Bird Field Sampling Methods

Collection of Tissues for Mercury Analysis

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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. The following sampling protocol is based on 22 years of live sampling over 37,000 landbirds and over 9,000 waterbirds by BRI.

April 2021

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1.0 Whole Blood Collection

The non-lethal 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., $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) 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 migratory passerines. Although blood can be drawn from any location on larger birds and can be determined by the biologist or veterinarian on site, BRI recommends drawing from the metatarsal (leg) 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 Sample Storage and 7.0 Sample Shipment). 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 sterile receptacles.** For ease of sample storage and shipping, blood dried on Whatman cards can be used for both contaminant and stable isotope analyses (Perkins and Basu 2018; Barst et al. 2020). **IMPORTANT: 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 procedure
Portable (~5L) cooler
Ice pack
Isopropyl alcohol pads
Dry cotton balls
Ultra-fine Sharpie™ permanent marker
Sandwich-size Ziploc™ plastic bags
Portable Sharps container
Optional: Whatman blood cards
Optional: Silica Gel Desiccant Packets (for Whatman card storage only)
Direct draw procedure using a manual syringe (for large birds only)
3 cc syringes with 21–25-gauge needles or 21–25-gauge butterfly needles with 7-inch tubing with blood collection tube holders
6 mL Lithium heparinized vacutainers (green top, for contaminant analyses only)
6 mL sterile vacutainers (for stable isotope analyses only)
Piercing procedure
22–25 gauge hypodermic needles (for large birds) or 26–27 gauge needles (for small birds)
Micro-hematocrit capillary tubes (use heparinized for contaminants, sterile for stable isotopes)
Leica Microsystems Critocaps™ or Critoseal™
6 mL archive vacutainer

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.

1. Separate feathers and sterilize the collection area with an isopropyl alcohol wipe.
2. Locate the desired vein.
3. Uncap needle.
4. After the alcohol has evaporated, insert needle parallel to the vein.
5. Begin drawing with manual syringe (Figure 1).
6. Draw 1 to 10 mL of whole blood and gently exit the vein.
7. Hold a fresh cotton ball on the collection area until bleeding has stopped (~10 seconds).
8. If not using Whatman cards, skip to step 13.
9. Use syringe to saturate circles on Whatman blood card (Figure 2)
10. Allow blood card to air dry before labelling with appropriate metadata (see 5.0 Metadata Requirements) using a permanent marker.
11. Store card in plastic bag with desiccant packets.
12. If not storing blood samples in capillary tubes, proceed to step 17.
13. Inject drawn blood into Lithium heparinized blood vacutainer(s) (green top) or sterile blood vacutainer(s) depending on desired analysis (see 4.0 Procedural Tables). IMPORTANT: AVOID FILLING VIALS COMPLETELY AS THE TOP WILL POP OFF UNDER PRESSURE. A TWO-THIRDS FULL VIAL IS PREFERRED.
14. Label the vacutainer(s) with appropriate metadata using a permanent marker (see 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 6.0 Sample Storage).
16. Dispose of used needles and excess wrapping in Sharps container.

Figure 1. Butterfly blood draw from a syringe.

