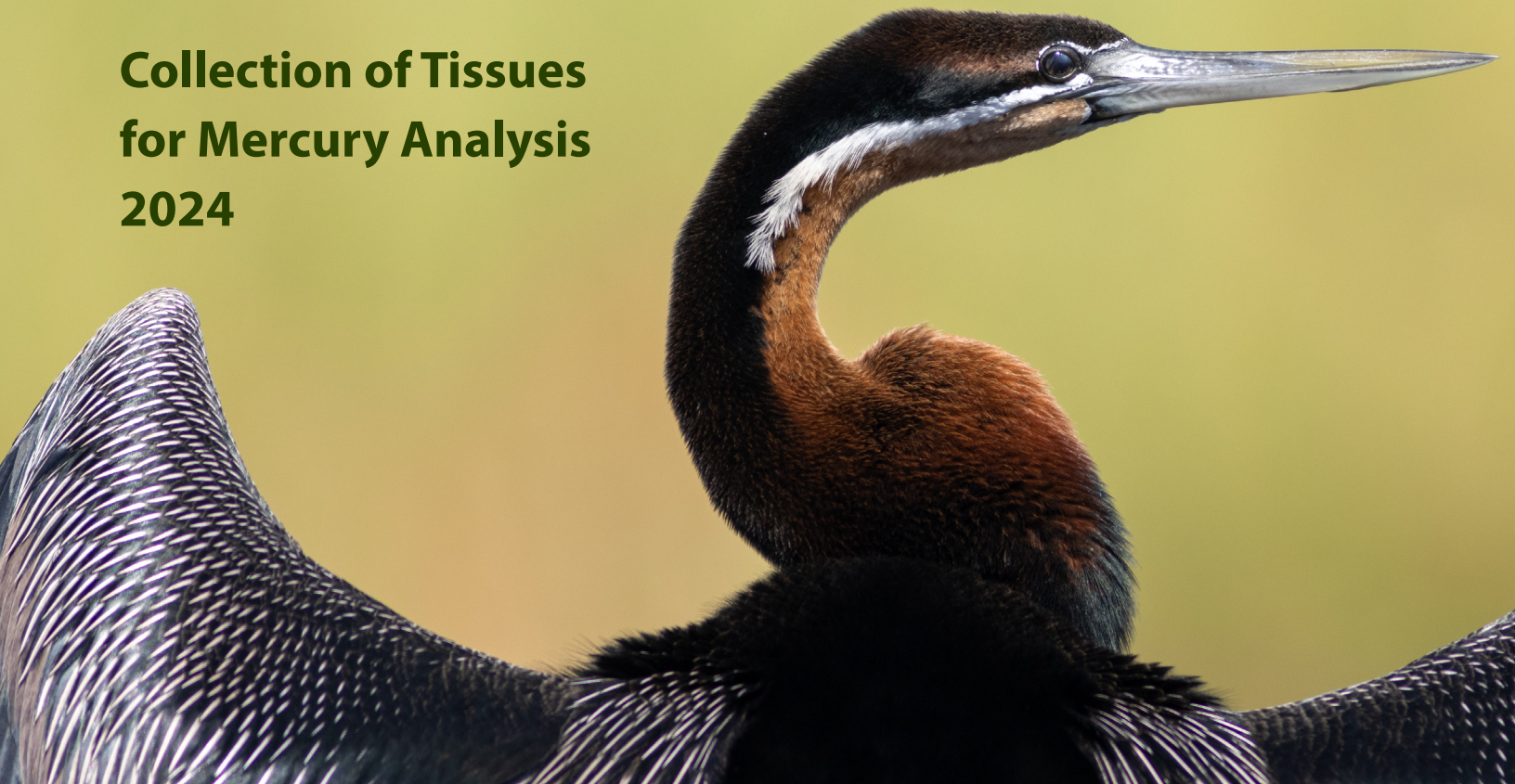
Bird Field Sampling Methods

Collection of Tissues for Mercury Analysis 2024



BIRD FIELD SAMPLING METHODS

Collection of Blood and Feathers for Contaminant, Stable Isotope, and Genetic Analyses

October 2024

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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.

TRACE 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. Contact us for more information.

