**Egg dissection protocol – updated 14/11/2022 by OC**

**Purpose:** separate yolk from albumin for use in yolk hormone assays

**Use:** High precision balance (to 0.001mg)

1. Eggs are collected Mondays, Wednesdays, and Fridays from the lizard colony. They are incubated at 28°C and dissected 24 +/- 2 hours following collection.
2. Record, the date, time, observer (the person dissecting the eggs), species (deli or guich), the sample ID (unique to each sample collected), egg ID (listed on egg cup), clutch ID (listed on egg cup), and enclosure (listed on egg cup).
3. Measure whole egg mass first. Make a small aluminum foil boat to mass egg. It should fit easily on the scale. Zero the scale to this boat. Mass egg on high precision balance to 0.001 mg in small aluminum foil boat. This should be done quickly. The eggs rapidly lose moisture when uncovered which means that the measured mass will continually (and quickly) decrease when the egg is on the balance.
4. Measure the egg length and width using calipers. With dial calipers, you will measure to a tenth of millimeter and estimate to a hundredth of a millimeter.
5. With egg on Kimwipe, make incision along the length of the egg with razor blade on the albumin side (if incision is made on yolk side most of the yolk will be lost). The albumin side can be identified by its more transparent nature compared to the yolk side. Also, the embryo will often be visible on the albumin side. Much of the albumin will leak out after the incision is made. You can use the same razor blade for 3-4 eggs, but they get dull and should be changed often.
6. Using the dissection scissors, increase the incision so it spans the length of the egg.
7. Place forceps inside incision and open to exposure the yolk.
8. With fingers, squeeze the egg so the incision gapes, then use a small spatula to scoop out yolk. It may work easiest to hold the egg in your fingers at this stage.
9. Using a pair of forceps, remove the embryo and on vessels from the yolk on the spatula.
10. Using a Kimwipe to dry any remaining albumin. Note: do not put Kimwipe directly on the yolk or it will stick to the wipe.
11. Mass egg yolk in balance in an Eppendorf tube. The balance first needs to be zero to the mass of the empty Eppendorf tube.
12. Add1.0mL of ddH20
13. Vortex thoroughly (count to 20)
14. Label the tube (on top) with an individual sample ID; on a paper label, write the enclosure, clutch, and date. Tape paper label with sticky tape.
15. Store on ice until you have dissected several eggs (3-4) and then transfer to -20°C freezer. This is done to minimize the number of times the freezer door is opened and closed which can dramatically warm up the freezer.
16. After each dissection, clean the spatula with ethanol.
17. After all dissections are finished for the day: 1) clean all dissection equipment with ethanol, 2) make sure the freezer door is closed before leaving the lab.