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# Preparation and Analysis Methods for Fish Tissue Collected from Lake Koocanusa, Montana

Open-File Report 2025–1034

U.S. Department of the Interior
U.S. Geological Survey

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# Conversion Factors

International System of Units to U.S. customary units

| Multiply | By | To obtain |
|---|---|---|
| | Length | |
| millimeter (mm) | 0.03937 | inch (in.) |
| micrometer (micron; µm) | 0.00003937 | inch (in.) |
| | Mass | |
| gram (g) | 0.03527 | ounce, avoirdupois (oz) |
| milligram (mg) | 0.0003527 | ounce, avoirdupois (oz) |
| kilogram (kg) | 2.205 | pound, avoirdupois (lb) |
| | Volume | |
| milliliter (mL) | 0.03382 | ounce, fluid (fl. oz) |

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

# Supplemental Information

Isotopes are atoms of an element having the same number of protons but a different number of neutrons within the atomic nucleus. This difference may cause differences in the physical and chemical behavior of less abundant isotopes when compared to the more commonly abundant isotope. Isotopic abundances are commonly expressed as ratios of the isotope of interest to the more abundant isotope in a sample to the ratio of those isotopes in a standard. The values are expressed in delta (δ) notation as parts per thousand (or per mil; ‰) differences between the isotopic ratios in the sample and the standard. Some isotopes are stable and do not change (decay) over time; other isotopes are unstable (radioactive) and change (decay) into different elements or isotopes with time.

# Abbreviations

| | |
|---|---|
| $\delta^{13}C$ | carbon-13/carbon-12 isotopic ratio |
| $\delta^{15}N$ | nitrogen-15/nitrogen-14 isotopic ratio |
| < | less than |
| ≤ | less than or equal to |
| EPA | U.S. Environmental Protection Agency |
| GSI | gonadosomatic index |
| ICP–MS | inductively coupled plasma-mass spectrometry |
| ID | identification |
| MTDEQ | Montana Department of Environmental Quality |
| MTFWP | Montana Fish, Wildlife and Parks |
| NBF | neutral-buffered formalin |
| QA | quality assurance |
| QC | quality control |
| QuEChERS dSPE | Quick, Easy, Cheap, Effective, Rugged and Safe dispersive solid phase extraction |
| USGS | U.S. Geological Survey |

# Preparation and Analysis Methods for Fish Tissue Collected from Lake Koocanusa, Montana

By Travis S. Schmidt,[1] Ashley M. Bussell,[1] Molly A. Moloney,[1], James L. Dunnigan,[2] Trevor M. Selch,[2] Jessica E. Brandt,[3] Craig A. Stricker,[1] A. Robin Stewart,[1] Veronika A. Kocen,[1] Danielle Cleveland,[1] Vicki S. Blazer,[1] Sarah E. Janssen,[1] Jacob M. Ogorek,[1] Meghan Dunn,[4] Theresa L. McBride,[4] Katie B. Adams,[4] Benjamin P. Colman,[5] Matt Young,[5] and Jennie Christensen[6]

## Abstract

Lake Koocanusa, a reservoir, receives mine wastes from metallurgical coal mines in the Elk River Valley of British Columbia, Canada. Selenium and other elements discharged by the mines into the waters of the United States can pose unknown risks to aquatic life. The U.S. Geological Survey Wyoming-Montana Water Science Center can collaborate with Montana Fish, Wildlife and Parks and other State and Federal agencies to design studies and to collect fish tissues to help fill this knowledge gap. This report describes the processes, techniques, and methods used to collect and analyze fish tissue collected from Lake Koocanusa; and procedures used to review and manage data, including quality assurance and quality control procedures used by the U.S. Geological Survey Wyoming-Montana Water Science Center and supporting analytical laboratories. These fish tissue collections began in 2021.

## Introduction

In 2012, the Montana Department of Environmental Quality (MTDEQ) identified Lake Koocanusa (also referred to as "Koocanusa Reservoir"), a transboundary reservoir in northwestern Montana and southeastern British Columbia, Canada (fig. 1), as threatened by selenium contamination from sources outside the State of Montana's jurisdiction or borders and subsequently listed the water body under section 303(d) of the U.S. Clean Water Act (33 U.S.C. 1251 et seq.) (MTDEQ 303(d) list [MTDEQ, 2018]). Research has determined that the dominant source of selenium to Lake Koocanusa is runoff from extensive metallurgical (nonthermogenic) coal mining operations (Storb and others, 2023), which are upstream from the reservoir in British Columbia. Selenium is biologically essential at low concentrations, but elevated environmental concentrations can cause toxicological and adverse biological effects (Lemly, 1999; Environment and Climate Change Canada, 2022; Montana Legislative Services Division, 2022). For example, trophic transfer and bioaccumulation of selenium in the aquatic food web can result in excess selenium being maternally transferred to eggs. In the egg, elevated selenium concentrations can impair egg viability and development of larval fish, resulting in physiological malformations (for example, spinal and fin deformities and teratogenic defects) and reduced egg survival and reduced growth, and survival of fish larvae and fry (Lemly, 1999; Johnson and others, 2020; Environment and Climate Change Canada, 2022). Fish populations within Lake Koocanusa, such as *Oncorhynchus clarki lewisi* (westslope cutthroat trout; Girard, 1856), *Oncorhynchus mykiss* (rainbow trout; Walbaum, 1792), *Prosopium williamsoni* (mountain whitefish; Girard, 1856), and Federally threatened *Salvelinus confluentus* (bull trout; Suckley, 1859; Hansen and others, 2002) declined considerably after the construction of the Libby Dam (Dunnigan and others, 2017; Presser and Naftz, 2020), while *Lota lota* (burbot; Linnaeus, 1758; Lee and others, 1980) populations have declined to near zero (Hardy and others, 2015; Dunnigan and others, 2017). The Kootenai River (spelled Kootenay in Canada) below Libby Dam supports the species described above and others including a population of the federally endangered *Acipenser transmontanus* (white sturgeon; Richardson, 1836; Duke and others, 2007). Moreover, burbot and westslope cutthroat trout are considered culturally important and have recreational value to Tribes in the region (Presser and Naftz, 2020). Considerable effort and resources have been dedicated to restoration of degraded habitats across the reservoir (Hardy and others, 2015), but water quality continues to contribute to declining populations (Dunnigan and others, 2023a).

[1]U.S. Geological Survey.

[2]Montana Fish, Wildlife and Parks.

[3]University of Connecticut.

[4]U.S. Environmental Protection Agency.

[5]University of Montana.

[6]TrichAnalytics, Inc.