Suggested Citation:

Evers DC, Savoy L, DeSorbo C, Regan K, Persico C, Sayers CJ II. 2024. Bird field sampling methods: collection of tissues for mercury analysis. Report BRI 2024-11, Biodiversity Research Institute, Portland, Maine, USA.

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1.0 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 6.0 Storage of Samples and 7.0 Shipment of Samples). If capillary tubes are unavailable, vacutainers are an adequate replacement and can store a larger sample volume.

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 1.2 through 1.4.

1.1 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. If blood will be stored in a
☐ 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
☐ 6mL blood collection tube (e.g. "Green top" Lithium heparinized Vacutainers®)	For contaminant analyses
☐ 6mL 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
☐ 6mL blood collection tube (e.g. "Green top" Lithium heparinized Vacutainers®)	For contaminant analyses
☐ 6mL 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
☐ 6mL archive Vacutainers®	For storage of sealed capillary tubes

For Whatman® Card Storage	
Item	**Purpose**
☐ Whatman® Proteinsaver Card	
☐ 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 (eg. Ziploc®)	For storing Whatman® card samples
☐ 6mL archive Vacutainers®	For storage of sealed capillary tubes

1.2 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 1.5 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 4.0 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 5.0 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 5.0 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 6.0 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)

1.3 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 4.0 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).
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 1.5 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 5.0 Metadata Requirements.
15. Place cotton pad into a paper coin envelope and label the envelope as described in Section 5.0 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 6.0 Storage of Samples).
17. Dispose of used needles and excess wrapping in Sharps container.

