SUMMER WORKING SCHEME

When I have a Rusty blackbird in my hand…..

Order of importance: If you do not have enough time or cannot get the requested amount of blood restrict your handling and collecting to the most important tasks which are the following (importance in descending order):
Feather for stable isotopes etc. -> blood sampling (mercury -> genetics -> parasites) -> sex -> age -> condition -> morphological measurements

1.) **Metal band** with number on right leg

2.) Individual **colour band** combination on left leg (at the moment, colour band combinations are shared between Jason, Claudia, Luke and Dave)

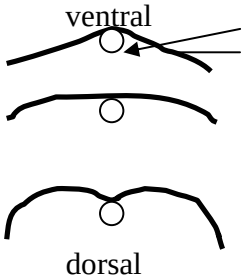
3.) **Sex**:
   a. Male body plumage glossy black
   b. Female body plumage gray or grayish brown
   c. Furthermore, measurements of males Wg >112, (tail >87; only valid for AHY?)
      measurements of females Wg <112, (tail <87; only valid for AHY?)

4.) **Age**: check wings and retrices for growth bars and underwing coverts for contrast
   a. < 1 year: - growth bars form a lane across the wing feathers indicating simultaneous growth
      - great underwing coverts pale brownish (females) or grayish (males) contrasting with the adjacent gray (F) or blackish (M) feathers (see figure)

   b. > 1 year: - growth bars differ between wing feathers indicating successive growth
      - great underwing coverts dark gray (F) or blackish (M) with little or no contrast between adjacent feathers (see figure)

5.) **Fat score**: - 8 steps interclavicula (see figure)
   - 8 steps abdomen (see figure)
   - Half steps e.g. 3.5 are also used. We score interclavicula fat and abdominal fat separately.

6.) **Body condition**: - 3 steps



   - lean — sternum breast muscle lower than sternum
   - average — breast muscle and sternum form a plateau
   - excellent — breast muscle higher than sternum
   - **use musclemeter** – place musclemeter on sternum in the middle of the thorax

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7.) **Molt score**: - 19 feather patches (see figure) in four intensities
   - 0: no molt
   - 1: <1/3 of the feathers in a patch in molt
   - 2: < ½ of a patch in molt
   - 3: < 2/3 of a patch in molt

8.) **Morphological measurements**:

Bill: - *Bill length*: from nares to tip of bill (distance between the anterior end of the nostril and the tip of the bill)
- *Bill widt*h: at anterior end of the nostril; keep the callipers oriented at a 90° angle to the axis of the bill
- *Bill depth*: at anterior end of the nostril; keep the callipers oriented at a 90° angle to the axis of the bill

Tarsus: - *Length* between the intertarsal joint and the distal end of the last leg scale before the toes emerge

Wing: - *Wing length **max***: measured with unflattened wing
- *Wing length **min***: Length of P1

Tail: - *Length* of central retrices: Hold the ruler parallel to the tail and insert it between the tail and the undertail coverts. Be sure that the end of the ruler coincides with zero!

9.) **Feather sample**: Take **P1** and **5-7 contour feathers** (from the back or the side) and store it in an envelope with the metal band number of the individual on it as well as sex, age, location and date. It is important that we get the entire feather because it will be used for three different analyses. Members of the IRBTG may already separate the different feather parts before sending them off.

a) Feather tip: snip off 1.5 cm of the feather tip (for stable isotopes) and put it in a new envelope marked with **'stable isotops' and** all other information (see above) on it.

b) Feather shaft: snip off the exposed part and put it (preferably without touching with hands or otherwise contaminating) in a Nunc-type tissue tube or sealable plastic bag if tubes are not available. The feather shaft should be OK at ambient temperature without being put into ethanol or lysis buffer. Write **'genetics' and** all information from the envelope on the tube or bag.

c) Middle part: place in a new envelope (for Hg analyses). Write **'mercury' and** all information from the envelope on it. Store it at a dry place at room temperature.

d) **Additional feathers**: take **10 feathers** from the **throat** or **crown** (see patches on molt figure) and place them in an **extra envelope** marked with '**stable isotopes from winter ground**' and all other information (see above).

