Periphyton Standard Operating Procedure

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Approvals:

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REVISION HISTORY

Revision No.	Date	Modified By	Sections Modified	Description of Changes
	02/18/11			Final Approved Document
1	12/23/2011	M. Suplee		Moved visual assessment of periphtyon to Chl*a* SOP.

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ACRONYMS

AFDW	Ash Free Dry Weight
APHA	American Public Health Association
DEQ	Department of Environmental Quality (Montana)
EDD	Electronic Data Deliverable
EDP	EQuIS Data Processor
EPA	Environmental Protection Agency (US)
ITIS	Integrated Taxonomic Information System
MDC	Montana Diatom Collection
MDEQ	Montana Department of Environmental Quality
PRA	Percent relative abundance
SOP	Standard Operating Procedures
UM	University of Montana
WQPB	Water Quality Planning Bureau (DEQ)

1.0 PERIPHYTON

Algae are ubiquitous in Montana surface waters, easy to collect, and represented by large numbers of species. As primary producers, algae are more sensitive to certain pollutants — like nutrients and herbicides — than other aquatic organisms. Different species are differentially sensitive to a variety of pollutants, and have been found to be useful indicators of nutrient and clean-sediment impacts. Measures of the structure of algal associations, such as species diversity and dominance, can be sensitive and useful indicators of water-quality impacts and ecological disturbance.

Periphyton are algae that live attached to or in close proximity to the stream bottom. Although other plants may occupy the stream benthos, notably mosses and vascular plants (macrophytes), algae contribute more to the diversity and productivity of Montana streams, particularly streams in the mountainous regions of the state.

Periphyton algae may form colonies or filaments that are visible to the unaided eye, or they may be one-celled, microscopic plants that are visible only in their accumulated growth. Two basic types of algae are found in Montana streams: diatoms (Division Chrysophyta, Class Bacillariophyceae) and soft-bodied algae. Soft-bodied algae are represented by four major divisions: green algae (Chlorophyta), blue-green algae or cyanobacteria (Cyanophyta), golden-brown algae (Chrysophyta), and red algae (Rhodophyta).

Pigmented growths of bacteria and fungi (i.e., iron bacteria, "yellow boy", and "sewage fungus") are sometimes found in Montana waters. These growths typically include one or more species of algae interspersed within their matrix. The diverse community of algae, fungi, bacteria and microinvertebrates (nematodes, protozoa, rotifers, etc.) that forms a slime or film coating the stream bottom is called the Aufwuchs. Sometimes this community of autotrophs and heterotrophs is also called "periphyton".

For more information about periphyton, the advantages of using benthic algae in stream surveys, and collection and bioassessment methods, there are a number of good sources (e.g., Britton and Greeson, 1989; Catteneo and Roberge, 1991; Porter et al., 1993; American Public Health Association, 1998; Barbour et al., 1999; Hering et al., 2006; Porter et al., 2008).

1.1 SCOPE AND APPLICABILITY

As of this version of the Standard Operating Procedures (SOP), periphyton biometrics are being used to determine probability of impairment to wadeable streams by two main pollutant types: nutrients and clean sediment. Montana Department of Environmental Quality (DEQ) has used benthic diatoms to assess water quality since the 1970s. Earlier approaches used diagnostic and descriptive biometrics based on quasi-universal ecological attributes of diatom species and observed structural characteristics of benthic diatom associations (Bahls *et al.*, 2008). The current approach, initiated in 2005, has used regional classification, stream reference sites, *a priori* knowledge of stressors in streams, and discriminant function analysis to identify “increaser” taxa that respond to specific stressors or combinations of stressors in a predictable way (Teply and Bahls, 2005; Teply and Bahls, 2006; Teply and Bahls, 2007; Bahls et al., 2008; Teply 2010). The current approach was undertaken because it was found that earlier metrics could not reliably discern impairment causes (Teply and Bahls, 2005; Teply and

Bahls, 2006). Development of diatom-based increaser taxa metrics has been restricted to the assessment of nutrient, sediment, and heavy-metals[1] impairments, or combinations thereof.