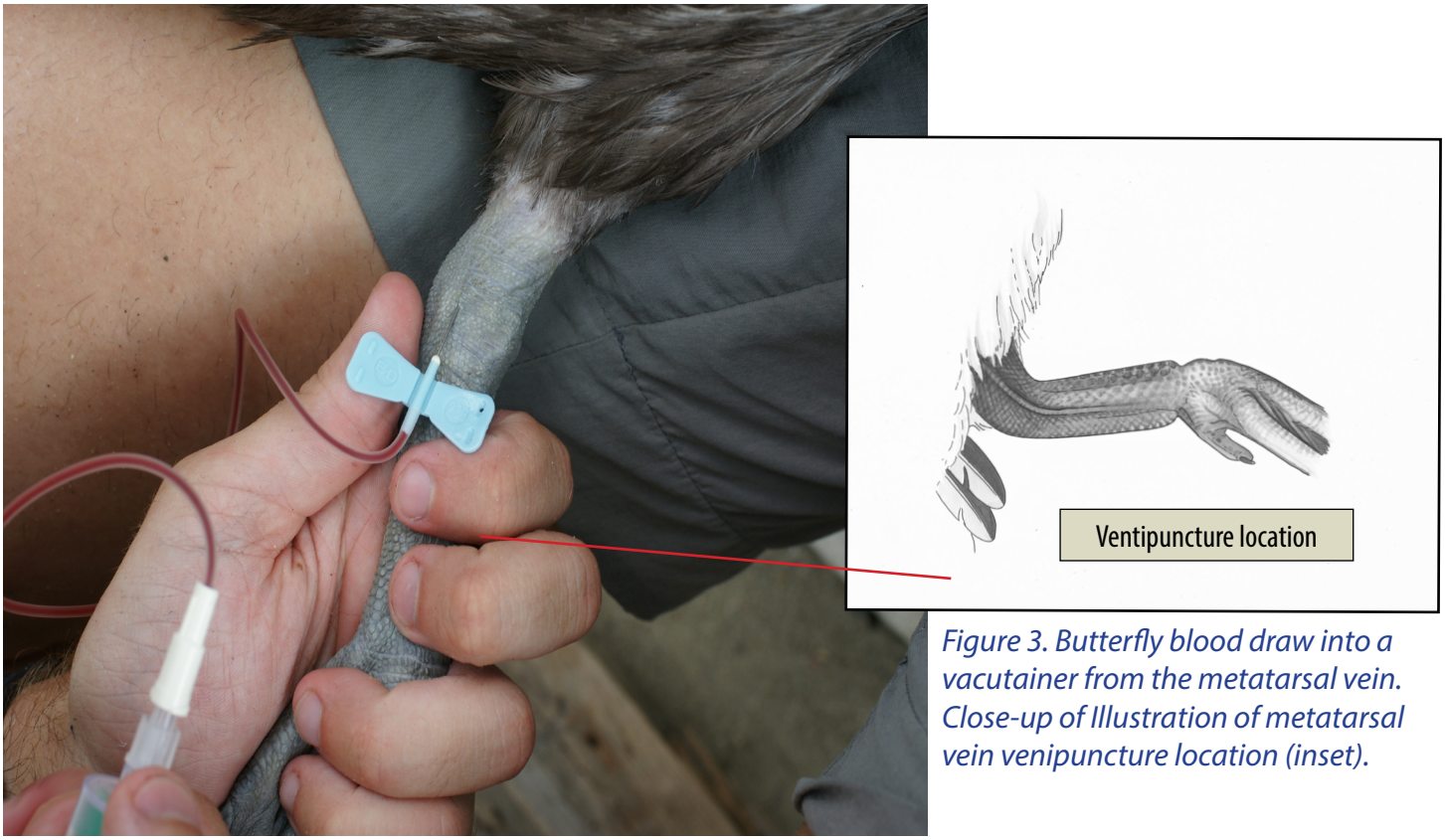

Figure 3. Butterfly blood draw into a vacutainer from the metatarsal vein. Close-up of Illustration of metatarsal vein venipuncture location (inset).

1.4 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 4.0 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 1.5 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 5. 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 6.0 Sample Storage).
17. Dispose of used needles and excess wrapping in Sharps container.

