Foraging Trials Manual

1. Figure out which bees you need: see schedule, Emily, or Carolyn
2. Turn on the foraging chamber to let it warm up
3. Capture appropriate bees from hives:
   - Always wear a lab coat and leather gloves when working with Bombus and Apis
   - If necessary, use small mesh bottomed tubes to collect Bombus, then transfer to Falcon tubes; all other bees can be collected with Falcon tubes
   - Only collect female Osmia and Megachile (fuzzy bellies, less hairy faces)
4. Put Falcon tubed Bombus and Osmia in the small refrigerator for 1 hour, Apis and Megachile for 30 minutes (use a post it to mark the time, the hive bees were collected from, and the number of tags needed)
5. While bees are chilling, assemble RFID tags and update the Google Doc:

   Update the Google Doc
   - Open Chrome and click on the "My Drive" bookmark, then "ForagingExptMetadata"
   - Update the gray boxes to reflect how many bees are in the trial, the replicate #, and how many bees have been used in a previous experiment
   - Use the bottom rows to stage your tag IDs before placing them in the red numbered rows (the "live" rows), then…

   Assemble the tags
   - Use refurbished tags when possible
   - Get 16 (or less if some bees already tagged) tags out and stick them to a post it note
   - Open iID DEMOsoft 2010, click "Read Identifier," and read the tags using the RFID pen
   - Copy/paste each tag ID individually into the Google Doc along with its correct colony ID (A, B, C, etc); use the staging rows, then transfer to the live rows
   - It may be necessary to correct the last 4 digits of the tag ID; double check using the program iID DEMOsoft 2013 in SCANdemo mode

   Back up the metadata
   - Copy all the data the from the gray boxes down to bee 16 ID on the ForagingExptMetadata file; paste it into the permanent metadata file (file name will change—see Emily or Carolyn at the start of a new round of trials), date it, note which hive the bees came from, and mark the bees that have already been in a trial
6. After the bees have chilled for 1 hour, glue the RFID tags to the bees (see image below)
   - Use a pin on either side of the waist to keep bees from moving
   - Use a very small dot of Elmer's glue to secure the tag to the bee's thorax
   - Move the tag using tweezers
   - Scoop the bee back into the Falcon tube and return her to the fridge for a few minutes until the glue sets up
   - Any bees that are already tagged can be read with the RFID pen while still in their tubes
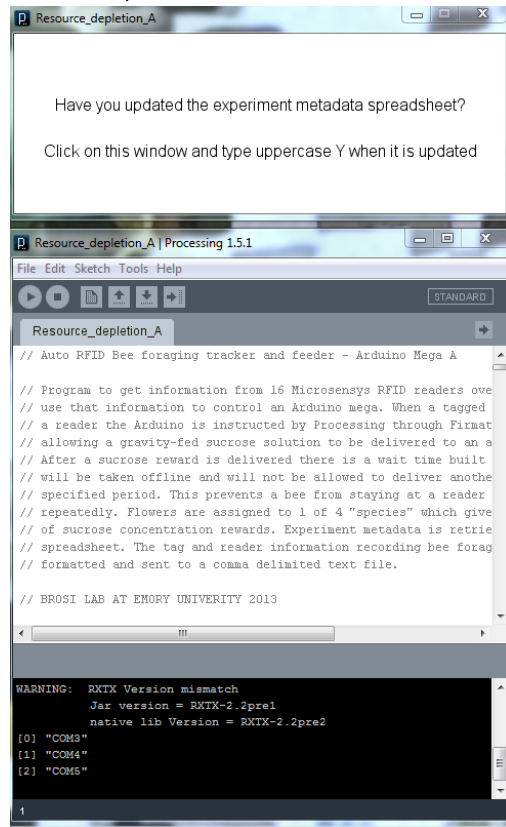
7. Starve bees in a dark cabinet for 1 hour; note the time they go into the cabinet on the post it
8. Starting a foraging trial:

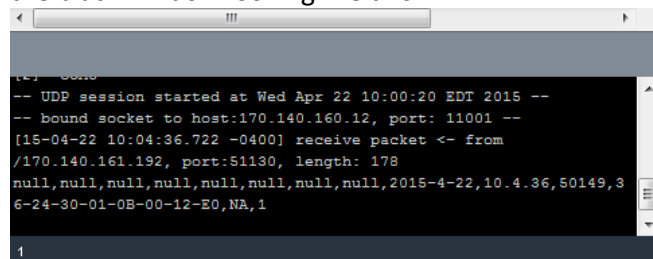   **Prepping the foraging chamber**
    - Double check your bees 5 minutes before their starving period is up to make sure all are alive and their tags are still attached
    - Wipe the green floor and flowers in the foraging chamber with EtOH;

    Processing
    - In the folders Resource_Depletion_A and Resource_Depletion_B, open the files Resource_depletion_A.pde and Resource_depletion_B.pde, and click "►" or "Run" in both programs
    - Text will appear in the black space and a window will open asking if you've updated the metadata, it should look like this:

    - Follow the instructions in the metadata window, then test the foraging chamber by inserting the test tag into a flower on the A side and the B side, which should show up in the black window looking like this:

    - Great! This means everything is working and bees can be added to the foraging chamber. If this series of messages doesn't appear, you have to restart the foraging chamber, by clicking "■" or "Stop," then exiting out of the program and starting again by reopening Processing

- If a pop up appears asking you to update Processing, Firmata, Arduino, or any program related to the foraging trials, DECLINE

Adding bees to foraging chamber

- Note the temperature in the foraging chamber; add this information to the metadata file
- Close all the doors, lock them using the pins, and tie a knot in all the sleeves except one
- Wearing a glove, pour bees one at a time from their Falcon tubes into the foraging chamber through the untied sleeve
- When all the bees are in the foraging chamber, lock and tie the door/sleeve and note the time the last bee went into the foraging chamber on the post it
- Flip down the blue tarp curtains

9. End of the trial:
    - After 75 minutes, end the by trial clicking "■" or "Stop," then exiting out of the program
    - Copy the name of the trial's .txt file from the Resource_Depletion_A and Resource_Depletion_B folders and add it to the permanent metadata file
    - Recapture the bees one at a time using the Falcon tubes
    - Place the bees in the small fridge with their post it for up to 1 hour to chill them before returning them to their hive; remove and save the tags from any dead bees
    - Put all chilled bees in one Falcon tube then return them to their hive

10. End of the day:
    - Refill sugar tubes up to the 10 mL line
    - Move all new .txt files in the Resource_Depletion_A and Resource_Depletion_B folders over to Google Drive; drag them to appropriate folder under "Carolyn 2015 Data" folder; repeat with the external hard drive "Foraging Chamber PC External"
    - Delete any mistake .txt files from Resource_Depletion_A and Resource_Depletion_B
    - Mark all completed trials on the Meta Checklist