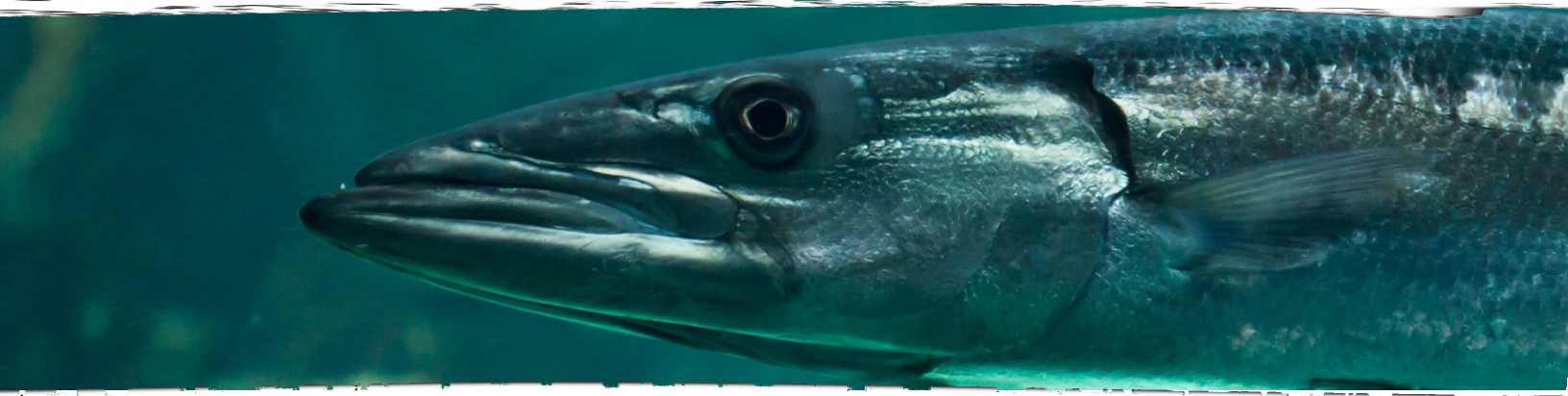




# Fish Field Sampling Methods



Collection of Tissues  
for Mercury Analysis

bri



## FISH 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.

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# 1.0 Protocol Overview

This sampling protocol is designed as a guide for the collection, processing, and shipping of fish 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 and exportation of fish and fish tissues.

## 2.2 Selecting a Sampling Site and Target Fish Species

Ideal sampling locations include freshwater and estuarine areas proximate to potential sources of emissions and releases of mercury. In addition, sensitive ecosystems such as wetlands and mangrove forests may also be appropriate for sampling. Target fish species and fish size should be selected based on at least one of two criteria: (1) the fish is an upper trophic level, predatory fish, and/or (2) is commonly consumed by local communities.

# 3.0 Fish Tissue 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

## COLLECTION OF TISSUES FOR MERCURY ANALYSIS

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each sample (e.g., fish species and total length) and collect fillet samples in plastic zip lock style bags. Samples will be frozen and shipped to Biodiversity Research Institute (BRI) for mercury analysis.

Sampling will focus on skin-off fillets of top predatory fish. Fish should be mature and of a similar size. Having a minimum of three individuals per species will allow for a more accurate assessment of mercury concentrations in the selected species.

It is important to note that at the market, fish may already be cut into fillets, so it is essential to avoid collecting more than one fillet from the same fish.

### 3.2 Equipment Needed for Sampling

Materials for fish tissue sample collection are listed below:

Item	Purpose
Ruler/tape measure	To measure the total length (cm) of the whole fish
Scale	To measure the total weight (g) of the whole fish*
Fillet knife	Needed only if subsampling fish from market or live caught fish
Plastic zip lock style bags and plastic wrap-food grade	For storing fish samples
Permanent marker & ballpoint pen	For labeling plastic zip lock bags
Small cooler with ice	To temporarily store fish 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

\* Total weight should be recorded only for live caught fish.

### 3.3 Fish Sample Collection

Standard protocols for the collection of fish fillet are outlined in this section. For each fish, there are a series of standard measurements that must be taken and recorded on the sample data sheet (Appendix I. Note: different steps are needed for sampling live caught fish versus fish from markets.

