Appendix B

Fish Tissue Sampling Standard Operating Procedure

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REVISION HISTORY

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ACRONYMS

Acronym	Definition
CFR	Code of Federal Regulations
COC	Chain of Custody
CVM	Center for Veterinary Medicine (FDA)
DEQ	Department of Environmental Quality (Montana)
DELTs	Deformities, eroded fins, lesions, and tumors
EDP	EQuIS Data Processor
EPA	Environmental Protection Agency (U.S.)
FDA	US Food and Drug Administration
FWP	Fish, Wildlife & Parks (Montana)
FWS	Fish & Wildlife Service (US)
HASP	Health and Safety Plan
HAZWOPER	Hazardous Waste Operations and Emergency Response
HUC	Hydrologic Unit Code
ID	Identification
INAD	Investigational New Animal Drug
JHA	Job Hazard Analysis
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
SAP	Sampling and Analysis Plan
SDS	Safety Data Sheet (formerly Material Safety Data Sheet)
SOP	Standard Operating Procedure
T/E	Threatened or Endangered
TMS	Tricaine methanesulfonate
U.S.	United States
USGS	United States Geological Survey
WQPB	Water Quality Planning Bureau (DEQ)
WQS	Water Quality Standards

DEFINITIONS

Word	Definition
Cervical Dislocation	Separation of the spinal column from the skull and/or brain.
Chain-of-Custody	Chain of custody (COC) is the protocol that provides a record of the persons having control of, and access to, a sample.
Composite Sample	A homogeneous mixture of samples from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample (U.S. Environmental Protection Agency, 2000).
Contaminant Reduction Zone	The location at a hazardous waste site where decontamination occurs.
Data Quality Objective	A statement of an acceptable quantitative criterion relative to the quality and quantity of the data to be collected and the ultimate use of the data.
Exclusion Zone	Also called the "hot zone," the area with the known suspected contamination and therefore the highest potential for exposure to hazardous substances.
Exophthalmia	An abnormal physical condition characterized by marked protrusion (bulging) of the eyeballs.
Formaldehyde	A colorless flammable gas with a pungent odor that is highly reactive with many substances and used as the basis of Formalin preservative solution.
Formalin	A solution of formaldehyde gas in water containing a certain percentage of methanol as a stabilizer; used as a disinfectant and as a preservative of biological samples
Health and Safety Plan	A document or collection of documents the objective of which is to assure that all work conducted as part of the project is done as safely as possible and with full consideration and awareness of potential risks.
Ichthyological Taxonomy	A branch of science dealing with the naming and identification of species of fish.
Introgression	The transfer of genetic information from one species to another as a result of cross-breeding.
Medical Monitoring Program	A schedule of periodic medical examinations performed for field personnel with the objective of preventing and identifying any degradation in health due to occupational exposure.
Neoplasm	An abnormal mass of tissue or a tumor.
Quality Assurance Project Plan	A document containing the elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for a project.
Safety Data Sheets	A document required by 29 Code of Federal Regulations (CFR) 1910.1200(g) to be provided by a chemical manufacturer, distributor, or importer the objective of which is to communicate the hazards associated with a chemical to the user.
Spawning	Manual stripping of eggs from live female fish.
Righting Response	The ability of a fish to correctly orient itself (dorsal side up) in water; an indicator of stress level or other conditions.
Taxon	A group of organisms determined by taxonomists to form a distinct unit.
Voucher	A preserved specimen representing selected species and maintained for validating taxonomic identifications and documenting spatial and temporal distributions (U.S. Geological Survey, 2002). May also be in photographic form.

1.0 SCOPE AND APPLICABILITY

Since 1998, the United States Environmental Protection Agency (EPA) has conducted or collaborated in ongoing freshwater fish contamination studies (U.S. Environmental Protection Agency, 2014b). At the drafting of this standard operating procedure (SOP), EPA is updating the national recommended aquatic life criteria for selenium. The updated criteria contain two water-column-based and two fish tissue-based elements, and EPA recommends that delegated authorities adopt all four elements (U.S. Environmental Protection Agency, 2014a). In anticipation of the continuing need for accurate data representing the levels of chemicals found in fish species in Montana, the Montana Department of Environmental Quality (DEQ) has drafted this SOP.

This SOP is intended to serve as a guide for and to assure integrity and consistency in the collection of tissue samples, including whole fish, fillet, and biopsy plugs, from fish populations in Montana waters by or for DEQ, and it should be referenced in development of project planning and design documents. Sample design elements, fish capture and collection methods (electrofishing, netting, etc.), and laboratory methodologies for analysis of chemicals in fish tissue samples are beyond the scope of this SOP.

2.0 FIELD SAMPLE COLLECTION METHODS

Sample collection procedures must be followed diligently to ensure the integrity of the information used in reporting and upon which any actionable decisions are based. Methods outside those described herein may be appropriate or necessary on a case-by-case basis, but any such deviations from the following methods should be included in event planning documents if anticipated and otherwise noted in sampling event documentation (e.g. logbooks) and final reporting.

2.1 PERSONNEL QUALIFICATIONS AND TRAINING

Fish tissue sampling teams must be staffed with personnel experienced in ichthyological taxonomy and fish handling, tissue sampling, and sample preparation and preservation. At a minimum, the sampling team should be composed of a fisheries biologist knowledgeable in local fish species, a field technician experienced with these sampling protocols, and a quality control person if the biologist and technician do not have the expertise to serve this role (Tetra Tech, 2000). Field personnel should be in adequate physical condition for the performance of sampling work in potentially adverse field conditions including extremes of heat, cold, elevation, precipitation; near dangerous wildlife; etc. **Note:** if the personnel performing fish specimen collection do not include a Montana Fish, Wildlife and Parks (FWP) representative, a fish collection permit must be obtained from FWP for the project.

All team members should be provided with a copy of any project documents pertinent to their role including but not limited to the sampling and analysis plan (SAP), the quality assurance project plan (QAPP), this SOP, field documentation and chain of custody (COC) forms, and sample labels. Team members should ensure they read and are familiar with the SAP, QAPP, and SOP. Personnel must be familiar with any health hazards associated with fixing or preserving agents to be used. Safety Data Sheets (SDS) for all chemicals used in field operations must be available to sampling team members. If

sampling will be performed in an exclusion or contaminant reduction zone of a hazardous waste site, sampling personnel are required to have Hazardous Waste Operations and Emergency Response (HAZWOPER) training.

Due to time or resource constraints, it may only be feasible to have one sampling team member fully trained or certified in all areas; typically this would be the team leader. However, pre-field orientation or training sessions for sampling team members should be conducted whenever possible. These sessions should emphasize strict adherence to sampling protocols, health and safety measures, and nuance specific to the project and should include hands-on activity in addition to classroom instruction. During field sampling activity, inexperienced sampling team members should be accompanied by technically proficient personnel until such time as they are able to perform tasks adequately on an individual basis.

2.2 HEALTH AND SAFETY

Personnel health and safety must be the highest priorities for any activity performed for DEQ, and these priorities are everyone's responsibility. Fish tissue sampling tasks are likely to occur in the vicinity of surface waterbodies and possibly in harsh outdoor environments. In such instances, refer to **Section 3.3** of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring," (Montana Department of Environmental Quality, 2012). If a health and safety plan (HASP) is available for the project or site, it should be reviewed and adhered to by sampling team personnel. The sampling team leader or project planner should complete the Job Hazard Analysis (JHA) form (WQPB-JHA-01), including project-specific information. All sampling team members must read and sign the form, and the form must be signed by a supervisor.

Sampling team members with potentially incapacitating medical conditions (e.g. MedicAlert medical issues) should provide enough information regarding such condition(s) to their team leader prior to entering the field to allow them to be prepared in the event of an emergency. Participation in a medical monitoring program may be appropriate if known or suspected hazardous materials will be encountered; however, it is not typically necessary for fish tissue sampling work. Additional safety precautions that should be taken specific to fish tissue sampling include the following:

- Proper handling of chemical fixatives (e.g. formaldehyde and formalin) to prevent inhalation or dermal contact issues;
- Safe use, storage, and transport of sampling sharps such as fillet knives, biopsy punches, and fish scalers;
- Use of gloves or pliers, as appropriate, to prevent cuts, punctures, or lacerations when handling fish species having sharp fins, spines, and teeth;
- Awareness of dangerous local wildlife that may be attracted to fish sampling activities; and
- Following correct lifting practices for moving heavy objects such as full sample coolers.

2.3 EQUIPMENT AND FIELD SUPPLIES

Attachment A of this SOP is a list of equipment and supplies specific to fish tissue sampling events that is intended to supplement field work checklist items such as those found in the general sections of **Attachment A** - Field Supply List of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring" (Montana Department of Environmental Quality, 2012).

Additions to or subtractions from this list may be appropriate dependent upon specific project objectives and conditions. Equipment and supplies should be inventoried and checked for condition and functionality prior to departure for the field and upon return. Restock, repair, and replacement of equipment as appropriate should occur immediately following field work to aid planning and scheduling for the next sampling team and/or event. Sample containers and labels should be prepared prior to sampling, to the extent possible, to increase efficiency in the field and reduce holding times.

2.4 FISH HANDLING

Maintaining sample integrity from the point of collection through sample processing and analysis is critical to meeting data quality objectives. All potential sources of extraneous contamination should be identified before sample collection and handling, if possible, and steps should be taken to ensure their elimination or minimization (Tetra Tech, 2000). Fish should be handled using clean nitrile gloves at all phases of sample processing.