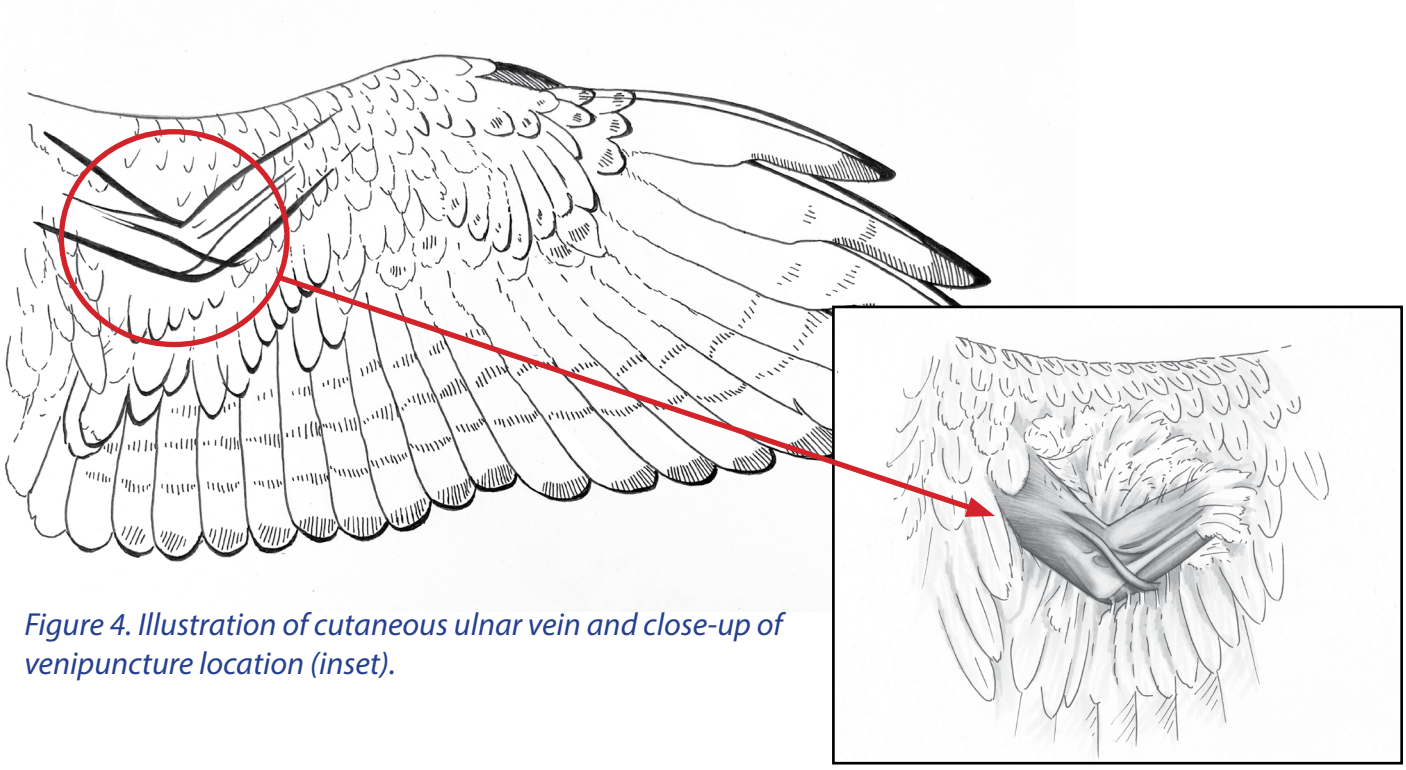

Figure 4. Illustration of cutaneous ulnar vein and close-up of venipuncture location (inset).

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)

1.5 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 5.0 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.

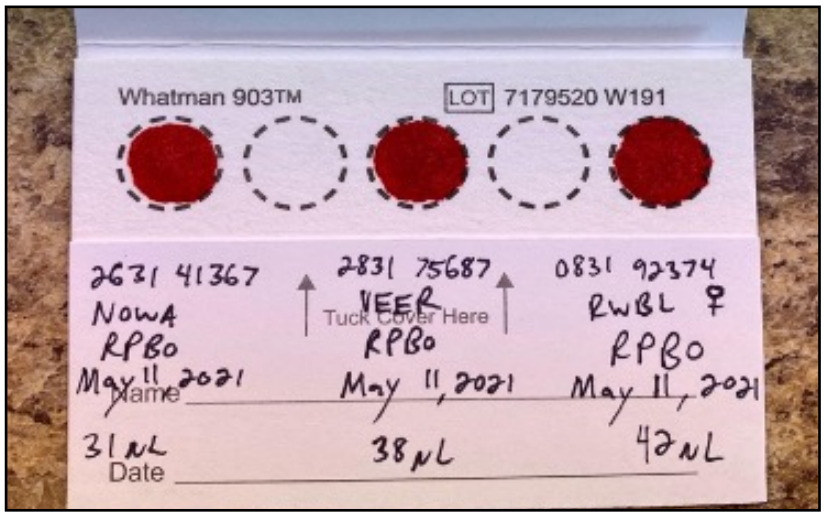

Figure 8. Completed Whatman® card with appropriate metadata: Unique ID #, species code, location code, date, and blood volume.

2.0 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. 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 8).
- 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.

