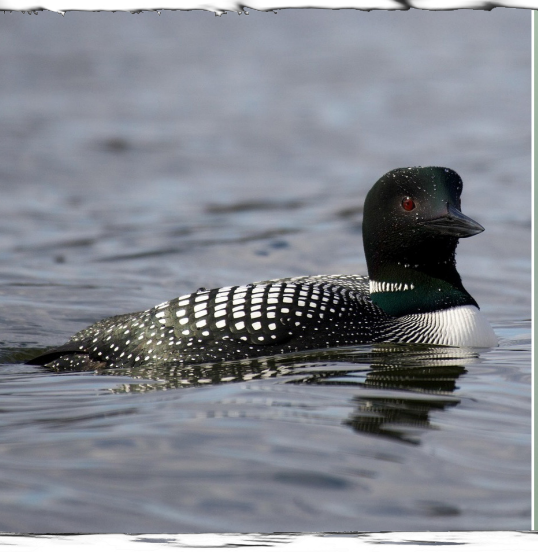
Bird Field Sampling Methods



BIRD FIELD SAMPLING METHODS

Collection, Storage and Analyses of Avian Tissue Samples

December 2025

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Biodiversity Research Institute (BRI) is a 501(c)3 nonprofit organization located in Portland, Maine, USA. Founded in 1998, BRI is dedicated toward supporting global health through collaborative ecological research, assessment of ecosystem health, improving environmental awareness, and informing science-based decision making.

For further questions or clarifications, or if you are interested in collaborating, feel free to contact Christine Gardiner by e-mail or visit our website for more information:

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1.0 Overview

Sampling of birds for blood, feathers, and other tissues can be informative for various aspects of diet, contaminants, exposure, movements, and health. The specific tissues chosen for these endpoints, however, will vary based on the life history of the focal species, season of sampling, and the research objective(s) to be addressed. For example, a songbird that breeds in wetlands and overwinters in other habitat types may have different contaminants in blood if sampled during summer versus winter. Further, at any given time, contaminants in blood may be wholly different than those sampled in feathers, as feathers are metabolically inactive tissues that contain contaminants present in the bloodstream at the time those feathers were grown while blood contaminant levels are generally representative of relatively recent dietary uptake.

Here, we outline a suite of common research questions for birds relating to sampling of blood, feathers, and other tissues. For each of these research questions, the specific tissue to be sampled, and the means of sampling, will vary. Feathers are metabolically fixed, so contaminant or stable isotope measurements will not change based on time of sampling for a single feather. In blood, however, the various components of blood (plasma, red blood cells, and white blood cells) all change at certain intervals as cells or proteins are replaced. These turnover rates impact the ability to interpret contaminant and isotope results, for example with a short half-life, plasma may be representative of contaminants and diet within the past few days, while whole blood may be a longer period of multiple weeks.

Another consideration is feather generations. Incomplete molts will result in a mix of feather generations that may have been grown at different times and in different locations. If distinct feather generations can be identified, then there may be potential for sampling of different time periods within the same individual, such as in migratory species where contaminant exposure may differ among sites or regions, for example. If this sort of feather variation does exist, then it is a possible source of error if not accounted for. It is therefore highly important to know molt cycles of focal species and sample specific feather groups that can be interpreted for the specific research question. See Appendices 1a-c for more details.

The Tropical Research for Avian Conservation and Ecotoxicology (TRACE) initiative is an international collaborative research network that generates scientific knowledge to inform tropical bird conservation through ecotoxicological monitoring. TRACE grew from more than a decade of bird sampling research by BRI throughout Central America and the West Indies and now supports and welcomes direct collaboration with researchers worldwide, especially students based in tropical institutions, that collect whole blood, feathers, or eggs from tropical birds. For more information, visit our website:

www.briwildlife.org/trace/

2.0 Objectives

BRI's protocols are designed to standardize sample collection, processing, and shipment to laboratories, and to train avian capture field crews and lab technician staff in sample collection and processing. Chosen analyses will vary depending on the specific objectives of a given study. The protocols and procedures described in this document enable BRI staff and trainees to (1) identify sampling equipment and supply needs, (2) conduct standardized collection, processing, and shipment of samples, and (3) maintain accurate field records of samples collected and shipped to laboratories.

Analyses of bird tissue samples can be used to address a diverse array of research questions including those concerning overall individual bird health, levels and physiological impacts of contaminant exposure, diet, movement, and population demographics. (See Appendix 1b for further description in addition to below.)

(1) Overall general health condition of the bird:

a. Complete Blood Count (CBC)
b. Chemistry Panel
c. Corticosteroids
d. Immunology
e. Presence of Fecal Parasites
f. Virology
g. Aspergillosis
h. West Nile Virus
i. Eastern/Western/Venezuelan Equine Encephalitis (EEE, WEE, VEE)

(2) Body burden of contaminants:

a. Trace Elements
b. Heavy Metals
c. Mercury
d. Organochlorinated Pesticides
e. Polychlorinated Biphenyls (PCBs)
f. Polybrominated Diphenyl Ethers (PBDEs)
g. Polycyclic Aromatic Hydrocarbons (PAHs)
h. Chemical dispersant (e.g., Corexit)

(3) Physiological impacts of contaminant exposure

a. Oxidative blood cell damage from contaminant exposure leading to hemolytic anemia:

i. Packed Cell Volume (PCV)
ii. Total Protein (TP)
iii. Heinz Body Analysis
iv. Hemoglobin (HgB), Ferritin, and Haptoglobin

(4) Genetic profiling:

a. Genetic sexing (sex specific difference in contaminant levels, population modelling)
b. Genoscape mapping

2.1 Basic Sample Collection Supplies

A suggested overall list of supplies should include the following:

- [ ] Dropcloth for working surface to lay out supplies and label/process samples.
- [ ] Clean towels or cloths for covering eyes/head and controlling wings/legs
- [ ] Bird bags for containing birds prior to processing and/or weighing bird
- [ ] Calibrated scale to weigh bird
- [ ] Alcohol-soaked cotton balls
- [ ] Critocaps® for sealing hematocrit tubes
- [ ] Heparin (1000U) – field supply in red top tube
- [ ] Cotton balls (dry)
- [ ] Syringes of varying sizes, depending on size of bird: 0.5cc insulin, 1cc TB, 3cc, 6cc, 12cc, and 20cc syringes or butterfly catheters for larger birds
- [ ] Needles of varying sizes: 21-27 gauge
- [ ] Red Hct tubes (heparin = anticoagulant) or manually pre-heparinized Blue Hct tubes
- [ ] Vacutainers of appropriate size for bird that is being sampled & for hematocrit tubes: red-tops, green tops
- [ ] Gauze pads (optional)
- [ ] "Kwik-Stop" powder
- [ ] Empty Ziploc bags (qt. and gal. sized)
- [ ] Sharps/biohazard container
- [ ] Fine point sharpies and pencils
- [ ] Coolers and ice packs; newspaper to separate from samples and/or seal in plastic bag
- [ ] Clipboard and Data Forms
- [ ] Banding supplies
- [ ] Federal and appropriate State collection and possession permits
- [ ] Storage container appropriate for volume of sampling supplies and mode of transportation
- [ ] First Aid Kit, radios, personal items, food
- [ ] Safety plans of vessel and SPOT tracker if appropriate

Supplies specific to a given analysis or study:

Analysis/Study	Additional Supply Item
Genetics	Filter paper, genetics cards, or cotton balls/cosmetic pads
CBC & Heinz Bodies	Frosted microscope slides
CBC & Heinz Bodies	Slide file box (10-25 slides)
Heinz Bodies	New Methylene Blue stain in Eppendorf tubes: Eppendorfs containing the NMB stain should be made prior to going to the field. Use a Hamilton syringe or pipette to measure 25uL of NMB stain for each Eppendorf tube. Thus, the only step to do in the field is to add the blood.
Heinz Bodies	Purple Top Microvette
Heinz Bodies	25uL pipette and pipette tip
Mercury, Isotopes	Paper envelope for feathers
Mercury, Isotopes	Sterile sample bags for feathers in coin envelopes
PAH	Aluminum foil for feathers
Migration, dispersal, tracking	Satellite or radio transmitters and receiver

Note: Sampling supplies in the field kit should be double-checked before going in the field to make sure there are enough. After every sampling trip, designate one team member to clean the field kit, bring samples to the lab, and replenish supplies as needed. For more supplies, notify BRI Field Laboratory staff or Program Director.

3.0 Whole Blood Collection

The nonlethal collection and analysis of whole blood samples in birds is used to identify the recent dietary uptake of contaminants, such as mercury (Evers 2018), and various stable isotopes (i.e., δ13C, δ15N, δ34S) that allow for diet reconstruction and identifying migratory origins. Blood samples can be obtained through two venipuncture techniques: direct draw and piercing. Direct draw is typically used on larger birds, such as waterbirds and raptors, and allows for a greater volume of blood to be collected.