Whether whole fish, fillet, biopsy punch sampling, and/or compositing will be employed, fish-to-sample processing should occur as quickly as possible following collection of fish from the waterbody. Despite efforts to limit handling and stress to fish, some mortality is likely. Mortality can be minimized by recognizing which species are sensitive to handling and prolonged confinement and processing them before others (U.S. Geological Survey, 2002). Shorter processing duration will typically result in increased sample integrity and survival rates for no-kill sampling specimens. **Threatened/Endangered (T/E) species should be released immediately upon capture unless included in the project, in which case they should be processed first and returned to the water as quickly as possible.**

2.4.1 Lethal vs. No-Kill

Lethal fish tissue sampling techniques may be appropriate in circumstances where determination of effects on aquatic life or human health warrant whole fish, fillet, or target tissue sampling. In such cases, fish should be euthanized as humanely as possible by anesthetizing or delivering a sharp, forceful blow to the head using a foil wrapped or inert, decontaminated instrument, followed by cervical dislocation while taking care not to break the skin. The force of the blow should be similar (slightly less) to that needed to drive a nail into wood (Erway et al., 2004). If this blunt trauma method is not feasible, asphyxiation by wrapping in foil and placing in a plastic bag on ice is an alternative (Parametrix, Inc., 2009) or an overdose of anesthetic may be administered.

When lethal sampling methods are not necessary or not possible (e.g. T/E species), the no-kill, biopsy punch method may provide data that meets quality objectives for certain analytes. This method involves inserting a biopsy punch into a de-scaled muscular section of a live fish. Following sample extraction, an antibiotic salve is placed over the excised area and the fish is released. In addition to eliminating the need to euthanize fish, biopsy punch sampling uses clean equipment and supplies each time a sample is collected, thus providing some reduction in risk of sample contamination (Syracuse Research Corporation, 2003). Mercury analysis concentration results have been demonstrated to be similar to fillet sample results (within 6%) under certain analysis techniques without causing fish mortality (Baker et al., 2004). See **Section 3.2.2** for details on performing biopsy punch sampling.

2.4.2 Anesthetizing

Anesthesia can facilitate sampling operations such as taking fish measurements and biopsy punch samples and may also lessen injury and/or mortality to fish. The following substances have been found to be effective anesthetizing agents for fish:

- Tricaine methanesulfonate (commonly as Tricaine methanesulfonate (TMS), MS-222, or Tricaine-S®) – TMS has been approved by the U.S. Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) for aquaculture purposes. Though an effective anesthetic, TMS has a very long withdrawal time (21 days) potentially making its use for field work impractical (Bowker et al., 2011). TMS should be used in accordance with the manufacturer's instructions.
- Eugenole (the active ingredient in clove oil and AQUI-S® E/20E) – AQUI-S® E and AQUI-S® 20E have been approved by FDA for use through the U.S. Fish and Wildlife Service's (FWS) Aquatic Animal Drug Approval Partnership (AADAP) as an Investigational New Animal Drug (INAD) on fish that will be released to public waters. AQUI-S® E contains 50% eugenole and AQUI-S® 20E contains 10% eugenole (U.S. Food and Drug Administration, 2015). The withdrawal period for AQUI-S® 20E is 72 hours (Bowker et al., 2011). AQUI-S® E/20E should be used in accordance with the manufacturer's guidelines and its FDA approval. Clove oil is typically 85-95% eugenole (U.S. Food and Drug Administration for Veterinary Medicine, 2007). Though many governmental and non-governmental organizations, including the U.S. Geological Survey (USGS) and FWS, provide information regarding methods of use of clove oil as a fish anesthetic, it has not been approved for use by the FDA.
- Carbon dioxide (CO_2) – CO_2 sedation can be accomplished using sodium bicarbonate at 142 to 642 ppm for 5 minutes, carbonated water, or gaseous carbon dioxide (Walsh and Meador, 1998). CO_2 may not be as effective in inducing deeper stages of anesthesia, making it less effective for sampling involving surgical procedures; however, recovery times are likely to be shorter (Peak, 1998). The following method of anesthesia using CO_2 is adapted from "Revised Protocols for Sampling Algal, invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program" (U.S. Geological Survey, 2002):

    1. Place subject fish in a 19 L (5 gal) bucket containing about 12 L of ambient water. (Only a few fish should be anesthetized at a time to minimize potential mortality resulting from prolonged sedation.)
    2. Add 350 mL of carbonated water or two CO_2-producing tablets to the bucket. Determining the CO_2 dosage in the field can be difficult because by the time the fish have responded to the sedation, the concentration of CO_2 may be too high (overdose can cause mortality). If the concentration is too high (onset of sedation is rapid), the fish should be moved to ambient water or processed immediately.
    3. Leave the fish in the bucket until the desired level of sedation is achieved (about 2 to 5 minutes).
    4. Recovery time for CO_2 anesthesia may be approximately 5 minutes (Peak, 1998).

Following sedation and sampling procedures, recovery time in a holding vessel of ambient water or an instream cage must be provided until full recovery is observed and the necessary withdrawal period has expired. Recovery time may vary depending on species, size, type of anesthetic, and time under

anesthesia. Release should occur downstream of the sampling reach to minimize the likelihood of re-sampling the same specimens.

2.4.3 Fish Welfare

Main stressors on fish during sampling include water quality, water temperature, duration of holding, and stress due to handling (U.S. Fish and Wildlife Service, 2012). Signs of captive fish stress include loss of righting response, gaping, gulping air, and exuding excessive mucus (Joy, 2013). The following handling techniques should be practiced and are important in the assurance of survival when handling T/E species:

- Proper body support of fish during handling (not holding by jaw or covering eyes or gills);
- Using wetted, gloved hands to reduce loss of protective mucus;
- Minimizing time out of the water;
- Ensuring temporary holding vessels are properly sized to fully submerge fish; and
- Taking special care to minimize any unsubmerged time when air temperatures are below freezing. Suspending sampling activity should be considering in such conditions (U.S. Fish and Wildlife Service, 2012).

If sample processing cannot occur immediately, fish should be kept in instream holding cages or aerated live-wells or tanks. If holding tank water cannot be continuously aerated, it should be changed frequently to prevent a stressful or lethal drop in dissolve oxygen level and holding time should be minimized. Some species, such as salmonids, are more sensitive to changes in water temperature and chemistry and parameters such as temperature, pH, salinity, and dissolved oxygen needs to be kept within fish tolerances in holding vessels. Ranges for holding water parameters should be specified in the SAP.

2.5 FIELD MEASUREMENTS AND DOCUMENTATION

Fish measurements associated with tissue sampling that are to be taken in the field will be dictated by the SAP and are likely to include the following parameters: species identification, length, weight, and examination for any external abnormalities. Some measurements, such as length and weight, may be performed by the personnel who capture fish specimens (collection team); alternatively, the personnel who take tissue samples (sampling team) may be the first project participants to take these metrics or it may be appropriate for this work to be duplicated by the sampling team. The SAP may specify a variety of sizes/ages of fish to be sampled depending upon the nature of the work (e.g. determination of a correlation between fish size or age and analyte concentration in fish tissue). If fish will not be returned to the water, euthanizing specimens (**Section 2.4.1**) prior to taking measurements may make field activities more efficient.

Sample collection activity is typically documented via a field logbook, a field sampling form, and a COC form. All field activities and observations should be documented in the field logbook. General information to be recorded in the logbook includes team member names and their affiliations, date of activity, daily activity start and end times, daily weather conditions, sample range or reach, and general accounting of field activities. Additionally, photograph descriptions, names and affiliations of visitors and

time of visit, and any deviations from the SAP and reasons for such deviations should be included in the field logbook. Typical logbook-keeping practices should be followed, including the following:

- Entries are made during activity or as soon after activity as possible;
- Entries are dark and indelible;
- Entries are factual and objective, subjective writing is rarely appropriate;
- Each new day's activities begin on a new page;
- Each page is dated and initialed by the author of the entries on it;
- Corrections are made by drawing a single line through the entry in error, allowing it to remain legible, and the corrector's initials and date of correction are included;
- No pages are removed from the logbook, regardless of legibility or accuracy;
- Field logbooks must be retained in accordance with DEQ Records and Information Management policies; and
- Generally, multiple projects should not share the same field logbook. An exception might be individual sampling tasks that are part of a larger project, in which case, the beginning and end points of the tasks should be clearly denoted.

Detailed sample metrics (see the following sections) will be documented on **Attachment B** – Fish Tissue Sample Form of this SOP. This information should be recorded as soon as possible following sample collection. A completed COC form must accompany each sample shipment package. The COC form may be provided by the sampling laboratory; however, a generic COC form is provided in **Attachment E**. See **Section 3.6** for more information on COCs.

2.5.1 Taxonomy

Ideally, fish will be identified to the species level (and possibly subspecies level, in accordance with the SAP) immediately following collection from the waterbody. Accurate identification is likely to be critical to data quality objectives, particularly if the SAP specifies composite sampling of certain species. Taxonomic identification should be performed by trained personnel knowledgeable of the local fish species. Taxonomic reference books and keys that contain species descriptions, ranges, illustrations, and photographs should be used to make field identifications. The nomenclature used to identify fish species should follow *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* (latest edition), American Fisheries Society, unless otherwise specified in the SAP.

