Common Themes in Reproductive Diversity
A501, Techniques in Reproductive Diversity
August 29, 2006

Lab#1: Working with wild animals – measuring molt in songbirds, collecting blood samples and feces, response to stressor

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Goals for the day:

1. Observe capture, handling, and banding of birds caught from the field.
2. Add color bands to already banded juncos, measure and weigh
3. Describe molt, note sex and age and recent experience
4. Take digital photos of tail feathers
5. Collect blood samples for DNA or hormones, at least 2 birds for DNA, 2 for hormones
6. Collect 'stress series' on birds for later hormone analysis
7. Collect feces from 1-2 birds for later extraction of hormones

Background:

Many of the techniques we will learn this semester will be based on bits and pieces of animals such as photos or videos of their behavior, images of their brains, or molecules such as DNA, hormones, immunoglobulins, hydrocarbons, or proteins extracted from their blood or other tissues.

The purpose of this lab is to begin with a whole live animal and to introduce you to basic field techniques employed in the study of birds. It could have been fish, insects, herps, or humans, but birds are my favorite and they are available, so we will use them as an example.

Materials:

Nets, traps, bait
Bird buckets
Banding supplies (numbered bands, pliers, colored bands, rulers, calipers, data sheets, clip boards)
Molt sheets to record molt data
Digital camera to record photos of feathers
Bleeding supplies (gloves, no. 26 needles, microhematocrit tubes, clay sealant, timer, cotton, eppendorf tubes, labeling marker, sharps container for waste, microhematocrit centrifuge, Hamilton syringe, distilled water to rinse syringe, also eppendorfs with Longmire's solution, blowdown jobbies, data sheet)
Feces collection (scraper, vials to store feces, labeling marker, data sheet)

Procedures:

1. Nets and traps will have been set in advance and we will begin the lab by walking around to see what we have caught. This may be a walk around exercise. We'll leave the nets open to see what else we catch during the afternoon.
2. In the building we will form into teams of 2. Each team will undertake each of the tasks listed above. Instructors will float to help you.
3. You will have a bucket with maybe 6 juncos in it.
4. Half of you will begin by bleeding birds in the outside room; the other half will begin by measuring birds and their molt in the office.

5. *If you work in the office first*, begin by adding color bands to birds that do not have them. Record the information about body mass and colors to a banding sheet. Measure the wing and the tail.
6. For each bird record its band number and state of molt. Examine the bird for molt on the wing and tail and the various tracts of the body. Ask for advice about how to determine which feathers are old, which are new, and which are growing. Rate the tracts of the body as beginning to molt, mid molt, or complete. There will be handouts to help you get oriented on the bird.
7. To measure tail white. We will use a digital camera to measure the amount of white in the tail Use a piece of black construction paper for the background with a scale (e.g., ruler). Smooth the feather you wish to photograph. Holding the bird in your left hand, slide the piece of paper between the feathers such that you can see as much of the feather as possible on the paper. Use your thumb to hold body feathers away from the tail feather. Zoom in to desired distance on the camera. Focus and shoot. Be as standardized as possible re distance and lighting conditions.
8. If there is time, have Jonathan teach us how to record the presence/absence of ectoparasites on the tail feathers.

9. *If you start in the outer room*, you will begin by bleeding the birds. A hard job will be made harder by the need to wear gloves.
10. To bleed a bird, hold it in your left hand, partially facing your palm. Use the fingers of your left hand to hold the wing in an extended position (your forefinger and middle finger are holding the birds wrist.
11. Use you right hand to pluck the small feathers that cover the birds 'elbow.' Use the thumb of your left hand to apply mild pressure to the bird's breast muscle or the base of the wing.
12. With your right hand prick the alar (wing vein) just wear it crosses the elbow. Be careful not to prick through the vein to form a hematoma. Be careful not to prick yourself (!). Using the thumb of your left hand maintain back pressure so the blood will bead as it emerges from the vein.
13. Hold hematocrit tubes to the bubble of blood and use gravity to let the blood fill the tube. Seal the tube with clay and move on to the next tube.
14. Wash hands when through.
15. Read on for instructions on what to do with the blood you have collected

PROCESSING PLASMA and FECES

(1) DNA

Birds bled for DNA only, we need two or three (no more) microhematocrit tubes of blood for each adult. Upon filling a microhematocrit tube with blood, add it to 0.5 ml of Longmire's solution (gravity feed). For the next tube of blood use a separate tube of Longmire's solution. [USE GLOVES when handling Longmire's] Note: A mixed up sample is a non-recoverable error; please take the greatest care to be sure that the blood sample corresponds to the band number of the label.

(2) Hormones

To process blood for hormones, the blood should have been sealed with clay sealant. Spin the microhematocrit tubes in the microhematocrit centrifuge for 4 minutes. PUT ON THE TOP OR YOU WILL LOSE YOUR SAMPLE!. Use enough clay sealant or your samples will leak out the bottom.

AND IF YOU ARE DOING MORE THAN ONE SAMPLE, BE SURE NOT TO GET THE TUBES MIXED UP. Write down the slots of the centrifuge that each sample goes in.

Take a microhematocrit reading (see below).