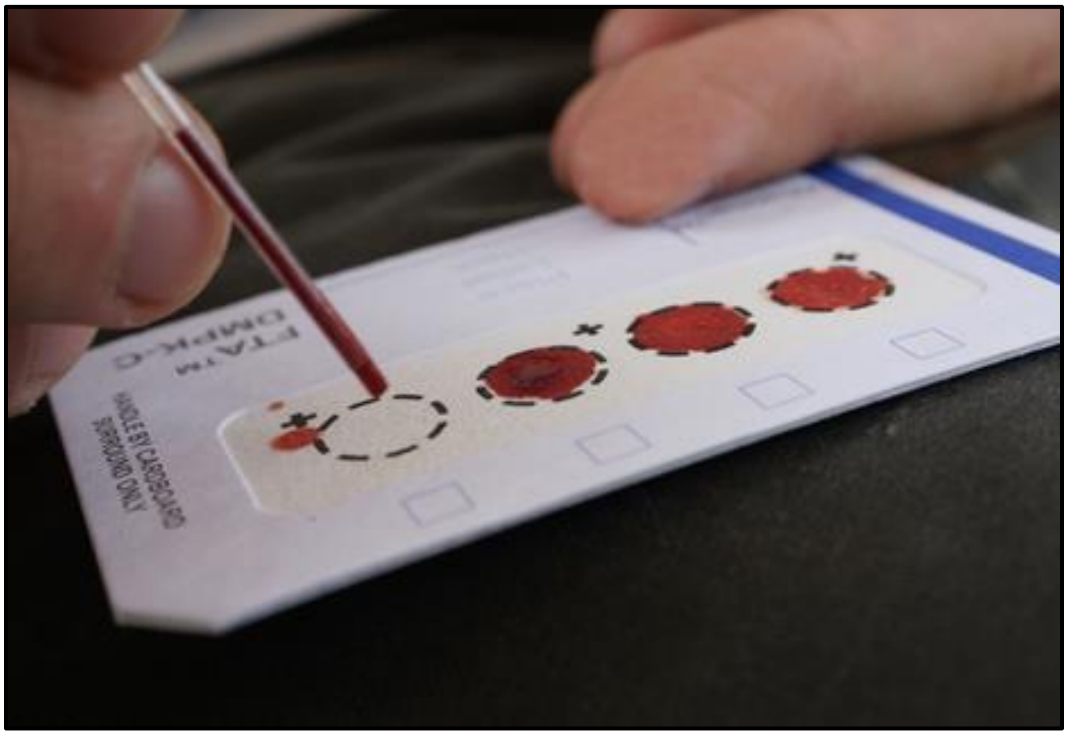

Figure 2. Filling Whatman blood card circles with a filled capillary tube.

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

1. Attach the butterfly needle to the blood collection tube holder.
2. Locate the metatarsal vein and sterilize the collection area using an alcohol wipe.
3. After the alcohol has evaporated, remove the plastic guard on the butterfly end of the needle.
4. Holding the needle in line with the vein, and the bevel facing away from the bone, penetrate the skin proximal to the tibiotarsal joint, 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.
5. Holding the barrel securely, insert a Lithium heparinized blood vacutainer (green top) or sterile blood vacutainer (depending on desired analysis, see 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. When the tube is filled to the desired amount, remove from the barrel and gently invert the vacutainer several times (Figure 3).
6. When all vacutainers have been filled, hold a cotton pad over the needle and gently remove the needle from the vein. Apply pressure to the puncture point with the cotton pad until the bleeding has stopped.
7. Use the blood in the tubing of the butterfly needle to fill four capillary tubes. Seal capillary tubes using Critoseal™ and place into a 6 mL archive vacutainer.
8. Properly label all vacutainers with appropriate metadata using a permanent marker (see 5.0 Metadata Requirements)
9. Place cotton pad into a paper coin envelope and label the envelope as described in section 5.0.
10. Store blood samples in a cooler with ice packs immediately in the field and transfer to a freezer within 24 hours of collection (see 6.0 Sample Storage).
11. Dispose of used needles and excess wrapping in Sharps container.