T/E species (unless included in the SAP), non-target species, or fish that do not meet size requirements should be immediately returned to the waterbody upon capture. Collection of a voucher specimen may be appropriate for each species type sampled (see **Section 3.4** for more information on vouchers). Obtaining a voucher is particularly important for any fish that are sampled but for which a positive identification has not been made. Adequate preservation and fixation (see **Section 3.3**) is critical when the laboratory must be relied upon for positive identification of the taxon for a sample.

2.5.1.1 Hybrids

Hybridized subspecies are known to exist among Montana fishes, particularly among the trout species. In such cases, an authoritative determination made by a qualified fisheries biologist or ichthyologist should be made if at all possible. Vouchering will be necessary if a qualified individual is not available in the field. If taxonomy is controversial, nomenclature should default to *Common and Scientific Names of*

Fishes from the United States, Canada, and Mexico (latest edition), American Fisheries Society, unless otherwise specified in the SAP. Genetic sampling may be appropriate if confirmation of hybridization cannot be determined through visual cues or if data regarding the degree of introgression is required.

2.5.2 Length

Total, fork, or standard length (see **Figure 2-1**) of fish specimens should be measured in accordance with the SAP and recorded on **Attachment B – Fish Tissue Sample Form**. To be consistent with the metric used by most U.S. fisheries biologists, total length should be used (U.S. Environmental Protection Agency, 2000).

The following procedures for measuring fish length using a measuring board are adapted from U.S. Geological Survey, 2002.

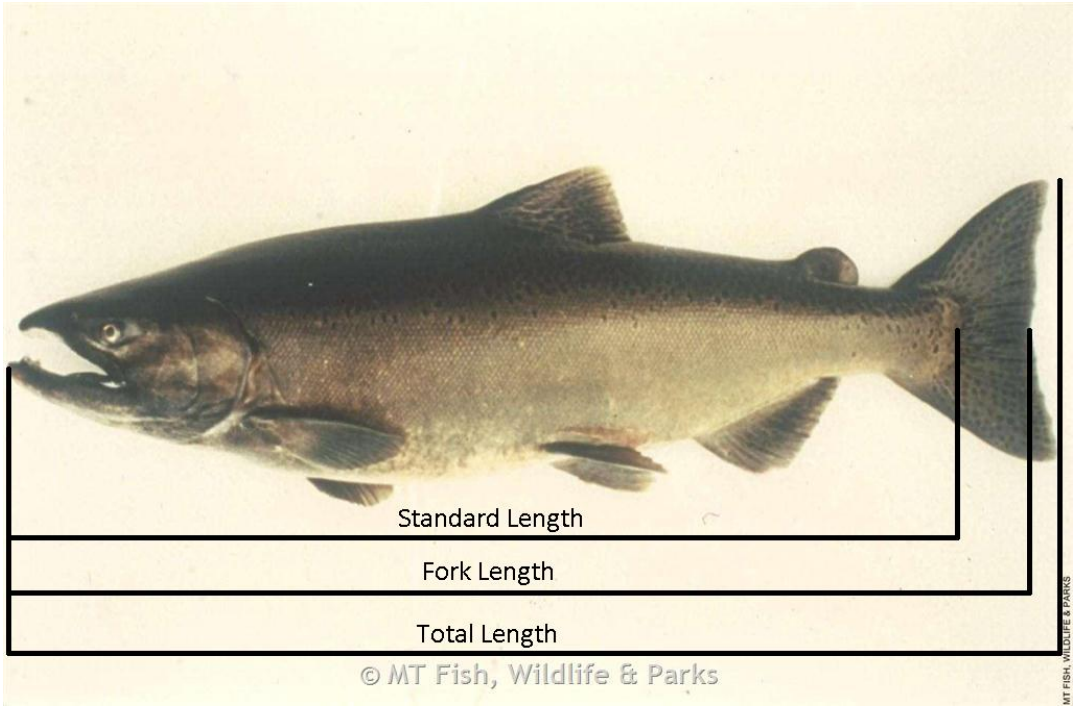

Figure 2-1. Measuring Fish Length.

Determining total length:

1. Place the fish with the body positioned on the right side, the head facing the observer's left, and the mouth closed.
2. Push the anterior end (snout) against the measuring board stop.
3. Measure total length as the distance from the closed mouth to the extreme tip of the caudal fin (see **Figure 2-1**), when the lobes of the caudal fin are compressed dorsoventrally, i.e. "un-splayed." Note: the caudal fin in **Figure 2-1** has not been compressed dorsoventrally.
4. Record total length to the nearest millimeter on **Attachment B** – Fish Tissue Sample Form.

The measuring board should be rinsed with ambient water between uses. It may be efficient to take photo vouchers while specimens are positioned on the measuring board as described in the steps above,

providing the benefit of visual representation of length as part of the voucher. Size requirements should be specified in the SAP; however, when grouping specimens into similar sizes, a general rule is that the smallest individual is at least 75% of the length of the largest individual.

2.5.3 Weight

Fish weights are to be recorded individually for larger fish; however, small species or juveniles may at times be weighed in batches; refer to the SAP for instructions. The following procedures for weighing fish specimens are adapted from U.S. Geological Survey, 2002.

Obtaining weights of individual fish:

1. Level, calibrate, and tare the specimen tray/pan and any covering (e.g. aluminum foil) on the scale.
2. Place the specimen on the scale. If the specimen overhangs the tray, ensure no parts of the specimen are supported by anything other than the balance platform.
3. Record the weight of the fish to the nearest gram on **Attachment B** – Fish Tissue Sample Form.

Obtaining composite/batch weights of fish:

1. Level, calibrate, and tare the specimen tray and any covering on the scale.
2. Place all individuals for a particular composite or batch sample on the scale.
3. Record the count (number of individuals weighed or contributing to the composite) and the total weight (batch weight) to the nearest gram on **Attachment B** – Fish Tissue Sample Form.

Scale calibration must be checked at regular intervals. Use of multiple weighing trays or trays of different sizes may aid weight measuring. Covering the scale with plastic wrap will help protect the instrument from weather and detritus. If samples will be filleted in the field, fillet weights should be measured and recorded as above, whether individually or in batches.

2.5.4 External Anomalies

External anomalies include deformities, erosions (typically in the fins), lesions, and tumors/neoplasms; often referred to as DELTs. DELTs may indicate the presence of sub-lethal environmental stresses, intermittent stresses, behavioral stresses, or chemically contaminated substrates (U.S. Geological Survey, 2002). Documentation of DELTs may be used to indicate a possible correlation between external anomalies and tissue contaminant concentrations; however, development of a statistical relationship may not be intended, appropriate, or possible.

All fish included in the sampling effort should be examined for DELTs and any observed external anomalies should be recorded on **Attachment B** – Fish Tissue Sample Form. Examination should occur while taking fish measurements to minimize handling fish. If possible, photographic record of extreme anomalies should be made. Injuries known or suspected to have occurred during collection (e.g. blackening or exophthalmia from electrofishing (Joy, 2013)) should not be included in records of DELTs for the specimen.

Attachment C – DELTs Examination has been adapted from U.S. Environmental Protection Agency, R10, 2005 and provides external features of fish that should be examined for anomalies. Consult U.S.

Geological Survey, 2002 for more information on external anomalies, including photographic examples of DELTs.

3.0 SAMPLE MANAGEMENT

Sampling of fish tissue involves a comprehensive process beginning with project planning and continuing through the field activities of the collection and sampling teams to the point of shipping to a laboratory for chemical analysis. During sampling activity, samples may pass through many processing stages and the hands of several personnel. The goal of sample management is the maintenance of sample integrity and identity through each stage of these activities, which is paramount to ensuring data quality objectives are met.

3.1 SAMPLE SELECTION

Sample selection criteria should be fully described in and followed strictly from the SAP. Typically, a minimum number of species examples of a certain length and/or weight will be required by the SAP, and species to be sampled may be divided into primary, secondary, or further tiers of targets. For example, all or certain target species of a sampling tier might be divided into three size classes: 1. <15 cm, 2. ≥15 cm – ≤30 cm, and 3. >30 cm. Particularly for composite sampling, the SAP may specify a minimum number of specimens to include in the composite (e.g. five) with enough mass to produce a sample for laboratory analysis (e.g. 500 g). The SAP should include a contingency section with instructions for instances when field conditions do not allow for achievement of the minimum sample selection requirements. Selection of specimens for sampling and categorization in accordance with the SAP is done during identification and field measurement of specimens to maximize sampling efficiency and to minimize handling of live fish. Field measurement procedures are included in **Section 2.5**.

3.2 SAMPLE PREPARATION AND HANDLING

Measures to prevent cross-contamination during sampling procedures are critical to meeting data quality objectives. All sample preparation beyond whole fish collection, non-lethal biopsy punch, or egg collection (i.e. filleting, organ harvesting, and sample compositing) should be performed in a laboratory cleanroom-type environment, free of metals or organics, and utilizing positive pressure filtered air, such as an offsite trailer designated and prepped specifically for sampling purposes. Certain factors such as parameter(s) of concern, large specimen sizes, limited cooler capacity, or lack of suitable staging sites may indicate collection methods such as filleting may be appropriate to perform in the field. However, rationale indicating how data quality objectives will be met under field conditions must be documented in the SAP, and any deviations from the SAP made during field activities must be recorded. Handling, labeling, storing, shipping, and disposal methods applicable to fish tissue samples are included in **Sections 3.4** through **3.6** and must be followed closely. Failure to follow these procedures may result in unusable data results or failure to meet project objectives such as development of translators.