Piercing is more suitable for smaller birds, such as passerines. Although blood can be drawn from multiple locations on larger birds and can be determined by the biologist or veterinarian on site, BRI recommends drawing from the metatarsal (leg), jugular vein (neck) or cutaneous ulnar veins (wing; Figure 1). On smaller birds, blood should only be drawn from the cutaneous ulnar vein (Figure 2).

Micro-hematocrit capillary tubes are preferred for laboratory analyses, but require specific storage and shipping arrangements (see 8.0 Storage of Samples and 9.0 Shipment of Samples). If capillary tubes are unavailable, vacutainers are an adequate replacement and can store a larger sample volume.

Recommended sampling sites for a variety of species are listed below.

- ***Passerines:*** Blood can be collected by puncturing the **brachial or median metatarsal vein** and collecting blood directly into a microcapillary tube. Use a 29-30 gauge insulin needle/syringe to collect blood from the jugular; or alternatively, 25-27 gauge needle to puncture the vein and collect blood using a heparinized hematocrit (Hct) tube.
- ***Long-legged wading birds, waterfowl, raptors, etc.:*** The **medial metatarsal vein** is readily accessed in most long-legged species and raptors. The brachial and right jugular veins are also suitable. Needles of 23-27 gauge appropriate to the size of the bird.
- ***Pelicans, Skimmers, Common Loons, Frigatebird, etc.:*** Branches of the **medial or dorsal metatarsal vein** are the best sites. Needles of 21-27 gauge and syringes or 21-23 gauge butterfly catheters appropriate to the size of the bird. ***Note: thick plumage can make access to the jugular and brachial veins difficult. Feathers should not be plucked to locate the vein as this may tear the skin. Dampening or wetting the feathers with alcohol is sufficient to expose the skin.***

The volume of blood collected depends on the species being sampled and the purpose for which blood is required (Appendix 2; Table A3). The American Ornithologists' Union recommends that no more than 10-20% of the total blood volume (TBV) be collected (TBV for birds is approximately 6-8cc per 100 g body mass). For the purposes of BRI's research projects, **the maximum blood collection volumes should be no more than 0.8% of the bird's body weight** (Table A3).

IMPORTANT:

Note that the blood collection volumes listed in Table A3 are maximum values for apparently healthy individuals.

However, the Field Crew Leader should adjust the actual volume of blood extracted to match the physical condition of the bird in hand.

Great care must be taken to avoid hematoma and bleeding, especially in very small birds where the loss of a couple of extra drops of blood can represent a significant proportion of the circulating blood volume and potentially prove fatal.

To reduce the risk of hematoma formation, ensure that the bird is carefully restrained so it cannot struggle, causing the vein to tear. Use the finest needle possible for the bird's size (21-27 gauge, even up to 30 gauge in passerines) and apply gentle, consistent pressure with cotton for 1-3 minutes to encourage clotting and stop bleeding.

After 1-3 minutes of pressure, check the site for bleeding. If it is still oozing, reapply pressure for an additional minute. A small amount of "Kwik-Stop" powder can be applied to the venipuncture site via cotton swab to aid clotting if needed.

When the bleeding stops, handle the bird carefully so that the clot is not disrupted. Check puncture site again before releasing bird.

IMPORTANT:

RESIST THE TEMPTATION TO CHECK THE SITE FREQUENTLY, which requires lifting the cotton and reducing pressure on the wound. This will prolong healing time.

IMPORTANT:

- Contaminant analyses require blood to be stored in Lithium heparinized receptacles, while stable isotope analyses require blood to be stored in non-heparinized receptacles. For ease of sample storage and shipping, blood can also be dried on Whatman cards and used for both contaminant and stable isotope analyses (Perkins and Basu 2018; Barst et al. 2020).
- If importing samples from countries with concerns of Exotic Newcastle Disease (END) or the H5N1 subtype of Highly Pathogenic Avian Influenza (HPAI) to laboratories that are not BSL-2 certified, BRI strongly recommends blood collection via Whatman cards to avoid import/export restrictions. However, BRI is a BSL-2 certified laboratory and can accept whole blood via any receptacle.

Rule of thumb for the maximum volume of blood: blood extraction should never exceed 1% of a bird's total body weight (Fair et al. 2010). Approximately 10% of a bird's weight is blood (McGuill and Rowan 1989); therefore, up to 10% of the volume of blood in a bird can be drawn. For example, a 5000 g adult Common Loon (Gavia immer) has approximately 500 g of blood. From this, a maximum of 50 g of blood can be taken (1 g = 1 mL). A 20 g songbird has 2 g of blood; therefore, a maximum of 0.2 mL or 200 µL of blood can be collected.

IMPORTANT:

BRI does not recommend collecting the maximum amount in any species. Specific blood sampling procedures for different taxonomic groups are reviewed in sections 3.2 through 3.4 and Appendix 2.

3.1 Supplies for Venipuncture

General Checklist	
Item	**Purpose**
☐ Datasheet	For recording data
☐ Copy of Sampling Protocol(s)	This ***Bird Field Methods Sampling SOP*** and project-specific sampling protocols
☐ Copies of all required permits	For example, state and federal banding and scientific collection permits
☐ Portable cooler w/ ice packs or Portable freezer	For storing collected blood while in the field
☐ Isopropyl alcohol pads or spray bottle	For clearing and sterilizing the puncture site
☐ Dry cotton balls or pads	For stopping bleeding at the puncture site post collection. Can also be used as an additional blood sample for some analyses
☐ Ultra-fine permanent marker (eg. Sharpie®)	For labelling samples. ***Note that some markers contain contaminants such as PFAS.***
☐ Portable Sharps Container	For containing used needles while in the field

For Direct Draw Using Manual Syringe (large birds only)	
Item	**Purpose**
☐ 3cc syringes with 21-25 gauge needles	Needle sizes will be species dependent
☐ 6 mL blood collection tube (e.g., "Green top" Lithium heparinized Vacutainers®)	For contaminant analyses
☐ 6 mL sterile blood collection tube (e.g., Vacutainers®)	For stable isotope analyses
☐ Micro-hematocrit capillary tubes	For use with Whatman® card blood storage technique
☐ Paper coin envelope	For storing cotton pad blood sample

For Direct Draw Using a Butterfly Needle (large birds only)	
Item	Purpose
☐ 21-25 gauge butterfly needles w/ 7 inch tubing	Needle sizes will be species dependent
☐ Blood collection tube holders	Can be re-used
☐ 6 mL blood collection tube (e.g., "Green top" Lithium heparinized Vacutainers®)	For contaminant analyses
☐ 6 mL sterile blood collection tube (e.g., Vacutainers®)	For stable isotope analyses
☐ Paper coin envelope	For storing cotton pad blood sample

For Piercing Procedure	
Item	Purpose
☐ 22-27 gauge hypodermic needles	Needle sizes will be species dependent. In general, 22-25 gauge for large birds and 26-27 for smaller birds
☐ Heparinized micro-hematocrit capillary tubes	For contaminant analyses
☐ Sterile micro-hematocrit capillary tubes	For stable isotope analyses
☐ Capillary tube sealing clay (e.g., Leica Microsystems Critoseal®) or other tube closure (e.g., Critocaps®)	For sealing capillary tubes
☐ 6 mL archive Vacutainers®	For storage of sealed capillary tubes

For Whatman® Card Storage	
Item	Purpose
☐ Whatman® Proteinsaver Card	For whole blood sample collection
☐ Silica gel desiccant packets	For keeping Whatman® card samples dry
☐ Calipers	For measuring the amount of blood in capillary tubes before deposition on Whatman® cards
☐ Sandwich-size, sealable plastic bag (e.g., Ziploc®)	For storing Whatman® card samples
☐ 6 mL archive Vacutainers®	For storage of sealed capillary tubes

3.2 Direct Draw Procedure Using a Manual Syringe (for Large Birds ONLY)

Typically, 1 to 10 mL of whole blood is collected from larger birds. A minimum of 0.5 mL should be collected for mercury analysis. For archival purposes, BRI recommends the collection of 1 mL, which can be useful for additional contaminant analyses.

