



## DATA COLLECTION PROTOCOL

*Detailed below are the data collection procedures, methods and materials used on the Red-tailed Hawk Project.*

Below is the order of operations following capture:

1. Photographs
2. Band
3. Measurements
4. Blood sampling
5. Transmitter attachment

If you have any questions, don't hesitate to reach out to Bryce ([bwr46@cornell.edu](mailto:bwr46@cornell.edu)) or Nicole ([npinicola@gmail.com](mailto:npinicola@gmail.com)).

## Photography

A standardized set of images are collected of each individual for objectively analyzing plumage characteristics, but their potential future value is virtually limitless. With images of appropriate quality, we can develop quantitative data on plumage traits like color and pattern, analyze fade and wear on feathers to study patterns of molt, measure relative morphometry of a bird, and much more.



Images displaying the following parts of the birds are collected, at minimum:

- 1: Ventral surface of bird's whole body and tail with left wing spread
- 2: Same as above, with right wing spread
- 3: Dorsal surface of bird's whole body and tail with left wing spread
- 4: Same as above, with right wing spread
- 5: Dorsal surface of spread tail including rump and upper tail coverts
- 6: Entirety of legging taken from the side
- 7: Ventral surface of tail including under tail coverts
- 8: Bird's head in profile, left side
- 9: Bird's head in profile, right side

Please consider using eBird to upload photos to the Macaulay Library.

Create a checklist with any appropriate protocol for your work. If operating a blind or stopping to process a bird at roadside, this may be a stationary checklist. When you upload your photos, be sure to include at least the band number in the notes for *each* photo, so this critical data remains linked with it.

## Lighting

Whenever possible, photographs are taken in even, neutral lighting to avoid heavy shadows, glossy reflectance from plumage, underexposure or overexposure. On sunny days, or overcast days with thin cloud layer (if sun direction can be determined) photos are taken entirely in the shade.

## Materials

Photos are taken against a board composed of plywood surfaced with light gray marine vinyl. Marine vinyl is an ideal material for field photography of raptors in the hand because of its durability and water-resistance for roadside or rugged field conditions, and smooth texture which is rendered virtually invisible in most images. It is also slightly stretchy, allowing it to be stapled taut across plywood to eliminate wrinkles if permanently fastened to a board to enable fast independent set up. While the vinyl is prone to slight creasing when fastened to a folding board, this can be lessened by offsetting the hinges to allow the vinyl to fold in a gap.

A passport color standard is attached to the board using Velcro, with one spot situated near the bottom corner of each side of the board to allow the standard to be moved to either side as needed.

Additionally, a small piece of dry-erase board material may be placed on the board with Velcro so that the bird's band number may be written on it. This ensures that the individual in all photos can always be identified, but may only be necessary in situations

where multiple birds may be captured in quick succession or at one time (like at a blind at a migration monitoring station) where the use of time stamps challenging or potentially fraught with error.

A DSLR camera is used to take photographs. Images are collected in RAW. Alternatively, a cell phone with the capability to take images in RAW may be used.

## Handling

The completeness of display of the bird's body and consistency in posture in each image is critical for maximizing the amount of information available in an image and for maintaining comparability between images for analysis.

Before taking photos, the bird is examined for ruffled or misplaced feathers which may have resulted from capture or handling. This is particularly important among flight feathers, where feathers may be tucked behind one another entirely.

Movements when handling birds for photographs are kept slow and low below the bird's head to keep the bird as calm as possible and achieve a consistent posture.

See Appendix 2 for example photos.

## Banding

A standard USGS band is placed on the bird's (right) tarsus, oriented to allow the band number to be read in the field if the bird is standing. Depending on project goals, darvic alphanumeric color band may be placed on the other (left) tarsus, oriented the same way.

## Measurements and data

All measurements are recorded in millimeters, unless otherwise stated.

### Age

If possible, please assess the age each bird according to the following plumage-based cycle codes:

- 1C – first-cycle plumage (juvenile plumage)
- A1C – after first-cycle (undetermined adult plumage)
- 2C – second-cycle (adult, bird has molted once)
- A2C – after second-cycle (adult, bird has molted at least once)
- 3C – third cycle (adult, bird has molted twice)
- A3C – after-third-cycle (adult, bird has molted at least twice), etc.

### Wing chord

Taken by resting the bird's wing on a ruler in a natural, folded pose without applying any pressure to flatten the wing. The measurement is taken from the 'wrist' to the longest primary.

### S1 chord

Taken by resting the bird's wing on a ruler in a natural, folded pose and gently holding all ten primaries slightly aside so that the next exposed flight feather is the first secondary. The measurement is taken from the 'wrist' to the tip of the first secondary, without applying any pressure to flatten it. This can be done right after taking the wing chord measurement by counting and gathering the primaries in one hand to hold them aside.

