

There are 3 different protocols. The first is carried out before an experiment, and the other 2 are carried out on alternating days of the experiment. The first protocol, dubbed Pre-Experiment, dry calibrates the sensors for duration of the experiment. The second protocol, dubbed, Bottles On, is carried out on the first day of the experiment then every other day. The last protocol, dubbed bottles Off, is carried out on the second day of the experiment then every other day.

Pre-Experiment

1. Turn the Arduino on.
2. Insert silicone stopper into the sipper tube of the first bottle to prevent the leakage of fluid.
3. Gently remove the rubber stopper such that it rests on the rim of the bottle, so as to disturb the sensor wire more than necessary.
4. Fill the tube to the top black line (where “-10” would be) with the desired solution.
5. Gently insert the rubber stopper into the top of the tube, some overflow may occur. Take care not to have the solution drip onto any electronic components.
6. Remove the silicone stopper from the sipper. If more than a short burst of water emerges from the sipper, check to see the rubber stopper is adequately inserted or search for cracks in the bottle. If a crack is found, replace the bottle and repeat steps 2 through 6.
7. Preemptively place a vessel under the sipper to catch the liquid that will be drained in the next step.
8. Remove the rubber stopper from the top of the bottle just like in step 3, liquid should start to leak from the sipper.
9. Push the ball bearing of the sipper further into the sipper to speed up the flow of water. This is done by gently pushing the end of the sipper.
10. Once the water has been evacuated, insert the rubber stopper into the top of the bottle like in step 5. Take extra care to keep the sensor wire straight.
11. Hold down the calibrate dry button (for about 3 seconds) of the corresponding sensor until a red flashing light appears. This button is found in the corner of the sensor box on the PCB board beneath “CALIBRATE DRY” inscribed in white. If in a dimly lit room, it is helpful to remember the dry calibration button is closest to the black wires that head to the Arduino as opposed to the sensor wire.
12. Repeat steps 2-11 for the remaining bottles.

Bottles On

1. Turn the Arduino on.
2. Insert silicone stopper into the sipper tube of the first bottle to prevent the leakage of fluid.
3. Gently remove the rubber stopper such that it rests on the rim of the bottle, so as to not disturb the sensor wire more than necessary.
4. Fill the tube to the top black line (where “-10” would be) with the desired solution.
5. Hold down the calibrate wet button (for about 3 seconds) of the corresponding sensor until a red flashing light appears. This button is found in the corner of the sensor box on the PCB board beneath “CALIBRATE WET” inscribed in white. If in a dimly lit room, it is

helpful to remember the wet calibration button is closest to the sensor wire as opposed to the wires that head to the Arduino.

6. Gently insert the rubber stopper into the top of the tube, some overflow may occur. Take care not to have the solution drip onto any electronic components.
7. Remove the silicone stopper from the sipper. If more than a short burst of water emerges from the sipper, check to see the rubber stopper is adequately inserted or search for cracks in the bottle. If a crack is found, replace the bottle and repeat steps 2 through 6.
8. Repeat steps 2-7 for the remaining bottles.
9. Record the first animal's weight and water bottle weight.
10. Place the sipper onto the cage so that the rodent may access it.
11. Simultaneously take mental note of the volume of the bottle and press the push button on the breadboard (not the one on the Arduino!). Record the volume in the bottle and the time the animals was given access to the sipper
12. Repeat steps 9 through 11 for the remaining animals.

Bottles Off

1. For the first animal, ensure 24 hours and 1 minute have passed since the animal was given access to the sipper.
2. Take mental note of the volume in the bottle, press the push button and remove the sipper from the cage such that the animals no longer has access to the sipper. Record the volume in the bottle and time the animal no longer had access to the sipper.
3. Repeat steps 1 and 2 for the remaining animals.
4. Remove the SD card from the Arduino, attempting to not remove it while the red LED on the data logging shield is flashing.
5. Insert card into a computer.
6. Find the SD card on the computer and change the name of "DATALOG.TXT" to a more descriptive title.
7. Drag the file to a place on the computer easily accessible by matlab for analysis
8. Eject the SD card from the computer.
9. Put the SD card back into the data logging shield of the Arduino.
10. Turn off the Arduino.