Follow this procedure:

1. Determine the volume of blood to be collected based upon mass guidelines and intended analyses.
2. Determine species appropriate needle size.
3. Lay sampling supplies out on a clean, level surface within easy reach (including extras of needles, tubes, cotton, etc.).
4. Generally, a second person will be required to hold the bird securely in a position that: a) restricts movement and protects against injury (to the bird and/or the sampling team); and b) gives the blood-sampler comfortable access to the chosen vein. For some species, a hood or other head covering can help to keep the bird calm and protect against injuries from the bill.
5. Locate the desired vein. This method can be used for blood draws from the jugular, subcutaneous ulnar, or metatarsal vein.
    - **Jugular vein** – located above the shoulder, near the front of the neck.
    - **Ulnar vein** – located at the elbow joint on the underside of the wing.
    - **Metatarsal vein** – located on the inside of the leg at the intertarsal joint. This vein can be difficult to see through the thickened skin of the leg.
6. Sterilize the collection area with an isopropyl alcohol wipe or spray.
7. Uncap the needle.
8. After the alcohol has evaporated, insert the needle parallel to the vein with the bevel facing up.
    - **Jugular vein** – insert the needle from the top down, approaching from the head with the needle pointing down toward the body.
    - **Ulnar vein** – insert the needle from the outside in, approaching with the needle pointing toward the body.
    - **Metatarsal vein** – insert the needle from the bottom up, approaching from the foot with the needle pointing toward the body.
9. Draw the amount of whole blood calculated in Step 1 (Figure 1). ***Note, if blood flow slows or stops, try gently adjusting the angle of the needle; often, angling slightly downward moves the bevel of the needle away from the vein wall and restores flow.***
10. Gently exit the vein and hold pressure on the collection area with a fresh cotton ball or pad until bleeding has stopped (~10 seconds).
11. If using Whatman® cards, use the syringe to partially fill 1 or more capillary tubes and follow the steps in Section 3.5 Whatman® Card Blood Storage Procedure before continuing to Step 12.
12. Inject drawn blood into "Green top" Lithium heparinized Vacutainer(s)® or sterile blood Vacutainer(s)® depending on desired analysis (see Section 6.0 Procedural Tables).

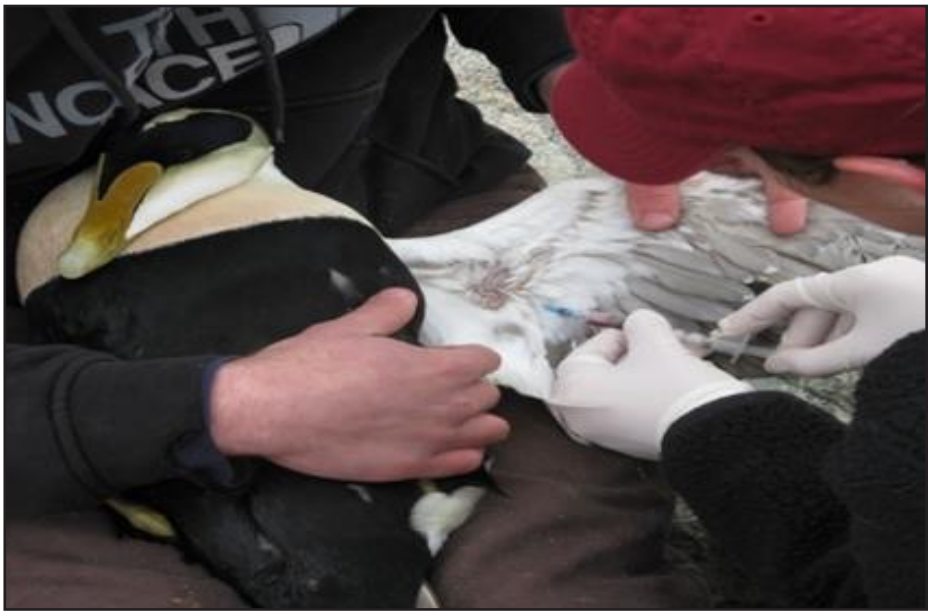

Figure 1. Direct draw blood collection from the cutaneous ulnar vein using a manual syringe.

IMPORTANT:

Avoid filling Vacutainers® completely as the top will pop off under pressure. BRI recommends filling Vacutainers® from one-half (1/2) to two-thirds (2/3) full.

13. Label each Vacutainer® with appropriate metadata using a permanent marker following the guidelines in Section 7.0 Metadata Requirements.
14. Place the cotton pad used to stop the bleeding at the puncture site in a paper coin envelope and label the envelope as described in Section 7.0 Metadata Requirements.
15. Store blood samples in a cooler with ice packs immediately in the field and transfer to a freezer within 24 hours of collection (see Section 8.0 Sample Storage).
16. Dispose of used needles and excess wrapping in Sharps container.

IMPORTANT:

All samples **MUST** have a unique sample identification number (I.D. #) that is labeled correctly and legibly.

Figure 2. The jugular vein of a Merlin, cleared of feathers and sterilized with alcohol in preparation for direct blood draw with a manual syringe. (Photo credit: Edward Jenkins)

3.3 Direct Draw Procedure Using a Butterfly Needle (for Large Birds ONLY)

Follow this procedure:

1. Determine the volume of blood to be collected based upon mass guidelines and intended analyses.
2. Determine species appropriate needle size.
3. Lay sampling supplies out on a clean, level surface within easy reach (including extras of needles, tubes, cotton, etc.).
4. Attach the butterfly needle to the blood collection tube holder ("barrel").
5. Locate the desired vein, either the subcutaneous ulnar vein or the metatarsal vein, and sterilize the collection area using an alcohol wipe or spray.
6. After the alcohol has evaporated, remove the plastic guard on the butterfly end of the needle.
7. Holding the needle in line with the vein with the bevel facing up (away from the bone) penetrate the skin and enter the vein in one smooth motion. Enter the vein so that the point of the needle is facing the bird.

> **IMPORTANT:** Do not go through both vein walls, just the top one. A small amount of blood should start to enter the tube of the butterfly needle.

8. Holding the barrel securely, insert a "Green top" Lithium heparinized Vacutainer® or sterile blood Vacutainer® (depending on desired analysis, see Section 6.0 Procedural Tables) into the large end of the barrel penetrating the stopper with the rubber coated needle. Blood should flow into the Vacutainer®. ***Note: if blood flow slows or stops, try gently adjusting the angle of the needle; often, adjusting the angle slightly downward moves the bevel of the needle away from the vein wall and restores flow.***
9. When the Vacutainer® is filled to the desired amount, remove from the barrel and gently invert the Vacutainer® several times to ensure the blood mixes with the heparin (Figure 3).

> **IMPORTANT:** If blood stops flowing to the Vacutainer before the desired/expected amount, another Vacutainer may be required. Vacuum in various Vacutainers may vary.

10. When all Vacutainer(s)® have been filled and removed from the barrel, hold a cotton pad over the needle and gently withdraw the needle from the vein.
11. Apply pressure to the puncture point with the cotton pad until the bleeding has stopped.
12. Use the blood in the tubing of the butterfly needle to fill four capillary tubes ½ to ¾ full.

> **IMPORTANT:** If using Whatman cards, select one of the capillary tubes and follow the steps in Section 3.5 Whatman Card Blood Storage Procedure before continuing to Step 13.

13. Seal capillary tubes using sealing clay or other closure (Critoseal®, Critocaps®, etc.) and place into a 6 mL archive Vacutainer®.
14. Label each Vacutainer® with appropriate metadata using a permanent marker following the guidelines in Section 7.0 Metadata Requirements.

15. Place cotton pad into a paper coin envelope and label the envelope as described in Section 7.0 Metadata Requirements.
16. Store blood samples in a cooler with ice packs immediately in the field and transfer to a freezer within 24 hours of collection (see Section 8.0 Storage of Samples).
17. Dispose of used needles and excess wrapping in Sharps container.