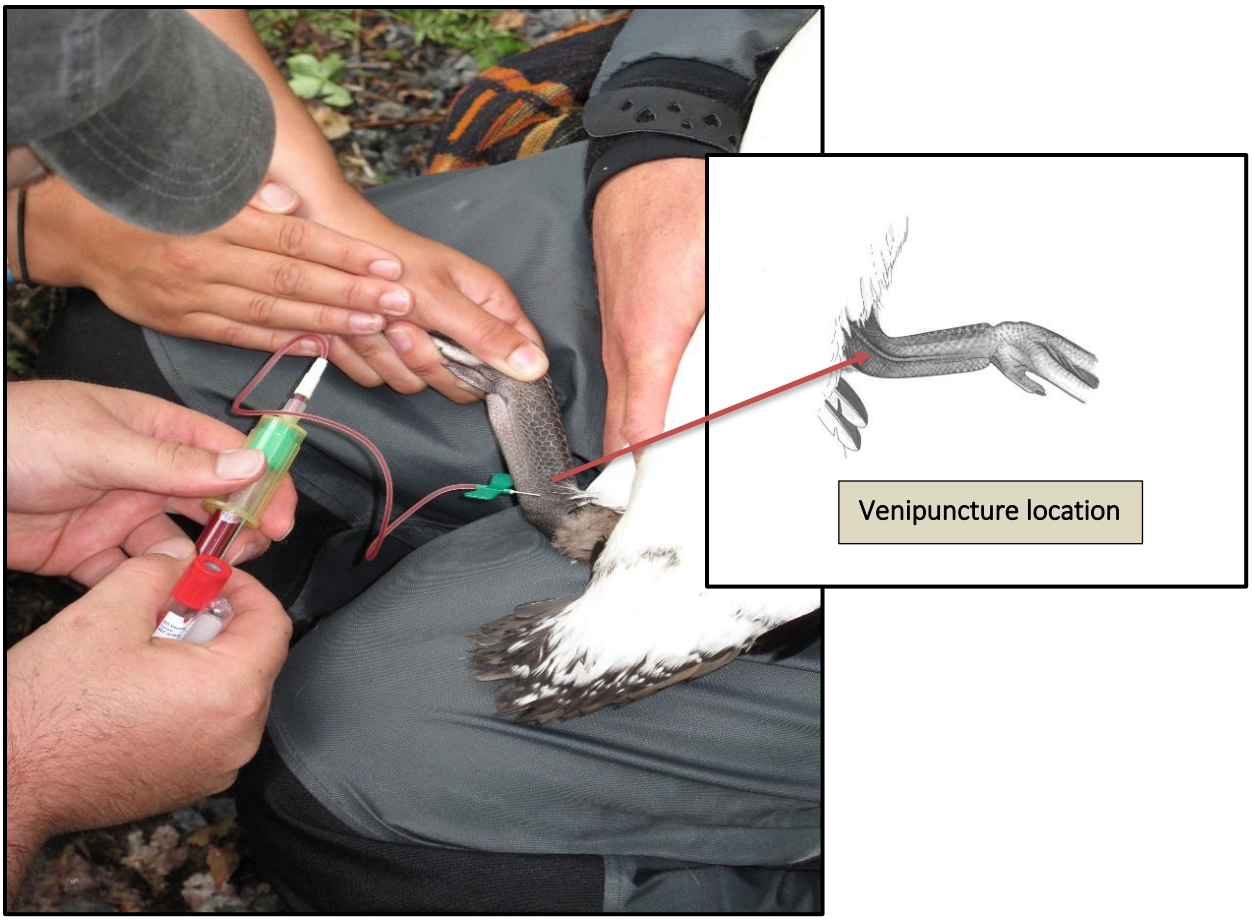

Figure 3. Butterfly blood draw into a vacutainer from the metatarsal vein. Close-up of Illustration of metatarsal vein venipuncture location (inset).

1.4. Piercing Procedure

1. Separate feathers and sterilize the collection area with an isopropyl alcohol wipe.
2. Locate the cutaneous ulnar vein in the wing (Figure 4 and 5).
3. Uncap needle.
4. <u>After</u> the alcohol has evaporated, prick vein with needle by gently entering parallel to the vein. **IMPORTANT: do not go through both vein walls, just the top one.**
5. Gently exit the vein and allow blood to pool (usually happens very quickly).
6. Collect blood by placing a Lithium heparinized or sterile capillary tube (depending on desired analysis, see 4.0 Procedural Tables) below the pooled blood. **TIP:** holding the tube at a downward angle will allow the blood to be more easily pulled into the tube via capillary action. **IMPORTANT: Fill the capillary tube at least ½ full, but no more than ¾ full.**

7. Collect 1–3 capillary tubes depending on the bird's mass.
8. Hold a fresh cotton ball on the collection area until bleeding has stopped (~10 seconds).
9. If not using Whatman cards, proceed to step 14.
10. Use filled capillary tube to saturate circles on Whatman blood card (Figure 2).
11. Allow blood card to air dry before labelling with appropriate metadata (see 5. Metadata Requirements) using a permanent marker.
12. Store card in plastic bag with desiccant packets.
13. If not storing blood samples in capillary tubes, proceed to step 17.
14. Use Critocaps™ or Critoseal™ to seal each end of the capillary tubes.
15. Place capillary tubes in a 6 mL archive vacutainer and properly label with appropriate metadata using a permanent marker (see 5.0 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 6.0 Sample Storage).
17. Dispose of used needles and excess wrapping in Sharps container.