10.) **Blood sampling**:

- for mercury (see instruction): use **heparinized** capillaries, do not forget bird ID, store at 4 F in freezer
- for diet analysis: fill one heparinized capillary with blood and blow the blood out of the capillary and into a small cryovial. Either add an equal volume of 70% ethanol or freeze without anything added.
- for genetics collect 40-80 µl in another **heparinized** capillary, write metal band number on tube and then put directly into ethanol or lysis buffer. The blood+ethanol or blood+buffer tubes are then OK at room temperature if necessary, but should be refrigerated (at about +4 degrees) for longer-term storage.
- for parasites make a **blood smear**. Place a small amount of blood at one end of a glass slide. Move a second slide up into the blood. The blood will creep along the second smear's edge and when the second smear is pushed to the other end of the initial slide the blood is pulled along making a smear that starts out thick but thins out as it moves down the slide. A thin smear is important. When the blood smear is dry put it into 100% methanol for approximately 2 minutes (time is not critical) to fix the cells. After removal from the methanol the dried smears can be sent to William Barnard.

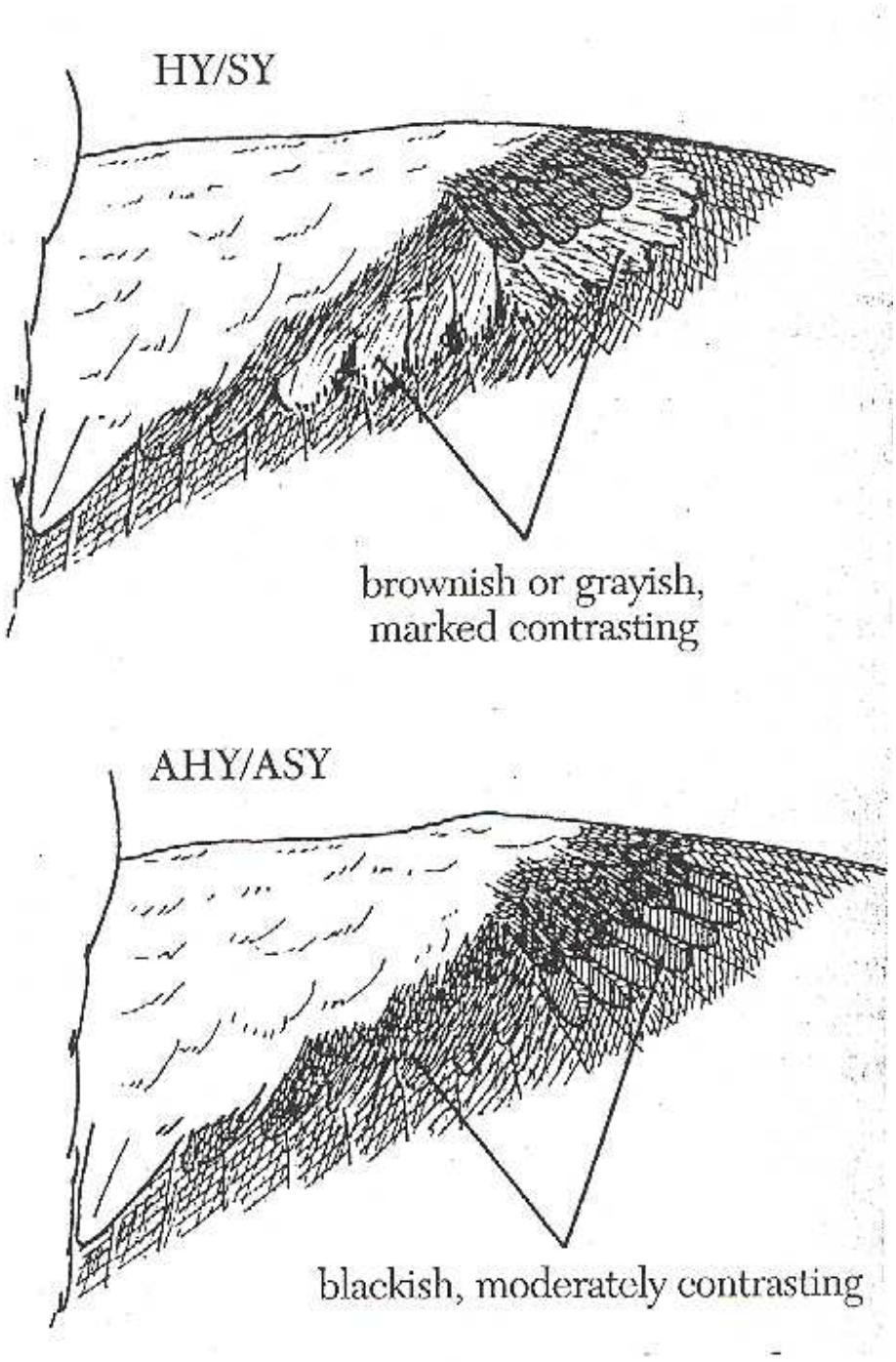
11.) **Faeces:** Collect faeces in tube with 70% ethanol

