. For the USDA to allow us to do our work it is very important that the crickets are contained and do not escape. Because they are not native here it is possible that it could change the ecosystem if they were to escape. For our own research it is very important that the cricket colonies don’t get mixed. Our rule is, “The second most detrimental thing you could do is mix cricket colonies. The first most detrimental thing you could do is mix colonies and then not tell us.” So, if anything happens, please let us know.

We routinely hold lab meetings to discuss the experiments being conducted in the lab and our role in them. In addition to your weekly cricket care responsibilities, you are required to be in attendance at the lab meetings.

Marlene Zuk is the primary researcher and our principal investigator. Susan Balenger is our post-doc and is in charge of the labs activities. Susan is your first contact person if there is ever a problem. Her office is located in the 330L, one hallway past our incubator room.

Throughout this manual we have tried to include pictures that would help your understanding of the procedures. We have also included pink sidebars with important information related to the topic on hand.

Welcome, once again. We are pleased to have you aboard!
Crickets are members of Orthoptera. They have a segmented body, and hind legs for jumping. They breathe through spiracles which are located on the sides of their abdomen. The palps are used to push food into their mandible. They use their antennae to feel their surroundings, their simple eye to detect light and dark, and their compound eyes to see in multiple directions.

Crickets molt their outer skeleton 6 or 7 times in their lifecycle. They do this by creating a new skin underneath their hardened exoskeleton and then they slough off their exoskeleton and emerge.

Female crickets can be identified by the existence of an ovipositor, which they use to place their eggs into the soil. Male crickets only have Cerci.

Male crickets use their wings to sing for a mate. And occasionally, our crickets will use their wings to fly.
Baby cricket food needs to be refrigerated and is kept in the fridge in the lab room. It contains more protein and is easier for the tiny crickets to eat.

Crickets are given baby food for approximately the first month of their development. Adult crickets are given rabbit pellets. This food is kept in the black receptacle near the fridge. Only one bag should be open at a time.

We keep a Tupperware container of each type of food in the refrigerator. You will refill these containers from the larger containers of food. Please keep an eye on the supply of food and write a note on the white board when the bag is half empty.

To minimize trips in and out of the incubator room please fill the two smaller containers of cricket food prior to entering the incubator room.
These cotton pads serve a dual purpose. First, they provide the crickets with a vital source of water. Secondly, female crickets lay their eggs in these pads. We collect used egg pads when we need to expand a colony. Egg-pads should be made of enough cotton so that when it is saturated with water it will fill to the line in the larger part of a petri dish. If the cotton pads are too small the females may damage their ovipositor when laying eggs, and the crickets will not have enough water. If they are too large it wastes cotton.

Excess cotton pads can be covered and left in the refrigerator.

At the beginning of your shift you will make egg-pads. This is done at the sink in the lab room, which is outside of the incubator room. We do this outside of the incubator room so that the cotton fibers in the air do not clog the filters on the incubators. We use de-ionized water to saturate the egg pads and then squeeze out excess moisture so that the crickets do not drown.
The incubator room is where the crickets are contained. We keep the basic supplies in the incubator room as well. It is very important that live crickets do not leave the incubator room unless contained for an experiment. Each incubator can hold 18 boxes of crickets, if placed 3 to a shelf. To place the boxes in a full cricket colony: position one box with it’s short dimension facing forward on the shelf, and then place two boxes next to it with their long sides facing forward. Each incubator is equipped with an alarm that will sound if the humidity deviates from the preset level. There is a toggle switch which you can use to momentarily silence the alarm. Before your leave for the day please double check that each incubator door is closed and that the humidity alarm is armed.
Everyday Supplies

- A plastic bag to put waste from the cricket boxes.
- Paintbrushes to clean the cage.
- A spray bottle with 10% bleach solution.
- Paper toweling, from the dispenser.
- Egg cartons, to create the crickets habitat.
- Petri dishes, both the large side and the small side
- Adult Cricket Chow.
- Baby Cricket Food.
- Cotton water pads.
- Clean Cricket boxes
Daily Chores Checklist

- Place day-old waste in autoclave bags and bring to 2nd floor autoclave
- Make Egg Pads
- Colony Maintenance (will be explained hereafter)
- Sweep/Mop floor
- Bleach counter top, and empty sink-catch
- Place Waste in freezer
- Change numbers on white board appropriately
- Sign off with date for the day
Each cricket colony has been given a specific color of tape. We use this tape to label the boxes.