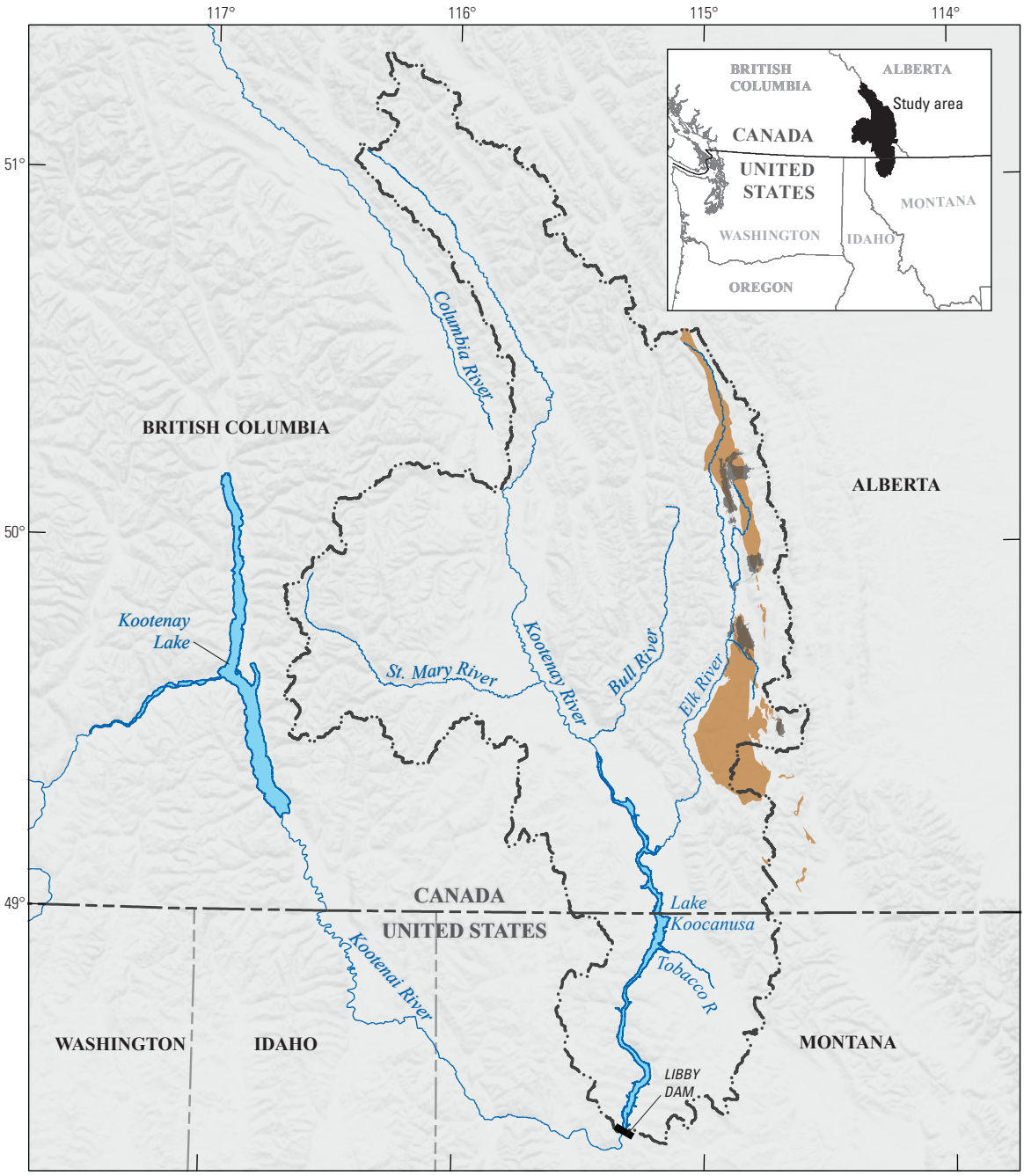


**Figure 1.** Map showing Lake Koocanusa, the Elk River Valley coal deposits and mines, and the Kootenai River Basin (spelled Kootenay in Canada), British Columbia, Canada, and Montana, United States.

## Purpose and Scope

This report describes the processes, techniques, and methods used for fish tissue collection on Lake Koocanusa, including descriptions of postcollection processes, techniques, and methods for the preparation and analysis of fish tissue. In addition, the process includes data management and review procedures, including quality assurance (QA) and quality control (QC) procedures used by the U.S. Geological Survey (USGS) Wyoming-Montana Water Science Center and supporting analytical laboratories. Fish tissue collections began in 2021. Changes to collecting fish tissue could include the fish species or exact tissue type targeted for collection, timing of fish collection, or chemical constituents of interest.

## Approach

The overall authority for the determination of fish collection and laboratory analysis of a tissue sample needs is collaborative decision between USGS Wyoming-Montana Water Science Center; Montana Fish, Wildlife and Parks (MTFWP); and the MTDEQ in coordination with the Koocanusa/Kootenai monitoring program. Fish species selected for sampling in 2021 included five or more individuals of the following fish species: rainbow trout, westslope cutthroat trout, *Richardsonius balteatus* (redside shiner; Richardson, 1836), *Ptychocheilus oregonensis* (northern pikeminnow; Richardson, 1836), *Catostomus macrocheilus* (largescale sucker; Girard 1856), and *Mylocheilus caurinus* (peamouth chub; Richardson, 1836; Page and others, 2023). The specific species targeted, timing and method of capture, and tissues harvested may change over time at the discretion of the researchers involved in this study. Fish collection is the responsibility of the MTFWP, leveraging existing fieldwork (Dunnigan and others, 2023b). MTFWP ensures all personnel who collect fish samples have appropriate training, are cognizant of onsite safety considerations, and maintain sample integrity throughout all collection procedures and field-processing steps. Postprocessing of fish, tissue sample preparation, sample submission for laboratory chemical analysis, tracking chains of custody, and data management is the responsibility of the USGS Wyoming-Montana Water Science Center.

