

**DRAFT - Sampling Protocol for
Westslope Cutthroat Trout Oncorhynchus clarki lewisi
in the Upper Missouri River Basin**

Richard A. Oswald
Montana Fish, Wildlife and Parks
Dillon, Montana

Robb F. Leary
Montana Salmon and Trout Genetics Laboratory
University of Montana
Missoula, Montana

Bradley B. Shepard
Montana Fish, Wildlife and Parks
Bozeman, Montana

James A. Brammer
Montana Fish, Wildlife and Parks
Dillon, Montana

Michael D. Enk
U.S.D.A. Forest Service
Lewis and Clark National Forest
Great Falls, Montana

William P. Dwyer
U.S.D.I. Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, Montana

David H. Kampwerth
U.S.D. I. Bureau of Land Management
Dillon Resource Area
Dillon, Montana

Brian W. Sanborn
U.S.D.A. Forest Service
Deerlodge National Forest
Butte, Montana

Ann Tews
Montana Fish, Wildlife and Parks
Lewistown, Montana

1995

Table fo Contents

Table of Contents ii

List of Tables ii

Introduction.....1

Population Surveys1

 Initial Surveys3

 Long-Term Monitoring.....3

 Special Studies4

Genetic and Disease Sampling4

 Genetics5

 Disease7

Reporting and Database Management7

Acknowledgements.....7

References7

List of Tables

Table 1. Level of hybridization which can be detected at the 95% confidence level between westslope cutthroat trout (WCT) and rainbow trout (RB) and between westslope cutthroat trout and Yellowstone cutthroat trout (YCT) at various sample sizes.4

Introduction

The Upper Missouri Westslope Cutthroat Trout Technical Committee (WCTTC) was convened by Montana Fish, Wildlife and Parks (FWP) in 1995 to review the status of westslope cutthroat trout *Oncorhynchus clarki lewisi* and recommend strategies for conserving this subspecies. In February 1998 the WCTTC broadened their scope at the request of FWP to include all of Montana. This document contains the WCTTC's recommended protocol for sampling in waters supporting westslope cutthroat trout.

Population Surveys

Population surveys fall into one of three categories: initial surveys; long-term monitoring; or special studies. These three types of monitoring are similar to the "baseline", "trend", and "project" monitoring types identified by MacDonald et al. (1991). We have detailed our recommendations for each of these categories below. We have recommended the minimum information that should be collected for "Initial Surveys" and expect that more information will be collected during "Long-Term Monitoring" and "Special Studies".

Initial Surveys

For waters that have not been surveyed, an initial survey is often conducted to assess the aquatic communities. Prior to conducting an initial survey, a review of information sources that might contain information on the target water must be completed. At a minimum, investigators should review information in the Montana River Information System (MRIS), files and reports of FWP including a contact with the local FWP fisheries biologist, and files and reports of the appropriate federal land management agency, if the water is within Forest Service or BLM administered lands. If this review finds that no information is available for the target water, an initial field survey should be done. All field surveys must be coordinated through the local FWP Fisheries Biologist or Regional Fisheries Manager under the authority of either a Memorandum of Understanding (MOU) or Collection Permit.

Sample section(s) should be selected to represent the portion of the water to be assessed. Sample section(s) must be described in detail to ensure that the section could be re-located for future field sampling, even by different samplers. At a minimum, the stream name, legal description and a narrative description of the sample section should be recorded on each data sheet. Narrative descriptions of the sample boundaries should reference permanent landmarks (ie. bridges, tributary mouths, etc.) and the distance each boundary is from these permanent landmarks. Distances should be measured by tape measurement, but may be paced or measured using vehicle odometer readings. Hand held global positioning systems (GPS) could be used for more accurate locations of sample sites. All sample sites must be plotted on maps for a permanent file record.