12.) **Digital picture:** you may take a digital picture of the bird for colour comparisons

13.) **Body mass**: weight bird

14.) DONE, release bird

Age – wing bars



Fat score

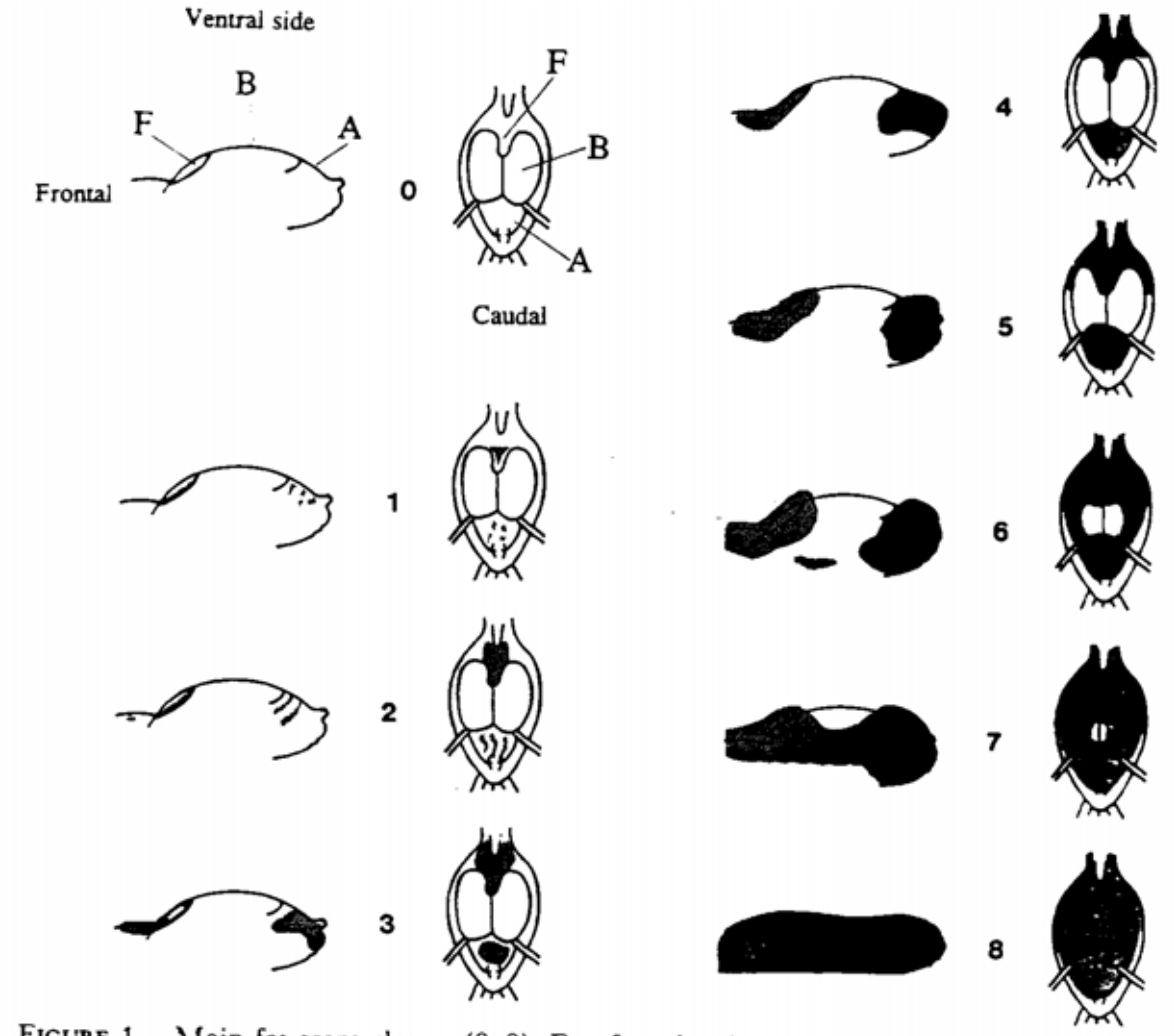
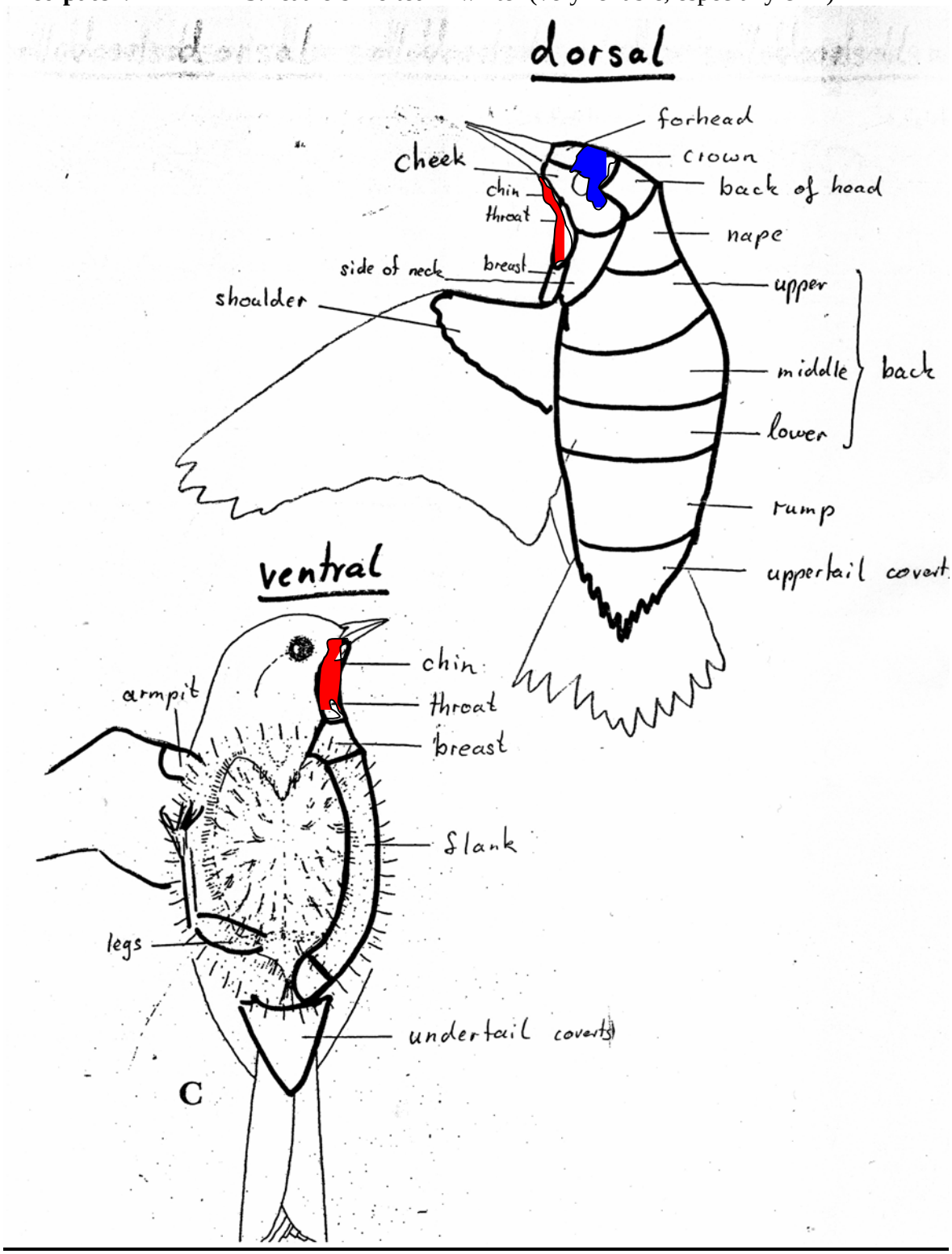