3.2.1 Filleting

The SAP should specify if fillets are to be prepared skin-on or skin-off, which may be dependent upon the chemicals being sampled for. Removal of skin may lower reported concentrations of organic chemicals while retention of skin may lower results for other analytes such as mercury (California

Environmental Protection Agency, 2005). Other factors such as cultural culinary preferences may influence the appropriateness of retention or elimination of other tissue or organs (e.g. fatty belly flap) or sampling specimens whole. Additional rationale for removal or retention of skin and other tissue on fillets can be found in U.S. Environmental Protection Agency (2000).

Fish should be resected into fillets under laboratory clean-room conditions within 48 hours of collection and ideally without freezing of tissue prior to resection. Freezing can cause rupture of neighboring anatomy, potentially contaminating the target tissue. If fillets are to be resected from frozen fish, fish should be thawed only to the point of allowing incision (U.S. Environmental Protection Agency, 2000). Specimens are ready to be filleted when the deepest portions of the target tissue still contain crystals but are no longer fully frozen (Kansas Department of Health and Environment, 2013).

The following steps must be taken in the filleting process:

1. Prior to any resection, all work surfaces (i.e. cutting-boards and tables) and cutting utensils must be washed with a mild detergent solution (phosphate- and scent-free) followed by multiple rinses with deionized water. Cutting boards must then be covered with unused aluminum foil with the dull side exposed.
2. Personnel must don new, nitrile, disposable gloves prior to any contact with specimens.
3. A decontaminated stainless steel, ceramic, glass, or titanium utensil will be used for filleting each specimen.
4. Specimens should be filleted using the procedures in **Attachment D**.
5. Avoid puncturing the gut and internal organs which could contaminate the fillet sample. If such puncturing does occur or the fillet comes in contact with any non-sterilized surface, immediately rinse the fillet with deionized water and record the event on **Attachment B** – Fish Tissue Sample Form.
6. Remove any bones remaining in the fillet following resection unless otherwise directed by the SAP.
7. The aluminum foil surface must be replaced between each specimen and cutting utensils must be decontaminated through deionized water rinsing and detergent use if necessary.
8. Depending on the requirements in the SAP, fillets may be:
    - weighed individually or per fish;
    - separated into routine samples and duplicate samples;
    - immediately homogenized for compositing purposes; and/or
    - wrapped in aluminum foil, labeled (see **Section 3.6**) and dated, placed in a waterproof plastic bag, and frozen at ≤-20°C for later processing (U.S. Environmental Protection Agency, 2000).

3.2.2 Internal Organs

To meet data quality objectives, harvesting of internal organs for sampling purposes can typically only be done in a laboratory clean-room environment. Fish should be dissected as quickly as possible and no more than 48 hours after collection if stored only cooled and not frozen. To remove organs for sampling, steps one through five under **Section 3.2.1** above should be followed carefully, leaving the intestinal tract and internal organs intact. At that point, the internal organs should be exposed. During removal of the organs to be sampled specified in the SAP, the gonads should be examined to determine or confirm the sex of the fish and gross pathology should be noted on **Attachment B** – Fish Tissue Sample Form.

The amount of time tissue samples are exposed to air should be minimized to reduce the chance of contamination and moisture loss.

Figure 3-1 includes a generalized representation of salmonid interior anatomy for reference. Detailed imagery of internal fish organs and methods of dissection can be found in the *Illustrated Field Guide for Assessing External and Internal Anomalies in Fish* (Smith et al., 2002).

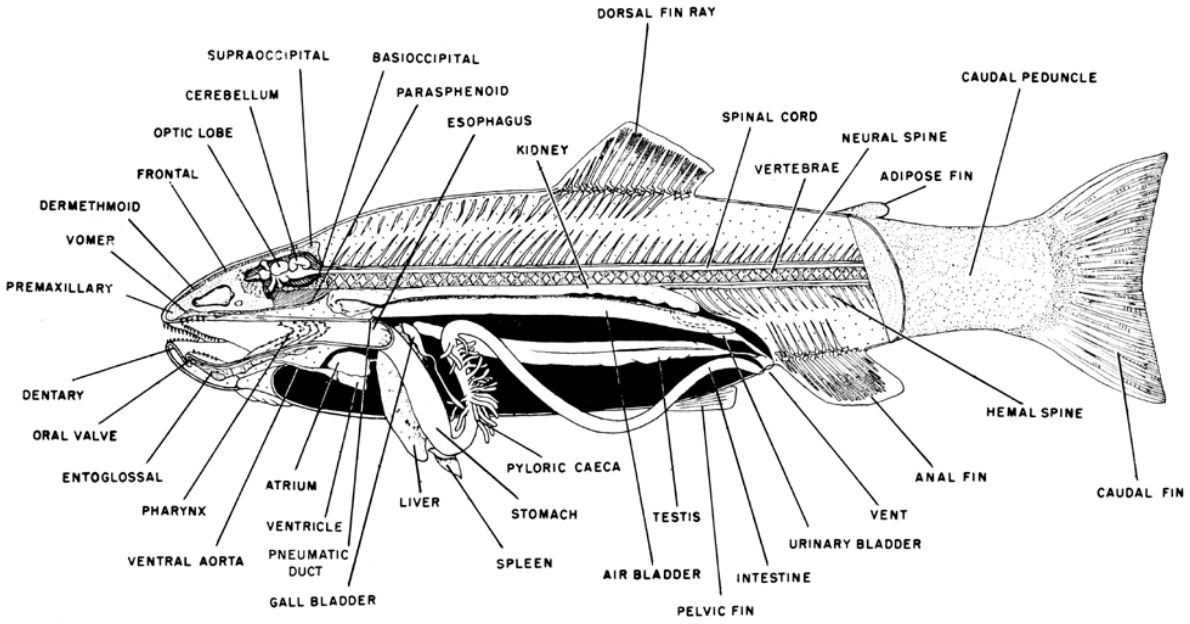

Figure 3-1. Salmonid Anatomy. (Leitritz, 1959)

Following removal and resection, steps seven and eight of **Section 3.2.1** should be followed for organ tissue or as otherwise specified in the SAP.

3.2.3 Eggs

Collection of fish eggs for sampling purposes may be desired for projects investigating concentrations of certain parameters. Field collection activities must coincide with the spawning times of the project's target species. Spawning times for species typical to Montana can be found in "Spawning Times of Montana Fishes," (Skaar, 2001). It may be difficult to achieve sufficient sample mass from small species or immature populations; therefore, SAPs should contain contingencies for these events.

If fish will be sacrificed, eggs can be harvested during dissection for collection of other tissues through the procedures for filleting and organ harvesting described above. Eggs are located along the gonads and typically form in rows. Manual stripping or spawning of eggs from ripe females should provide sufficient mass of eggs for sampling purposes and allow return of fish to the environment. Spawning activities should be performed in a manner that minimizes handling of fish and anesthetics should be used to reduce stress (Piper et al., 1982). Eggs themselves are delicate and can be damaged if fish are handled and/or spawned too roughly (Shrable et al., 1999).

Manually strip eggs as follows:

1. Anesthetize fish if appropriate (see **Section 2.4.2**).
2. Ready clean sample glassware. One large container to collect eggs in and distribute them to smaller containers may be appropriate.
3. Rinse fish in ambient water, particularly in the area of the vent (see **Figure 3-1)** to remove any foreign matter and potential contaminants.
4. Hold the fish about the head with one hand while positioning the vent over the receiving container and tilted slightly downward. Two individuals may be necessary to sample very large specimens–one person holding the fish and one person performing the stripping.
5. Apply gentle pressure with the other hand to the ventral side beginning well behind the pectoral fin and moving slowly toward the vent. Direct the resulting stream of eggs into the sampling container.
6. Room must be left in final sampling glassware for sample expansion upon freezing (Murphy, 2012).
7. Label the samples in accordance with **Section 3.6**, complete **Attachment B** – Fish Tissue Sample Form, freeze the samples as quickly as possible, and store at ≤ -20°C.

If the eggs are not easily stripped with gentle pressure, the fish is likely not ripe. Do not attempt to force stripping with excess pressure as harm to the fish and damage to the eggs may occur. Under this circumstance, the fish should be returned to the water without sampling or it will need to be sacrificed to obtain eggs.

3.2.4 Compositing

Compositing samples allows for cost-effective estimation of average concentrations of analytes in target species and helps ensure adequate sample mass for analysis of a suite of target analytes. However, composite samples by nature will not allow for discovery of the extremes of analyte concentrations in target media. The following guidelines should be followed when compositing fish tissue specimens (whole fish, fillets, biopsy punch, etc.); more details and any deviations from these procedures should be detailed in the SAP:

- Specimens should typically be all of the same species due to species-specific bioaccumulation potential, and of the same sex, if possible.
- Specimens should be collected during the same sampling event and no longer than one week apart.
- Specimens should be of legally-harvestable and at least consumable size and weight when sampling for human health purposes.
- Size of specimens should be within a range so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and samples should be classed according to any length-based requirements in the SAP (see **Section 3.1**).
- Ideally, each composite sample for a specific species will contain the same number of individual fish – a minimum of three, and preferably, five individual specimens.
- Enough sample mass (usually 200-500 grams) must be generated for the target analyte suite and equal amounts of mass should be used from each individual specimen in the composite.