After locating a sample section the length of the sample section should be measured using a tape measure, but may be paced. Length of sample sections should be a minimum of 75 m or about 35 times the wetted width of the stream, whichever is longer, to ensure that all habitat types are represented. Lyons (1992) found that the number of fish species captured in Wisconsin streams by a towed electrofishing unit approached or exceeded an asymptotic level when a length of stream 35 times the width was sampled. If fish densities are so low that few fish are captured (observed) in a continuous sample section, additional spot sampling of high quality habitats may be conducted, but this should be noted on field data sheets (ie. "Sampling conducted only in 5 pools from point A to point B"). In addition to sample section length, other information which must be recorded includes: sampling equipment used (shocker model number) and settings (waveform and voltage); wetted width; Rosgen channel type; substrate composition [proportion of silt, sand, small gravel (sand to 0.25"), large gravel (0.25 to 3.0"), cobble (3.0-10.0"), small boulder (10-24"), large boulder (>24"), and bedrock]; dominant riparian vegetation and features; stream channel and stream bank condition (bank stability) rated as "poor" to "excellent"; stream habitat composition (proportion pool, riffle, run and pool forming structure); presence and location of known barriers to fish movement; and air and water temperature along with time of measurement. Optional habitat information which should be considered includes: water conductivity; water pH; instream cover; bank cover; substrate fines by depth; substrate embeddedness; frequency of woody debris within the stream channel by size class; average water depth; thalweg depth; pool volume; residual pool volume; and other variables which investigators wish to compare to fish abundance or use to characterize the habitat. A sample data sheet is provided in Appendix A for reference.

Biotic information that should be collected includes observations of amphibian and benthic macroinvertebrate species presence and fish abundance data. Fish abundance may be assessed using one or more of several different techniques including electrofishing, underwater observation, angling, seining, and trapping. Use of a non-invasive technique such as underwater observation is preferred where this technique is appropriate. When using electrofishing, we recommend that pulse rates and waveforms which will minimize potential for injury (straight DC, CPS, or pulsed DC with frequencies under 40 Hz and voltages as low as possible to remain effective) be employed. The method of capture and sampling gear should be recorded on the data sheet. We recommend making population estimates using either a mark-recapture or depletion estimator to quantify capture efficiencies. However, in some cases where population estimates cannot be made due to gear or time constraints, relative catch per unit effort must be reported (number captured per length or area of stream or time of sampling). During electrofishing all captured fish, but especially westslope cutthroat trout, should be transferred to a live car/bucket as soon as they are captured to minimize repeated electroshocks. Temporary fish-holding gear should not be over-crowded with fish to minimize stress. Holding facilities must have adequate water depth and flow to ensure survival of held fish. Electrofishing should be avoided during the spawning season, if possible, and over spawning sites when westslope cutthroat trout embryos are still within the substrate. Exceptions to the prohibition of electrofishing during spawning would be to collect gametes for restoration and/or conservation

efforts. Electrofishing and fish processing should not be conducted at higher water temperatures (>62°F) to minimize stress to fish.

At a minimum all captured fish must be identified to species and counted. If investigators are unsure of the identification of any species, several specimens of that species should be preserved and sent to Dr. William Gould, Montana State University for identification. Specimens sent to Dr. Gould should be stored in labeled containers with 10% formalin. Containers should not be glass. Either nalgene bottles or zip-lock bags with a small amount of formalin will preserve specimens. Labels should indicate the water body, a legal description of sample location, date of sample, and the name of the person and their agency who did the collecting. A cover letter to Dr. Gould should indicate whether reference specimens are needed back. He recommends larger specimens have the right side of the body cavity opened to allow preservative to enter the body cavity. For best results, specimens should be sent as soon after collection as possible to limit the washing out of coloration by the preservative. Specimens can be sent in an alcohol solution, however, they need to be sent immediately after collection and a note must included to alert Dr. Gould to transfer the specimens to a formalin solution when he receives them.

Lengths (either fork or total) must be recorded for all westslope cutthroat trout. Genetic samples should be collected (see below) for verification of genetic status of the population. We recommend recording lengths for all fish species collected, but lengths must be recorded for all westslope cutthroat. Measuring weights and removal of scales for aging are optional. Minimize stress on westslope cutthroat trout by processing all cutthroat first when working fish. Immediately after processing, gently transfer fish to live holding facility located in relatively calm water with adequate depth and flow to minimize stress to held fish. Re-distribute fish back into the sample section to as near to capture locations as possible ensuring that fish are released into low velocity waters.