All fish tissue samples are shipped to USGS or partner (non-USGS) laboratories for analysis; other laboratories may be added as needed. USGS laboratories described in this document include the Eastern Ecological Science Center Leetown Research Laboratory in Kearneysville, West Virginia; the Geology, Geophysics, and Geochemistry Stable Isotope Laboratory in Denver, Colorado; the National Research Program Ecology and Contaminants Project Laboratory in Moffett Field and Menlo Park, California; the Upper Midwest Water Science Center Mercury Research Laboratory in Madison, Wisconsin; and the Columbia Environmental Research Center in Columbia, Missouri. Non-USGS laboratories include the Center for Environmental Sciences and Engineering at the University of Connecticut in Storrs, Connecticut; Brooks Applied Laboratory in Bothell, Washington; the U.S. Environmental Protection Agency (EPA) Region 10 Manchester Environmental Laboratory in Port Orchard, Wash.; the Environmental Biogeochemistry Laboratory at the University of Montana in Missoula, Montana; and TrichAnalytics, Inc., in Saanichton, British Columbia, Canada. The addresses and contact information for USGS and non-USGS laboratories include the following:

- U.S. Geological Survey, Eastern Ecological Science Center, Leetown Research Laboratory
  11649 Leetown Road
  Kearneysville, WV 25430

- U.S. Geological Survey, Geology, Geophysics, and Geochemistry Stable Isotope Laboratory
  Denver Federal Center
  Building 95, MS 963
  Denver, CO 80225–0046

- U.S. Geological Survey, National Research Program Ecology and Contaminants Project Laboratory
  345 Middlefield Road
  Mail Stop 496
  Menlo Park, CA 94025

- U.S. Geological Survey, National Research Program Ecology and Contaminants Project Laboratory
  Building 800450
  Severyns Avenue
  Moffett Field, CA 94035

- U.S. Geological Survey, Upper Midwest Water Science Center
  Mercury Research Laboratory
  1 Gifford Pinchot Dr
  Madison, WI 53726

- U.S. Geological Survey, Columbia Environmental Research Center
  4200 New Haven Rd
  Columbia, MO 65201

- Center for Environmental Sciences and Engineering, University of Connecticut
  3107 Horsebarn Hill Road
  U–4210
  Storrs, CT 06269

- Brooks Applied Laboratory
  13751 Lake City Way NE, Suite 108
  Seattle, WA 98125

- U.S. Environmental Protection Agency, Region 10,
  Manchester Environmental Laboratory
  7411 Beach Drive E
  Port Orchard, WA 98366

- Environmental Biogeochemistry Laboratory
  Charles H. Clapp Building, Room 365
  University of Montana, Geosciences
  32 Campus Drive 1296
  Missoula, MT 59812–1296

- TrichAnalytics, Inc.
  207, 1753 Sean Heights
  Saanichton, British Columbia, Canada V8M 0B3

## Field Sampling and Design

The MTFWP leverages existing programs to collect desired fish species and sample numbers deemed necessary to address exposure and uptake of elemental contaminants by fish and into various fish tissues (for example, liver, ovary, and muscle). The sampling design, methods used, and data collected as part of fish collection in Lake Koocanusa and the Kootenai River downstream from Libby Dam have been previously described in part here and elsewhere (Dunnigan and others, 2023b) including descriptions of sampling season(s), sampling locations, collection methods, and required field data (that is, fish length, weight, sex, and maturity). The MTFWP is responsible for providing all field personnel with required training.

Fish collection efforts are categorized into two primary collection periods. The first collection period occurs during the MTFWP's annual standardized fish population assessment, which monitors trends and status of the fish community in Lake Koocanusa. This effort uses gill nets placed at 14 standardized locations on the reservoir. This collection period is when MTFWP has historically collected the most fish for tissue analysis. Sexually mature fish are not targeted for collection but are identified once fish are captured. The targeted sample size during this collection period may be as many as 10 females of each species containing eggs to serve as a trend indicator of contaminant levels.

The second collection period employs a combination of gill netting, electrofishing, and angling to collect the targeted species at approximately weekly intervals leading to the peak of sexual maturity for each species. Sampling dates are identified annually based on water temperatures, which affect the timing of spawning activity and sexual maturity of the targeted fish species. Timing of sexual maturity can help provide a comprehensive evaluation of representative samples collected from the same water body over multiple years. Five female specimens of each species can be targeted for collection during each sampling event during this sampling period. Captured fish are humanely euthanized, minimally processed in the field, and placed immediately on ice until later frozen.

If field collections fail to achieve the target number of females or collect a male fish, these fish can be processed and analyzed according to methods and procedures outlined in this report. The target number of fish specimens per species is meant to minimize the number of fish harvested and provide enough data to develop an average concentration per sampling period. For example, burbot are purposely not targeted during some years but could be inadvertently collected. When possible, burbot are targeted by hoop nets for regular population estimates, and some burbot could be processed as described in this report.

## Initial Sample Handling and Field Documentation

MTFWP maintains sample integrity for all steps of collection through field sample processing (Dunnigan and others, 2023b). Fish are handled with powder-free nitrile gloves at all phases of the sampling process. Sample collection information and field observations are documented using a field sampling form. Identification of fish to the species level are performed in the field by a MTFWP biologist. Samples that cannot be definitively identified in the field can be frozen for later identification in the laboratory. The MTFWP collection team measures total length and weight and notes the presence of any external abnormalities (for example, parasites, deformities, lesions, tumors, and eroded fins) on the field sampling form. A metric measuring board on a plastic or wooden base is used for length measurements. Total length is recorded to the nearest millimeter with the fish's body positioned on its right side, mouth closed, and facing the observer's left. Weight is recorded to the nearest gram by placing the fish into a precleaned plastic container set on a portable electronic balance. Standard field cleaning procedures are followed between each fish collected for all field equipment (EPA, 2015). When possible, gonads are removed in the field, weighed, and preserved to calculate the gonadosomatic index (GSI). The GSI is calculated by the formula (*gonad weight*/ [*body weight–gonad weight*]) (Jan and Jan, 2017). Individual gonads with sufficient weight are split equally into two parts—half are frozen to less than or equal to (≤) −20 °C for analytical chemistry analysis, and the other half are fixed in 10 percent neutral-buffered formalin (NBF) for histological analysis. Gonads identified for histological analysis are placed in a plastic, labeled collection jar, and covered with NBF to 10 times the tissue volume for initial tissue preservation. Prior to shipping, excess NBF volume is poured off leaving enough NBF to sufficiently cover the specimen. Frozen specimens cannot be used for histopathological analysis. Note that NBF is a hazardous material and should be handled with care by trained staff.

When fish size could limit available gonad tissue sample weight, such as when working with redside shiners, gonads may not be removed in the field, and pairs of fish may be captured so that 5 females are available for chemical analysis and 5 females for histological evaluation. Any deviation from standard sampling practices is documented on the field sampling form.

Whole fish (ovary already dissected) are double bagged in sealable plastic zipper bags and placed on ice until delivered to MTFWP offices in Libby, Mont., where they are frozen to ≤−20 degrees Celsius (°C). Once frozen, fish should not be allowed to thaw until time of dissection by the USGS Wyoming-Montana Water Science Center. Fish may remain frozen for as much as 1 year before dissection. When not field extracted, gonads are removed in the WY–MT WSC labs, weighed, and preserved to calculate the GSI as described above.