1.1.1 Index Period

Although stream periphyton may be sampled anytime of the year, the recommended time is early summer through early fall (July 1 through September 30th). This is a time of stable flows, peak periphyton diversity, and standing crop in most Montana streams. Summer is also the season when most reference data have been collected**. Periphyton samples that will be used to derive increaser diatom metrics per Teply (2010a, 2010b) should be collected during the July 1st to September 30th index period.**

High flows and turbid waters should be avoided because they limit access to and obscure visibility of the stream bottom. Assessments should be delayed for at least two weeks following high, bottom-scouring stream flows to allow for recolonization by algae and succession to a mature periphyton community. If monitoring for year-to-year trends, perform data collection about the same time each year.

1.1.2 Sampling Design

Development of a sampling design depends largely on the objectives of the study. For MT DEQ's typical water-quality assessment monitoring, the physical boundaries of the study (i.e., the sampling frame) are the waterbody assessment unit (smallest unit for which an impairment decision is made). The number of sampling locations and frequency of sample collection (to achieve representative sampling) in the sampling design will vary and should be described in the project Sampling and Analysis Plan. The reader should consult the particular assessment methodology document associated with the stressor of concern (e.g., nutrients) to help determine the best sampling design. It is beyond the scope of this SOP to address all sampling design permutations. Rather, the intent of this document is to describe appropriate sample collection and evaluation methods, along with appropriate quality control.

1.2 DATA RESOLUTION

DEQ collects data at two general levels of resolution; a visual assessment in the field, and quantitative/semi-quantitative identifications and counts of algae samples sent to approved laboratories. Each type provides information that can help with interpretation of the other.

- Field-level Visual Assessment: Aquatic Plant Visual Assessment Form. This form and its associated appendices are found in Section 7.0 of the chlorophyll *a* SOP (WQPBWQM-011).

- Identification and Counts: Identification of soft-bodied algae to genus; estimated relative abundance of cells in each genus; estimated rank of each genus according to biomass. Identification of diatoms to species; proportional count yielding percent relative abundance of each species; calculation of diatom metrics.

[1] Although diatom-increaser metrics to assess metals impacts were evaluated (Teply, 2010a), no significant stand-alone models were developed. Note however that the presence of some metals contamination in a stream will not confound the assessment of, say, nutrient impact assessment. The assessment tools have been designed to function properly in the presence (or absence) of the other stressor types.

2.0 FIELD SAMPLE COLLECTION METHODS

2.1 SAMPLE COLLECTION FOR ALGAE IDENTIFICATION AND COUNTS

For identification and enumeration of algae, one of two sample collection methods should be used, each being more applicable to specific types of projects than the other. These are: PERI-1, and PERI-1mod. Each is described below, with suggestions as to which types of streams or studies the method may be most applicable to.

PERI-1:
PERI-1 is appropriate for assessing non-flowing streams where the collector may be restricted to extant pools, and is also appropriate for flowing streams where a defined reach is not being established. PERI-1 does not require a defined reach length; rather, it requires that the reach is represented by the sample. The collector should observe conditions about 50 m up- and downstream from the initial arrival site (~100 m total), to assure that the collection area is fairly typical of the site in question. In a high gradient stream, for example, a PERI-1 sample would typically be dominated by rock scrapings from gravels and cobbles.

Microalgae are collected from natural substrates in proportion to the approximate rank of those substrates at the study site. Collection of microalgae involves scraping the entire upper surface of several rocks (small gravel through cobbles), lifting the algal film off of near-shore sediments, scraping submerged branches, and sucking up fine sediment in depositional areas. Collection tools should include a toothbrush or test-tube brush, a small pocket knife, a turkey baster (to suck up fine sediments), a small stainless steel spoon, and a plastic tray to place the material in prior to transfer to the storage bottle. The standard storage container, a 50 cm^3 centrifuge tube, is fairly small and will fill quickly; do not over-add any particular batch of sampled material. To aide in this, material collected in the plastic tray can be sub-sampled and the subsample transferred to the centrifuge tube. Thoroughly mix the material in the tray prior to sub-sampling.

Macroalgae are picked by hand in proportion to their abundance at the site. In selecting macroalgae for sampling, the sampler should attempt to visually distinguish between the various growth forms that represent different algal taxa. Macroalgae are collected both for determining community composition and as substrates for microalgae, and are included with the microalgae composite. The goal is to collect a single composite sample that is a miniature replica of the stand of algae which are present at the study site.