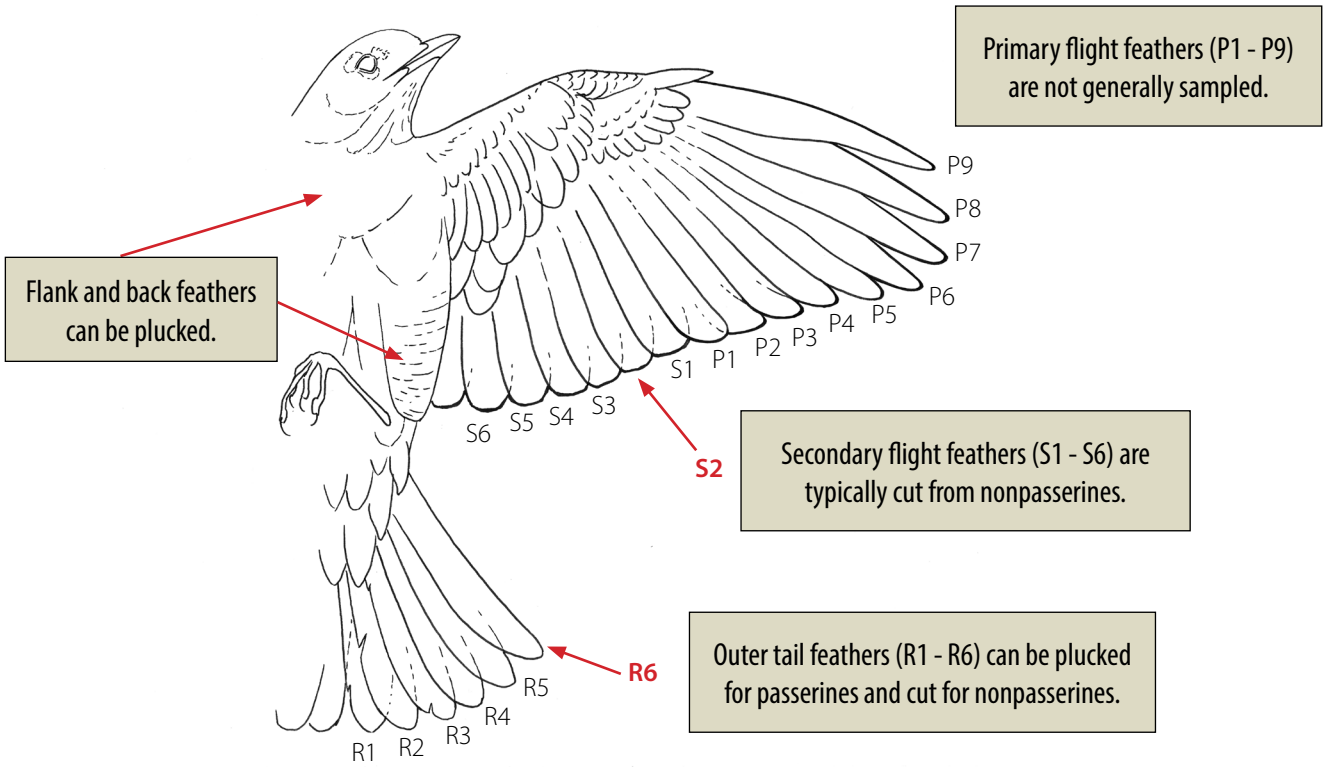

Figure 9. Illustration of standardized feather sampling locations on a typical passerine.

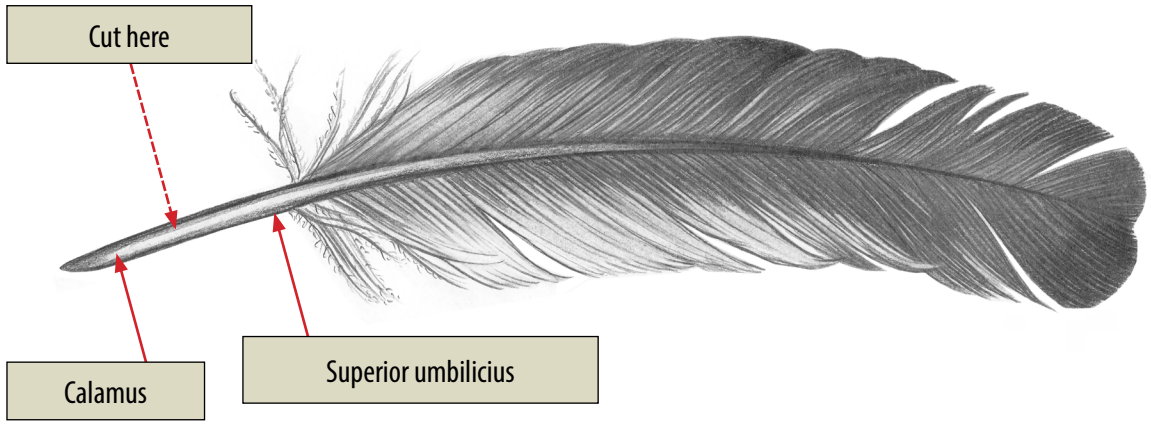

Figure 10. Standardized field clipping of secondary flight feathers.

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 5.0 Metadata Requirements).

2.1 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

3.0 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).

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 next 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. Label a waterproof sample tag with appropriate metadata (see 5.0 Metadata Requirements) and place it with the egg inside a plastic bag.
4. 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 6.0 Storage of Samples). The handling of inviable eggs follows the same procedures as viable eggs.

3.1 Supplies for Egg Collection

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

4.0 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

5.0 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-2023/download/)
- ☐ Date (please use letters for the month instead of numbers, i.e., Mar 11, 2024)
- ☐ 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. Further information on forms and submissions is found in the Appendix.

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).

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.

6.0 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.

- 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.

7.0 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 7.0. 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 7.0. 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**.

7.1 Required Permits

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

- A copy of the collector's federal USFW Threaten and Endangered permit
- 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.

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Appendix A.