## Preparing for Dissection

Fish tissue resection and processing is performed by USGS WY–MT WSC staff. All fish processing activities are recorded in a laboratory tissue processing log data sheet (app. 2). Dissected fish tissue are weighed on a 4-place analytical balance if greater than 200 grams or a 5-place analytical balance if the weight is smaller than 200 grams. Analytical balances are certified annually and verified daily upon use. All surfaces, equipment, and cutting tools are decontaminated as appropriate between each fish tissue sample to avoid cross-contamination of samples. Clean plastic sheeting is placed on cutting surfaces, trays, and balances prior to placing the sample on the work area (EPA, 2015; Queensland Department of Environment and Science, 2018). Plastic covers and nitrile gloves are replaced after working with each fish or tissue sample to prevent cross-contamination between fish samples. Procedures for filleting and dissecting fish follow the EPA document “Technical Standard Operating Procedure for Fish Handling and Processing” (EPA, 2001), and additional details of the steps involved are provided in the next section. Any deviations from these procedures should be noted on the laboratory tissue processing log data sheet. All tissue samples are placed in a clean, labeled vial or zipper bag (according to procedures supplied by the USGS or supporting laboratory performing the analysis), and then placed into a second cleaned zipper bag and immediately placed in the laboratory freezer. Prior to dissection, a naming convention procedure should be created to identify each tissue sample and relate the sample back to each individual fish. Labeled sample containers are kept with the associated field and laboratory tissue processing sheets and chain of custody forms for the entirety of the project or shipped following laboratory-specified shipping procedures.

## Fish Dissection

1. Put on clean powder-free nitrile gloves.
2. Lay fish flat on one side with the dorsal fin facing away from you.
3. Remove the liver and egg/ovary (when not previously removed in the field) and weigh.
4. Place each sample (liver, egg/ovary) into its individual labeled sample container.
5. Remove the left filet of the fish from the tail to the dorsal fin, remove the skin, and record the filet weight.
6. Proceed to the opposite side and remove the right filet of the fish, remove the skin, and weigh, if necessary, to recover sufficient muscle weight for characterization. Note that each laboratory provides a minimum working weight for a specific analysis or analytical suite.
7. Place filet(s) into its individual labeled sample container as specified by the laboratory analyzing that subsample.
8. Place all labeled sample containers into a zipper bag and keep frozen at −20 °C until shipment to the designated laboratory for analysis. Holding time recommendations for frozen wet fish tissue (including egg/ovary) is 6 months but can be up to 2 years for most elemental analyses (EPA, 2000; Queensland Department of Environment and Science, 2018). Note that smaller sample sizes may lose moisture more rapidly than larger samples during extended freezer storage. If moisture is a critical component, it may be desirable to freeze-dry the tissue in a timely manner, which extends the holding time for elemental analysis indefinitely.

## Sample Documentation and Chain of Custody

All collected fish tissue samples should be labeled with a unique alphanumerical sample identification (ID) number that relates back to the individual fish collected. Labels should contain, at a minimum, the sample ID number, species identification, sample date, and resection time. Tissue samples are maintained and stored at the USGS WY–MT WSC laboratory until shipped to an analytical laboratory for future analysis. Frozen samples are shipped to laboratories on dry ice and by the fastest shipping method possible. Refer to appendix 1 for the collection and processing flowchart.

A record of all fish tissue samples collected and shipped to other laboratories are maintained by the project chief or assigned project team member, with status updates to include

received dates and laboratory assigned ID numbers in an electronic spreadsheet, to ensure the complete and timely receipt of analytical results.

## Sample Preparation

Fish tissue samples are dried and homogenized by the WY–MT WSC in Helena, Mont., or by the individual laboratories receiving the samples depending on project and laboratory needs. Fish tissue is dried by freeze-drying or by dehydrator at a temperature no higher than 55 °C. The percentage of moisture (the quantity of water contained in the tissue) is calculated using the equation, *moisture content* (*in percentage*)=(*wet weight of sample–dry weight of sample*)/(*wet weight of sample×100*) (EPA, 2001). When freeze-drying the Wyoming-Montana Water Science Center will use a Labconco FreeZone® freeze-dry system with bulk tray dryer. The percentage of moisture calculations is notated in the WY–MT WSC laboratory notebook, and calculations and results should be provided as a machine-readable data file to other laboratories upon request.

## Sample Analyses

The procedures for laboratory analyses of tissue samples are shown in table 1. Sample and QC results are compiled by the Wyoming-Montana Water Science Center and reported in a USGS ScienceBase data release (https://www.sciencebase.gov/catalog/). Redundancy of analytes among laboratories may be due to the need to use different methods to address the large differences in the weight of fish tissues analyzed and (or) changes in the availability of various laboratories to support this project.

### U.S. Geological Survey Eastern Ecological Science Center, Leetown Research Laboratory, Kearneysville, West Virginia

The USGS Eastern Ecological Science Center Leetown Research Laboratory performs histopathological analysis on tissue samples (table 1) to characterize the reproductive health developmental stage of fish gonads. Each gonad (ovary) sample are given a unique histology laboratory ID number upon receipt. This ID number is maintained throughout the analytical and reporting processes and allows the histopathologic data to be linked back to the original sample. Formalin-fixed gonads are cross-sectioned, dehydrated, paraffin-embedded, sectioned at 5 micrometers, and stained with hematoxylin and eosin for light microscopic examination (Luna, 1992). For QA/QC purposes, slides are read by two Eastern Ecological Science Center histopathologists. Gonad stage, and any abnormalities, such as atresia, abnormal yolk, parasites, fibrosis, or intersex, are documented as described by Blazer (2002).

