APPENDIX IV. SAMPLING PROTOCOL, BRI, 2009.

2009

PROTOCOL FOR SAMPLING BIRD AND MAMMAL TISSUE FOR BLOOD FOR CONTAMINANT ANALYSIS

BIODIVERSITY RESEARCH INSTITUTE

PROTOCOL FOR SAMPLING BIRD AND MAMMAL TISSUE FOR CONTAMINANT ANALYSIS
2009

WILDLIFE SCIENCE CHANGING OUR WORLD

SUBMITTED BY:

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BioDiversity Research Institute (BRI) is a 501(c)3 nonprofit organization located in Gorham, Maine. 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.

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FRONT PHOTO CAPTION: Osprey (*Pandion haliaetus*) young and Indiana Bat (*Myotis sodalis*). Photos provided by BRI staff.

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BIRD SAMPLING

The following sampling protocol is based on 21 years of live-sampling over 6,000 birds including over 3,000 loons by BioDiversity Research Institute.

1.0 FEATHER COLLECTION

The collection of feather samples for mercury (Hg) analysis is useful in identifying the body burden of Hg in birds. The symmetrical collection of two feathers is useful for measuring fluctuating asymmetry. Any feather can be analyzed for Hg (including shed feathers), but adult secondaries are a useful standard. Secondaries can be pulled from songbirds but should be cut on all other birds (unless the bird is molting and it is easily removed). For some species, such as raptors, secondaries may not be feasible and therefore symmetrical collection of tail feathers is recommended. For unfledged birds, only body feathers are recommended (see Appendix A).

1.1 Secondary Flight Feathers

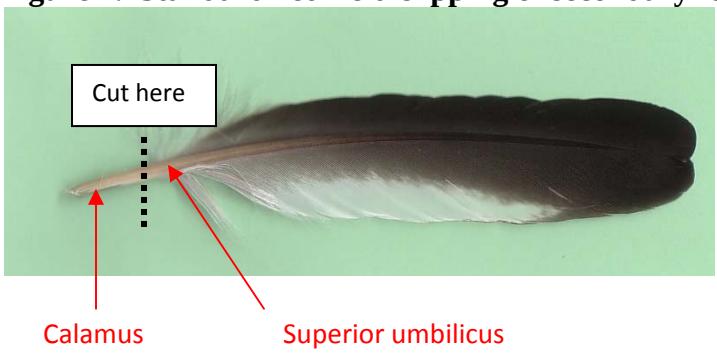
The second secondary feather should be clipped from each wing (i.e., two total feathers). The second secondary feather should be determined from where the primaries and secondaries meet in the middle of the wing (if difficult to determine, most birds have 10 primaries, grebes have 11, and songbirds 9 or 10). Each feather should be clipped along the calamus (shaft), well below the end of the feather vane (i.e. below the superior umbilicus on the calamus), located near the skin (Figure 1). Samples should be labeled and properly stored (see section 4.0 Label and Storage of Samples).

1.2 Supplies for Sampling Birds

The following supplies are needed for collecting feather samples:\

- Small cutting pliers
- 3/8 x 6 clasp envelopes
- Permanent marker (i.e., Sharpie®)

Figure 1. Standardized field clipping of secondary feathers



2.0 BLOOD COLLECTION

The collection of blood samples is used to identify the recent dietary uptake of Hg and can be obtained through two venipuncture techniques: direct draw and pierce. Direct draw is typically used on larger birds while piercing is suitable for small birds (see 2.1 Whole Blood; see Appendix A).

2.1 Whole Blood

Blood can be collected directly using syringes and butterfly needles with vacuum tubes (direct draw) or through piercing a vein and using capillary tubes or microtainers. Blood should be collected from either the caudal tibial vein (Figure 2) or the cutaneous ulnar (Figure 3). Samples should be labeled and properly stored (see section 4.0 Label and Storage of Samples).