When assigned to work with a colony of crickets you will be responsible for their care. This requires that you clean each box and replace the egg pad, food, and carton pieces twice a week. The days should be spaced 2 to 3 days apart.

Your responsibility also includes population control, collecting egg pads, and harvesting baby crickets as needed. Basic cleaning up after yourself is also required.

Being mindful of the crickets, remove the lid so that you may change the egg pad and food dish. If you are worried about losing crickets, work inside a large plastic container.
The cotton egg pad is always placed in the large side of a petri dish. Please wipe the bottom of the petri dish dry before placing it in the box with baby crickets to avoid drowning the babies. This cotton egg pad gives the crickets the moisture they need. It also serves as a place for the female crickets to lay their eggs. The food is placed in the small side of the petri dish. Crickets are fed Rabbit Pellets from the time that they are about one month old. When they are very young we have a special powdered mix that we feed them. It is easier to chew and gives them more protein. When crickets are very small they like to hide in the food dish. It is necessary to sweep through the food with your paintbrush so that you do not throw any baby crickets away. Your next task will be to clean the egg cartons that we use for the cricket’s habitat. The crickets use the egg carton spaces to hide. Younger crickets need more egg carton.
When you are cleaning a box it helps to leave one piece of egg carton in the box at all times so that the crickets have some place to hide.

We can usually use the same egg carton for a few weeks before it needs to be changed. Please check carefully that there are no crickets hiding in it before you throw it away.

When the egg cartons are cleaned you can brush all of the debris into a corner using a paintbrush or piece of paper toweling. You can use a small piece of cardboard to scoop the debris out of the box.

You may discard used food, paper toweling, and egg cartons into a plastic bag, they do not need to be frozen before they are autoclaved. The egg pads (used water cottons) however should be frozen first so that no baby crickets escape.

All containers and paint brushes should be sprayed with bleach, then washed and dried.

When you finish with your shift sweep the floor, bleach the counter tops, and dispose of any waste.
Transferring Crickets to a Clean Box

When the box itself becomes dirty.

Image 1: Label a fresh box
Image 2: Place a fresh food and water container into the new box. Some crickets may be easily transferred because they like to hide underneath the egg carton.
Image 3: Gently tip the full box of crickets onto its side so that you can place one corner of the old box inside of the new box. The crickets can then jump into the new box on their own.
Image 4: This is a variation if you are not comfortable with the crickets yet, or if you have an unusually lively bunch. You can clean the box, removing all of the frass, and then tip the whole box upside down on top of the old box.
We bleach containers, and the countertops, and wash our hands between colonies. We do not want to risk transmitting disease between colonies. Cricket Paralysis Virus is deadly to our colonies. The symptoms include paralysis, twitching, and cannibalism within the colony. If you see signs of this virus you should notify Susan right away! The disease is a cricket virus, it does not pass to humans beings.

Occasionally you will see a white crystal-like mold growth on the egg cartons of infected colonies. If you see this you should remove the egg cartons and replace them with fresh egg cartons. It is a simple step toward preventing this disease. We also disinfect all of the equipment that comes in contact with the crickets.

All containers used for cricket care must be washed and then sprayed with bleach water. The containers must be completely dried before they are used for another cricket colony. Paintbrushes and petri dishes should also be bleached and washed after use.

We use 10% bleach solution, which we make ourselves. When a bottle is empty you can add 2.5 ounces of bleach to a spray bottle and fill the bottle up with water to the 25 ounce mark. Our spray bottles are labeled in 5 ounce increments and the 5 ounce mark is near the bottom of the spray bottle. Bleach solution must be labeled with the date it was made. Try to use the oldest bottle up first.