General procedures for compositing samples include the following steps which should be performed in a laboratory clean-room environment. Modification of these procedures may be necessary depending on SAP requirements, available equipment, and site-specific conditions.

1. Work surfaces should be prepared in accordance with the procedures in **Section 3.2.1**.
2. Samples should be partially thawed as described in **Section 3.2.1** to facilitate homogenization.
3. Fillets or other tissue to be homogenized should be reduced to cubes of a size (e.g. ~2.5 cm) able to be easily handled by the available homogenization apparatus. Additional manual processing may be necessary for whole fish compositing.
4. Fish tissue should be homogenized through use of a food-grade grinder, high-speed processor, or a laboratory homogenizer.
5. Repeated cycles through a homogenizer and/or hand-mixing may be necessary to achieve final consistency.
6. Final consistency of the homogenate should resemble a fine paste of uniform color and texture (U.S. Environmental Protection Agency, 2009) with no remaining structure greater than 1 mm in size (U.S. Environmental Protection Agency, 2005).
7. Samples should be containerized in pre-cleaned glassware with lids lined with non-reactive materials.
8. Label the sample in accordance with **Section 3.6** and complete **Attachment B** – Fish Tissue Sample Form for the sample.
9. Composite samples must be frozen as quickly as possible and stored at ≤ -20°C.

Ideally, parts of the homogenizing apparatus that contact the sample (e.g. blades) will be made of titanium or tantalum as stainless steel has been found to be a potential source of nickel or chromium contamination due to high speed abrasion. Additionally, it may be helpful to chill these contact points prior to use (chips of dry ice may serve this function) to prevent the sample from sticking to the apparatus (U.S. Environmental Protection Agency, 2000).

3.2.5 Biopsy Punch

As described in **Section 2.4.1**, biopsy punch sampling methods provide a no-kill sampling alternative to lethal techniques. When biopsy punch sampling is implemented, the following steps should be taken. These procedures are more easily accomplished by two or more personnel so fish and tissue sample handling tasks can be performed concurrently.

1. If anesthesia will be used, fish should be anesthetized in accordance with **Section 2.4.2** and their welfare maintained as described in **Section 2.4.3**.
2. Work surfaces should be prepared in accordance with the procedures in **Section 3.2.1**.
3. Specimen measurements, particularly weight, should be obtained prior to biopsy punch sampling.

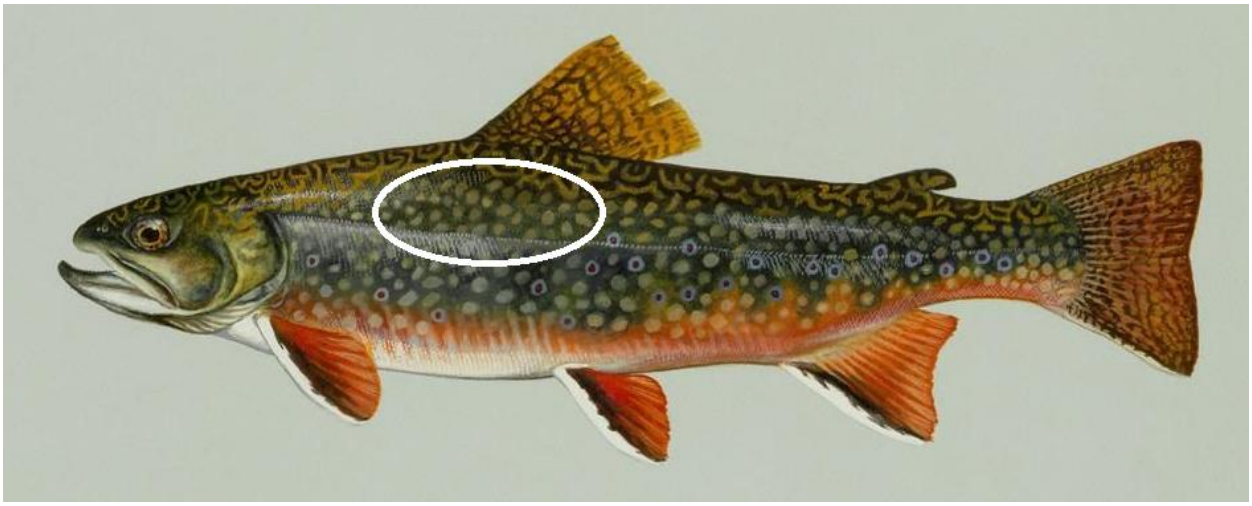

Figure 3-2. Biopsy Punch location.

4. Using a pre-cleaned utensil, a small area of scales (if present) must be cleared from one side (typically the left) of the dorsal area. The white oval on **Figure 3-1** represents the area within which to make the incision. It may be possible to also remove the skin of the specimen in the area to be biopsied at this time.
5. Insert the biopsy punch through the skin at the descaled area with a slight twisting motion to the prescribed depth of the punch, penetrating the skin (if remaining) and muscle tissue.
6. Using a slight tilting motion to sever the tissue at the end of the punch while leaving the sample intact, remove the punch.
7. Place a laboratory pipette bulb on the opposite end of the biopsy punch from the tissue sample. Squeeze the bulb quickly and firmly, to discharge the tissue sample onto unused aluminum foil if it must be further processed (e.g. removal of skin or compositing) or directly into pre-cleaned sample glassware. If necessary, use clean forceps to remove the tissue from the punch.
8. If the tissue plug comes in contact with any non-clean surface, immediately rinse it with deionized water and record the event on **Attachment B** – Fish Tissue Sample Form.
9. Liberally apply antibiotic salve to the biopsy area on the fish and return it to the waterbody as quickly as possible, first allowing a safe recovery location if anesthesia was used.
10. Label the sample in accordance with **Section 3.6** and complete **Attachment B** – Fish Tissue Sample Form for the sample.
11. Freeze samples as quickly as possible and store them at ≤ -20°C.
12. Do not attempt to reuse a biopsy punch that has contacted a specimen or any non-clean surface.

3.3 VOUCHERS

Vouchers may or may not be called for in the SAP. Voucher specimens may not be necessary when species to be sampled are well known and easily identifiable. Alternatively, voucher specimens may be critical when dealing with visually similar species and/or hybrids, making taxonomic identification in the field difficult. Vouchering is accomplished either via direct collection and preservation of individual fish, or through photographic record. Vouchering may be imperative for quality assurance/quality control

(QA/QC) purposes and may have important additional functionality beyond the scope of the project such as documentation of the existence of invasive or exotic species in a waterbody.

Specimen Vouchers

Where species size and abundance allow, the SAP may call for preservation of individuals of the project sample species. Also, if T/E, or otherwise protected species expire during sampling activities, they should be preserved as vouchers. The following steps should be taken in preparing specimen vouchers:

1. Take and record appropriate field measurements (see **Section 2.5**) and euthanize the specimen humanely (see **Section 2.4.1**).
2. Place the specimen in an appropriately-sized sample container with a 10% solution of buffered formalin (see **"Voucher Preservation"** and **"Caution"** below). Multiple individuals of the same species or suspected species may be placed in the same container; however, ensure that specimens are not too tightly containerized – anatomy should not be contorted and fish biomass should not be greater than 40% of the container contents by weight (U.S. Environmental Protection Agency, 2006a). Use enough preservative to completely submerge all specimens.
3. Specimens larger than 10 cm should receive a small incision along the right ventral area to aid preservation.
4. Complete a label with appropriate information (see **Section 3.6**) and affix it to the voucher bottle securely. The SAP may require multiple labels or identification (ID) tags for voucher specimens and may require placement inside sample containers. Vouchers must be clearly identified so they are not mistakenly used for sampling purposes.
5. Two to seven days in the solution are required to ensure proper preservation of the voucher (U.S. Geological Survey, 2002).

Voucher Preservative

A buffered formalin preservative solution can be prepared by the addition of 400 g of borax to a 20 L container of 100% formalin and checking to ensure a pH of 6.5 to 7.0 (U.S. Environmental Protection Agency, 2006b; Lazorchak, 2000). A smaller volume of the solution can be prepared by adding 100 mL of formaldehyde to 900 mL of water, separately mixing 3 g of borax with 10 mL of water, and then mixing the two solutions together (U.S. Geological Survey, 2002).

Caution: Formalin and formaldehyde are caustic, potentially carcinogenic, hazardous substances and must be handled in accordance with Federal regulations found at 40 CFR 1910.1048. Wear proper personal protective equipment (e.g. gloves and safety glasses) when handling these chemicals. Reserve solution should be stored in a safety cabinet or cooler lined with absorbent materials and transported outside the passenger area of vehicles if at all possible (Lazorchak, 2000).

Photographic Vouchers

Photographic vouchers may be the appropriate or only course of action such as in cases when examples of a target species are too large for specimen preservation or represent T/E species. The following guidelines should be followed when using photographs as vouchers:

- The equipment used should have sufficient macro capability to capture details of external characteristics when photographing small species or anatomical features.