Long-Term Monitoring

A study plan detailing the objectives of long-term monitoring and the sampling design must be completed in coordination with FWP prior to initiation of a long-term monitoring program. Long-term monitoring should be conducted to assess trends in the distribution and abundance of westslope cutthroat within the upper Missouri River basin over time. Long-term population monitoring should be done in a few drainages selected to "typify" a particular set of conditions within each major river drainage. Population estimates should be made periodically within sample sections stratified to represent each type of drainage. These data would also be useful for model truthing (ie. habitat relationships, extinction risk assessments, etc.). Genetic status should also be monitored.

FWP should provide the lead for long-term monitoring efforts with land management agencies assisting. Local biologists from agencies outside FWP will likely conduct long-term

monitoring; however, these monitoring efforts must be coordinated through FWP. Sampling frequencies and sites should be based on clearly identified objectives to answer specific research or management questions and the desired level of precision with an over-riding consideration of potential impacts to monitored populations of westslope cutthroat. We recommend that for most population monitoring programs sampling frequencies be limited to, at most, every other year unless it is clearly demonstrated that more frequent sampling is necessary. Sampling should occur within permanently marked sample sections that are at least 300 m long or 35 times the average wetted width, whichever is longer. Population estimates must be conducted using either a mark-recapture or depletion estimator. Genetic sampling should not be repeated at a frequency shorter than every five years unless repeated annual sampling is necessary to obtain an adequate "single" sample size (see "Genetics and Disease Sampling" below).

Special Studies

Special studies are monitoring which would occur over relatively short durations (3-20 years) to assess specific population or habitat treatments. Population treatments might involve removal of competitive or potentially hybridizing exotic species where populations of westslope cutthroat trout exist; removal of competitive or potentially hybridizing exotic species and replacement with westslope cutthroat trout; assessing angling regulation changes to enhance populations of westslope cutthroat trout; and removal of individuals or gametes for brood or re-founding of westslope cutthroat trout populations. Habitat treatments might involve watershed reclamation efforts; in-channel habitat enhancement; riparian restoration (ie. livestock enclosure); land management actions; mining reclamation; road reclamation; etc. FWP will be the lead agency in coordinating population treatments and monitoring treatment effects including preparing environmental documentation under MEPA regulations. Fish Research Extension, Fish and Wildlife Service (FWS), and/or land management agencies can propose population treatments to FWP and assist with the treatment and environmental review. For habitat treatments, either FWP or land management agencies may take the lead in coordinating the treatment or preparing environmental documentation; however, fish population monitoring will be coordinated through FWP.

A study plan which details the objectives of the treatment and monitoring program, including the sampling design, must be completed prior to initiation of the treatment. All study plans must be on file with FWP. Within each study plan the following areas must be addressed: treatment proposed and schedule of treatment; monitoring plan and methods including sampling frequency and sites; expected outcome of treatment; and measure for assessing success of treatment. In addition the plan must commit to annual coordination between FWP and treatment sponsors to ensure that proposed monitoring is conducted.

The first phase for any of these treatments would be collection of baseline data prior to treatment. Baseline data would be collected at a frequency and duration to adequately document population conditions prior to implementing the treatment. Sample sites should be selected

following a treatment-control study design protocol. All fish and habitat sampling would, at a minimum, collect the information detailed above ("Initial Surveys"). The second phase would be post-treatment evaluation. Post-treatment evaluation duration would depend upon the treatment being evaluated.

Genetic and Disease Sampling

Genetics

For the first genetic and/or disease sampling of a stream, at least ten fish should be sampled, however, in streams which support extremely low populations, five fish may be all that should be sacrificed due to small population considerations. We recommend that samples be taken from at least three locations within the stream (low, middle and high in the drainage) to assess distribution of genetic status within the drainage. If low population considerations make it impossible to sacrifice ten fish, we recommend collecting fish over two to three consecutive years to obtain a sample of ten fish. In streams supporting relatively large populations, a sample size of 25 will provide a more reliable assessment of genetic status (Table 1). For those populations where individuals appear obviously hybridized, we recommend sending in a sample of 5 fish to confirm the genetic status as introgressed.

