

Sampling and Analysis Plan 2005 for the University of Montana Watershed Health Clinic, Wadeable Stream Reference Study

I. Task 1: Wadeable Stream Reference Study

Task 1 General Description: Between July 1 2005 and September 30 2005 approximately fourteen wadeable stream sites will be sampled in two general areas of Montana: SE Montana (Tongue and Powder river basins, and further to the east); and in SW Montana around Dillon. (Some non-biological sampling such as geomorphic measurements may occur in October.) Sampling effort in each region will be equally weighted so approximately 7 sites will be sampled in SE Montana and approximately 7 sites in the Dillon area. An effort will be made to select primarily reference sites, based on best professional judgment and the MT DEQ's previous work identifying and screening reference sites. Staff of the University of Montana Watershed Health Clinic will work closely with staff of the MT DEQ on the selection of sites; final site selection will be approved by the MT DEQ prior to sampling. Sampling site locations will be photo-documented and georeferenced with GPS units. Sites should preferably be located on BLM lands or within watersheds containing BLM lands. BLM regional hydrologists will be contacted regarding sites on their jurisdictional lands for the purpose of agency coordination.

Timing of assessments

Each stream site will be visited according to the following schedule:

Once per season measurements:

Each stream site will be characterized once during the study for physical habitat/morphological parameters using the following methodologies:

- EMAP Western Pilot physical habitat assessment for a stream reach (EPA 2002)
- NRCS riparian and stream assessment form (2005 version)
- Rosgen Level II stream classification (which includes entrenchment ratio, W/D ratio, sinuosity, slope, and bankful to bankful pebble count).

Multiple sampling measurements:

Stream sites will be sampled three times between late spring and early fall for periphyton parameters, including chlorophyll *a*, phaeophytin, and algal C:N:P content. Water samples will be collected for various analyses (including nutrients). Chlorophyll, phaeophytin, ash-free dry weight (AFDW), and algal P content will be analyzed by the UM Watershed Health Clinic. The state of Montana DPHHS Environmental Lab (State lab) will analyze the water samples for TDS, pH, alkalinity, carbonate and bicarbonate, chloride, sulfate, potassium, calcium, magnesium, sodium, turbidity & TSS. Periphyton C & N content will be analyzed by the MSU Laboratory in Bozeman, MT. The UM Flathead Lake Biological Station will analyze the water samples for various nutrients (total N, NO_{2/3}, TP and SRP).

Periphyton (diatoms and soft-bodied algae) will be sampled once for community composition analyses. Samples will be collected in July and/or August. Vicki Watson of the U of M Watershed Health Clinic will provide the periphyton samples to a MT DEQ selected contractor.

Aquatic macroinvertebrates will be sampled from late spring to early fall. Vicki Watson of the U of M Watershed Health Clinic will provide the macroinvertebrate samples to a MT DEQ selected contractor.

Field protocols

Deviations from the following general sampling plan may arise and will be discussed between the University of Montana Watershed Health Clinic and the MT DEQ prior to implementation, for final approval by MT DEQ.

A reach will be laid out and divided into ten subreaches with 11 transects, based upon the wetted width of the stream, or a minimum of 150 m length (EPA 2002).

To calculate discharge, water velocity will be measured up to three times during the study (if streams are flowing and wadeable) at one transects located within the designated reach that will have been selected for ease of measuring discharge (i.e., most weir-shaped). Velocity will be measured at 20 equally spaced intervals along these transects at 0.6 depth.

Macroinvertebrates will be sampled following Western Pilot EMAP protocols (EPA 2002). A reach-wide composite and a targeted-riffle sample (EPA 2002) will each be collected one time during the first sampling event (early season) and once each during the last sampling event (late season). In cases where it is not possible to collect targeted riffle samples because the stream lacks riffles such samples may be skipped. One modification to EMAP methods will be applied to minimize resampling of sites. During any subsequent sampling after the first sampling, collection will occur either 1 meter above or 1 meter below the original reach-wide or targeted-riffle sampling sites. A replicate of each method will be undertaken at one site during one of the sampling events.