### U.S. Geological Survey Geology, Geophysics, and Geochemistry Stable Isotope Laboratory, Denver, Colorado

The USGS Geology, Geophysics, and Geochemistry Stable Isotope Laboratory analyzes muscle tissue subsamples for the stable isotopes of carbon-13/carbon-12 isotopic ratio ($\delta^{13}C$) and nitrogen-15/nitrogen-14 isotopic ratio ($\delta^{15}N$; table 1), which can be used as an indirect indicator of fish trophic position in a food web (Post, 2002). Samples consist of 1±0.1 milligram (mg) dry weight of homogenized sample material weighed into a 5- by 9-millimeter tin crimp-sealed capsule (Costech Analytical, Inc.). Each sample is given an internal laboratory ID number. Samples are analyzed for $\delta^{15}N$ and $\delta^{13}C$ using a flash isotope ratio mass spectrometry elemental analyzer (ThermoFisher Scientific; Waltham, Massachusetts), interfaced to a Delta V Plus mass spectrometer (ThermoFisher Scientific; Waltham, Mass.), and operated in continuous flow mode (Johnson and others, 2018). Results are reported in standard delta (δ) notation in parts per thousand (or per mil; ‰) relative to the respective isotopic scales. Data are normalized to reference material USGS40 (L-glutamic acid; Qi and others, 2016) and USGS41a (L-glutamic acid enriched in $^{13}C$ and nitrogen-15; Qi and others, 2016). Laboratory standards are analyzed as unknowns, and duplicate samples are analyzed to provide estimates of accuracy and precision (typically less than [<] 0.2 per mil for both isotopes). Secondary laboratory standards are analyzed to provide supporting estimates of precision and data quality. The mass fractions of sample nitrogen and carbon are reported (as percentages) where appropriate following normalization to the accepted concentrations of certified reference materials (Qi and others, 2016).

### U.S. Geological Survey National Research Program Ecology and Contaminants Project Laboratory, Menlo Park, California

The USGS National Research Program Ecology and Contaminants Project Laboratory analyzes tissue samples for selenium (table 1). Specialized laboratory analysis is required when tissue sample weights are below that specified by standard methods. Consequently, some small weight samples of <10 mg dry weight are analyzed for selenium using a specialized method performed by the USGS National Research Program Ecology Contaminants Project Laboratory (table 1). Small weight samples are analyzed using a three-step nitric-perchloric acid reflux procedure with selective hydride generation atomic absorption spectrophotometry and oxidative

**Table 1.** Summary of procedures for laboratory analyses of tissue samples.

[Refer to the "Sample Analyses" section of report for additional analytical information. mg, milligram; mg/kg, milligram per kilogram; USGS, U.S. Geological Survey; ww, wet weight; NA, not applicable; $\delta^{13}C$, carbon-13/carbon-12 isotopic ratio; $\delta^{15}N$, nitrogen-15/nitrogen-14 isotopic ratio; dw, dry weight; ‰, parts per thousand or per mil; EPA, U.S. Environmental Protection Agency; ICP–MS, inductively coupled plasma-mass spectrometry; ICP–MS/MS, inductively coupled plasma-tandom mass spectrometry; Se, selenium; Hg, mercury]

| Analyzing laboratory | Analysis type | Analysis method or method reference | Minimum required sample weight (mg) | Method detection limit (mg/kg) | Method reporting limit |
|---|---|---|---|---|---|
| USGS Eastern Ecological Science Center, Leetown Research Laboratory, Kearneysville, West Virginia | Histopathology | Light microscopic examination (Blazer, 2002) | Variable (ww) | NA | NA |
| USGS Geology, Geophysics, and Geochemistry Stable Isotope Laboratory, Denver, Colorado | Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) | Isotope ratio mass spectrometry (Johnson and others, 2018) | 2 (dw) | NA | 4.5–6.4 ‰ |
| USGS National Research Program Ecology and Contaminants Project Laboratory, Moffett Field and Menlo Park, California | Se | Selective hydride generation atomic absorption spectrophotometry (Stewart and others, 2004) | 2–10 (dw) | 0.01–0.03 | 0.03 mg/kg |
| USGS Upper Midwest Water Science Center Mercury Research Laboratory, Madison, Wisconsin | Methylmercury | Gas chromatography and cold vapor atomic fluorescence spectroscopy (Hammerschmidt and Fitzgerald, 2006) | 10 (dw) | 0.0013 | NA |
| | Total Hg | Cold vapor atomic absorption spectrophotometry (EPA Method 7473; EPA, 1998a) | 10 (dw) | 0.0038 | NA |
| USGS Columbia Environmental Research Center, Columbia, Missouri | Multiple elements, including Se | ICP–MS; similar to EPA Methods 3050B (EPA, 1996a) and 6020B (EPA, 2014) | 20 (dw)[1] | Variable | Variable[2] |
| Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, Connecticut | Vitamin E (alpha-tocopherol) | Ultra-high performance liquid chromatography-mass spectrometry (Yu and others, 2008; Sony and others, 2019) | 100 (ww) | NA | 0.0152 mg/kg |
| | Vitamin A (retinol) | | 100 (ww) | NA | 0.006 mg/kg |
| Brooks Applied Laboratory, Bothell, Washington | Se | ICP–MS/MS (EPA Methods 3052 and 6020B; EPA, 1996b; 2014) | 50 (ww) | 0.005–0.013 | 0.009–0.025 mg/kg |
| | Methylmercury | Gas chromatography and cold vapor atomic fluorescence spectroscopy (EPA Method 1630; EPA 1998b) | 50 (ww) | 0.001–0.009 | 0.0028–0.0114 mg/kg |
| | Total Hg | ICP–MS/MS (EPA Methods 3052 and modified 6020B; EPA, 1996b, 2014) | 50 (ww) | 0.003–0.008 | 0.008–0.023 mg/kg |

**Table 1.** Summary of procedures for laboratory analyses of tissue samples.—Continued

[Refer to "Sample Analyses" section of report for additional analytical information. mg, milligram; mg/kg, milligram per kilogram; USGS, U.S. Geological Survey; ww, wet weight; NA, not applicable; $\delta^{13}C$, carbon-13/carbon-12 isotopic ratio; $\delta^{15}N$, nitrogen-15/nitrogen-14 isotopic ratio; dw, dry weight; ‰, parts per thousand or per mil; EPA, U.S. Environmental Protection Agency; ICP–MS, inductively coupled plasma-mass spectrometry; ICP–MS/MS, inductively coupled plasma-tandom mass spectrometry; Se, selenium; Hg, mercury]

| Analyzing laboratory | Analysis type | Analysis method or method reference | Minimum required sample weight (mg) | Method detection limit (mg/kg) | Method reporting limit |
|---|---|---|---|---|---|
| EPA Region 10 Manchester Environmental Laboratory, Port Orchard, Washington | Multiple elements including Se | ICP–MS (EPA Methods 3052 and 6020B; EPA, 1996a, 2014) | 2.5 (ww) | Variable | Variable |
| | Total Hg | Cold vapor atomic absorption spectrophotometry (EPA Method 7473; EPA, 1998a) | To be determined | To be determined | Approximately 0.040 mg/kg |
| Environmental Biogeochemistry Laboratory, University of Montana, Missoula, Montana | Multiple elements, including Se and total Hg | ICP–MS/MS (Fatemian and others, 1999; EPA, 2007; Wolf and Adams, 2015) | 2–50 (dw) | Se: 0.03–0.1<br>Hg: 0.06–0.1 | NA<br>NA |
| TrichAnalytics, Inc., Saanichton, British Columbia, Canada | Multiple elements, including Se and total Hg | Laser ablation ICP–MS (LaBine and others, 2021) | 10 (dw)[1] | Se: 0.1–0.5<br>Hg: 0.01–0.03 | Se: 0.3–1.5 mg/kg<br>Hg: 0.03–0.1 mg/kg |

[1]Minimum required sample weight can be lowered if required.