#### 3.3.1 Sampling Live Caught Fish

1. Set up clean plastic cutting surface with a clean fillet knife (Figure 1).
2. Record fish species and common name on the data sheet. Photograph fish and note the photo number on the “comments” column of the data sheet.
3. Record information about where the fish was caught (specify latitude and longitude) and record on the data sheet.
4. Rinse the whole fish with purified or clean water to remove any dirt and debris.
5. Use the scale to record the weight of whole fish (with guts intact) by first placing a clean piece of plastic wrap on the tared electronic scale. Weigh the fish in the tared piece of wrap to nearest 0.1 g and record on the data sheet (Figure 2).
6. Measure the total length of the fish to nearest 0.1 cm and record on the data sheet (Figure 3).



**Figure 1.** A clean working area for processing fish samples.



**Figure 2 (left) and 3 (right).** Taking the total weight (to the nearest 0.1 g) and total length (to the nearest 0.1 cm) of a fish. These data are used to interpret the results of mercury analysis and allows for comparisons across multiple sampling sites.

7. Remove a small section of scales from the dorsal part of the fish, directly behind the gills.
8. Position the knife perpendicular to the fish and cut through skin, stopping when the knife blade hits the backbone of the fish.
9. Slice through the fillet moving towards the ventral part of the fish. Be sure not to cut into the central cavity of the fish where body fluids could contaminate the sample (Figure 4). Remove the fillet from the fish body with a slicing motion towards the tail-end of the fish.



**Figure 4.** The first cut into the fish is behind the gills. The slice goes into the tissue and stops at the spine. Following this, a horizontal cut is made to separate the fillet from the bone and rest of the fish.

10. Place the skin/scale side down on the cutting board. Place the knife near the tail area and cut through the fillet without slicing through the skin. Angle the knife flat, in between the fillet and the skin, and slowly separate the fillet from the skin.
11. Rinse the fillet with purified water or clean water and drain the water from fillet.
12. Take a pre-labeled plastic zip lock bag and tare the field scale with the bag. Place the fillet in the bag and weigh to the nearest 0.1 g and record on the data sheet (Figure 5).
13. Place the sample bag in a second, pre-labeled bag, remove air, and seal.
14. Double-check all information on the data sheet.
15. Transfer the sample to the freezer until ready for shipment.





**Figure 5.** It is important to record the sample ID clearly and accurately. Place the cleaned fillet into the labeled bag. Remove all air from the bag and fold. Place the fillet sample on the tared, pocket-size scale. Record the fillet weight on the sample data sheet.

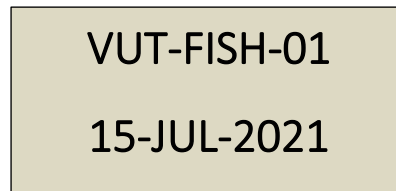
### 3.3.2 Sampling Fish from Market

1. Record fish species and common name on the data sheet.
2. Obtain information about when and where the fish was caught. Specify the location (latitude and longitude) on the data sheet, noting if the information is where the fish was caught or (if unavailable) note the market location.
3. Measure total length of the fish to nearest 0.1 cm (record on the data sheet).
4. If possible, use a scale to record the weight of whole fish (with guts intact).
5. Collect a tissue sample from the fillet and store in a labeled plastic zip lock bag.
6. Temporarily store labeled sample bags in a cooler while in the field.
7. Post-field collection and prior to storing samples in a freezer, please rinse all fish samples with purified water to make sure no surface contamination is present.
8. After rinsing, samples can be pat dry with a paper napkin. Do not reuse napkins between samples.
9. Take a pre-labeled plastic zip lock bag and tare the scale with the bag. Place the fillet sample in the bag and weigh to the nearest 0.1 g and record on the data sheet.
10. Be sure all rinsed samples are returned to their original, labeled plastic zip lock bag. Transfer the sample to the freezer until ready for shipment.