Rule of thumb for the maximum volume of blood: 10% of a bird's weight is blood, therefore up to 10% of the volume of blood in a bird can be drawn. For example, for a 5000g adult loon, 500g of that weight is blood. From this, a maximum of 50g of blood can be taken (1g = 1cc). For a 10.g songbird, 1g is blood therefore a maximum of 0.1cc of blood can be taken. **BRI does not recommend taking the maximum amount in any species**.

2.2 Direct Draw Technique

- Sterilize collection area with a cotton swab and alcohol (if possible);
- Insert needle parallel with the vein;
- Begin drawing with manual syringe or insert vacuum vial into the butterfly tube;
- Draw 0.5-3.0 ccs of blood, depending on species and objectives;
- Remove needle and hold a fresh cotton swab on the collection area until bleeding has stopped (typically within 10 seconds);
- If vacuum tubes are not used, slowly place blood into heparinized vials, preferably not through the needle;
- Rock vials several times to adequately mix blood with heparin (IMPORTANT);
- Be sure to properly label and store samples (see section 4.0 Label and Storage of Samples).

Figure 2. Direct draw of blood from the caudal tibial vein.

2.3 Pierce Technique

- Locate the cutaneous ulnar vein in the wing (Figure 3 and 4);
- Prick vein with a needle by gently entering parallel to the vein (do not go through both vein walls, just the top one – IMPORTANT);
- Gently exit the vein and allow blood to pool (usually happens very quickly);
- Collect blood by placing the capillary tubes or microtainer below the pooled blood (a downward angle will allow the blood to be more easily pulled into the capillary tube or microtainer);
- Collect 2-3 capillary tubes; for birds that weigh at least 20 grams, at least 0.2 cc's in a microtainer;
- Fill capillary tubes at least ¼ full, but no more than ¾ full (IMPORTANT);
- Hold a fresh cotton swab on the collection area until bleeding has stopped (typically within 10 seconds);
- Use Critocaps® to seal each end of the capillary tube;
- Place capillary tubes in a vacuum vial and properly label all vials (see 2.1.3).

Figure 3 and Figure 4. Collecting blood from the cutaneous ulnar using a capillary tube.

2.4 Supplies

The following supplies are needed for blood collection, by technique:

For Direct Draw

- 21-27 g needle
- Green top plastic vacutainer (1-5 cc)
- Cottonballs, Cooler, Freezer Pack,
- Reclosable plastic bags (i.e., Ziploc® bags)
- Permanent marker (i.e., Sharpie®)

For Piercing

- 26-27 g needle
- Heparinized Mylar® Wrapped 75mm Hematocrit Tubes
- Critocaps ® Disposable Micro-Hematocrit Tube Closure
- Cottonballs, Cooler, Freezer Pack,
- Reclosable plastic bags (i.e., Ziploc® bags)
- Permanent marker (i.e., Sharpie®)

3.0 EGG COLLECTION

The collection of egg samples for Hg analysis is useful in identifying the body burden of Hg in females. 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" in pencil. Return the following day. If the "X" is still in the same position, indicating the egg has not been turned in 24 hours, collect the egg. Samples should be labeled and properly stored (see section 4.0 Label and Storage of Samples). The handling of viable eggs follows the same procedures as inviable eggs.

3.1 Supplies

The following supplies are needed for collecting eggs:

- Reclosable plastic bags (i.e., Ziploc®) – sandwich size, reclosable plastic bags (i.e., Ziploc®) – quart size
- Permanent marker (i.e., Sharpie®), Waterproof sample tags (i.e., Rite-in-the-Rain®), Pencil

4.0 LABELING AND STORAGE OF SAMPLES

4.1 Feather Samples:

- Store all labeled feathers in 3 3/8” x 6” clasp envelopes
- Each sample should be labeled using a permanent marker, with the following information: 1) date of collection; 2) species; 3) age and sex; 4) band # (if appropriate); 5) sampling location (i.e.; river or lake name); and 6) state or province.
- Feather samples should be stored at room temperature and out of sunlight.

4.2 Blood Samples:

- Each sample should be labeled using a permanent marker, with the following information: 1) date of collection; 2) species; 3) age and sex; 4) band # (if appropriate); 5) sampling location (i.e.; river or lake name); and 6) state or province.
- Store all labeled blood samples for mercury analysis in a **FREEZER** (IMPORTANT).