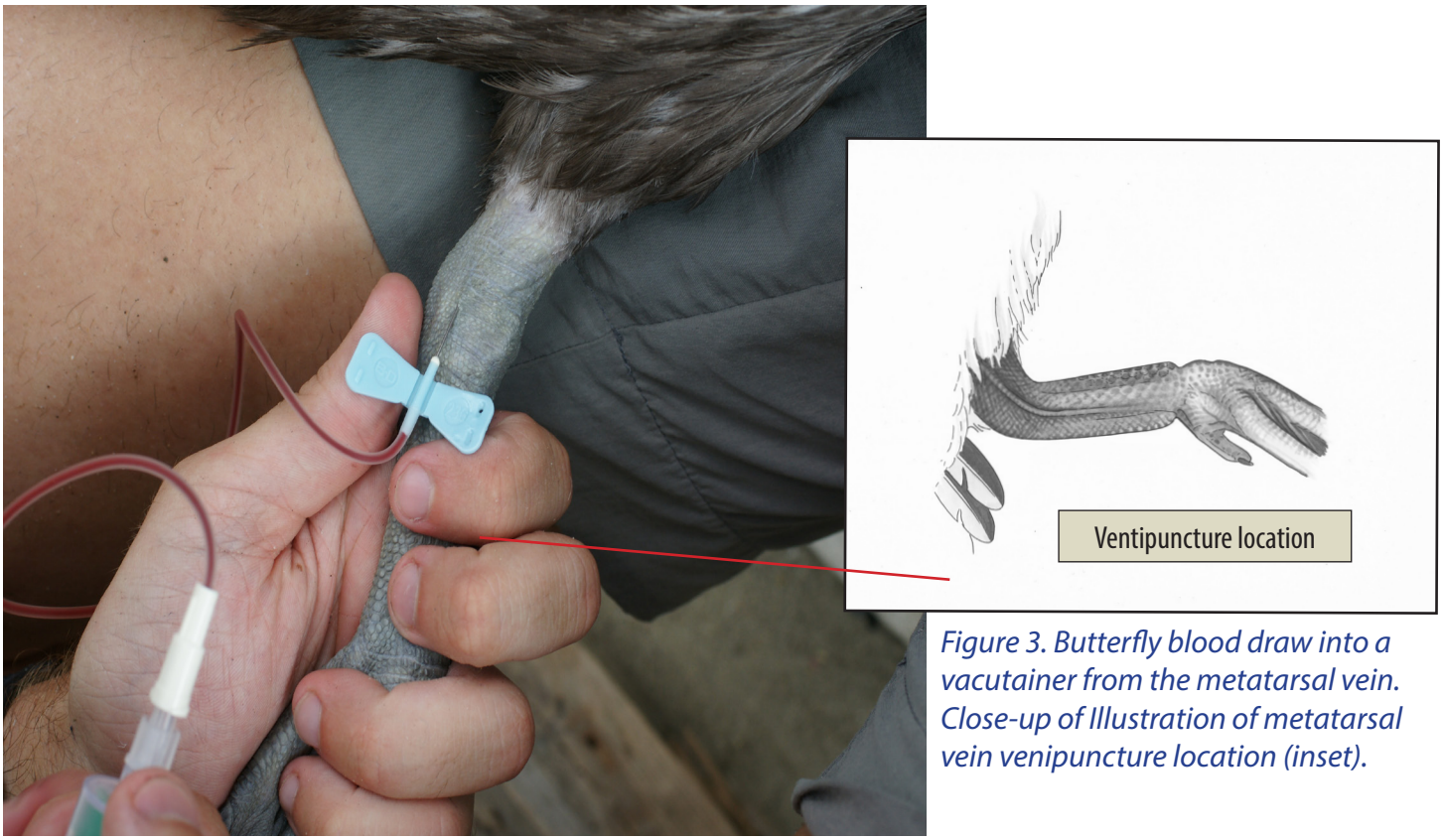

Figure 3. Butterfly blood draw into a vacutainer from the metatarsal vein. Close-up of Illustration of metatarsal vein venipuncture location (inset).

3.4 Piercing Procedure

Follow this procedure:

1. Determine the amount of blood to be collected based upon mass guidelines and intended analyses.
2. Determine species appropriate needle size.
3. Lay sampling supplies out on a clean, level surface within easy reach (including extras of needles, tubes, cotton, etc.).
4. For smaller birds, the blood-sampler can hold the bird in their non dominant hand in a modified bander's grip exposing the underside of the bird. The index and middle finger can be used to extend and hold the wing open, pinching at the carpal joint, to expose the cutaneous ulnar vein. For larger birds, a second person can hold the bird securely in one hand and extend the wing with the other.

IMPORTANT:
Ensure that the grip on the bird and the wing is secure enough that it cannot pull the wing away during sampling.

5. Separate feathers to locate the cutaneous ulnar vein in the wing (Figure 5). ***Note: if the vein appears small and/or constricted, applying gentle pressure on the vein above the puncture site (proximal to the body) can raise and plump the vein to make it easier to puncture.***
6. Sterilize the collection area with an isopropyl alcohol wipe and ensure that the collection area is free of feathers as these will quickly clot the blood.
7. Uncap needle.
8. After the alcohol has fully evaporated, position the needle parallel to the vein, bevel up, and gently prick the vein. Be careful not to go through both vein walls, just the top one. ***Note: if the alcohol has not completely dried, it can disperse the blood and make it difficult to collect.***
9. Gently exit the vein and allow blood to pool/bead; this usually happens very quickly.
10. Collect blood by placing a Lithium heparinized or sterile capillary tube (depending on desired analysis, see 6.0 Procedural Tables) below the pooled blood. Holding the tube at a downward angle will allow the blood to be more easily pulled into the tube via capillary action.
11. Collect the number of capillary tubes determined in Step 1, filling each ½ to ¾ full (Figure 6).

IMPORTANT:
Watch the progress of the blood in the tube carefully. Some will fill very quickly and must be changed out rapidly while others will fill very slowly, requiring a steady hand and patience. If blood is no longer flowing into the tube, you can try gently bending and/or flexing the wing to encourage blood flow. *Note: if the bird remains in good condition, additional blood can be sampled from the other wing if blood flow stops before reaching the required sample volume.*

12. Apply pressure to the collection area with a fresh cotton ball or pad to stop bleeding and reduce hematoma (~10 seconds).
13. If using Whatman cards, follow the steps in Section 3.5 Whatman Card Blood Storage Procedure; otherwise, continue to Step 14.

14. Seal capillary tubes using sealing clay or other closure (Critoseal, Critocaps, etc.).
15. Place capillary tubes in a 6 mL archive Vacutainer® and properly label with appropriate metadata using a permanent marker (see Section 7.0 Metadata Requirements).
16. Store capillary tube blood samples in a cooler with ice packs immediately in the field and transfer to a freezer within 24 hours of collection (see Section 8.0 Sample Storage).
17. Dispose of used needles and excess wrapping in Sharps container.

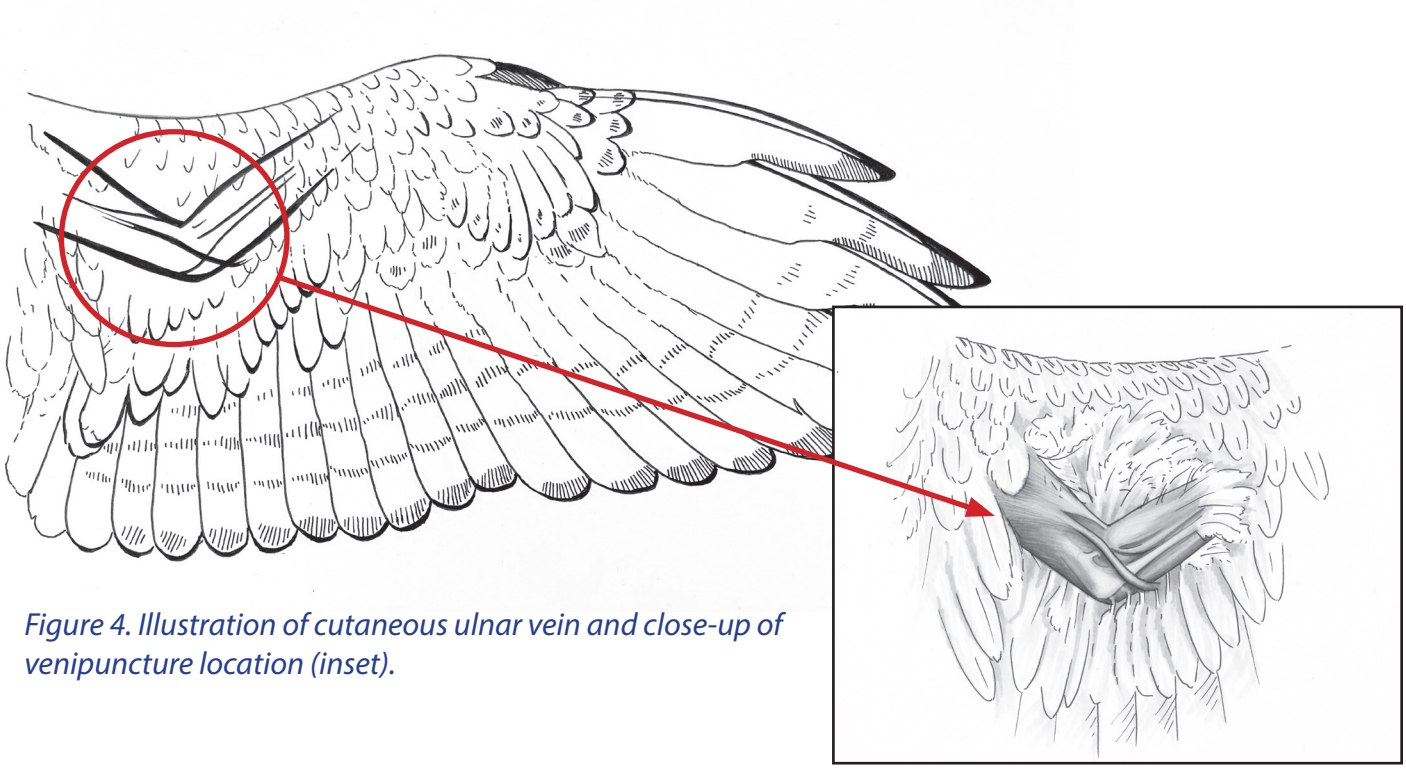

Figure 4. Illustration of cutaneous ulnar vein and close-up of venipuncture location (inset).

Figure 5. A "bead" of blood forming after puncturing the subcutaneous ulnar vein. The bird is held in a modified bander's grip by the blood-sampler. (Photo: Lauren diBiccari)

Figure 6 Approaching the subcutaneous ulnar vein of an Upland Sandpiper holding the needle parallel to the vein and bevel up. The area has been cleared of feathers and sterilized with alcohol. (Photo: Lauren diBiccari)

3.5 Whatman® Card Blood Storage Procedure

1. Use calipers to measure the amount of blood in the capillary tube in millimeters. **Record this value.**
2. Use the capillary tube to saturate a circle on the Whatman® proteinsaver card (Figure 7).