## 4.0 Sample Labeling Format

It is imperative that all samples have a unique sample label ID that is labeled correctly and legibly. Each country is assigned a unique three-letter code, following the country codes developed by the International Organization for Standardization (ISO). These codes can be found at <https://www.iso.org/obp/ui/#search/code/>

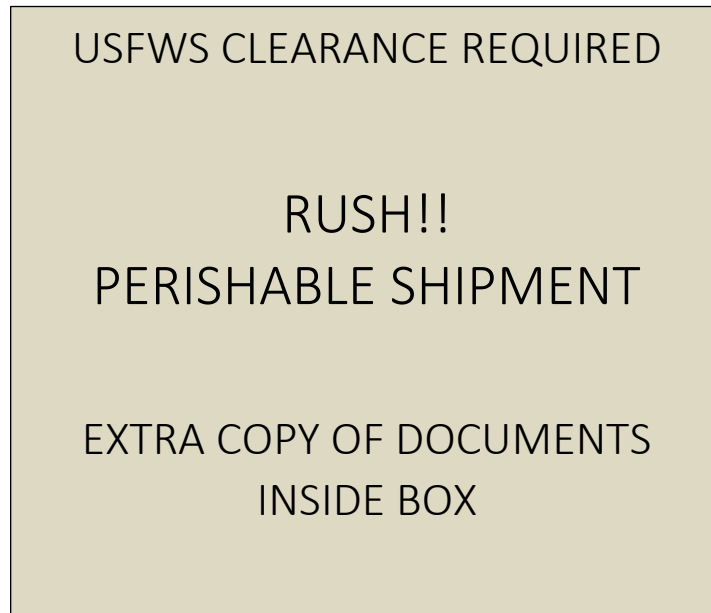
When labeling each fish fillet sample, please use the following convention: the three-letter country code followed by the word FISH, and the two-digit, sequential number of the sample (e.g., 01, 02, 03, ...). Below the label, please put the date the sample was collected, using the format of DD-MMM-YYYY. As an example, the first fish sampled from Vanuatu on July 15, 2021, could be labeled as follows in Figure 6:



**Figure 6.** Correct labeling convention for individual shipped samples.

## 5.0 Sample Shipment

Fish samples are to be stored frozen solid (deep freeze) prior to shipment. Use a small foam cooler to secure and store samples in during shipment. Place freezer packs in the shipment cooler. The cooler should then be sent in a cardboard box, properly labeled on the outside (see Figure 7) and include all documentation. For advice on packaging your samples, contact BRI ([mark.burton@briloon.org](mailto:mark.burton@briloon.org)).



**Figure 7.** On the outside of the cardboard box holding the small foam cooler with samples, write clearly or affix a label with the above language.

International shipment of samples requires all paperwork to be filed correctly with the appropriate authorities and that all export and import permissions are granted. Please follow the steps below to mail samples:

1. Email your fish pictures and a copy of your data sheet to BRI ([mark.burton@briloon.org](mailto:mark.burton@briloon.org)) and await further instruction. Keep in mind that fish samples will need to be shipped on a **Monday** or **Tuesday** to avoid weekend delays. Please email this information to BRI at least two weeks prior to shipping to allow sufficient time to prepare the necessary forms.
2. BRI will verify your pictures and the information on your data sheet. Once verified, BRI will send you a completed USFWS 3-177 via email.
3. Once your USFWS import documentation is prepared, print three copies of the form. Include one inside the package and put two in a DHL pouch on the outside of the cardboard box containing the foam cooler with samples.
4. BRI will schedule your shipment with DHL. You will receive an email from BRI with instructions and DHL's paperwork for shipment of your package.

**Important Shipping Notes**

1. Please keep samples frozen until the time of pickup or drop off at your DHL location.
2. Every effort will be made to schedule shipments early in the business week (i.e., Monday or Tuesday) to avoid possible delays at U.S. customs.
3. When asked by the shipping representative if you are shipping anything perishable, answer NO!

## 5.1 Customs Export Paperwork

Each collaborating organization must provide any certificate or proof of scientific research and exporting authorization, if necessary, prior to conducting the sampling.

All tissue and whole fish samples must be cleared by all appropriate governmental agencies and customs offices. It is imperative that shipments be accompanied by all the necessary export permits.

Some countries may require an export permit to ship wildlife samples. Contact country national wildlife authorities (for ex. Ministry of Environment) for information. If a country requires an export permit, be sure to make copies of the permit and include one copy on the outside of the package (cardboard box) and one inside the package (foam cooler).

**Shipments arriving in the United States may be denied entry, destroyed, or returned if they do not include the appropriate, country-specific export permits.**

If you have any questions or concerns about securing permits, please contact Mark Burton at BRI ([mark.burton@briloon.org](mailto:mark.burton@briloon.org)).