- Fish should be photographed on a measuring board or with a rule or some other measure appearing in the photograph for scale. Conventionally, the left side of the fish is photographed (Walsh and Meador, 1998).
- Multiple shots of the same specimen should be taken if necessary or helpful in characterizing/identifying the species (e.g. a full-body, a lateral shot, and a close-up(s) of features inherent to the species). Some staging, if possible, (e.g. spreading and pinning of fins of fixed specimens) may aid in the use of the photograph for identification purposes.
- It is recommended that a tag with at minimum a voucher ID number be included in the image.
- A log of the voucher photographs containing information such as the following must be maintained:
    - project and/or site ID
    - date of photograph
    - known or suspected species name and/or common name
    - image file name
    - description of the specimen or other notes regarding the photograph.
- Whether or not a project target species, invasive/exotic and T/E species should be photo-documented, provided the welfare of T/E species can be maintained. FWP may prohibit return of some invasive/exotic species to the waterbody.
- Backup image files as soon as possible.

3.4 SAMPLE LABELING AND CUSTODY

All collected samples must have labels. The method of identifying and labeling samples should be provided in the SAP and strictly followed. At a minimum, sample labels should include the following information:

- The collecting organization name;
- The project name/number;
- The sample ID number;
- The date the sample was taken; and
- The time the sample was taken.

Additional information that may be recorded on labels includes but is not limited to:

- Sample location information (e.g. reach code);
- The sampler's name and/or team member identification number;
- Species name or code (see **Attachment F** for codes, common names, and abbreviations for fish species used by FWP);
- Analysis to be performed;
- Additional sample ID numbers (e.g. composite or individual specimen numbers); and
- Preservative used (e.g. formalin)

Further guidance on sample labeling can be found in Appendix D of the *Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring* (Montana Department of Environmental Quality, 2012).

All samples must be maintained, stored, and transferred under chain of custody (COC). COC procedures begin when the sample is collected and remain the responsibility of a sampling team member until the samples are relinquished to a laboratory or disposed of. In general, to maintain chain-of-custody, samples must remain in the physical possession (i.e. being carried by or within view) of the COC form signatory unless COC-sealed and locked in a restricted access location. Use of a COC form ensures that samples are traceable from the time of collection through processing and analysis until final disposition (Parametrix, Inc., 2009) and may be required by the analyzing laboratory. The COC form originates with the personnel who performed the sampling and remains with the samples (typically sealed in a plastic bag attached to or in the sample cooler) as it passes to other personnel for additional processing, shipping, etc. A generic COC Form suitable for use for fish tissue samples is provided as **Attachment E**.

3.5 SAMPLE HOLDING, STORAGE, AND SHIPPING

Unless further resection and/or sample analysis will occur within 24-48 hours, samples should be frozen as quickly as possible after collection and processing using dry ice or other means of cooling samples to ≤-20°C. Samples should be shipped to the laboratory within 48 hours and preferably, 24 hours from time of collection. Once frozen, actual maximum laboratory holding times may range from 28 days to one year (U.S. Environmental Protection Agency, 2000) and samples should not be allowed to thaw until time of analysis. If samples cannot be frozen while being stored in the field, they should be stored on wet or blue ice and shipped to the laboratory within 24 hours of collection. Samples must be packaged such that they will not come into contact with each other or preservative melt water. This should be accomplished through use of an appropriate combination of aluminum foil, plastic bags, and plastic tubing depending on how the samples have already been packaged or containerized. If sample compositing will be performed by the laboratory, individual specimens composing a composite should be held, stored, and shipped together. As stated in **Section 3.4**, when not in the physical possession of a sampling team member, samples should be locked in a restricted access location such as a trailer, vehicle, or building room.

During storage and prior to shipping, it must be ensured that an adequate amount of wet, blue, or dry ice covers and is layered between samples to prevent degradation. Sample volume in shipping coolers/containers should be 60-70% with the remainder being coolant, depending on ambient conditions. If voids remain within coolers after all samples and coolant have been added, the additional space should be filled with clean material (e.g. bubble wrap or paper) to prevent the samples and coolant from shifting. Following packing, coolers/containers should be taped shut securely and COC seals should be added.

Dry ice has the potential to off-gas and build up pressure inside a tightly sealed cooler and it will damage skin if handled without protection such as gloves. A Class 9 Dangerous Goods label is required for shipments containing dry ice and a cooler with vents or drains which will remain open during shipping should be used to prevent pressure build-up. The weight of dry ice included in the shipment will need to be known for completion of the information required on this label. Special containerization, labeling, and shipping forms may be required for shipments containing formalin if not in conformance with the small quantity exemptions found in 49 CFR 173.4. It may be appropriate to line the bottom of the cooler/container with absorbent material, particularly if preservatives are used, to prevent hazardous materials from leaking from shipments.

The most current shipping requirements for both the U.S. Department of Transportation and shipping carrier, if being used, should be consulted when shipping samples. When using a carrier for shipping, it may be helpful to inform the laboratory of the carrier name and anticipated arrival date and time for the samples. If the shipment contains multiple parcels, the carrier should also be alerted as to how many cooler/containers to expect and they should be labeled appropriately (i.e. “1 of 3,” “2 of 3,” etc.). The sampling schedule should be set to ensure the laboratory is prepared to process sample shipments immediately (e.g. weekend delivery may need to be avoided).

3.6 SAMPLE DISPOSAL

It is the sampling team members’ responsibility to prevent the release of hazardous substances into the environment from sampling activities. Sampling-derived waste must be disposed of in accordance with federal, state, and municipal regulations. Recyclable and non-recyclable materials should be sorted into their appropriate waste streams and transported offsite for processing or disposal, respectively. Disposable sharps such as scalpels and biopsy punches should have cutting edge guards replaced, if so equipped, and be disposed of in a safe manner. All chemical wastes must be disposed of properly; preservatives and dry ice are potentially hazardous and should not be discharged in the field. Acids should be properly neutralized prior to disposal.

Caution should be taken in the collection and disposal of offal from field sampling activities such as filleting due to the attraction of other wildlife such as bears. It may be acceptable to dispose of whole fish carcasses in the waterbody following puncture of the swim bladder. FWP should be consulted regarding when, where, and how fish remains can be left in the field.

4.0 QUALITY CONTROL

Quality control (QC) procedures for field activities primarily consist of precautionary measures that should be taken to prevent contamination of samples and equipment, and preparation of replicate and blank samples. The following subsections describe these QC procedures, but sampling team members should also be familiar with and refer to the QC section(s) of the SAP and the Quality Assurance Project Plan (QAPP) for project-specific QC tasks requirements.

In addition to the measures described below, the following basic QC tasks should be performed during field sampling:

- Measurement units should be used consistently as specified in the SAP or recommended in **Section 2.5**.
- Taxonomic references must be standardized and documented across sampling teams and for the life of the project. The services of a qualified taxonomist may be necessary and vouchers should be obtained.
- Holding times must be adhered to as specified in the SAP or recommended in **Section 3.5**.
- Sample integrity must be maintained through preservation, packaging, storage, and COC procedures.
- Field logs, sample and COC forms, labels, and any other project records must be completed promptly, thoroughly, and accurately.

Sections 4.1 and **4.2** describe the typical QC sampling that most projects will include; QC sampling requirements should be detailed in the SAP. The purpose of QC sampling is to:

1. Verify samples were not contaminated during the sample collection process;
2. If found, determine the origin of contamination; and
3. Assess the accuracy and precision of the methods of analysis (U.S. Environmental Protection Agency, 2014c).

More information on replicate and blank sampling can be found in **Sections 5.4** and **5.5** of DEQ's "Water Quality Planning Bureau Field Procedures Manual for Water Quality Assessment Monitoring," (Montana Department of Environmental Quality, 2012).

4.1 REPLICATE SAMPLES

Collection and retention of replicate (duplicate) samples, even when resources do not allow immediate analysis, preserve the possibility of QC review at a later time (U.S. Environmental Protection Agency, 2000). Wherever sufficient numbers of fish are available, replicate samples should be collected at a rate of ≥ 10% (i.e. at least one replicate sample for every 10 samples collected) unless otherwise specified in the SAP. Likewise, for composite samples, at least every 10^{th} sample should be split into a routine and a replicate sample; therefore, it may be prudent to factor available tissue mass into the decision of which composite sample to duplicate. A minimum of one replicate sample should be collected regardless of the number of sampling locations or composite samples prepared. Replicate samples should be collected in the same manner as routine samples, without deviation, and may be collected as blind replicates (i.e. labelled in a manner such that the laboratory is unaware of which routine sample they are a duplicate of) if called for by the SAP. Unless otherwise specified in the SAP, replicate samples should be taken from the same matrix preparation (i.e. taken from the same fillet as the routine sample to be replicated or the same composite should be divided into a routine and a replicate or a biopsy plug sample should be split into two samples provided enough sample mass will be available in both halves).

4.2 BLANK SAMPLES

Blank samples taken in the field generally consist of the following:

- Rinsate blanks – samples of unused rinsate and
- Process blanks – samples of rinsate used on equipment, utensils, containers, supplies, etc. which are already in a "clean" state.

Blank samples are processed, stored, handled, and shipped just as routine samples. The purpose of field blank samples is to evaluate sources of contamination introduced to the sample through field sampling methods. Detection of an analyte in a blank sample could indicate that routine sample results are biased-high. Blank samples should be prepared once at the beginning of a sampling event and then at a rate of 5% (i.e. at least one blank sample per every 20 samples processed) or one with every batch of samples, whichever is more frequent (U.S. Environmental Protection Agency, 2000), unless otherwise specified in the SAP. It may also be appropriate to prepare another blank at the end of a sampling event or end of each day. Being a solid medium, fish tissue is not easily contaminated by most analytes, and it may be difficult to quantify a magnitude of a contaminant that has been transferred to a tissue sample from rinsate, containers, supplies, or equipment (Idaho Department of Environmental Quality, 2008).