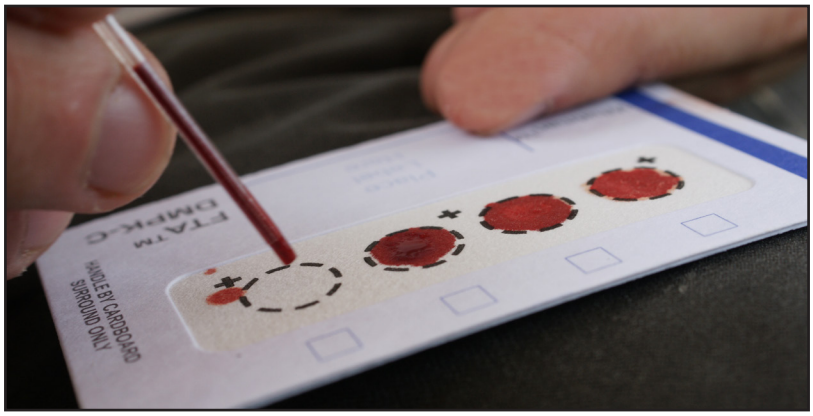

Figure 7. Filling Whatman® proteinsaver card circles with blood from a capillary tube.

3. If using one capillary tube to fill two circles, measure the amount of blood remaining in the tube after the first circle was filled before filling the second circle. **Record this value.**
4. Allow the Whatman® card to air dry before labelling with appropriate metadata (see Section 7.0 Metadata Requirements), including the amount of blood deposited in each circle, using a permanent marker (Figure 8).

Calculating blood volume (µL) from caliper measurements:

- For a 70µL, 75mm capillary tube, multiply the caliper measurement by a conversion factor of 0.933µL/mm to calculate the blood volume in microliters (µL).
- If two circles were filled from one tube, calculate the amount of blood deposited in the first circle by subtracting the amount recorded in Step 3 from the initial amount recorded in Step 1 and then multiplying by 0.933µL/mm.

5. Store card in a plastic bag with desiccant packets.

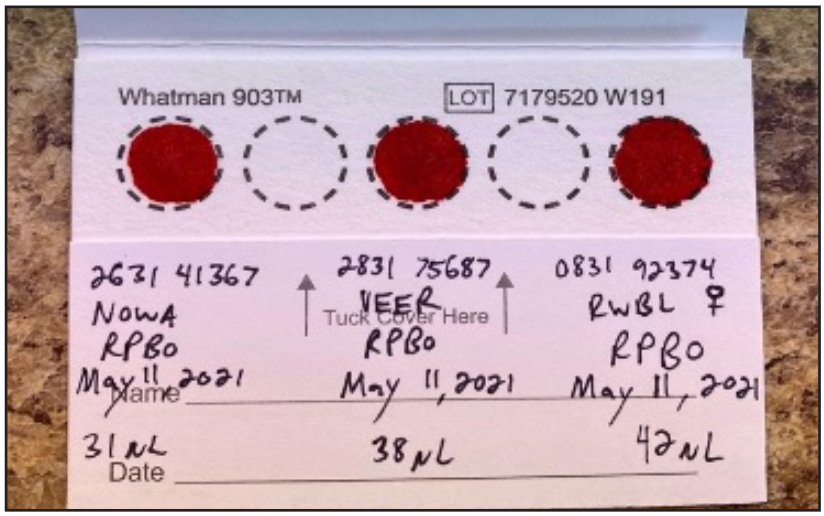

Figure 8. Completed Whatman® card with appropriate metadata: Unique ID #, species code, location code, date, and blood volume.

4.0 Feather Collection

The collection of feather samples is useful in identifying the body burden of heavy metals, such as mercury, because methylmercury is typically transferred to feathers during feather growth (Evers 2018). The symmetrical collection of two feathers is useful for measuring fluctuating asymmetry. Feathers are also commonly analyzed for stable isotopes to provide insights on natal or molting origins, trophic level, and dietary habits. Feather samples may also be used for genetic analyses as when feathers are plucked, a small of amount of skin cells remain attached to the calamus. As different feathers may be molted and regrown during different times of the year, study objectives should inform feather selection.

- Any feather can be analyzed for mercury, but secondary flight feathers, tail feathers (rectrices), back feathers, and flank feathers are useful standards depending on the target species.
- For larger birds, such as seabirds, BRI usually collects two second secondaries (S2), two outer tail feathers (rectrices, R6), and 10 flank feathers, whenever possible (Figure 9).
- For migratory raptors and passerines, the removal of flight feathers may have a negative impact on flight efficiency, particularly during migration, so only rectrices and flank feathers should be collected in these taxa.
- Back feathers are also commonly sampled from raptors because differences in feather wear and age are relatively conspicuous.
- Flank feathers are especially useful when conducting retrospective analyses of methylmercury concentrations in museum specimens, since museum curators generally do not approve of the removal of flight and tail feathers.

Since methylmercury concentrations comprise 95% or more of the total mercury in feathers, analysis of total mercury, rather than methylmercury, is typically sufficient for evaluating mercury exposure and risk (Evers 2018). However, feathers from museum specimens are likely compromised by mercury-based preservatives routinely used by museum curators. To avoid such interference from external mercury contamination, all feathers from museum specimens need to be analyzed for methylmercury concentrations (Perkins et al. 2019).

To determine the location of the second secondary feather (S2), examine where the primaries and secondaries meet in the middle of the wing (if difficult to determine, most birds have 10 primaries, grebes have 11, and songbirds have 9 or 10). In larger birds, clip the S2 feather from each wing (i.e., two total feathers) along the calamus (shaft) above the superior umbilicus (Figure 9).

For some species, secondaries may not be feasible or recommended to collect and therefore symmetrical collection of outer tail feathers (R6) is recommended. R6 and flank feathers can be collected by plucking for songbirds. For larger birds, R6 feathers may need to be cut.

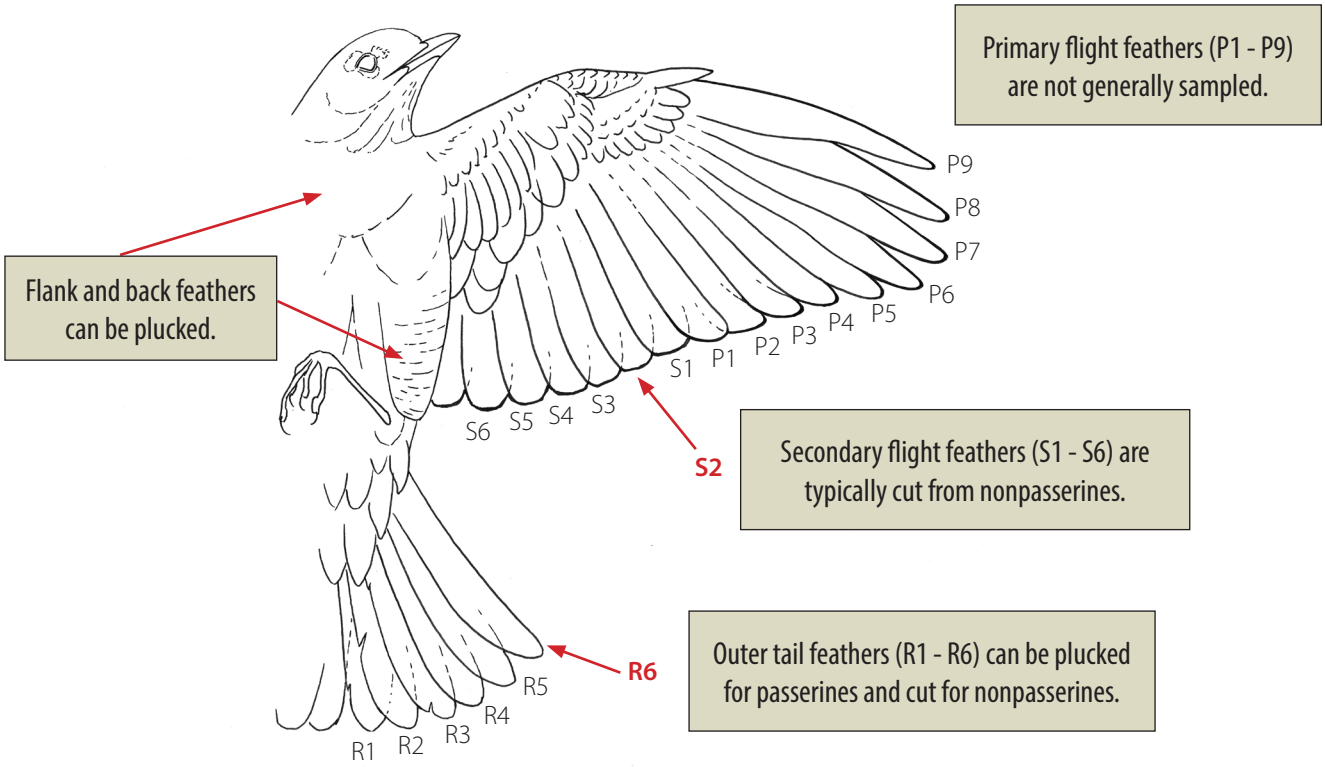

Figure 9. Illustration of standardized feather sampling locations on a typical passerine.