[2]Results censored at the limit of quantification; a preset method reporting limit is not used.

digestion (Stewart and others, 2004). Residue is redissolved after the evaporation of the nitric acid in 4 molarity hydrochloric acid, and then stored until final selenium analysis. Teflon boiling stones, diluted digest solution, 2 percent persulfate solution, and concentrated hydrochloric acid are added to a 400-milliliter glass beaker, covered, and brought to a boil. Once boiling, heat is reduced to maintain a constant rolling boil for 30 minutes. Samples are analyzed using hydride generation after being cooled overnight. Accuracy verification is completed by the digestion of selenium in oyster tissue standard from the National Institute of Standards and Technology with each group of 10 samples and corrected for salt content by measuring sodium concentrations using flame atomic absorption spectroscopy. To ensure accuracy and precision, standards are analyzed as unknowns in triplicate (typical recovery is 97.2 percent), and the duplicates are processed (typical standard deviation divided by mean is <4.5 percent). All selenium concentrations are reported on a dry weight basis.

## U.S. Geological Survey Upper Midwest Water Science Center, Mercury Research Laboratory, Madison, Wisconsin

The USGS Upper Midwest Water Science Center Mercury Research Laboratory analyzes tissue samples for methylmercury, total mercury, and the percentage of moisture (table 1). Once received, frozen samples are immediately stored at −20 °C, assigned unique sample identifiers, and entered into the laboratory information management system. Samples are lyophilized and homogenized before analysis. In addition, frozen tissue samples are weighed before and after lyophilization to calculate the percentage of moisture (refer to the "Sample Preparation" section). Samples are analyzed for total mercury and methylmercury following procedures described in EPA Method 7473 (EPA, 1998a) and Hammerschmidt and Fitzgerald (2006), respectively. Briefly, samples are analyzed for total mercury using combustion (to a maximum temperature of 850 °C) coupled to detection via cold vapor atomic absorption spectroscopy using a Nippon MA-3000. Tissues for methylmercury analysis are pretreated using a 4.5 molar nitric acid extraction and heated for 8 hours at 55 °C prior to analysis. Methylmercury extracts are then analyzed for concentration by derivatization (using sodium tetraethylborate) and coupled to detection by gas chromatography and cold vapor atomic fluorescence spectroscopy using a Brooks Rand automated methylmercury analyzer. For total mercury and methylmercury analyses, QA and QC measures include the use of certified calibration standards purchased from the instrument manufacturer, triplicate analysis of 10 percent of the total sample count, analysis of certified reference materials such as International Atomic Energy Agency 407-fish homogenate and 436-tuna fish flesh at a 10 percent sample frequency, and routine analysis of instrument- and method-blank samples.

## U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri

The Environmental Chemistry Branch at the USGS Columbia Environmental Research Center analyzes tissue samples for multiple elements (23 or more, number of elements may change over time) including selenium (table 1). Tissues are lyophilized, microwave digested with high-purity concentrated nitric acid and hydrogen peroxide, and then analyzed using inductively coupled plasma-mass spectrometry (ICP–MS; PerkinElmer NexION 2000); concentrations are reported on a dry weight basis. These methods are similar to EPA 3050B (EPA, 1996a) and EPA 6020B (EPA, 2014), and the analytical approach is intended to represent the biologically or environmentally accessible (that is, "total recoverable") elemental fraction. The QC includes laboratory control standards, analysis duplicates and spikes, method replicates and spikes, interference check samples, and the use of certified reference materials.

## Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, Connecticut

The Center for Environmental Sciences and Engineering at the University of Connecticut analyzes tissue subsamples for vitamin E (alpha-tocopherol) and vitamin A (retinol; table 1). Samples are prepared by Quick, Easy, Cheap, Effective, Rugged and Safe dispersive solid-phase extraction (also referred to as "QuEChERS dSPE") techniques. Fish tissue and QC samples are analyzed by ultra-high performance liquid chromatography with mass spectrometry (Waters Acquity coupled with Acquity TQD tandem mass spectrometers; Waters Corporation, Milford, Mass.) using an ultra-high performance liquid chromatography ethylene bridged hybrid C18 column at 25 °C for analyte separation (Yu and others, 2008; Sony and others, 2019). Alpha-tocopherol, retinol, surrogates, and an internal standard compound are detected and quantified in positive electrospray ionization mass spectrometry mode using the Waters Corporation IntelliStart software. Analyte signal optimization and statistical analyses for results calibration and quantification are performed using the Waters Corporation QuanLynx application manager (MassLynx software version 4.2; Yu and others, 2008; Sony and others, 2019). All peaks are quantified against the internal standard of testosterone-d3. Laboratory QA procedures are followed, including the analysis and evaluation of QC samples (duplicate, method-blank, matrix-spike, and laboratory-control samples).

## Brooks Applied Laboratory, Bothell, Washington

Brooks Applied Laboratory analyzes tissue samples for selenium, methylmercury, and total mercury (table 1). Analyses are performed using a microwave digestion (modified EPA Method 3050B; EPA, 1996a) with a mixture of concentrated nitric acid, hydrochloric acid, and hydrogen peroxide, and inductively coupled plasma-tandem mass spectrometry using a modified version of EPA Method 6020B (EPA, 2014). Samples analyzed for methylmercury are extracted using a mixture of potassium hydroxide and methanol in accordance with established procedures (EPA Method 1630; EPA, 1998b). Gas chromatography and cold vapor atomic fluorescence spectroscopy is then used to quantify methylmercury in the extracts. Analysis of total solids begins with each sample being measured into preweighed vessels. Vessels are reweighed at the end of the lyophilization process, then a percentage of dried solid is calculated as weight remaining after moisture removal. Internal data review includes, at minimum, a review by an analyst, QA specialist, and two project managers. QC samples analyzed in each analytical batch includes laboratory duplicate samples, certified reference samples, laboratory blank spike samples, and method-blank samples.