PERI-1mod:
The PERI-1mod method (i.e., *modified* PERI-1) is used at a stream site that has a defined longitudinal length. This will usually be the 11-transect reach, established as 40X the wetted width at the reach midpoint, or 150 m long at a minimum (MT DEQ 2011a). Like PERI-1, it is a single composite sample that is a miniature replica of the stand of algae which are present at the study site. Both micro- and macroalgae are collected.

Starting from the most downstream transect, at each of the 11 transect sampling locales algal material should be collected from substrate representative of the right, left, or center locale. Collection tools should include a toothbrush or test-tube brush, a small pocket knife, a turkey baster (used to suck up fine sediments), a small stainless steel spoon, and a plastic tray to place the material in prior to transfer

to the storage container. The standard storage container, a 50 cm^3 centrifuge tube, is fairly small and will fill quickly; do not over-add any particular batch of sampled material. To aide in this, larger volumes of material collected in the plastic tray can be sub-sampled and the subsample transferred to the centrifuge tube. Be sure to thoroughly mix the material prior to sub-sampling.

As the collector works his/her way upstream, it should be noted whether or not any substrate type that is common along the site has been precluded from sampling due to the manner in which the 11 transects happen to have fallen along the longitudinal length. If an important substrate type has been precluded the sampler should, after completing the uppermost transect, return to the substrate in question and collect algae in an amount approximately proportional to the substrate's presence in the reach.

2.2 Sample Preservation and Management

All collections of microalgae and macroalgae are placed in a single sample container (50 cm^3 centrifuge tube). In the field, enough ambient water should be added to cover the collected material and achieve a volume of 45-48 ml. The sample is then preserved with formalin (i.e., 40% formaldehyde solution) to bring the final sample solution strength to about 2-4%. This equates to adding about 2-5 ml of formalin to the sample centrifuge tube. (The purpose of the formalin is to retard bacterial decay.) ParaFilm wax is stretched around the tightened lid of each centrifuge tube to minimize leakage. The centrifuge tube is gently inverted to distribute the preservative. An identifying label should be affixed to the outside of the container. The label should include stream name and location, the name of the collector, and the date, per instructions in the current Field Manual.

After preservation with formalin, samples can be transported without refrigeration, but they should be protected from light until the time that they are processed. Samples stored for a long time should be checked and may be replenished with formalin, if needed. Samples are returned to the Monitoring and Assessment Water Lab in the Last Chance Gulch building in Helena, MT, after which the collector's Site Visit Forms/Chain of Custody are submitted via normal channels.

Submittal of samples to approved laboratories (for algae ID and counting) is handled by DEQ's Monitoring and Assessment Section. MT DEQ will send the samples to the Biological Contractors at least once a month. A Sample Submittal Form for all samples in the batch will accompany the samples. This form contains metadata and an "Activity ID Number" for each unique sample. The Activity ID number will be used to track the sample through data storage and processing.

2.3 Quality Control – Field Sampling

Periphyton sampling methods are largely qualitative procedures used to determine taxon abundance. Therefore, typical field sampling controls such as replicate sampling are likely to be influenced by the judgment of the sampler, what the sampler considers to be a representative sample, and the variability of taxon within the communities. To measure this, replicate samples may be collected and then submitted to the same taxonomist (to minimize counting and identification differences) in order to determine Percent Taxonomic Difference. If PERI-1mod has been used, the sampler should collect the replicate PERI-1mod sample via one of the 2 remaining longitudinal sampling patterns (see MT DEQ 2011a). For example, if the sampler began on river Right at transect A, then the sampler should begin to collect the replicate sample at transect A on river Left or river Center, following the applicable collection

pattern in the upstream direction. As for the original sample, any important substrate types precluded during collection of the replicate PERI-1mod sample should be collected proportionally.