### Tail

Taken from the base of the tail to the tip of the longest tail feather.

### Footpad

Length of the pad of the foot, taken from the base of the rear (hallux) and middle talon with each of these respective toes fully extended flat. Toes may be relaxed by holding base of hallux and pushing in on toe to 'uncurl' it by unlocking this tendon. This will cause the front middle toe to also relax. See Appendix 3 for photos.

### Hallux

Length of the rear talon, taken from base of talon where it meets toe to tip. See Appendix 3 for photos.

### Culmen

Length of the exposed culmen, taken from the outer edge of the cere to the tip of the bill. See Appendix 3 for photos.

### Total bill (culmen with cere)

Length of the entire bill, taken from where the base of the cere meets the skull to the tip of the bill. See Appendix 3 for photos.

### Weight

Weight of the bird, recorded in grams.

### Crop

The fullness of the bird's crop. Recorded as 0 (empty), 0.5 (half full) or 1 (full).

## Observations and breeding information

A very important part of our work is to potentially connect the data we are collecting to a breeding location. Because of the importance of breeding location, it is imperative to note a breeding confidence code using the following structure:

- 1- Bird associated with occupied nest containing nestlings or eggs, or a pair attending fledglings, or a combination of two or more code 2 parameters
- 2- Bird associated with a mate and nest, during breeding period (15 May – 31 July)\*, or presence of a brood patch, no visible signs of nestlings or eggs
- 3- Bird paired in territory, during breeding period (15 May – 31 July)\*, but no sign of nest structure
- 4- Bird of appropriate phenotype (ssp.) in breeding distribution during breeding period (15 May – 31 July)\*
- 5- Bird during non-breeding period (1 August – 14 May)\*

\*Use discretion for dates. If you feel that for your area, these dates do not match known breeding phenology, please adapt your code accordingly and make a note in the comments of your data sheet. If you are attaching a transmitter, please record the breeding code at time of capture, not for the breeding provenance eventually obtained through the tracking effort (these should likely all be code 5 if deployment focuses on the wintering area).

## Blood and Feathers

A blood sample of 0.1 to 0.2mL is collected from the brachial vein with a 25 to 27-gauge beveled needle and a 1 mL syringe. Blood is stored in lysis buffer (see Appendix 1), with 1 part blood to 4 parts buffer in a 1.5mL vial, which is pre-filled with 0.5mL of buffer and immediately inverted upon adding blood to mix.

Four contour feathers may be collected, two from the breast, and two from the bird's back. Feathers are stored in manila envelopes.

Finally, please make any comments you see fit in the comments section of your datasheet. Let us know anything you find peculiar, interesting, etc.

## Appendix 1 - Lysis Buffer Protocol

### BLOOD LYSIS BUFFER

Lovette-Evo Bio lab

White, P.S., and L. D. Densmore. 1992. Mitochondrial DNA isolation, p. 50-51. *In* A.R. Hoelzel [ed.], Molecular genetic analysis of populations: a practical approach. IRL Press at Oxford University Press, Oxford, England, New York, U.S.A.

100 mM Tris pH 8; 100 mM Na<sub>2</sub> EDTA, 10 mM NaCl, 2.0% SDS

Working soln.: Final Concentration	Stock Concentration	Dilution of stock	amount of stock to make 100 ml working soln (store at 20°C)	amount of stock to make 1000 ml working soln (store at 20°C)
100 mM Tris pH8	1 M (1000 mM)	1:10	10 ml	100 ml
100 mM Na <sub>2</sub> EDTA	500 mM	1:5	20 ml	200 ml
10 mM NaCl	5 M	1:500	0.2 ml (200 µl)	2 ml
0.5 %* SDS ( <u>sodium dodecyl sulfate</u> )	20 %	1:40 (or 1:10 for 2%)	2.5 ml (10 ml for 2%)	25 ml (100 ml for 2%)
Deionized water			67.3 ml (59.8 for 2%)	673 ml (598 ml for 2%)

- 2% SDS if blood is collected out of the US and being brought back, as per USDA permit.

Use 4 parts lysis buffer to 1 part blood. Aliquot 0.5 ml per 1.5 ml tube and add up to 1 full hematocrit tube of whole blood, mix immediately by shaking and inverting and store at room temp. (~20°C) for up to a year (or more). NOTE: Use heparinized hematocrit tubes!

This collection buffer will solidify at 4°C, due to the SDS. If this happens, warm to room temperature before using. Store all reagents at room temperature.

1 M Tris pH 8.0 (100 ml of stock) Dissolve 12.11 g of TRIZMA base (F.W. 121.1) in 80 ml water, adjust pH to 8.0 with HCl, then bring final volume to 100 ml. (approximately 6 ml per 100 ml Tris).