4.3 INTERFERENCES AND PRECAUTIONS

Interferences such as cross-contamination and improper sample selection can result in biased, inaccurate, or erroneous data results. Several precautions to avoid these interferences are built into the procedures and methods described above. The following precautionary measures should be kept in mind during sampling activity:

- Use wetted, gloved hands to prevent contamination transfer to fish.
- Ensure entries on forms, labels, COCs, and in logbooks are accurate and legible to prevent transfer of erroneous values to electronic systems or sample misidentification.
- Use standardized units of measure, measuring tools, and taxonomic references across sampling locations and for the duration of the project.
- Follow the sample species, size, age, sex, and mass requirements specified in the SAP precisely.
- Follow all calibration methods and frequencies specified in equipment manuals or the SAP.
- Follow the steps for decontamination of surfaces and equipment as described in **Section 3.2.1**.
- Ensure vouchers are properly preserved in accordance with the procedures in **Section 3.3**.
- Check sample integrity regularly while holding (e.g. ensure samples do not come into contact with melt water, frozen samples do not thaw prior to lab delivery, etc.).
- Maintain chain-of-custody at all times.

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ATTACHMENT A – EQUIPMENT AND FIELD SUPPLIES

Qty	Item	√
1	First aid kit	
1	Cold weather kit (seasonal)	
1	Collection permit (if required)	
1	Sampling and Analysis Plan (SAP) copy	
1	Quality Assurance Project Plan (QAPP) copy	
1	Health and Safety Plan (HASP) copy	
1	Fish Tissue Sampling Standard Operating Procedure (SOP) copy	
1	Project field logbook	
1	Taxonomic key	
NA	Field sample collection form	
NA	Sample labels (set)	
NA	Chain of custody (COC) (one for each sample cooler plus two extra)	
NA	COC seals (one for each sample cooler plus two extra)	
NA	Carrier airbills/shipping forms (one for each sample cooler plus two extra)	
1	Field clipboard/form case	
3	Indelible writing device (e.g. pencil, Rite-in-the-Rain® pen)	
2	Chemical splash goggles	
1	Nitrile gloves (100ct. box)	
2	Paper towels (roll)	
2	Anesthetization/holding vessel (e.g. live-well or bucket)	
1	Inert or foil-wrapped blunt instrument (for euthanizing fish)	
1	Live cage (for containing fish in surface waterbody)	
1	Minnow net (for collecting small species from livewell/bucket)	
2	Holding tray	
2	Inert (e.g. glass) cutting board	
1	Fish measuring board (min. precision – ±1mm)	
1	Measuring tape (mm increments)	
1	Portable balance (min. precision – ±1g)	
1	Calipers (min. precision – ±1mm)	
2	Heavy duty aluminum foil (roll)	
4	Plastic Bags (2 gallon, box of)	
4	Plastic bags (1 gallon, box of)	
4	Plastic bags (1 quart, box of)	
6	Plastic bags (33 gallon)	
1	Plastic wrap (roll)	
2	Packing/strapping tape (roll)	
NA	Cooler/ice chest	
NA	Wet/blue/dry ice	
NA	Bubble wrap, absorbent packing material	
2	Forceps	
2	Fillet knife	
2	Scaling knife or scalpel	
2	Pliers	
2	Scissors or utility knife	

Qty	Item	√
3	Deionized water (gallons)	
1	Camera – macro-capable, electronic storage media, extra batteries (for voucher photographs)	
NA	Preservative/fixative[1] (typically 10% borax-buffered formalin or 37% buffered formaldehyde, enough volume for samples & vouchers)	
NA	Reagents - acetone, hexane, methanol, 20% nitric acid (volumes sufficient for cleaning equipment and blank samples)	
2	Food-grade poly tubing (rolls)	
1	Plastic cable ties (30-50 count package)	
Additional Equipment/Supplies for Composite Sampling		
2	Stainless steel bowl (4 or 6 quart, for holding/hand-mixing samples)	
1	Homogenization apparatus (blender, grinder, laboratory homogenizer)	
NA	Lab sample glassware (for composite samples)	
Additional Equipment/Supplies for No-Kill Biopsy Sampling		
NA	Class 9 Dangerous Goods diamond placard (dry ice shipments greater than 2.5kg/5.5lbs)	
NA	Sterile disposable biopsy punch (Acu-Punch® or equivalent brand in boxes of 25 or 50)	
2	Pipette bulb (for discharge of sample from biopsy punch)	
NA	Antibiotic salve (volume enough to cover all planned biopsy punch sampling)	
NA	Scintillation vial (or equivalent sterile laboratory glassware, for punch sample)	
NA	Anesthetizer (e.g. package of Alka-Seltzer® or equivalent CO_2 tablets, clove oil)	

1. NA = not available. Refer to the SAP.
2. Formalin and formaldehyde are potentially human carcinogens and should be handled only in well-ventilated areas while wearing chemical-resistant gloves and approved eye protection.

ATTACHMENT B – FISH TISSUE SAMPLE FORM

Affix sample label here

Date: _____	**Time:** _____	**Personnel:** _____		
Waterbody: _____		**Location:** _____		
Station ID: _____	**Visit No:** _____	**HUC:** _____		**County:** _____
Lat: __ __.__ __ __ __ __	**Long:** __ __ __.__ __ __ __ __	**Datum:** _____	**Elevation:** _____ m ft	**Geo Method:** _____

Sample ID No.				**Composite Sample**	☐	**Composite No.**	
Genus species				**Min Length** (mm)		**Max Length** (mm)	
Collection Method				**Min Weight** (g)		**Max Weight** (g)	
Sample Type (circle) **Whole Fish** **Fillet** **Biopsy Punch** **Eggs** **Voucher** **Organ(s) _____**		**Length** (mm**) TF FL SL**		**Batch** (Total) **Weight** (g)		**Count**	
		Weight (g)		**DELTs**			
		Size Class					
		Sex (M or F)		**Preservative:** wet/blue ice dry ice other _____			

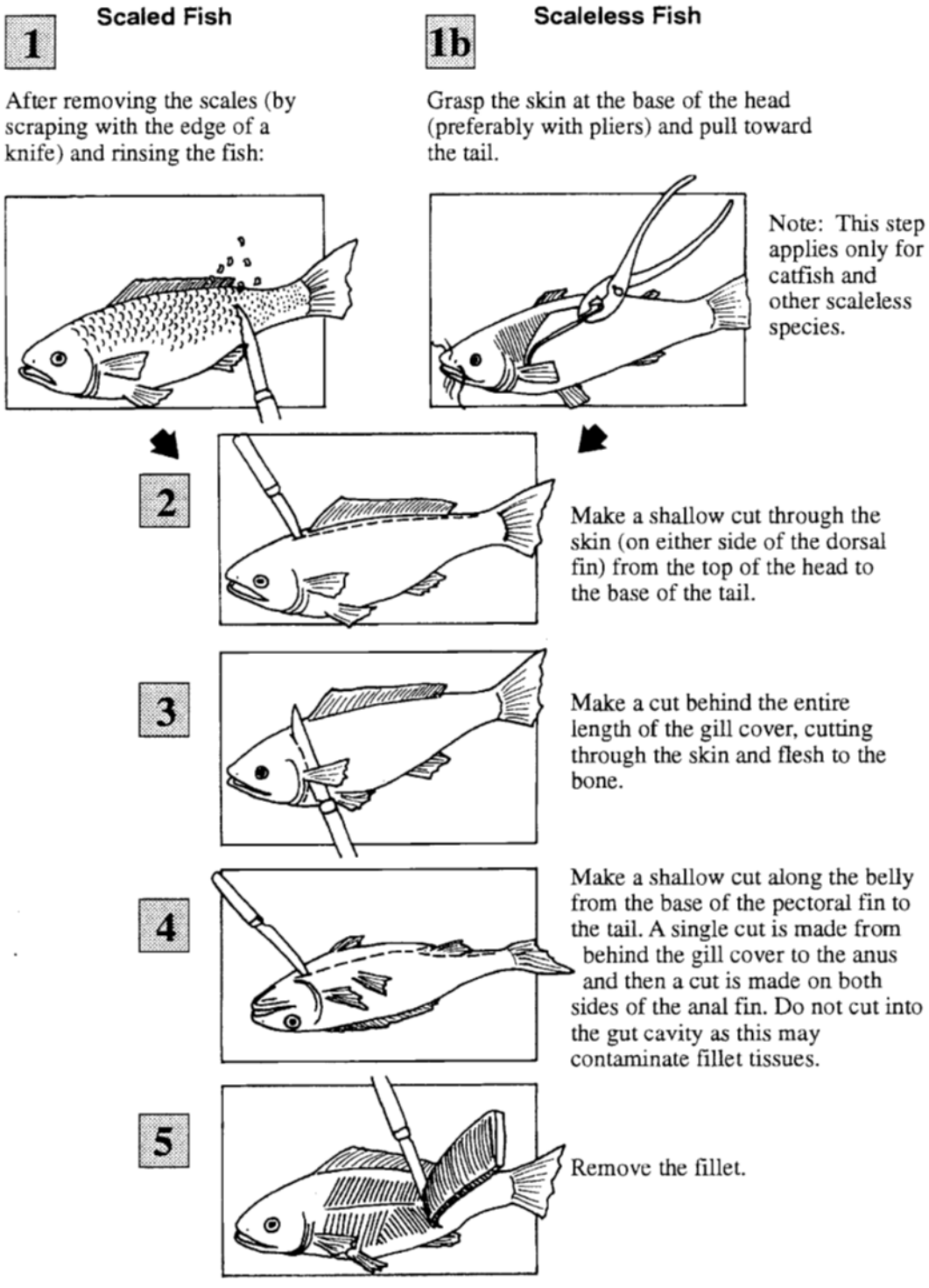
Comments:

ATTACHMENT C – DELTS EXAMINATION

Adapted from US Environmental Protection Agency, R10, (2005)

External Feature	Condition	Description
Body Structure	Deformities	Skeletal anomalies of the head, spinal vertebrae, and fins
Fin Erosion	No active erosion	Normal-appearing fins
	Mild active erosion	Active erosion, no hemorrhage or secondary infection
	Severe active erosion	Active erosion with hemorrhage and/or secondary infection
	Other	Any other observations of special significance
Eyes	Normal	No aberrations evident – clear eye
	Exophthalmia	Swollen, protruding, bulging
	Hemorrhagic	Bleeding in the eye
	Blind	Opaque
	Missing	Less than two
	Other	Any abnormalities not included above
Skin	Normal	No apparent aberrations (discolorations, blisters, cysts, tumors, scale anomalies)
	Mild	Mild aberrations present
	Moderate	Moderate aberrations present
	Severe	Extreme aberrations present
Gills	Normal	No apparent manifestations in gills
	Frayed	Erosion of tips of gill lamellae – ragged appearance
	Clubbed	Swelling of tips of gills
	Marginate	Light discolored margin along distal tips of lamellae or filaments
	Pale	Very light in color
	Pseudobranchs	Obviously convex in aspect rather than flat or concave, aberrations
	Other	Any abnormalities not included above
Thymus	Hemorrhage	None, mild (two or three spots), severe (many pin-point spots, some coalescing, swollen appearance
Opercles	Normal	No shortenings, gill completely covered
	Slight shortening	Small portion of the gill exposed
	Severe shortening	Considerable portion of gill exposed
Parasites	Infection	None, mild (few), moderate (some), numerous (many) – copepods, lice, leaches, grubs, fungus

ATTACHMENT D – FISH FILLET PROCEDURES



Source: USEPA, 1991

ATTACHMENT E – CHAIN-OF-CUSTODY FORM

Affix Chain-of-Custody Label here.

Project Name/Number	**Collecting Agency** (name, address, phone)	**Sample Date**
Sampling Personnel (print and sign)		Container _____ of _____
Waterbody (name)	**Location** (reach code and/or lat/long)	**Station ID**

Sample ID No.	**Composite No.**	**Sample Time**	**Chemical Analysis**						**Comments**

Delivery/Shipment □ hand □ shipped		**Deliver/Ship to:** (org., address, phone)		**Date/Time Shipped**	
Relinquished by: (*sig.*)	**Date/Time**	**Received by:** (*sig.*)	**Relinquished by:** (*sig.*)	**Date/Time**	**Received by:** (*sig.*)
Relinquished by: (*sig.*)	**Date/Time**	**Received by:** (*sig.*)	**Relinquished by:** (*sig.*)	**Date/Time**	**Received by:** (*sig.*)
Account/Contract No.		**LAB USE ONLY** **Delivery temp.** Wet Ice: _____ Dry Ice: _____		**Remarks:**	

ATTACHMENT F – MONTANA FISH, WILDLIFE & PARKS
FISH SPECIES CODES AND ABBREVIATIONS

Code	Common Name	Abbr.	Code	Common Name	Abbr.
001	Rainbow Trout	RB	046	Sturgeon Chub	ST CH
002	Cutthroat Trout (unk. sub. sp.)	CT	047	Emerald Shiner	EM SH
003	Brook Trout	EB	048	Sand Shiner	SD SH
004	Brown Trout	LL	049	Redside Shiner	RS SH
005	Bull Trout	BULL	050	Creek Chub	CR CH
006	Lake Trout	LT	051	Pearl Dace	P DC
007	Golden Trout	GT	052	Fathead Minnow	FH MN
008	Kokanee	KOK	053	Golden Shiner	G SH
009	Coho Salmon	SS	054	Sicklefin Chub	SF CH
010	Arctic Grayling	GR	055	River Carpsucker	RC SU
011	Rainbow x Cutthroat Hybrid	RBxCT	056	Longnose Sucker	LN SU
012	Westslope Cutthroat Trout	WCT	057	White Sucker	W SU
013	Yellowstone Cutthroat Trout	YCT	058	Largescale Sucker	LS SU
014	Whitefish (unspecified)	WF	059	Blue Sucker	B SU
015	Lake Whitefish	L WF	060	Bigmouth Buffalo	BM BUF
016	Sculpin (unspecified)	COT	061	Smallmouth Buffalo	SM BUF
017	Largemouth Bass	LMB	062	Shorthead Redhorse	SH RH
018	Bass (unspecified)	BASS	063	Mountain Sucker	MT SU
019	Sunfish (unk. centrarchid)	SUN	064	Stonecat	S CAT
020	Yellow Perch	YP	065	Black Bullhead	BL BH
021	Crappie (unspecified)	CR	066	Yellow Bullhead	YL BH
022	Sauger/Walleye	SAWE	071	Brook Stickleback	BR SB
023	Northern Pike	NP	072	White Bass	W BS
024	Channel Catfish	C CAT	073	Smallmouth Bass	SMB
025	Bullhead	BLHD	074	Bluegill	BG
026	Burbot	LING	075	Pumpkinseed	PUMP
027	Sturgeon	STRG	076	Green Sunfish	G SUN
028	Paddlefish	PF	077	Black Crappie	BL CR
029	Peamouth	PEA	078	White Crappie	WH CR
030	Goldfish	GDF	079	Rock Bass	R BS
031	Sucker (unk. catostomid)	SU	081	Sauger	SGR
032	Common Carp	CARP	082	Walleye	WE
033	Northern Pikeminnow	N PMN	083	Iowa Darter	IOWA
034	Goldeneye	GE	085	Mountain Whitefish	MWF
035	Utah Chub	GILA	086	Pygmy Whitefish	PWF
036	Freshwater Drum	DRUM	087	Chinook Salmon	CK SAL
037	Minnow (unk. cyprinid		088	Splake (Brook x Lake Trout)	SPLK
038	Shortnose Gar	GAR	089	Salmon (unspecified)	SAL
039	Longnose Dace	LN DC	090	White Sturgeon	W STRG
040	Buffalo (unspecified)	BUFF	091	Pallid Sturgeon	P STRG
041	Redbelly/Finescale Dace	NRB/F DC	092	Shovelnose Sturgeon	S STRG
042	Brassy Minnow	BR MN	099	Rainbow Smelt	RB SM
043	Western Silvery/Plains Minnow	WS/P MN	100	Trout-perch	TR PR
044	Flathead Chub	FH CH	103	Plains Killifish	PKF
045	Lake Chub	LK CH	106	Mosquitofish	MQF

Code	Common Name	Abbr.	Code	Common Name	Abbr.
108	Sailfin Molly	SFM	134	Spoonhead Sculpin	SP COT
109	Shortfin Molly	SHM	135	Rocky Mountain Sculpin	RM COT
110	Rainbow x Westslope Cutthroat	RBxWCT	136	Clark Fork Sculpin	CF COT
111	Rainbow x Yellowstone Cutthroat	RBxYCT	137	Columbia Slimy Sculpin	CSL COT
112	Variable Platy	VPF	140	Western Silvery Minnow	WS MN
113	Rainbow x Yellowstone x Cutthroat	RBYCTWCT	141	Plains Minnow	PL MN
115	Green Swordtail	GST	142	Finescale Dace	FC DC
118	Trout (unspecified)	TRT	143	Northern Redbelly Dace	NRB DC
119	Trout/Salmon (unk. salmonid)	TR SAL	144	Peamouth x N. Pikeminnow	PEAxNPMN
120	Rainbow x Golden	RBxGT	145	Spottail Shiner	SP SH
121	Upper Missouri Cutthroat	UMCT	146	Peamouth x Redside Shiner	PEAxRSSH
122	Native Rainbow Trout	NRB	147	Redbelly x Finescale Dace	NRBxFCDC
123	Cutthroat x Golden	CTxGT	148	Northern Pike x Muskie	NPxMK
124	Brook x Bull	EBxBULL	149	Sauger x Walleye	SGRxWE
125	Cisco	CIS	150	Golden x Rainbow x Cutthroat	GTxRBxCT
126	Atlantic Salmon	AL SAL	152	Sunfish Hybrid	SUN HY
128	Native Redband x Westslope	NRBxWCT	153	Central Mudminnow	CM MN
129	Native Redband x Rainbow	NRBxRB	154	Brook x Brown	EBxLL
130	Mottled Sculpin	M COT	155	Striped Bass	ST BS
131	Slimy Sculpin (unspecified)	SL COT	156	Gizzard Shad	GZ SHAD
132	Torrent Sculpin	T COT	157	Redbelly Pacu	REDB P
133	Shorthead Sculpin	SH COT	158	Grass Carp	GS CARP