4.3 Egg Samples:

- Each sample should be labeled using a permanent marker, with the following information: 1) date of collection; 2) species; 3) age and sex; 4) band # (if possible); 5) sampling location (i.e.; river or lake name); and 6) state or province.
- Place eggs in a reclosable bag with a waterproof sample tag with the egg sample. Use a pencil to identify sample with the above information, including any comments.
- Store all labeled egg samples in a refrigerator if processing in <3 weeks or in a freezer if processing >3 weeks.

5.0 SAMPLE SHIPMENT

- All samples should be shipped in a plastic cooler with an enclosed master BRI packing list. Use attached form (Appendix I).
- If vacutainers are glass it is IMPORTANT d in such a way as to avoid breakage (i.e., use bubble wrap or put in a reclosable plastic bag and pack the cooler with newspaper).
- If samples are shipped Next Day Air, use frozen blue ice packs for blood and egg samples. The receipt of these samples after two days is acceptable. Contact BRI to make sure the office is open.
- Feathers do not need special packing and do not need to be kept cold (if shipped within 3-4 months after collection).
- For all samples, use packing or duct tape to seal the cooler.

Send to: BioDiversity Research Institute, 19 Flaggy Meadow Road, Gorham, Maine, USA. (207-839-7600/7655 tel/fax)

6.0 LABORATORY ANALYSIS

Tissue sample analysis for mercury in blood and eggs is most often conducted using a Cold Vapor Atomic Spectroscopy (CVAA) or direct mercury analyzer (DMA). For feathers CVAA, DMA and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) are used. The ICP-MS does not have the detection limits needed for blood and eggs. Proper homogenization techniques are critical for standardizing comparative tissue mercury concentrations.

BAT SAMPLING

The following sampling protocol is based on nine years of live-sampling over 3,000 bats and over 500 furbearers by BioDiversity Research Institute.

7.0 SAMPLE COLLECTION FOR BATS

The collection of blood samples is used to identify recent dietary uptake of Mercury (Hg) in bats.

7.1 Blood Collection

VENIPUNCTURE TECHNIQUE:

- Locate the acute ulnar vein in the wing or uropatagium vein (near foot) of the wing membrane
- Prick vein with a needle (do not go through both vein walls, just the top one – IMPORTANT);
- Allow blood to pool (usually happens very quickly);
- Collect blood by placing the capillary tubes or microtainer below the pooled blood (a downward angle will allow the blood to flow into the tube or microtainer through capillary action (Figure 5 and 6);
- Collect 1-2 capillary tubes or at least 0.2 cc's in a microtainer;
- Only fill capillary tubes a minimum ¼ to ½ maximum (IMPORTANT);
- Hold a fresh cotton swab on the collection area until bleeding has stopped (typically within 10 seconds – styptic powder may be used if necessary);
- Use either Critocaps (preferred) or Critoseal to seal each end of the capillary tube;
- Place capillary tubes in a vacuum vial and properly label all vials.

Figure 5 and Figure 6. Collecting blood from the acute ulnar using a capillary tube.

7.2 Supplies

The following supplies are needed for collecting bat blood samples:

- 27 or 27.5g needles
- 10 cc vacutainers
- Heparinized Mylar® Wrapped 75MM Hematocrit Tubes
- Critocaps ® Disposable Micro-Hematocrit Tube Closure
- Cottonballs, Cooler, Freezer Pack, Ziplock® bags

7.3 Bat Fur Removal

Clip hair from the belly and back, as close to skin without cutting the bat; use other locations as needed. Take 4-12 scissor cuts (~0.01 grams of hair, fill hair bag ¼ full). Small plastic bags or sample tubes can be used to store the samples. The hair follicle does not need to be harvested. To avoid cross-contamination, ensure scissors are cleaned between each bat sampled; this can be done by wiping the scissors with alcohol swabs and visually inspecting them to make sure there is no hair remaining from a previous bat. Samples should be labeled and properly stored (see section 8.0 Label and Storage of Samples).