Phytoplankton will be sampled in duplicate from well-mixed portions of the water column at all stream sites using 25 mm glass fiber (GF/F) filters, and then frozen in the dark on dry ice or a portable freezer. When streams have become intermittent, samples will be collected from up to two different pools when possible. The collected material will be analyzed for chlorophyll *a*, phaeophytin and AFDW within 60 days of collection according to the lab protocols of the UM Watershed Health Clinic.

Periphyton will be sampled between late spring and fall for both quality and quantity at each stream. During each subsequent sampling after the first sampling, collection will occur either 1 meter above or 1 meter below the transect line to avoid resampling the same location. Quality samples will be collected for community composition analyses and for C, N, and P content of algae, using one of four different techniques: transect reach wide composites; targeted pool/depositional composites; algal CNP samples; and macrophyte & filamentous algae cover.

Quality sampling methods are detailed below:

a. Transect Reach-Wide Composites—Collection of multihabitat algae composites (for organism identification) will be undertaken at each transect following the multihabitat methods outlined in Table 6.1 of Barbour et al. (1999). These methods closely overlap those used by MT DEQ outlined in its SOP manual and both literature sources should be consulted (the main difference is that MT DEQ uses a turkey baster to sample fine depositional areas). The composited material will be chopped with a scissors and mixed, and then preserved with Lugol's solution or 2% buffered formalin.

b. Targeted Pool/Depositional Composites—Results from the Nutrient Pilot Study indicated that pools and depositional habitats are the best location in prairie streams to collect diatoms to assess nutrient impacts (Bahls 2003). Therefore, a composite sample of algal films on fine sediments from pools and other depositional areas (for organism identification) will be collected from any/all depositional areas within the reach using the “turkey baster” method (DEQ SOP). If 100% of the transects are depositional/pool habitats, a subsample from the Transect Reach-Wide Composite sample can be also labeled as a Targeted Pool/Depositional Composite sample.

c. Samples for Algal C, N, and P Content—Samples for the C, N, and P content of algae will be collected as two separate groups; filamentous & nostoc algae composites, or algal films on hard substrate (rocks) composites. These will be collected simultaneously with the collection of the Transect Reach-Wide Composite samples (described above). Small grab samples of filamentous/nostoc algae will be composited together from all transects where they are found, while algal film scrapings of hard surfaces will be composited together from transects where they are found. Prior to storage, the filamentous/nostoc composites will be rinsed with tap water to clean them of mud and debris. The composites will then be kept cool or on ice (but *not* frozen) and held in the dark until laboratory preparation.

d. Macrophytes & Filamentous Algae-- A visual estimate of the proportion (by areal coverage) of submergent & floating leaf macrophytes and filamentous algae covering the stream bottom will be recorded during each sampling at each transect using Western Pilot EMAP protocols (EPA 2002) and the “fish cover/other” assessment procedure (see Fig. 6.5 of manual). Examples of all macrophyte species observed throughout the reach will be collected in a 1000 ml HDPE bottle and preserved with 2% buffered formalin.

Quantity sampling methods are detailed below:

a. Periphyton Quantity (biomass/area) samples will be collected from a known area. Up to 11 replicates will be collected, one from each of 11 transects where sampling is possible. Where rocky substrate exists, the template method will be used for diatom or Nostoc films, or short uniform growth of attached filaments. The hoop sampling method will be used where floating filamentous algae, very heavy attached filamentous algae, heavy macrophyte growth, or combinations

thereof occur. Macrophytes growth collected will be of the above ground parts only. These methods are described on pages A-68 to A-70 of EPA's Nutrient Criteria Technical Guidance Manual (EPA 2000) and in the DEQ SOP manual. The collected material will be analyzed within 60 days of collection for chlorophyll *a*, phaeophytin and AFDW according to the lab protocols of the UM Watershed Health Clinic. In cases where filamentous algae and macrophytes are growing together, the two plant types will be physically separated, rinsed in a sieve to remove mud, and then stored separately on ice or frozen. In cases where algae and macrophytes were intermixed, notes will be recorded concerning the relative contribution of each plant type to the sample. A 5-category system will be used indicating the by-weight proportion of each in the sample, ranging from 100% macrophytes to 100% algae: 100/0 75/25 50/50 25/75 0/100. If moss is a part of the mix, its proportional contribution will be noted as well.