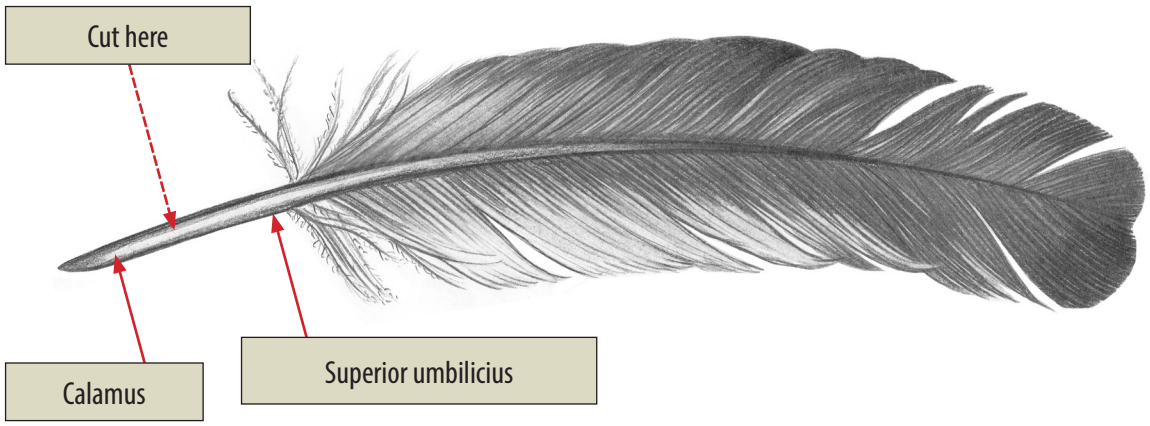

Figure 10. Standardized field clipping of secondary flight feathers.

To pluck feathers, pinch the calamus firmly, relatively close to the base, and pull gently away from the skin. Place samples of different feather types into separate paper coin envelopes and label with appropriate metadata using a permanent marker (see 7.0 Metadata Requirements). If collecting feathers for genetic analyses, be careful not to touch the tip of the calamus when plucking or placing the feathers in their envelopes.

4.1 Supplies for Feather Collection

- ☐ Small cutting pliers
- ☐ $3^{3}/_{8}$ inch x 6 inch paper coin envelopes
- ☐ Ultra-fine Sharpie™ permanent marker
- ☐ Sandwich-size Ziploc™ plastic bags

5.0 Egg Collection

The collection of egg samples for contaminant analyses, especially mercury, is useful in identifying female body burden, since methylmercury can be transferred to developing eggs during the laying period (Heinz et al. 2010). BRI **typically collects only nonviable eggs**, but collection of viable eggs (with permits) follows the same procedure.

Procedure for collecting eggs:

1. Whole eggs are often collected when it is certain they have failed. If the egg is cold or putrid smelling, mark it with an "X" using a permanent marker.
2. Return to the nest the following day. If the "X" is still in the same position as you left it the previous day—indicating the egg has not been turned in 24 hours—collect the egg.
3. Wrap the egg in standard-weight aluminum foil to minimze risk of external contamination.
4. Label a waterproof sample tag with appropriate metadata (see 7.0 Metadata Requirements) and place it with the egg inside a plastic bag.
5. While in the field, store egg samples in a cooler with ice packs, then transfer to a refrigerator or freezer as soon as possible (see 8.0 Storage of Samples).

5.1 Supplies for Egg Collection

- ☐ Sandwich-size Ziploc™ plastic bags
- ☐ Waterproof sample tags (i.e., Rite-in-the Rain™ pages)
- ☐ Ultra-fine Sharpie™ permanent marker
- ☐ Standard-weight aluminum foil

6.0 Procedural Tables

Table 1. Tissue sampling procedural flowchart for mercury analysis. Tissue selection is dependent on the focal taxonomic group(s) and research objective(s).

Desired tissue	Collection method	Suitable focal taxa	Receptacle	Minimum Amount	Storage
Blood	Direct draw	Waterbirds, raptors	Lithium heparinized vacutainer	0.5 mL	Freezer
			Whatman card	1 card	Refrigerator or room temperature
	Piercing	All taxa	Lithium heparinized capillary tube	25 µL	Freezer
			Whatman card	1 card	Refrigerator or room temperature
Secondary feathers (S2)	Clipping	Waterbirds	Paper coin envelope	2 symmetrical feathers	Freezer
Tail feathers (R6)	Clipping	Waterbirds, raptors	Paper coin envelope	2 symmetrical feathers	Refrigerator or room temperature
	Plucking	Songbirds	Paper coin envelope	2 symmetrical feathers	Refrigerator or room temperature
Flank feathers	Plucking	All taxa	Paper coin envelope	10 feathers	Refrigerator or room temperature
Back feathers	Plucking	Raptors	Paper coin envelope	5 feathers	Refrigerator or room temperature
Eggs	NA	All taxa	Plastic bag	1 egg	Freezer

Table 2. Tissue sampling procedural flowchart for stable isotope analysis. Tissue selection is dependent on the focal taxonomic group(s) and research objective(s).

Desired tissue	Collection method	Suitable focal taxa	Receptacle	Minimum Amount	Storage
Blood	Direct draw	Waterbirds, raptors	Sterile vacutainer	0.5 mL	Freezer
			Whatman card	1 card	Refrigerator or room temperature
	Piercing	All taxa	Sterile capillary tube	25 µL	Freezer
			Whatman card	1 card	Refrigerator or room temperature
Secondary feathers (S2)	Clipping	Waterbirds	Paper coin envelope	2 symmetrical feathers	Freezer
Tail feathers (R6)	Clipping	Waterbirds, raptors	Paper coin envelope	2 symmetrical feathers	Refrigerator or room temperature
	Plucking	Songbirds	Paper coin envelope	2 symmetrical feathers	Refrigerator or room temperature
Flank feathers	Plucking	All taxa	Paper coin envelope	10 feathers	Refrigerator or room temperature
Back feathers	Plucking	Raptors	Paper coin envelope	5 feathers	Refrigerator or room temperature
Eggs	N/A	All taxa	Plastic bag	1 egg	Freezer

7.0 Metadata Requirements

Please clearly print at least the following information on each archive vial, paper coin envelope, or sample tag using a permanent marker:

- ☐ Band number, or other unique identification number
- ☐ Species common name or standardized species code (please also include species Latin name if collected outside of the United States). It would be helpful to update taxonomic names in line with the eBird/Clements checklist: (https://www.birds.cornell.edu/clementschecklist/introduction/updateindex/october-2024/)
- ☐ Date (please use letters for the month instead of numbers, i.e., Mar 11, 2025)
- ☐ Sampling location name, state or province, and country
- ☐ Feather type, if applicable
- ☐ Age and sex of individual (i.e., "After Second Year, Male"), if applicable

Please organize all metadata using the preferred templates.

IMPORTANT:

Identification number for unbanded birds:

If a bird is unable to be banded in the field, the individual and the corresponding sample must be given a unique identification number (i.e., "Organization-Country-Unbanded0001"), which should be available on both the sample receptacle and data sheet(s). For all birds sampled in the US and Canada, use the format UN-2 letter state or province - 2 letter year - 4 letter species - 4 digit identifier, e.g., UNME25COLO0001.

Sending samples to BRI or TRACE

If contributing samples to BRI or the Tropical Research for Avian Conservation and Ecotoxicology (TRACE) initiative for collaboration, at the earliest convenience, please also send any accompanying banding data for all sampled birds as an excel spreadsheet or .csv file.

Our preferred data management and banding system follows that of the MAPS and MoSI programs.

8.0 Storage of Samples

Please follow these requirements:

- Whole blood samples in capillary tubes or vacutainers should be immediately stored in a cooler with ice or ice packs in the field.

IMPORTANT:
Blood samples should then be transferred to a freezer ***AS SOON AS POSSIBLE***, or within 24 hours of collection, and should remain frozen until analysis. While heavy metals, such as mercury, in blood are stable, freezing samples prevents blood degradation. **Subsamples should be taken as soon as feasible after collection but before freezing**, e.g., plasma for PFAS analyses.

- Dried blood spots on Whatman cards should be stored with a desiccant packet in separate, sealed plastic bags (to reduce the influence of moisture). Ideally, these should be stored in a 4°C refrigerator (to prevent molding), but can also be stored at room temperature away from direct sunlight prior to shipping and analysis.

9.0 Shipment of Samples

If you are submitting samples to BRI or to the TRACE initiative for collaboration, thank you for contributing!