Once you have bleached and washed the containers it is acceptable to leave the clean containers on the drying cart for the next Cricket Technician to put away. All supplies must be clean before they are put away, and they should be put away neatly.
Advanced Care: Dividing Boxes

First you need to set up a new box with labels, food, water, and egg cartons.
If you move egg cartons from the original box to the new box remember to look for crickets that are hiding inside the egg carton spaces.
Gently tip the full box so the corner fits inside the new colony box.
Then you coax the desired number of crickets into the new box.

When the crickets are very young you can fit about 500 in a box without any problems. When they get older, and larger, they need more space. Juvenile crickets are large enough to be eating the adult food (which is to say that, they are about as big as the food pellets themselves). At this time the box should be divided until there are 30-60. When they get a little larger you will divide them again. And when they get a little larger you will divide them again, until there are 20-40 large adult crickets in each box.
Advanced Care: Collecting Egg Pads

The crickets have three life stages that we are concerned with: infant, juvenile, and adult. This picture is a representation of all three life stages. The first is an egg-pad box, the second holds juvenile crickets, and the largest box holds adults. Juvenile crickets are at least ¼ of an inch long, and are big enough to eat adult food. Adult crickets can be distinguished from juvenile crickets because adult crickets receive their wings in their penultimate or final molt.

- We maintain our colonies by harvesting egg pads. Collecting egg pads is necessary to revitalize your colony's population.
- Retrieving the egg pad from a box of adult crickets, rinse it out, squeeze the water out, and place it in a small Gladware box.
- Label the box with the name of the colony and the date that you collected the egg pad.
- Give the adult cricket box a fresh water cotton to replace the one that you saved.
One female cricket can lay up to 200 eggs. It takes about two weeks for the eggs to hatch, and the pad will continue to produce crickets for another 1-2 weeks. When they are young a colony box can hold 500 or more infant crickets. If you are just beginning a new colony box you may not have enough crickets to transfer them to a larger container. In this instance, use another small Glad box as a colony box. Don’t forget to place a food dish and a water dish into this smaller container. You transfer the crickets by brushing them off of the cotton pad with a paintbrush. They are fragile! Please be gentle!

The small boxes hold moisture that the crickets need, but too much moisture could drown a baby cricket. Please wipe the box out before you replace the egg pads!
Maintaining Colony Genetics

- It is not only necessary to care for your current colonies, but for future colonies too. We must be careful not to inbreed crickets, as they serve as analogs to their wild counterparts.

  This can be done multiple ways:
  - Using babies from multiple egg pads per baby box
  - Mixing boxes of juveniles or adults together
  - Collecting egg pads from multiple adult boxes
Communication through the White Board

The White Board is located in our Lab 330, to the left of the main door. It should be the first thing that you see when you walk in. It is how we communicate basic information within the lab.

There is a section to identify how many boxes of crickets are in each of your colonies. This number will need to be changed every time you combine or divide boxes, and also when you save egg pads or freeze used egg pad boxes.

There is also a section to write any needed supplies. When you open the last bag of cricket food or cotton, or if we need egg cartons or plastic bags, please write this on the white board under Cricket Supplies. If you do not inform us of this then it could create problems for the next person.

The final section is where you write the date you cared for the crickets in your colony. This is necessary so that we may keep track of the last time each colony was cleaned.
Disposal of Waste

- Waste is autoclaved after being frozen overnight
- To autoclave something:
  - Place waste in autoclave bags, then put 1-3 bags into the autoclave bin
  - Place autoclave tape on each bag
  - Bring down to 240 via the elevator
  - Use the log near the autoclave to sign up for a time to use it
- When the autoclave is available:
  - Place the bin into the autoclave, and gently shut the door
  - Use the touchscreen to select PV30
  - Waste can then be deposited into the black waste bin near the white board
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