Bat 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.

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1.0 Protocol Overview

This sampling protocol is designed as a guide for the collection and processing of bat tissue samples for the measurement of total mercury. Sample collection following these general protocols will allow comparisons to be made across sampling sites and assist in identifying potential mercury hotspots posing risk to both human and ecosystem health.

2.0 Field Planning and Logistics

Sufficient planning and preparation will ensure that data collected are of the highest quality and will provide accurate information for regional resource managers.

2.1 Permits and Permissions

It is important to obtain all necessary ministry and/or national collection permits and licenses for the collection of bat and bat tissues.

3.0 Fur Sample Collection

3.1 Sampling Overview

Collected samples will provide important information in developing awareness about potential risks associated with mercury pollution. It is important to make sure all samples are collected in a safe and clean manner. Collaborating organizations are asked to record basic information for each sample. Sampling bat fur is indicative of chronic mercury exposure in bats.

3.2 Equipment Needed for Sampling

Materials for bat fur tissue sample collection are listed below.

Item	Purpose
Stainless steel cuticle scissors	To clip fur
Coin envelope, small plastic zip lock style bags, vials	For storing fur samples
Permanent marker & ballpoint pen	For labeling plastic zip lock bags
Data sheet	To be filled out for every sample collected
Map of country/sampling site	Use to mark the location of each sampling site

3.3 Bat Fur Collection

Standard protocols for the collection of bat fur are outlined in this section. For each bat, there are a series of standard measurements that must be taken and recorded on the sample data sheet.

3.3.1 Sampling Bat Fur

1. Record bat species and common name on the data sheet.
2. Collect a fur sample using small stainless steel cuticle scissors
3. Clip fur from the belly and back and other locations as needed. Clip as close to skin as possible without cutting the bat. The fur follicle does not need to be harvested. It will take 4-5 scissor swipes (~0.01 grams of fur).
4. Store fur in individually labeled sample container (e.g., coin envelope, plastic zip lock style bags, vials).
5. Ensure scissors are cleaned between each bat sampled by wiping the scissors with alcohol swabs and visually inspecting them to make sure there is no fur from a previous bat to avoid cross-contamination.
6. Samples do not need to be refrigerated or frozen

3.4 Fur Sample Labeling Format

It is imperative that all samples have a unique sample label ID that is labeled correctly and legibly. Use an alpha-numeric code of species [2-letter code], country, year, and individual number), species (Latin name), Location (site, town, county, state, country), length of forearm (mm), body mass (g), age (adult, juvenile), sex (M=male, F = female), reproductive condition for females (P = pregnant, L= lactation, PL = post-lactation, NR, non-reproductive), reproductive condition form males (length and width of testes), location, and date.

As an example, a bat sampled in Maine, United States on June 7th, 2008, could be labeled as follows in Figure 6:

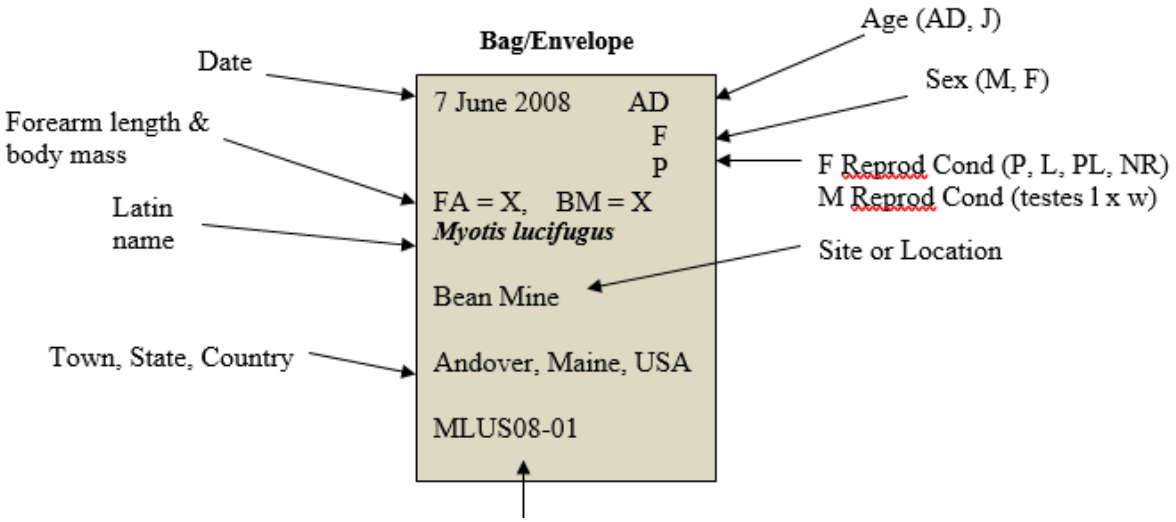

Figure 6. Correct labeling convention for individual shipped samples.

4.0 Blood Sample Collection

4.1 Sampling Overview

Collected samples will provide important information in developing awareness about potential risks associated with mercury pollution. It is important to make sure all samples are collected in a safe and clean manner. Collaborating organizations are asked to record basic information for each sample. Sampling bat blood is indicative recent dietary uptake.

4.2 Equipment Needed for Sampling Blood

Materials for bat blood tissue sample collection are listed below.

Item	Purpose
27, or 27.5g needle	To draw blood

10 cc vacutainer	Container for storing the blood sample tube
Hepranized Mylar® Wrapped 75MM Hematocrit Tubes	Tube for storing blood samples
Critocaps ® Disposable Micro-Hematocrit Tube Closure	Seals end of capillary tubes
Cotton balls	To stop bleeding after drawing blood
Permanent marker & ballpoint pen	For labeling plastic zip lock bags
Plastic zip lock style bags	To organize and store samples
Cooler and Freezer Packs	To keep samples cold in the field
Data sheet	To be filled out for every sample collected
Map of country/sampling site	Use to mark the location of each sampling site

4.3 Bat Blood Collection

Standard protocols for the collection of bat blood are outlined in this section. For each bat, there are a series of standard measurements that must be taken and recorded on the sample data sheet.

4.3.1 Sampling Bat Blood

1. Locate the acute ulnar in the wing or a vein in the tail portion of the wing membrane (**preferred**)
2. Prick vein with a needle—IMPORTANT—> **do not go through both vein walls in patagium, only the top one**; (only in uropatagium can you prick needle through both walls)
3. Allow blood to pool (usually happens very quickly)
4. 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)
5. Collect 1-2 capillary tubes or at least 0.2 cc's in a microtainer
6. IMPORTANT—> **only fill capillary tubes a minimum ¼ to ½ maximum**
7. Hold a fresh cotton swab on the collection area until bleeding has stopped (typically within 10 seconds)
8. Use either Critocaps (**preferred**) or Critoseal to seal each end of the capillary tube
9. Place capillary tubes in a vacuum vial and properly label all vials
10. Store all blood samples in cooler with ice packs while in the field
11. Transfer samples to a **freezer** for storage as soon as possible; they must remain frozen until analysis


Figures 1 and 2. Collecting blood from the acute ulnar using a capillary tube.

4.4 Blood Sample Labeling Format

It is imperative that all samples have a unique sample label ID that is labeled correctly and legibly:

1) date of collection
2) species
3) age and sex
4) ID #
5) sampling location (i.e.; river or lake name)
6) state or province.