## U.S. Environmental Protection Agency Region 10 Manchester Environmental Laboratory, Port Orchard, Washington

The EPA Region 10 Manchester Environmental Laboratory analyzes tissue samples for multiple elements, including selenium and mercury (table 1). Samples are subdivided, with a portion of wet material reserved for mercury analysis by EPA Method 7473 (EPA, 1998a) using a Milestone direct mercury analyzer based on atomic absorption analysis. The bulk of the samples are homogenized, freeze-dried in a Labconco FreeZone® freeze-dry system with bulk tray dryer, particle-size reduced, and microwave digested in a CEM MARS 6 microwave. Microwave digestion follows a modified version of EPA Method 3052 (EPA, 1996b) using concentrated nitric acid, hydrochloric acid, and hydrogen peroxide. The microwave digestates are analyzed for multiple elements (possibly 18) including selenium by ICP–MS following EPA Method 6020B (EPA, 2014) on an Agilent 7700x ICP–MS (table 1). Preparation batches for both ICP–MS and direct mercury analyzer includes the following QC samples: method blanks, laboratory-control samples (blank spikes), laboratory duplicates, matrix-spike and matrix-spike duplicate samples, and certified reference materials. Preparation for freeze-drying (moisture content) includes method-blank and laboratory duplicate samples. All analytical results undergo two levels of technical review before release.

## Environmental Biogeochemistry Laboratory, University of Montana, Missoula, Montana

The Environmental Biogeochemistry Laboratory at the University of Montana analyzes tissue samples for multiple (greater than 50) elements using inductively coupled plasma-tandem mass spectrometry, including selenium and total mercury (Fatemian and others, 1999; Wolf and Adams, 2015; table 1). Samples between 5 and 50 mg dry weight are digested by microwave digestion in Teflon vessels with ultrapure nitric acid and hydrogen peroxide using a modified version of EPA Method 3051A (EPA, 2007). To stabilize mercury, gold chloride is added immediately postdigestion (Lo and Wai, 1975). Selenium isotopes (selenium-77, selenium-78, selenium-80, and selenium-82) are analyzed using oxygen as a reaction gas and a mass shift of 16. Mercury is analyzed on mass using oxygen as a reaction gas for mercury-200, mercury-201, and mercury-202. Laboratory QC standards include blank, spike, and duplicate samples that have either been generated through the digestion method and subsequent dilutions or in the laboratory after digestion, as well as independent calibration verification standards and certified reference materials.

## TrichAnalytics, Inc., Saanichton, British Columbia, Canada

TrichAnalytics, Inc., analyzes tissue samples for multiple elements, including selenium and mercury (table 1). Each sample is given a unique laboratory identifier upon receipt. If samples are not lyophilized when received, the wet samples are weighed, dried in a dehydrator at <55 °C, and then reweighed to calculate percentage of moisture. Dried samples are homogenized into a fine powder with a stainless-steel pestle and mortar. The powder is then placed in a die press to create a pellet for analysis. Samples are analyzed using laser ablation solid sampling coupled with ICP–MS detection (LaBine and others, 2021). This approach allows for dry sample weights as low as 10 mg to be analyzed without compromising detection limits (table 1). Sample preparation is adapted for tissues smaller than 10 mg. The sample metal concentrations (30 elements, including selenium and mercury) are determined using certified reference materials. Each sample set is assessed for accuracy and precision using the certified reference materials as unknown test samples to ensure the analytical system is suitable for meeting data quality objectives. If accuracy and precision data quality objectives are not met, the data from that sample set is not reported, and the sample set is reanalyzed. A duplicate sample is analyzed for every 10 samples to assess homogeneity. Results are reported in milligrams per kilogram dry weight.

## Quality Assurance and Quality Control Procedures

The QA/QC procedures applicable to fish tissue processing, preservation, and preparation by the USGS WY–MT WSC include the following:

1. The USGS WY–MT WSC laboratory operates under the quality management system required of all USGS laboratories. The quality management system establishes a standard by which all laboratory operations are performed in accordance with established policy, procedures, and documentation that ensure the laboratory continually meets a defined standard of quality (U.S. Geological Survey, 2023).

2. Key components of the QA/QC plan for this project include, but are not limited to, following standard operating procedures for decontaminating equipment, dissecting of fish for tissue analysis, and using skilled personnel for laboratory preparation and processing. All deviations from standard protocols are documented in the physical or electronic log sheets and (or) laboratory notebook, and photographic documentation is completed, when possible, then stored in electronic data files.

3. Total holding time and freezer temperature monitor records from relinquishment (from MTFWP) to resection and delivery to each respective laboratory is also documented for each sample.

## Data Quality Objectives

The data quality objectives for this study closely follow guidelines outlined by the U.S. Department of the Interior (2008) to ensure data of known and acceptable quality are obtained. Documentation of basic information such as compatible monitoring objectives and program design features, metadata (when, where, and how data will be collected and who collected and analyzed the data), ancillary information (explanatory variables and study-site characteristics), and QA/QC data released from the analytical laboratories is used to evaluate data quality and uncertainty.

## Data Quality Assessment

Laboratory QC samples and results are reviewed and compared with those of the co-collected primary samples. Other factors considered in each data review include compliance with sample laboratory hold times and receipt conditions (samples arrive at the lab at the appropriate temperatures), results of laboratory blank sample analyses, laboratory detection limits, and adherence to laboratory control standards. Estimated values may be used for evaluation purposes, whereas rejected values are qualified, flagged, and not used in any interpretive analyses.

## Data Management and Reporting

Any preparation and processing information, metadata, and (or) ancillary information recorded onto paper forms by the USGS WY–MT WSC laboratory personnel is scanned into electronic project files and considered original records. These data are combined with analytical data from the laboratories in electronic data files to maximize their preservation. The USGS WY–MT WSC site administrator maintains backups of all locally stored electronic information. Nonelectronic information, if any, is retained by the project chief or designated personnel. Analytical results and metadata that have been reviewed and approved is disseminated to the public through a USGS data release, interpretative report(s), and (or) other published documents.

## Data Processing and Validation

Sample metadata, laboratory preparation and processing notes, and any ancillary information is combined with laboratory analytical data into electronic data files. Analytical data are any chemical, physical, or biological determination results released from the laboratory. All analytical results are reviewed for completeness, and questionable values are noted by the project chief or designated personnel. Analytical results from each laboratory are released in a Microsoft Excel spreadsheet format to the project chief and (or) designated personnel. These analytical results include any laboratory data qualifiers and (or) explanations in the laboratory note section of the spreadsheet. If data from more than one tissue sample are available, the results can be compared with previous results to identify obvious errors, such as decimal errors, and sample outliers. The analytical results and any comparisons noted are reviewed and recorded in the electronic project file by the project chief or designated personnel. “Reviewed and approved” results can be included in the USGS data release, and any laboratory data qualifiers can be indicated in the associated metadata.