Where fine sediments exists, a core of the top one cm of sediment will be collected using a cut-off 60 cc syringe (DEQ SOP). The collected material will be stored in 50 cc centrifuge tubes (DEQ SOP) and frozen for later analysis. Fine sediment samples will be analyzed within 60 days of collection using the laboratory protocols of the UM Watershed Health Clinic, and will **only** be analyzed for chlorophyll *a* and phaeophytins.

Water quality samples in the field

Unfiltered Samples: Water samples will be collected as near the middle of the thalweg as safety permits in well-mixed water. TSS samples will be collected using a DH-48 suspended sediment sampler. Other samples will be collected by grab sample. An attempt will be made to depth integrate the sample by allowing the bottle to fill as it is lowered from the surface to the bottom of the stream. One 250 ml unfiltered sample will be kept in the dark on ice or in a refrigerator until analyzed for pH, alkalinity, conductivity, chloride, sulfate, other ions, TSS, and turbidity. Another 250 ml unfiltered sample will be frozen until analyzed for TN and TP. Sample bottles will be rinsed with stream water before collecting unfiltered samples.

Field-filtered Samples: For soluble nutrients (SRP, NO_{2/3}), 500 ml will be collected in wide mouth bottles, then filtered through a 0.45 um filter. 130 ml of filtrate will be placed in a HDPE bottle and frozen until analyzed. Filtration will be accomplished with a large syringe connected to a disposal filter capsule or a reusable filter holder. A small amount of deionized water followed by a small amount of the sample will be wasted through the filter before the filtered sample is collected. All reusable gear will be acid washed (10% HCl) and triple rinsed in deionized water between uses.

All sample bottles will be high density polyethylene (HDPE). All sample bottles will be new or will be acid washed in 10% HCl & triple rinsed in deionized water. Samples bottles will be rinsed with a small amount of the filtered sample before collecting the final filtered sample. Field blanks and filter blanks will be made on each sampling run and included with the samples to evaluate contamination and detection levels under field conditions.

Special Water Quality Samples: In addition to the water quality samplings detailed above, stream sites in the Tongue and Powder river basins will also be sampled for ammonia and metals (for a metals scan). Ammonia samples will be field filtered at 0.45

µm as described above and then frozen, while metals-scan samples will be collected in 150 ml bottles and preserved with nitric acid to a pH < 2.0.

Task 1 Products & Acceptance Criteria: For each sampling event: 1. Completed copies of site visit forms filled out by field staff, delivered to DEQ within 30 days of sampling. 2. An electronic data deliverable of water chemistry results provided to DEQ from the State Environmental Lab via email at: WQPBSIMDeliverables@mt.gov. 3. An electronic data file (Microsoft Access or Excel 2000), preferably in a normal (not cross-tab) layout, containing the analytical results for Chl a, phaeophytins, AFDW and algal P analyses for samples collected from the sampled streams. 4. A copy of the final chain-of-custody forms for all samples collected and submitted to the State Environmental Lab (post login), and other laboratories (UM Watershed Health Clinic and Flathead Lake Biological Station). 5. Signed, hardcopy analytical reports for each analysis deliverable generated by the laboratories (MSU Laboratory in Bozeman, State Environmental Lab, Um Watershed Health Clinic and Flathead Lake Biological Station). Hardcopy analytical results are to have the following:

- i. State Environmental Lab – standard LIMS report
- ii. UM Watershed Health Clinic, Flathead Lake Biological Station and MSU Laboratory in Bozeman – minimum information required on printout from their Excel file as follows:
 1. **Name, function and signature of person authorizing the test report.**
 2. **Title** (e.g., “Test Report” or “Laboratory Results”);
 3. **Name, Address, and phone number of Laboratory** where the samples were processed and results reported. Results from subcontracted analyses must be indicated and copy of subcontracted analytical report attached.
 4. **Unique identification of the test report.** Suggest using the COC ID. The total number of pages included in the report must be clearly shown so that the extent of the report can be known and any missing pages determined (example: page 1 of 15).
 5. **The name and address of the client and project name:**
 Client: Department of Environmental Quality
 Water Quality Standards Section
 1520 E. Sixth Avenue
 P.O. Box 200901
 Helena, MT 59620
 Project: 2005 Lakes Assessment
 6. **Identification of the analytical method used.** This would be the Reference Method from the laboratories Method SOP (e.g., EPA 200.8). Lab SOP numbers may be used for methods developed by the laboratory, (e.g. Performance Based Methods).
 7. **A description of the client’s sample** and unambiguous identification of the sample(s) including the client identification code.
 8. **Dates:** Date and time of sample collection, date and time of sample receipt, date and time of sample preparation, date and time of sample analysis. Used to determine if holding times were met.

9. **The results from the environmental test.** This includes: Identification of parameter measured, the result obtained from the test (“nd” used to indicate non-detect at MDL), the method detection limit (corrected for dilution/concentration calculation), the units of measurement (e.g., ug/l, mg/kg, NTU), identification of whether dry weight reporting was used for solid samples, any qualifiers associated with the results and explanation of their meaning.

Task 1 Anticipated Completion Timeframe: September 30, 2005.

Task 2. Laboratory Analysis of Samples Collected in Task 1

Laboratory Protocols and QA/QC plan

Watershed Health Clinic protocols – chlorophyll, phaeophytin, AFDW, and algal CNP

Periphyton quality samples will be on filters and/OR stored frozen in petri dishes or plastic zip-lock bags, while biomass/area samples will be small masses stored frozen in petri dishes or zip-lock bags.

CNP of algae from the composite “filamentous/nostoc” and the “algal films on hard substrate” samples will be processed immediately upon return from the field. The filamentous/nostoc samples will be chopped and mixed using a blender, tissue homogenizer, or scissors. Six subsample replicates of the homogenized material will be removed; three will be immersed in 12% HCl until they stop fizzing (to remove inorganic carbonates) and then rinsed in a nylon net in tapwater to remove the acid. The material will then be dried at 105 °C. The “algal films on hard substrate” samples will be homogenized in a blender with 500 ml tap water and then filtered onto 0.45 µm GF/F filters until they are plugged, and the volume recorded. A total of 6 filters will be collected from each homogenized sample. Three of the filters (for C & N analysis) will be immersed in 12% HCl until they stop fizzing (to remove inorganic carbonates). These will then be replaced on to the filter holder and 50 ml tap water pulled through them to remove the acid, and dried at 105 °C. The filters and dried filamentous/nostoc samples will then be sent to the MSU analytical laboratory for C and N analysis via the high temperature induction furnace method (American Society of Agronomy 1996). **For the algal P analysis**, the remaining three filters (from algal film on hard substrates and/or the three remaining filamentous/nostoc subsamples) will be immersed in 0.17 M Na₂SO₄ solution, drained, and then dried (or analyzed immediately). Algal P content will be analyzed using the method of Solarzano and Sharp (1980). Recovery efficiency of the method will be checked using dried tomato leaves having an NIST certificate of analysis of 0.216% P. All aliquots will have their wet weights and dry weights determined, as well as the tare weight for the filters used, to allow for interrelation of sample analyses.

Chlorophyll/phaeophytin analysis: Both samples on filters and the small masses will be handled in a similar way. Both filters and masses will be allowed to thaw in their petri dishes at room temperature in the dark, removed from the petri dish and placed in a mortar. The samples will be ground for one minute in 95% ethanol alcohol using a pestle. Just enough solvent will be used to achieve a light green color (lab workers are trained to recognize the range of acceptable shades). The solvent is then drained into a

small graduated cylinder and measured, and the solvent and sample are placed in a vial which is stored in the refrigerator. Once all the samples have been ground, the vials are warmed in a water bath to 75 degrees C and held there for 2 minutes. Then the chlorophyll extracts are centrifuged to clarity (absorbance at 750 nm < 0.01). Each vial of extract is handled in the following way: a 3ml aliquot of extract is removed from each vial, placed in a glass cuvette and read in a split beam, 2nm spectrophotometer at 664, 665 and 750 nm. Then the extract in the cuvette is acidified to 0.003M HCl (0.1ml of 0.1N HCL), mixed, held for 90 seconds and read again at the same wavelengths. Note: if the initial absorbance at 664 nm exceeds 0.8, the sample is diluted until absorbance is below 0.8. If absorbance at 750 nm is greater than 0.01 (or greater than 10% of the 664 reading), the sample is recentrifuged.