**Or buy it from Fisher Scientific (cat# BP1758-500 for 500 ml; -100 for 100 ml )**

500 mM EDTA (Sodium ethylenediamine tetraacetic acid F.W. 372.2) pH 8.0 (100 ml of stock)

Dissolve 18.61 g of EDTA in 70 ml of water. Adjust pH to 8.0 with ~ 5 ml of 10 M NaOH. Add water to 100 ml.. \*\*Note: EDTA will not go into solution until it is at or near pH 8. You can also add NaOH pellets, a bit at a time until dissolved, but this is a bit more risky. You need ~2.5 gm of NaOH pellets per 100 ml of solution.

**OR buy it from Fisher Scientific (cat# BP2482 -500 for 500 ml, -100 for 100 ml )**

5 M NaCl

29.2 g NaCl

Water to 100 ml

(heating the water first in the microwave helps this go into solution)

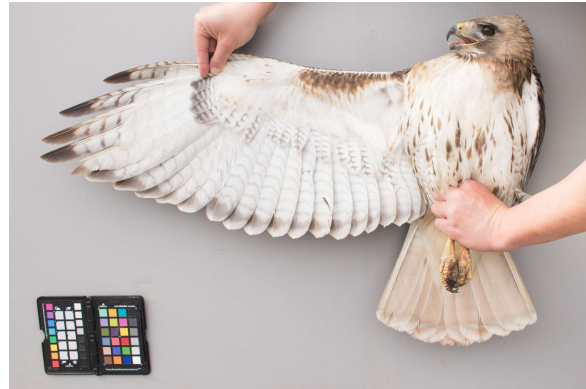
**OR buy it from Fisher Scientific (Cat# NC9846712)**

**20% SDS (sodium dodecyl sulfate) solution from Fisher Scientific (cat#BP1311) .**

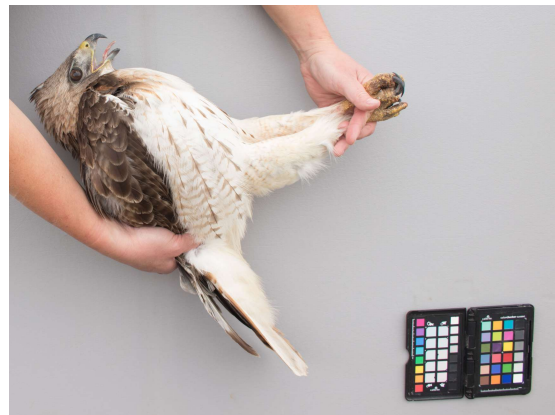
## Appendix 2 – Example photos



a. Left: ventral surface of bird's whole body and tail with left wing spread.  
b. Same as above, with right wing spread.

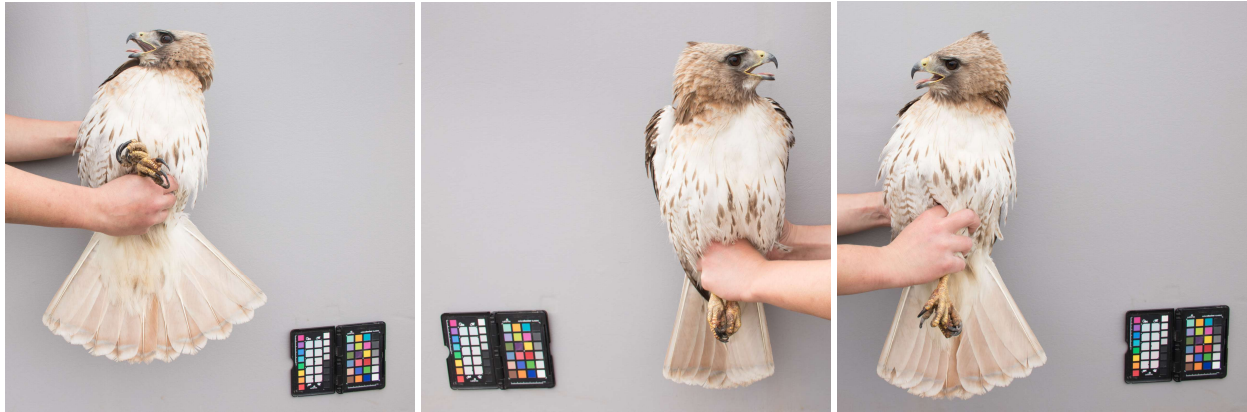


c. Dorsal surface of bird's whole body and tail with left wing spread.  
d. Same as above, with right wing spread.



e. Dorsal surface of spread tail including rump and upper tail coverts  
f. Entirety of leggings taken from the side





f. Ventral surface of tail including under tail coverts

g. Bird's head in profile, left side

h. Bird's head in profile, right side

### Appendix 3 - Morphometrics



a. Left: footpad measurement

b. Right: hallux measurement

c. Bottom left: culmen

d. Bottom right: total bill