7.4 Supplies

The following supplies are needed for collecting fur blood samples:

- Small stainless steel surgical, cuticle, or nose hair (curved) scissors.

8.0 SAMPLE COLLECTION FOR FURBEARERS

8.1 Blood Collection

After obtaining a total body weight by weighing the animal in its holding box, transferring to another holding box, and subtracting the weight of the original box, caught animals are administered tranquilizers. A mixture of Ketamine (2.5 mg/kg) and Metetomidine (0.025 mg/kg) sedatives via hand injection to the rump calm the animal during the tissue sample collection. Approximately three to five minutes following injection, the animal is fully sedated. Following sedation, blood can be obtained by using a 21-gauge needle and a green top-heparinized vacutainer, approximately 7 cc of whole blood can be drawn from the jugular vein or the brachial vein using a manual syringe. The blood can then be transferred to the green-top-heparinized vacutainer tube through the rubber gasket in the cap.

Following blood collection, the animal is administered the antiseden, Atipamezole (0.10 mg/kg). Mink and otter required approximately 5-10 minutes to fully recover following the injection of Atipamezole. The total time anesthetized is approximately 45 minutes.

8.2 Supplies

The following supplies are needed for collecting mink and otter blood samples:

- 21-19guage needles
- Green top and red top vacutainer
- Disposable Syringes -10mL
- Cotton balls and alcohol

8.3 Furbearer Hair Removal

A small patch of fur is clipped from the area located just above the animal's hind foot. Take 4-5 scissor cuts (~0.01 grams of hair) and stored in a small plastic bag or sample tube. The hair follicle does not need to be harvested. To avoid cross-contamination, ensure scissors are cleaned between each animal sampled; this can be done by wiping the scissors with alcohol swabs and visually inspecting them to make sure there is no hair remaining from a previous animal. Samples should be labeled and properly stored (see section 8.0 Label and Storage of Samples).

8.4 Supplies

The following supplies are needed for collecting mink and otter fur samples:

- Fur clippers or small scissors
- Small plastic bags or sample tubes

9.0 LABELING AND STORAGE OF SAMPLES

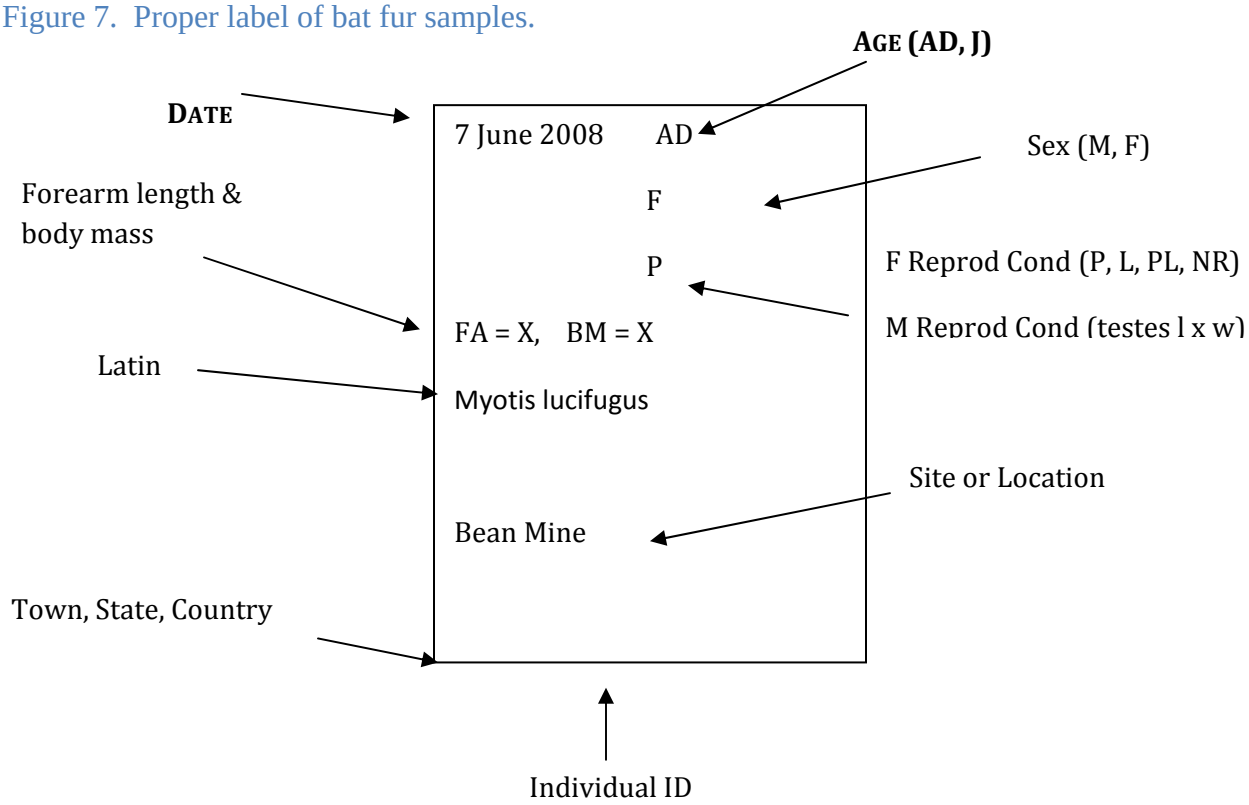
9.1 Fur Samples:

- Samples should be placed in small paper (glassine) envelopes, Ziploc bags, or small plastic screw-cap vials. Each sample should be labeled with a permanent marker, with the following information: (1) individual ID number (use an alpha-numeric code of species [2-letter code], country, and year), Species (Latin name), (2) location (site, town, county, state, country), (3) length of forearm (mm), (5) body mass (g), (6) age (adult, juvenile), (7) sex (M=male, F=female), (8) reproductive condition for females (P = pregnant, L= lactation, PL = post-lactation, NR, non-reproductive), (9) reproductive condition for males (length and width of testes) and (10) location, and date (Figure 7).

- For mink and otter, all general labeling criteria will be the same. Length of forearm (mm) and (8 + 9 reproductive conditions) may be omitted for these larger animals.

- Fur samples should be stored at room temperature and out of sunlight.

Figure 7. Proper label of bat fur samples.



9.2 Blood Samples:

- Each sample should be labeled using a permanent marker, with the following information: 1) date of collection; 2) species; 3) age and sex; 4) sample ID # or tag # (if appropriate); 5) sampling location (i.e.; river or lake name); and 6) state or province.
- Store all labeled blood samples for mercury analysis in a cooler with Blue ice and transfer to a **FREEZER AS SOON AS POSSIBLE** (IMPORTANT).

10.0 SAMPLE SHIPMENT

- All samples should be shipped in a plastic cooler with an enclosed master BRI packing list. Use attached form (Appendix I).
- If vacutainers are glass, it is IMPORTANT to pack in such a way as to avoid breakage (i.e., use bubble wrap or put in a reclosable plastic bag and pack the cooler with newspaper).
- If samples are shipped Next Day Air, use frozen blue ice packs for blood and fur samples. The receipt of these samples after two days is acceptable. Contact BRI to make sure the office is open.
- Fur does not need special packing and does not need to be kept cold (if shipped within 3-4 months after collection).
- For all samples, use packing or duct tape to seal the cooler.

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11.0 LABORATORY ANALYSIS

Tissue sample analysis for mercury in blood and fur is most often conducted using a Cold Vapor Atomic Spectroscopy (CVAA), Cold Vapor Atomic Florescence (CVAF), or direct mercury analyzer (DMA). Proper homogenization techniques are critical for standardizing comparative tissue mercury concentrations.

Appendix I. Example of Master Packing List for Samples.

BRI Master Packing List

Sample Type (egg, blood, feather, fur)	Date Collected	Species	Band Number	Age	Sex	Location	Town/State	Location (lat)	Location (long)