At least two weeks prior to shipping any samples, please complete the metadata submission form to allow sufficient time for BRI to file the proper permits. BRI will send a completed USFWS 3-177, USFWS MBTA, and a USDA 16-3 VS permit via email once the permits have been issued. After all other permits have been approved (see 9.1 Required Permits), please schedule a shipment with your carrier service of choice.

To package the tissue samples, please follow these instructions:

- Use a small cooler to secure and insulate all blood and egg samples during shipment.
- Place ice pack(s) inside the cooler to insulate the frozen blood and eggs.
- If archive vials are glass, or egg samples are included in the shipment, it is IMPORTANT to pad the samples with bubble wrap or newspaper inside the cooler to avoid breakage.
- Feathers and dried blood spots on Whatman cards do not need special packing and do not need to be kept cold (if shipped within 3–4 months after collection).
- Place the cooler in a cardboard box and fill the empty space with additional packing materials, such as bubble wrap or newspaper, to secure the cooler during shipment.
- Include a set of all necessary permits and forms at the top of the packing material before sealing the package with packing tape (see 9.1 Required Permits).
- Attach a second set of permit copies in a plastic pouch on the exterior of the package.

Please include the following details on the shipping label and package exterior:

Biodiversity Research Institute
276 Canco Road
Portland, Maine 04103, USA

WILDLIFE :: USFWS :: MBTA

EXTRA COPIES OF DOCUMENTS INSIDE BOX

IMPORTANT:
To avoid postal or customs delays, ship samples on a Monday or Tuesday, and never just before a federal holiday.

When asked by the shipping representative if you are shipping anything perishable, answer **NO**.

9.1 Required Permits

For shipments within the United States, please include the following:

- A copy of the collector's federal USFWS Threatened and Endangered Species permit, if applicable
- A copy of the collector's federal banding permit (blood and feather samples)
- A copy of the collector's USFWS Scientific Collections Permit (eggs and tisssues other than blood or feathers)
- A copy of the collector's state scientific collecting permit
- A copy of the importer's USDA import permit* (not required if samples were collected within the U.S.)

For shipments outside of the United States, please include the following:

- A copy of the importer's USDA import permit*
- A copy of the importer's CDC permit
- USFWS Form 3-177 (Declaration of importation or exportation of fish or wildlife)
- FedEx Declaration of Biological Shipments*
- FedEx Commercial Invoice
- CITES export permit, if applicable
- A copy of the origin country's export permit, if applicable

**Forms provided by BRI via email*

IMPORTANT:
Shipments arriving in the United States may be denied entry, destroyed, or returned if they do not include the appropriate permits. For further questions or clarifications, or if you are interested in collaborating, feel free to contact BRI via phone, email, or visit us at our website.

For further questions or clarifications, or if you are interested in collaborating, feel free to contact BRI via phone, email, or visit us at our website:

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Appendix 1a. Conceptual approach to sampling birds for various potential research applications, relative sample masses required, and potential storage methods

Table A1: Bird sampling methods and corresponding applications and considerations.

Research Objective	Example	Medium	Relative mass or volume required	Storage method	Major considerations
Population genetics	DNA or RNA extraction	DNA (blood or follicles)	A few drops of blood or follicle tip	Buffered or frozen (-20 C)	Storage medium and DNA viability
Heavy metals analysis	Hg or Pb analysis	Blood, organs, muscle, bone, feathers	≥1 g or mL	Air dried or frozen (-20 C)	External contamination
Volatile organic compounds	PCBs, dioxins, PAHs	Serum or plasma	≥1 mL plasma or serum	Frozen (-80 C)	Sample degradation at warmer temperatures
Stable organic compounds	PFAS	Serum or plasma	≥0.5 mL plasma or serum	Frozen (-20 C)	External contamination
Bulk stable isotopes	d15N, d13C	Any	≥0.5 g dry mass	Air dried or frozen (-20 C)	Tissue half-life
Compound-specific stable isotopes	d15N in specific amino acids	Any	≥1 g dry mass	Air dried or frozen (-20 C)	Tissue half-life
Microplastics	Dietary plastics exposure, phthalates	Gut or fecal samples	More is better	Frozen (-20 C)	External contamination
Biomarkers	Health metrics	Serum or plasma, blood slides	≥0.5 mL plasma or serum, 1 slide	Frozen (-80 C), air dried	Sample degradation at warmer temperatures
Disease	HPAI	Swabs	1 swab	Buffer solution, frozen (-80 C) or air temp	Storage medium can impact utility
Parasites	Hemoparasites, helminths	Swabs or blood	1 swab or blood slide	Air dried, in alcohol, or frozen (-20 C)	Storage method is sample-specific

Appendix 1b. Description of possible analyses

Complete Blood Count (CBC):

The CBC measures the three main blood cell types: red and white blood cells and platelets. The red blood cell count can indicate anemia or dehydration. The white blood cells are counted as a whole (absolute count) and differentiated into subtypes (differential count) to indicate infection, stress, endoparasitism, allergies, and even certain types of cancer. A low platelet count can prevent effective blood clotting. The morphology of cells, evaluated by looking at a smear of blood under a microscope, can provide more details regarding the cause of any abnormalities in overall cell count.

Chemistry Panel:

The chemistry panel provides assessment of liver and kidney function, glucose levels (indicative of such conditions as stress, starvation, liver disease, and diabetes), electrolytes, and metabolic status.

Corticosteroids:

Stress hormones, such as cortisol, become elevated when an animal is undergoing either acute or chronic stress such as disease, contaminant exposure, environmental extremes, and disturbance from predators and/or humans.

Immunology:

The immune function of birds has high potential to be affected by a variety of stressors, such as contaminant exposure, disease, and parasitism. Suppression of the immune system makes wildlife highly susceptible to secondary and opportunistic infections.

Genetics - profiling and gender ID:

Male and female birds have potential to have different exposures and responses to contaminants due to differences in their sizes, behaviors, and diets. However, particularly in monomorphic species, the gender of the bird can be difficult to confirm by body morphometrics or coloration. Thus, identifying the gender of the birds in the affected population through genetic analysis provides essential information to evaluate the long-term population impacts of a contaminant, including survival and reproductive success. Genetic profiling of populations exposed to a contaminant provides valuable information regarding the structure of metapopulations (regional populations), local populations, colonies and sub-colonies (breeding and non-breeding) that have potential to be affected by the contaminant.

Fecal Parasites:

A wide variety of internal parasites can infest wild birds. Parasites are ubiquitous in wild avian populations, as birds are exposed regularly through their diet. However, parasite loads may not be pathogenic unless a bird is stressed directly or is exposed to additional stressors, such as a contaminant and inability to find suitable (uncontaminated) food. A heavy or specific parasite load can lead to anemia, gastric ulceration, and nutritional malabsorption, with the potential to facilitate opportunistic infections and/or a lower tolerance to secondary environmental stressors.

Virology:

Avian Adenovirus Avian Influenza, Avian Encephalomyelitis, Avian Laryngotracheitis, Avian Paramyxovirus Type 1, Avian Paramyxovrius Type 2, Avian Paryamyxovirus Type 3, Infectious Bursal Disease) are common avian viruses which can be an additional stressor in a contaminated bird.

Aspergillosus:

Opportunistic ubiquitous fungal infection which affects stressed, and therefore immunocompromised, birds. Exposure is through environmental contamination with no vertical/horizontal transmission.

West Nile Virus:

West Nile virus causes inflammation of the brain in birds, and has high potential to be fatal and heavily impact avian populations, although asymptomatic/subclinical infections can also persist in avian populations. It is readily transmitted via mosquito vectors, but also can be transmitted through feather-picking, cannibalism, oral secretions, and feces.

Eastern/Western/Venezuelan Equine Encephalitides (EEE, WEE, and VEE, respectively):

These encephalitides are transmitted primarily via mosquito vectors, but they can also be spread by feather-picking, cannibalism, vertical transmission, interspecies bridge transmission (via mosquito vector). Birds are a primary reservoir for EEE and WEE, and most avian species are

generally asymptomatic. Symptoms include a high fever, muscle pain, and neurologic symptoms associated with inflammation of the meninges of the brain, such as altered mental status, headache, meningeal irritation, photophobia, and seizures.

Trace Elements (calcium, chloride, cobalt, copper, iron, magnesium, molybdenum, phosphorous, potassium, sodium (plus K/Na ratio), selenium, zinc):

Indicators of heavy metal environmental contamination. Changes in trace elements and electrolytes can reflect altered metabolic function. Levels can be assessed from blood, organ and muscle tissue (liver, lungs, kidneys, brain, pectoral muscles).

Heavy Metals (Arsenic, lead, mercury, cadmium, thallium, +/- selenium):

Indicators of environmental contamination.