Draw off the plasma using a Hamilton syringe that has been rinsed (3 times with distilled water) and dried (bore all the way down, tip placed against a kimwipe, so no chance of dilution). Measure the volume to the microliter with the syringe. Be sure not to dilute the sample with water – it needs to be pure plasma.

Store the plasma in a carefully labeled 0.5 ml (i.e., small) Eppendorf tube (sample #, band #, top and side). **Be sure that the caps are tightly sealed** (push down hard, wrap with parafilm). Freeze the plasma samples.

If you also need the red blood cells, then after drawing off the plasma, break off the clay seals and blow the red blood cells (not easy) into Longmire's solution using the devise 'designed' for that purpose. Process as you normally would process a blood sample for DNA.

(3) Hematocrit

For blood collected for hormones, it is possible to get a reading of the % of blood that is made up of red blood cells. This is known as a hematocrit reading and is accepted by some as a measure of overall condition. In general (people too), males have higher hematocrit readings than females and castrates have lower readings than intact males. On the other hand, birds infected with blood parasites may have lower hematocrit readings.

To get these readings, line up the swivel reader so that zero is at the top of the clay seal, and 100% is at the top of the column of blood (meniscus at 100%). Then read the % red blood cells (RBC).

(4) Feces

Scrape feces from aluminum foil, store in labeled Eppendorf tube. Record on data sheet.

Instructions for Stress Series

Measuring hormone concentrations in the field is a challenge, primarily because catching a bird affects its hormonal state. Males of many species, for example, respond to a challenge such as a staged intrusion (a playback or lure) by elevating their testosterone. And probably most birds respond to handling by elevating their corticosterone.

Questions for 2004: Do molting birds respond more strongly to handling stress than non-molting birds (or birds that are early in molt vs. later in molt)?

Taking a blood sample for "stress series"

Your goal is to bleed the bird 3 times in succession, immediately upon capture, 15 min after capture, and 30 min after capture. Be sure to obtain a FULL tube of blood each time. Anything less and the series is a failure. But too much bleeding is hard on birds too, so we are trying to get just the right amount.

You will need a stopwatch, ice bucket, data sheet, and bleeding supplies.

- Remove a freshly caught birds from the bucket
- Record the time you did this to the second using a stopwatch (see data sheet).
- Begin to bleed **immediately**
- Record the time to the second.
- As fast as possible, collect 1.5 full tubes of blood, seal with clay (two half tubes can add up to one full tube, but air bubbles don't count. Your job is **collect at least 50 ul of blood.** No need to collect more.
- Complete bleeding in < 1 min if possible, record the time required to the second.
- Stop blood flow with a tiny wad of cotton.
- Note circumstances surrounding the capture, e.g., caught right away, took a long time to bleed, etc.
- 15 min later (from exact beginning of when began to bleed), collect 1 more tube, seal with clay **(be sure you have 50 ul of blood).** No need to collect more.
- Record the time to the second.
- 30 min later (from exact beginning of first bleed), collect 1 more full tube of blood and seal with clay (**be sure you have 50 ul of blood**). No need to collect more.
- Record the time to the second.
- Check to see that you have recorded the times of capture, onset and end of first blood sample, onset and end of second blood sample (exactly 15 min after onset of first), and onset and end of third blood sample.

- Spin tubes in microhematocrit centrifuge (for god's sake put the top on).
- Take a hematocrit reading on each and record the value if desired.
- Draw off the plasma with a Hamilton syringe that has been rinsed with distilled water and dried with a kimwipe
- Measure the plasma as you draw it off and record on data sheet.
- Introduce plasma into a 500 ul labeled (!) Eppendorf microtube, samples are numbered stress

series C-06-1a, c-061b, C-031c, C-06-2a, etc.

- Rinse the syringe.
- Place labeled microtubes in the *freezer* in a Nunc box. Be sure tops are tight,. Wrap with parafilm. We don't want plasma to evaporate!

STRESS SERIES DATA SHEET

SAMPLE #_____

DATE_____ TIME_____

BAND #_____ COLORS_____

SEX_____ AGE_____

BLEEDER_____ CAUGHT BY_____

STAGE OF REPRO/#NESTLINGS/NESTLINGS'AGE_____

NEST ID or LOCATION_____

TIME NET OPENED_____ TIME OF CAPTURE_____

'MENTAL STATE' OF BIRD IN 15 MIN PRIOR TO CAP (0 = undisturbed, 2 = mildly disturbed, 3 = highly disturbed, integrate) _____

TIME BLD1 BEGUN (0-3' after cap)_____TIME BLEED1 ENDED_____

TIME BLD2 BEGUN (15'after cap)_____ TIME BLEED2 ENDED_____

TIME BLD3 BEGUN (30' after cap)_____TIME BLEED 3 ENDED_____

MASS OF BIRD (bleed before you process) _____

PLASMA VOLUME 1 (ul)_____

PLASMA VOLUME 2 (ul)_____

PLASMA VOLUME 3 (ul) _____

SPECIFIC COMMENTS (include details of how bird was caught and other important observations, but please be sure to fill out info above and not use this narrative as a substitute for that)_____

_____

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(please store aspirated samples in *tightly sealed* eppendorfs , labelled C (for cort)-06-xy, e.g., C-06-1.a, .C-06-1.b, C-06-1.c, etc., to indicate cort, year, sample number in year, and order of sample in the stress series)