Table 1. Level of hybridization which can be detected at the 95% confidence level between westslope cutthroat trout (WCT) and rainbow trout (RB) and between westslope cutthroat trout and Yellowstone cutthroat trout (YCT) at various sample sizes using allozyme techniques.

Species Cross	Sample Size					
	1	5	10	15	20	25
WCTxRB	22%	5%	2.5%	1.6%	1.3%	1.0%
WCTxYCT	12%	2.5%	1.5%	0.9%	0.7%	0.5%
RBxYCT	14%	3.0%	1.5%	1.0%	0.8%	0.6%

An attempt should be made to collect multiple size (age) classes, however, try to collect mostly smaller (minimum size of 75 mm; 3 inch) size classes. We recommend that, if possible, larger, mature individuals not be sacrificed, especially in small populations; however, when non-lethal fin clip DNA analyses are planned, adults can be sampled. We suggest that fish sacrificed for allozyme sampling also be used to obtain other information such as: disease status (see

protocol below), age by removing otoliths, and sex ratio and state of maturity by assessing condition of gonads. If additional information will be collected from sacrificed fish, the Salmon and Trout Genetics Laboratory should be contacted prior to the collection to ensure that specimens sent to them for genetic analyses are suitable. **Do not puncture the stomach** because stomach contents could invalidate allozyme genetic testing.

Each individual fish or fin sample should be placed in an individual container. For fish a zip-lock bag should be used and for fins individual glass or plastic vials containing alcohol should be used. It is recommended that adipose fins not be used for a fin tissue sample due to the amount of fatty tissue in this fin. Small sections of anal or pectoral fins can be excised and placed in separate vials. For each location within a stream, samples should be labeled and stored together. Labeling for each sample location should include the stream name, collector name, date of collection, legal description of collection site, narrative description of collection site, and number of fish collected at the site. All samples from within each stream should be bagged together in a large zip-lock bag and labeled with stream name, county, mountain range, legal description, date of sample, collector name, agency of collector, and number of fish in total sample (n).

Whole fish samples should be held on ice immediately post-mortem in the field and transferred as soon as possible to a freezer set well below freezing point. After one month or less, fish should be shipped or delivered to the Salmon and Trout Genetics Laboratory at the University of Montana after contacting their staff. Samples should be shipped in a cooler with dry ice.

Additional samples should be collected for genetic analysis within a year following the initial sample collection if the results from the first genetic sampling are inconclusive or based on a questionable sample size (<10), or more reliable results are needed for management purposes (n=25) or for consideration as a donor source (n=50). When additional samples are deemed necessary, these samples should be obtained as soon as possible and from as near to the original collection sites as feasible.

Disease

Periodic fish health sampling should be done periodically for feral fish populations to determine presence of fish pathogens and parasites. These inspections should be conducted:

1. Prior to moving fish and/or eggs from one location to another;
2. During routine fish sampling when fish must be sacrificed for any reason, such as genetic testing; or
3. To investigate potential causes of a fish kill or population decline.

All samples should be from fresh fish. Select fish of the appropriate species for which

are testing. If it is difficult to collect a large enough sample size from the target species (ie. cutthroat trout), other trout species inhabiting the same water may be used to supplement the sample, or replace the cutthroat trout. Any sized fish can be used for the samples, however, for certain pathogens age does make a difference in detecting the disease. For example, to sample for whirling disease the fish should ideally be between 4 to 12 months of age.

A sample size of 60 fish will provide a 95% confidence of detecting most diseases in a total fish population of 2,000 or more fish. A sample size of less than 60 will still provide useful information and should be submitted, especially if the fish were sacrificed for other reasons.

It is strongly recommended that field biologists contact Montana FWP's Fish Health Lab (Jim Peterson, Montana FWP Fish Health Lab, P.O. Box 2163, Great Falls, Montana 59403, phone: 406-452-6181) prior to collecting field samples. Try to give the Fish Health Lab staff as much lead-time as possible. The Lab will try to provide staff to meet field crews in the field to collect fish health samples. Personnel from the Fish Health Lab can coordinate fish health sampling with the Salmon and Trout Genetics Lab at U of M, provided you give each of these labs prior notice.