The amounts of pigments in the sample are calculated using the following formulae:

Chlorophyll a in mg

$$= DF \times [(A664b - A750b) - (A665a - A750a)] \times V \times [R / (R - 1)] \times k / L$$

Phaeophytin in mg

$$= DF \times R [(A665a - A750b) - (A664b - A750a)] \times V \times [R / (R - 1)] \times k / L$$

where A664b = absorbance at 664 nm before acidification

A665a = absorbance at 665 nm after acidification

R = acid correction ratio (maximum ratio of A664b:A665a,
i.e. for an extract containing no pheophytin) = 1.72

k = absorbance coefficient of chlorophyll a at 664 nm in 95% alcohol = 11.99

V = total volume of the extract in liters

L = length of the light path in cm

DF = dilution factor

To convert this amount of pigment to amount per sq. meter of stream bottom, divide by the area sampled in sq. meters.

After chlorophyll analysis is complete, the extracts are placed in aluminum weigh boats and dried for AFDW analysis.

QA/QC for the spectrophotometer:

The spectrophotometer is first zeroed against a blank of the same alcohol used for the extraction. Throughout the run the spec is checked against the blank to insure that there has been no drift. If the absorbance reading of the blank has drifted by more than 0.005, the spec is rezeroed. In addition, at the beginning of each run, an internal lab standard (a piece of clear green plastic) is read at 664, 665, & 750 nm to determine that the spec is reading consistently from day to day.

Periphyton AFDW samples will be in two forms: frozen onto glass fiber filters and dried chlorophyll extracts in weigh boats. The filters are thawed & transferred to aluminum weigh boats. Boats are dried to constant weight, stored in a dissector and weighed on an analytical balance. Then the samples & boats are ashed at 500 degrees C for an hour, cooled to room temperature, spritzed with water to rehydrate clays, dried again to constant weight, stored in a dissector and reweighed.

Ash free dry weight of the samples is computed by:

$$AFDW = \text{dry weight} - \text{ashed weight.}$$

To convert the above weight to biomass/area, divide by the area sampled.

Products & Acceptance Criteria: An electronic data file (Microsoft Access or Excel 2000) containing the analytical results for chlorophyll *a*, phaeophytins, AFDW analyses, and algal P-content analyses. Signed, hardcopy analytical reports for each analysis deliverable generated by the laboratories (UM Watershed Health Clinic and Flathead Lake Biological Station). Hardcopy sheets shall contain the same information as outlined above in “**Task 1 Products & Acceptance Criteria**”.

Task 2 Anticipated Completion Timeframe: November 30, 2005.

REFERENCES

American Public Health Association. 1998. Standard methods for the examination of water and wastewater, 20th edition. American Public Health Association, Washington, D.C.

American Society of Agronomy. 1996. Methods of soils analysis part 3. Chemical methods. Soil Science Society of America, Inc., Madison WI. Chapter 34. High temperature induction furnace method, pp 971-977.

Bahls, L. L. 2003. Algal indicators of nutrient availability in selected northeastern Montana streams June-August 2002. Prepared for MT Department of Environmental Quality under contract No. 200012-7.

Barbour, M.T., Gerritsen, J., Snyder, B.D., and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish. 2nd edition. United State Environmental Protection Agency, EPA 841-B-99-002.

EPA. 1993. EMAP surface waters lake field operations volume I resource group manual. Environmental Monitoring and Assessment Program. Draft.

EPA. 2000. Nutrient criteria technical guidance manual, rivers and streams. United States Environmental Protection Agency, EPA-822-B-00-002.

EPA. 2002. Western Pilot Study: Field operations manual for wadable streams. Environmental Monitoring and Assessment Program (EMAP).

Solorzano, L., and J. H. Sharp. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.* **25**: 754-758.