## Health and Laboratory Safety

A job hazard analysis has been prepared for this study and is provided to USGS WY–MT WSC laboratory personnel and reviewed prior to sample handling and processing activities. The job hazard analysis includes an assessment of job tasks; potential on-the-job exposures to chemical

and biological hazards; unsafe acts, or conditions; required personal protective equipment and job responsibilities; and telephone contacts. A copy of the job hazard analysis is provided in appendix 2.

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# Appendix 1. Collection and Processing Flowchart

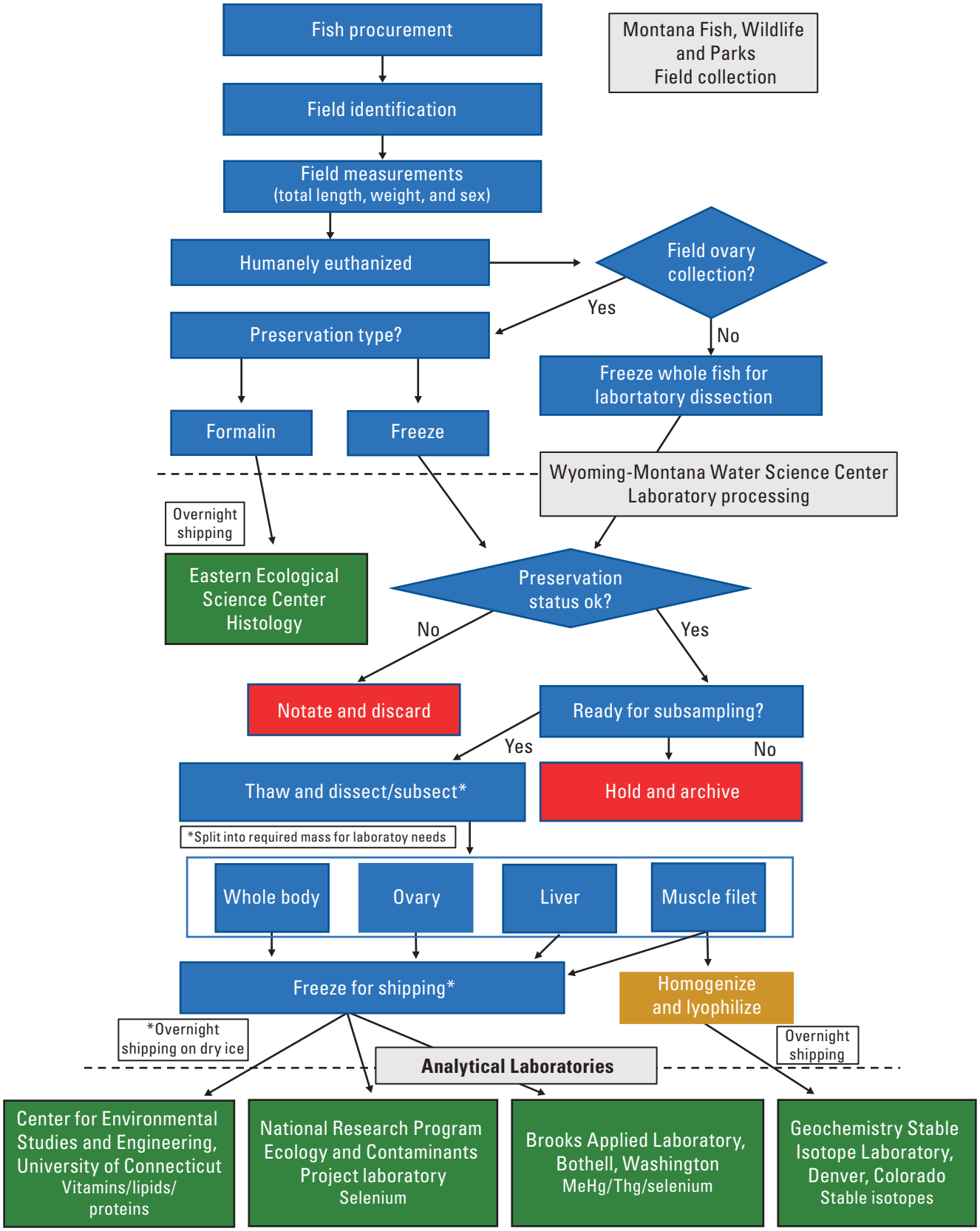


**Figure 1.1.** Flowchart showing collection and processing procedures.

# Appendix 2. Job Hazard Analysis

## Emergency Contacts

In an emergency, dial 911 or contact the sheriff or police department listed in table 2.1. For nonemergency help, call the U.S. Geological Survey (USGS) Helena, Montana, laboratory contacts in table 2.2.

## Laboratory Safety Requirements

1. A chemical hygiene plan shall be developed for any USGS laboratory handling hazardous material.
2. All laboratory personnel shall be provided with the appropriate personal protective equipment and mandated use for handling of hazardous materials.
3. Laboratory personnel shall be provided with material safety data sheets and any needed training for handling hazardous materials.
4. Hazardous materials and waste are handled and disposed of in accordance with the transportation and handling procedures outlined in laboratory Chemical Hygiene Plan.

## Potential Laboratory Hazards

1. Physical hazards from sharp objects, electrical shock, freeze-dryers, and ovens are present.
2. Chemical hazards from storing and handling of acids and solvents are present.
3. Chemical hazards from exposure via inhalation of formalin are present.

## Medical Emergencies

All USGS laboratory staff are responsible for necessary medications and personal requirements. USGS personnel are required to be current in CPR and first aid. Laboratory staff should be aware of each other's medical conditions and treatments for those conditions. Examples of medical conditions may include severe allergic reactions, diabetes, and coronary problems. If a medical emergency occurs, call 911.

**Table 2.1.** Sheriff and Police contacts.

| Location | Contact | Telephone number |
|---|---|---|
| Lewis and Clark County, Montana | Sheriff department | (406) 447–8204 |
| Helena, Montana | City police | (406) 442–3233 |

**Table 2.2.** Laboratory emergency contacts.

| Laboratory | Emergency contacts | Telephone number |
|---|---|---|
| U.S. Geological Survey Wyoming-Montana Water Science Center ecology laboratory | Travis Schmidt | (406) 594–9788 |
| U.S. Geological Survey Wyoming-Montana Water Science Center water-quality laboratory | Lindsey King | (406) 465–7021 |

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