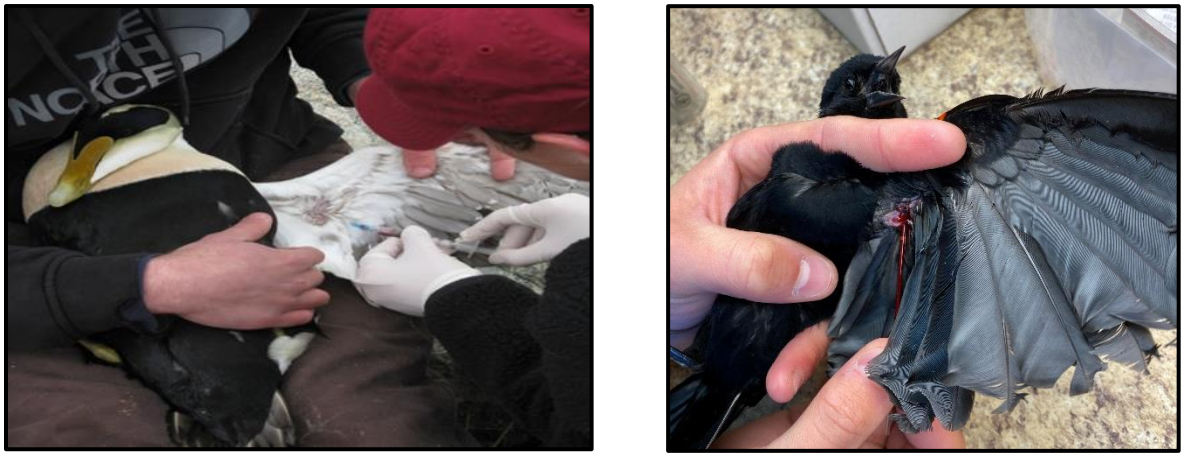

Figure 4. Direct draw blood collection from the cutaneous ulnar vein using a manual syringe (left) and a capillary tube after piercing the vein (right).

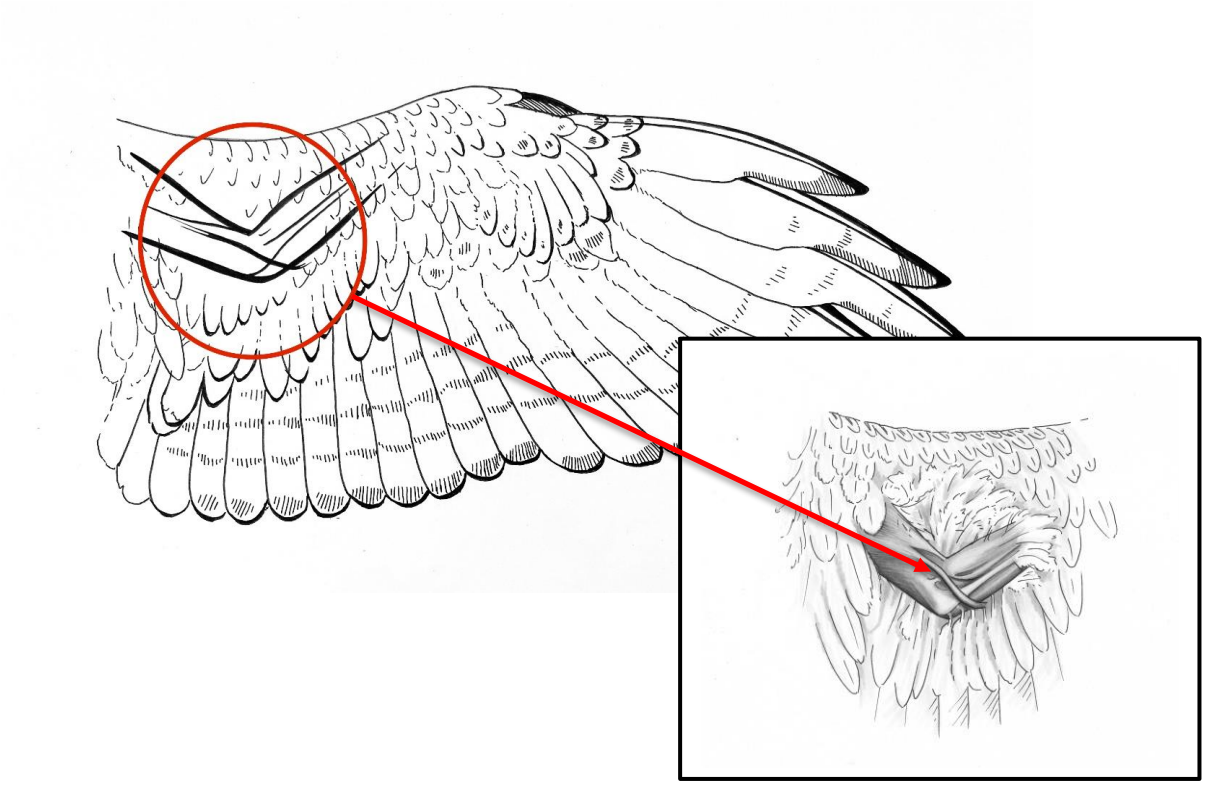

Figure 5. Illustration of cutaneous ulnar vein and close-up of venipuncture location (inset).