Record length, weight, location (water, site, legal), date, collector, and agency on a data form and send a copy of this form in each type of the following samples. Specific sample protocol for different disease testing follows:

1. Whole heads (whirling disease). Cut off heads and place in plastic bags. Five heads per bag. Label each bag with species, age or size of fish, date, and bag number. Record the bag number with the site information above for each fish.
2. Culture from kidney (furunculosis, redmouth). Collect a kidney culture with a sterile inoculating loop. It is important that this be done in a sterile setting. Should send in fish to lab for this test.
3. Kidney and spleen samples (IPN, VHS). Place a piece of kidney and piece of spleen in small plastic tube (provided by Fish Health Lab) with saline solution. Pool kidney and spleen from fish fish in each tube.
4. Kidney tissue sample (kidney disease). Place all kidney (or remaining kidney if samples taken for IPN and VHS) in a small plastic bag or whirl pack.
5. Ovarian, or seminal, fluid sample. When spawning fish to move fertilized eggs, sampling of ovarian and seminal fluids can be used to test for several diseases without sacrificing any more fish. During spawn taking, collect ovarian and seminal fluids in separate cups. Fluids from five females and five males can be pooled. After fluid from five fish have been collected in a cup, pour fluid into plastic tubes with getocin (these tubes of getocin will be provided by the Fish Health Lab prior to sampling). Do not mix ovarian and seminal fluids.

If you want to separate the fish health samples from the genetic samples in the field, you can remove tissue samples from each fish for genetic sampling prior to doing the fish health sampling. For genetic sampling you will need to take: 1) a whole eye; 2) a piece of the liver; and 3) a piece of white muscle tissue. Special care must be taken when removing the fish's eye to ensure it does not burst. Only whole eyes can be tested. When taking the piece of liver for genetics testing, it is extremely important to keep the liver sterile for disease testing. Try not to touch the liver with any instrument or your fingers if they have not been sterilized. Use alcohol to sterilize your knife and tweezers. You may wish to try to remove fish health tissue, especially if you are only doing whirling disease sampling, and send the rest of the fish to the genetics lab with an eye. Contact the Fish Health and Salmon and Trout Genetics labs prior to doing field sampling to ensure that correct field procedures provide the tissues each lab needs.

All fish health sampling must be done on Monday, Tuesday, or Wednesday to ensure that they arrive in the lab prior to the week's end. Viral samples must be placed on the cells within 72 hours after sampling. Samples must be carefully packed for shipping to the appropriate labs. Samples must be shipped in coolers with frozen blue ice packs or dry ice using some type of Express Mail. Samples should be insulated from dry or blue ice to prevent direct contact. Label each sample prior to shipping and send in completed field sampling forms. FWP's Fish Health Lab will provide disease sample supplies, including data forms and labels.

Reporting and Database Management

Data should be summarized each year it is collected. Summarized data must include methods of assessment; lengths and locations of sample sections; population estimates by sample section, species, size class, and standardized to length or area; average length and length range sampled; and genetic status and sample size for genetic analyses. These summaries must be entered into the MRIS, the official repository for fisheries information in Montana. Local biologists should also share these summaries with their counterparts in other agencies within the local area. Formats for these shared data summaries at the local level can be coordinated between local biologists. It is recommended that local information be stored in two formats. One format would store all summarized information by creek name alphabetized for easy access. The other format would be a tabled format of all streams sampled which includes date sampled, location sampled, species collected, and genetic status of westslope cutthroat trout including percent purity and sample size, where available. For each "Special Study" annual reports and a final report must be completed. All reports must detail all results and include project identification, location, and objectives. The final report must assess the success of the treatment by comparing measured results with pre-determined measures of success.

Acknowledgements

J. Peterson of Montana FWP provided the disease sampling protocol. Dr. William Gould provided the sampling protocol for submitting whole fish for species identification.

References

- Lyons, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. *North American Journal of Fisheries Management* 12: 198-203.
- MacDonald, L.H., A.W. Smart, and R.C. Wissmar. 1991. Monitoring guidelines to evaluate effects of forestry activities on streams in the Pacific Northwest and Alaska. U.S. Environmental Protection Agency Report EPA 910/9-91-001. Region 10, U.S. EPA, Seattle, Washington.

Appendix A

Field Data Sheet
(Tentatively Approved for MRIS)