FIGURE 1. Main fat score classes (0–8). F = furcular depression (interclavicular depression), B = breast muscles, A = abdomen, stippled = fat.

Molt

blue patch: MALES: feathers molted in winter (especially around the ear; select feathers that look fresh as not all feathers in this patch may be molted)
red patch: **FEMALES**: feathers molted in winter (very reliable, especially chin)



Molt Scheme

Specimen													
Chin													
Throat													
Breast													
Flank													
Legs													
Undertail cov													
Armpit													
Cheek													
Side of neck													
Shoulder													
Forehead													
Crown													
Back of head													
Nape													
Upper back													
Middle back													
Lower back													
Rump													
Uppertail cov													

STANDARD OPERATING PROCEDURE FOR TISSUE COLLECTION AND SHIPPING FOR MERCURY ANALYSIS IN SONGBIRDS

TISSUE SAMPLE IDENTIFICATION

This nomenclature approach incorporates sample attributes (e.g., location, collection matrix, date) within the field sample identifiers (ID) to provide concise sample information to the end-user. The ID is recorded on the sample container label and Chain of Custody (COC) forms. If tissue samples are from an individual that is banded, then use the band number as the ID code and link that band number with the following information: species, state, study location, matrix, and date of sample collection. Your standard banding sheets will generally suffice. Information on age, sex, and measurements are of great interest for interpretation purposes.