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 re-grown 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 2 second secondaries (S2), 2 outer tail feathers (rectrices, R6), and 5–6 flank feathers, whenever possible (Figure 6). 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).

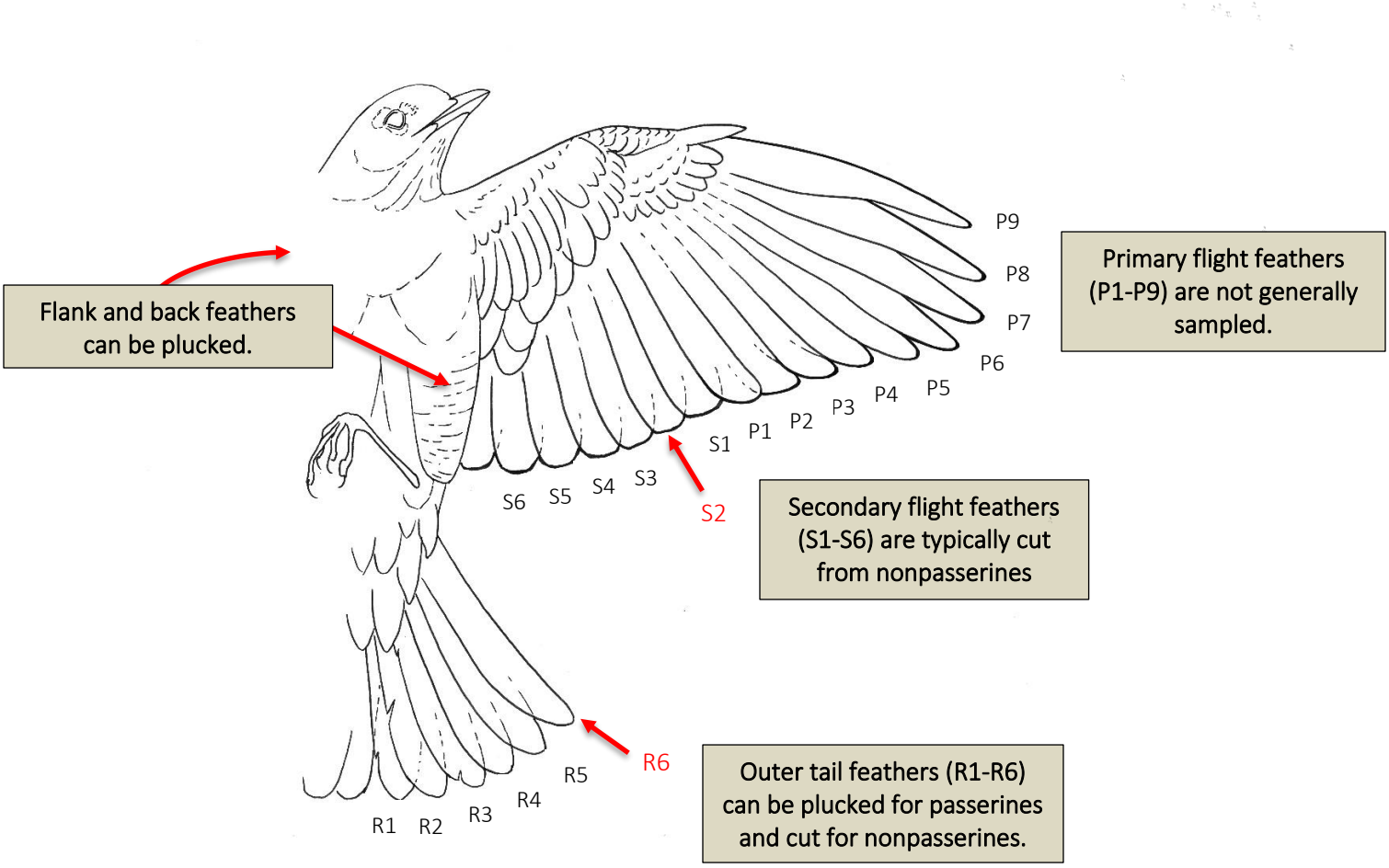

Figure 6. Illustration of standardized feather sampling locations on a typical passerine.

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 7). 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.

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).