Mercury:

Mercury is a toxic metal deposited in the environment due to incineration of fossil fuels (e.g., from coal-fired power plants) and is also associated with exposure to oil contamination. In certain conditions, elemental mercury stored in sediments in aquatic habitats is readily converted to methylmercury, the toxic form that magnifies up the food web, with potential to affect wildlife at top trophic levels. At elevated levels, mercury causes sublethal neurologic effects, and has been associated with decreased reproductive success in a variety of piscivorous species in aquatic ecosystems, and potentially in insectivorous species in terrestrial habitats as well.

Organochlorinated Pesticides:

Organochlorine pesticides, such as DDT, aldrin, and dieldrin, can affect the neurologic, cardiac, and endocrine systems. They are very persistent in the environment and bioconcentrate up the food web, accumulating at higher levels in higher trophic taxa.

Polychlorinated Biphenyls (PCB's):

An organochlorine compound formerly used in transformers, PCBs have adverse endocrine effects, can cause dermatoses, liver damage, cancer, developmental, and teratogenic effects. The variable number and position of the chlorine atoms on the phenyl rings creates 209 congeners with variable mechanisms and severity of toxicity.

They bioaccumulate to higher trophic levels, particularly in species associated with estuary marshlands. Additional stressors, such as loss of habitat, make the clinical impact of PCBs more severe.

Polybrominated Diphenyl Ethers (PBDEs):

Polybrominated diphenyl ethers are a class of flame retardant with two phenyl rings separated by an ether linkage. Like chlorine atoms in PCBs, the variability of bromine atoms on the rings results in 209 PBDE congeners with variable mechanisms and severity of toxicity. PBDEs are environmentally persistent and biomagnify to higher trophic levels. They are endocrine disruptors with effects on the thyroid axis, reproductive, and neurologic systems.

Polycyclic Aromatic Hydrocarbons (PAH):

Exposure to oil from a spill results in significant sublethal physiologic impacts to wildlife. Changes in plasma biochemistry and the presence of total circulating levels of PAHs after short- and long-lasting exposure to fuel oil include decreased glucose (negatively correlated with total PAH levels) and inorganic phosphorus levels, oxidative damage and hemolytic anemia, reduced creatinine, and variable responses of asparatate aminotransferase and gamma-glutamyl transferase enzymes, dependent on the sex of individuals and the temporal pattern of exposure (Alonso-Alvarez et al., 2007a and 2007b). These physiologic changes indicate damage to vital organs, including the liver and kidneys. Determination of circulating PAH levels in birds exposed to an oil spill provide a deeper understanding of the long-term sublethal impacts to avian populations.

Hemoglobin, Reticulocyte Count, Packed Cell Volume (PCV), and Total Protein (TP):

Hemoglobin is the oxygen carrying protein of vertebrate red blood cells; packed cell volume (hematocrit) is the proportion of blood volume that is occupied by red blood cells; and total protein is the concentration of serum proteins in the blood. Mean corpuscular hemoglobin concentration is a value calculated from hematocrit and hemoglobin which further defines anemic conditions. The number of immature red blood cells (reticulocytes) is indicative of bone marrow stimulation in response to the anemia. Determining the PCV, reticulocyte count, total hemoglobin, and mean corpuscular hemoglobin concentration provides an assessment of whether exposure to a contaminant, such as crude oil and/or PAHs, has caused signs of hemolytic anemia.

Heinz Body, Ferritin, and Haptoglobin:

These physiological indicators of oxidative damage and hemolytic anemia provide insight into whether birds manifest physiological impairment related to exposure to polycyclic aromatic hydrocarbons from an oil spill. Heinz bodies (Heinz-Ehrlich bodies) are inclusions in red blood cells resulting from oxidative damage to and precipitation of hemoglobin. Ferritin is an iron storage protein that is elevated in cases of hemolytic anemia, and haptoglobin is an acute phase protein that functions to sequester hemoglobin leaked from injured red blood cells. These metrics of physiological response are significant to organismal health, and are established signs of toxicity in birds exposed to crude oil and/or PAHs (Leighton et al., 1983; Troisi and Borjesson, 2005; Troisi et al., 2006 and 2007).

Hemolytic anemia has been demonstrated in several species exposed to crude oil (Leighton et al., 1983; Fry and Lowenstine, 1985; Leighton, 1986; Couillard and Leighton, 1993; Yamato et al., 1996). Anemia causes reduced availability of oxygen to tissues, leading to anaerobic metabolism, altered cell membrane permeability, cellular and tissue dysfunction, and ultimately organ failure. While the pathophysiology following exposure to crude oil is multifactorial, oxidative hemolytic anemia has high potential to play a key mechanistic role in morbidity and mortality. Thus, determining the character and severity of anemia, presence of Heinz bodies, and changes in haptoglobin and ferritin assists in predicting survival in oiled birds.

Chemical Dispersants:

To mitigate the effects of the surface oil resulting from a spill, chemical dispersants, such as Corexit 9500/9500a, may be applied at the spill source and throughout the area of the spill. However, little is known about the impacts of these dispersants on wildlife and aquatic ecosystems, particularly through subsurface applications, including the potential for direct toxicity of the dispersant, its bioaccumulation potential, differences in toxicity of dispersed vs. non-dispersed oil, effects on bioavailability of oil, and effects on PAH uptake in biota. Determining if wildlife is directly exposed to a dispersant by analyzing for its presence in avian blood will significantly enhance scientific and clinical knowledge of the potential biotic impacts related to dispersant use in oil spills.

Appendix 1c. Guidance for sample selection for various research applications*

Table A2: Matrix of advised guidance for research application and sample types.

Type	Blood			Bone	Keratin				Organ				Swab	
Subtype	Whole blood	Red blood cells	Plasma/ serum	Bone	Body contours	Flight feathers	Tail feathers	Nail/toe	Skeletal muscle	Gut	Liver/ kidney	Skin	Oral or cloacal swab	Fecal swab
Population genetics	Ideal	Ideal	Not advised	Not advised	Ideal	Follicle	Follicle	Not advised	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal
Heavy metals analysis	Ideal	Ideal	Not advised	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal	Not advised	Ideal	Ideal	Ideal	Ideal
Voltatile organic compounds	Not advised	Not advised	Ideal	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Ideal	Not advised	Not advised	Not advised
Stable organic compounds	Not advised	Not advised	Not advised	Not advised	Possible, high sample mass required	Possible, high sample mass required	Possible, high sample mass required	Possible, high sample mass required	Possible, high sample mass required	Not advised	Ideal	Ideal	Ideal	Ideal
Microplastics	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Ideal	Not advised	Not advised	Not advised	Possible
Stable isotopes	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal	Ideal	Not advised	Ideal	Not advised	Not advised	Not advised
Biomarkers	Ideal	Not advised	Ideal	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised
Disease	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Ideal	Not advised
Parasites	Ideal	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Not advised	Ideal

**This list is not meant to be exhaustive, but as a guide for how to approach sampling efforts for various applications. Blood and keratin-based tissues can be sampled noninvasively, while bone and organs are typically used from carcasses (harvested by hunters or birds found dead by other means).*

Appendix 2. Guideline using body weight to measure of how much blood can be safely taken

Table A3: Avian Blood Collection Quantity, Needle, and Container Guidelines***

Body Weight (gms)	Needle or Butterfly Catheter Size	Safe Max Amt Blood (cc) 0.8% body wt	ABSOLUTE Max Amt Blood (cc) 1% body wt	Collection Container #1	Collection Container #2	Collection Container #3	Collection Container #4
<100	25G - 28G needle	0.1-0.2	0.25	Hct tube EDTA microvette	--	--	--
100*	25G - 28G needle	0.8	1	Hct tube EDTA microvette	--	--	--
500**	25G - 28G needle	4	5	Hct tube EDTA microvette	1-3cc hep syringe >> green microtainer	--	--
1000	25G needle	8	10	Hct tube EDTA microvette	3-5cc hep syring, >> green microtainer	--	--
1500	23 or 25G needle	12	15	Hct tube EDTA microvette	3-5cc hep >> green vacutainer	4-6 cc green vacutainer	4-6 cc green vacutainer
2000	23 or 21G butterfly	16	20	Hct tube EDTA microvette	4-6 cc green vacutainer	4-6cc green vacutainer	4-6cc red vacutainer
2500	23 or 21G butterfly	20	25	Hct tube EDTA microvette	4-10cc green vacutainer	4-6cc green vacutainer	4-6cc red vacutainer
3000	23 or 21G butterfly	24	30	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
3500	23 or 21G butterfly	28	35	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
4000	23 or 21G butterfly	32	40	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
4500	23 or 21G butterfly	36	45	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
5000	23 or 21G butterfly	40	50	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
5500	23 or 21G butterfly	44	55	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
6000	21G butterfly	48	60	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
6500	21G butterfly	52	65	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer
7000	21G butterfly	56	70	Hct tube EDTA microvette	4-10cc green vacutainer	4-10cc green vacutainer	4-6cc red vacutainer

** 27g needles on 1cc heparinized syringe; ** 25g needles on 3 or 6cc heparinized syringe. *** Butterfly catheter-vacutainer set-ups are recommended for birds >1000g.*