If a tissue sample does not have a band ID then the sample ID needs to include state, study location, matrix, date of sample collection, and depth (where applicable).

The Field Sample ID will be at least 15 characters and be composed of at least five (5) parts (Matrix ID is b=blood, f=feather, e=egg, etc.):

Part 1	**Part 2**		**Part 3**		**Part 4**		**Part 5**
XXXX	XX	-	XX	-	XXXXXX	-	X
AOU Code	State	-	Study Location	-	Date	-	Matrix ID

An example is RUBL-AK-KR-081505-b

TISSUE SAMPLE COLLECTION

If birds are banded with a USFWS band, use the band number as the identification number for the tissue sample. If a tissue sample is collected from an unbanded bird, use the above coding procedure.

Use a small gauge (#25 or 27) needle to puncture a hole in the cutaneous ulnar vein (wing vein) located at the bend of the wing. Use water to part the feathers to create a suitable venipuncture location. One the vein is lightly punctured, a bubble of blood will form. Place a heparin coated 75 mm capillary tube on the bubble. Capillary action will collect the blood. Do not fill the capillary tube. Preferred amount is 1/3 to ½ of the tube. Two to three tubes of blood should be collected. Seal tubes with critoseal capillary tube sealant or plastic capillary tube caps on both ends to avoid blood from escaping. Place all capillary tubes from the individual in one plastic 10 cc vacutainer to prevent breakage and permit labeling.

Place blood samples in a secure freezer until shipped. All tissue samples submitted for analysis will be analyzed for total mercury (and potentially for calcium using blood tissues only) at Texas A&M Trace Element Research Lab.

SAMPLE SHIPPING

Samples should be packaged for shipment as follows:

1. Check sample container to determine if it is adequately identified. Compare sample labels with chain-of-custody form (COC).
2. Check if containers are secure.
3. A picnic cooler (such as a Coleman or other sturdy PLASTIC cooler) is typically used as a shipping container. In preparation for shipping samples, tape the drain plug shut from the outside. Place approximately 3 inches of inert packing material, such as Styrofoam beads or "peanuts," on in the bottom of the container. Other commercially available shipping containers may be used. Vermiculite is NOT to be used.
4. Place sample containers upright in the picnic cooler and covere with bubble wrap in such a way that they do not touch and will not touch during shipment (VERY IMPORTANT).
5. Cardboard separators may be placed between the bottles at the discretion of the shipper.
6. All samples should be shipped to BRI on ice or blue ice paks.
7. Place additional inert packing material in the cooler to partially cover the sample containers (more than halfway). Blood samples, which are required to be shipped to the BRI with ice, should have ice in bags placed around, among, and on top of the sample containers. Surrounding the sample containers with blue ice paks is also adequate. The cooler should then be filled with inert packing material and the liner taped shut.
8. Place paperwork to BRI inside a plastic bag. Seal and tape the bag to the inside of the cooler lid. Include the COC form. Nofity BRI when samples are being sent. Seal cooler with packing tape.
9. Use an overnight carrier and have delivered next business morning. Avoid shipping on a day where samples may not reach BRI by the next business day.
10. Ship all samples to:

    BioDiversity Research Institute
    19 Flaggy Meadow Rd.
    Gorham, Maine 04038
    (207-839-7600/7655 phone/fax)

    Contact David Evers (x110) or Melissa Duron (x102) when sending samples.

Addresses

1.) Mercury: David Evers
BioDiversity Research Institute
19 Flaggy Meadow Rd.
Gorham, Maine 04038
(207-839-7600/7655 phone/fax) x 110
david.evers@briloon.org

2.) Genetics: Terry Chesser
National Museum of Natural History
Washington, DC 200013-7012
ChesserT@si.edu

3.) Parasites: William Barnard
Biology Department
Norwich University
Northfield, VT 05663
barnard@norwich.edu

4.) Feathers: Russell Greenberg
Smithsonian Migratory Bird Center
NZP
3001 Connecticut Ave., NW
Washington, DC 20008
GreenbergR@si.edu

5.) Measurements: Claudia Mettke-Hofmann (preferentially email!)
MPI for Ornithology
Von-der-Tann-Str. 7
82346 Andechs
Germany
Mettke@orn.mpg.de