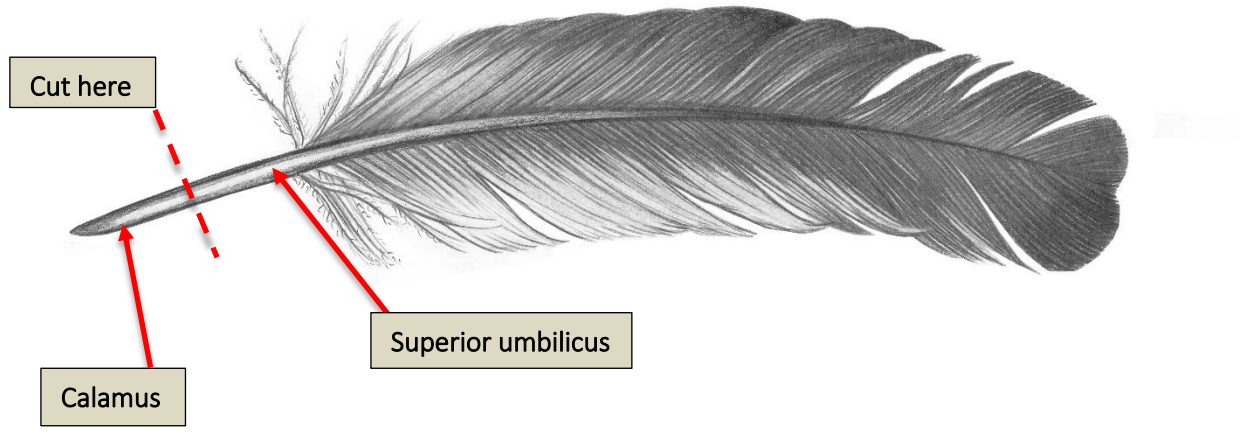

Figure 7. Standardized field clipping of secondary flight feathers.

2.1. Supplies for Feather Collection

- Small cutting pliers
- 3 ⅜" x 6" 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). 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. If the "X" is still in the same position on the following day — indicating the egg has not been turned in 24 hours — collect the egg. Label a waterproof sample tag with appropriate metadata (see 5.0 Metadata Requirements) and place it with the egg in a plastic bag. Store egg samples in a cooler with ice packs in the field and transfer to a refrigerator or freezer as soon as possible (see 6.0 Sample Storage). 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	0.4 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	5 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	0.4 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	5 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

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, if applicable
- Species common name (please also include species Latin name if collected outside of the U.S.)
- Date (please use letters for the month instead of numbers, i.e., Mar 11, 2021)
- 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 and submit to BRI via this form. **IMPORTANT: If contributing samples to BRI or the Tropical Research for Avian Conservation & 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. Example data sheets can be found here.**

IMPORTANT: 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).

6.0 Sample Storage

- 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.

- Feather sample envelopes should ideally be stored in a sealed plastic bag in a 4°C refrigerator (to prevent molding), but can also be stored in a sealed plastic bag at room temperature away from direct sunlight prior to shipping and analysis.
- Egg samples should be stored in a sealed plastic bag with an accompanying waterproof sample tag. Egg samples should be stored in a 4°C refrigerator if processing in <3 weeks or in a freezer if processing >3 weeks.

7.0 Sample Shipment

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 7.1. Required Permits), please schedule a shipment with your carrier service of choice. To package the tissue samples:

- 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 7.1. Required Permits). Attach a second set of permit copies in a plastic pouch on the exterior of the package.
- IMPORTANT: To avoid postal or customs delays, samples need to be shipped on a Monday or Tuesday, and never right before a federal holiday. When asked by the shipping representative if you are shipping anything perishable, answer "NO!"

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

7.1. Required Permits

For shipments **within the U.S.**, please include:

1. A copy of the collector's federal bird banding permit
2. A copy of the collector's state scientific collecting permit
3. A copy of the importer's USDA import permit * (**not required if samples were collected within the U.S.**)

For shipments **outside of the U.S.**, please include:

1. A copy of the importer's USDA import permit *
2. A copy of the importer's USFWS Migratory Bird Treaty Act import permit *
3. USFWS Form 3-177 (Declaration of importation or exportation of fish or wildlife) *
4. A copy of the collector's federal and provincial bird banding and scientific collecting permits, as necessary
5. FedEx Declaration of Biological Shipments
6. FedEx Commercial Invoice
7. CITES export permit, if applicable
8. 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:

+1 (207) 839–7600

bri@briloon.org

www.briloon.org

TRACE Project - Biodiversity Research Institute | Portland, ME USA (briloon.org)

8.0 References

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