3.0 PERIPHYTON PREPARATION AND IDENTIFICATION

3.1 PREPARATION AND IDENTIFICATION

Diatom algae. Each periphyton sample must be processed in a manner that will yield a permanent strewn mount slide suitable for a diatom proportional count and containing a representative subsample of the diatoms present in the original sample (distinction between living and dead diatom cells will not be required from the prepared slide). Larger samples (greater than 25 ml) may be subsampled before cleaning by vigorously shaking the sample for several minutes and immediately pouring off a subsample. The "cold" sulfuric acid-potassium dichromate method is recommend for sample cleaning, but any method that adequately dislodges, separates and randomizes diatom frustules and clears them for identification, is acceptable. (Burnt mounts are not acceptable.) Each permanent strewn mount shall be prepared in Hyrax™. (Mounts in immersion oil or similar media are not acceptable.) Each mount shall be labeled with a sample identification name assigned by MT DEQ (activity ID number). Mounts shall be made in the center of a microscope slide. A diatom proportional count will be performed according to Standard Methods (Section 10300 C; APHA, 1998). The contractor will identify and enumerate 800 diatom valves (400 cells) on each diatom slide at a minimum of 900 X to the lowest practical taxonomic unit (minimum genus).

Non-diatom algae. Laboratory contractors shall use one of the two following approaches, as specified in each task order. 1. Quantitative. The contractor shall use methods described in Section 6.0 (page 6-7) of Barbour et al. (1999), and in Standard Methods (Sections 10200 F and 10300 C; APHA, 1998). The contractor will homogenize each algal sample with a tissue homogenizer or blender. For identification and enumeration of soft algae, the sub-sample will be shaken vigorously and an aliquot will be removed to a Palmer-Maloney Counting Cell. A minimum of 300 cell units will be identified to the lowest practical taxonomic unit (minimum genus) and counted at 400X. If algae are too sparse or too small to allow for counting 300 cells using the Palmer counting chamber, a Sedgwick-Rafter cell will be used instead. A "cell unit" is either a single algal cell or a 10 micrometer section of thallus or filament from algae that grow in such forms (e.g., *Oscillatoria*). 2. Qualitative. (**Note: Unless directed otherwise, the following method is MT DEQ's default non-diatom algae counting method.)** The sample is poured into a shallow pan and small portions of different macroalgae are removed to a microscope slide. The remainder of the sample is returned to the sample jar and agitated to dislodge epiphytic algae and randomize algal cells and colonies. Then, using a soda straw or large-bore pipette, a several-drop subsample of microalgae is added to the fragments of macroalgae on the glass slide. A coverslip is placed over the algae subsample, completing a composite wet mount. The wet mount is scanned under a compound microscope at 200X.

Soft-bodied algae are identified to genus, stepping up the magnification to 400X if necessary. After all of the common soft-bodied algae are identified, each genus is ranked according to its estimated contribution to the total algal biomass at the site, taking into account the remaining macroalgae and microalgae in the original sample. The genus with the most biomass is ranked number 1; the genus with the next most biomass is ranked number 2, and so on. Diatoms are included, but they are ranked as a group (Class Bacillariophyceae) and not as individual genera. Genera that are rated as rare are not ranked.

Genera of soft-bodied algae and diatoms as a group are also rated as to the relative abundance of their cells:

R (rare)	Fewer than 1 cell per field of view at 200X, on the average;
C (common)	At least one, but fewer than five cells per field of view;
VC (very common)	Between 5 and 25 cells per field of view;
A (abundant)	More than 25 cells per field of view, but countable;
VA (very abundant)	Number of cells per field too numerous to count.

These designations have no counterpart in terms of cells per unit area of stream bottom. Although the density of algae material in each wet mount shall vary, a certain degree of standardization is achieved by the need to provide sufficient separation of cells and passage of light through the mount to allow for the identification of genera and estimation of cell numbers.

3.2 QUALITY CONTROL-LABORATORIES

To assure quality control, five percent (5%) of samples will be randomly selected and will be split, so that two different contracting laboratories may analyze them. The split will be undertaken by laboratory A and sent to laboratory B. The independent laboratory (laboratory B) shall report directly back to MT DEQ on their results rather than back through the original laboratory.

4.0 DATA INTERPRETATION

As mentioned in **Section 1.1**, over the years MT DEQ has used a variety of periphyton-based biometrics to help interpret stream water quality[2]. MT DEQ's current approach uses pollutant-diagnosing biometrics based on stressor-specific increaser diatom taxa, as described in Teply (2010a; 2010b) and earlier documents (Teply and Bahls, 2005; Teply and Bahls, 2006; Bahls et al., 2008). Currently there are increaser-taxa biometrics available for nutrients and sediment in both the mountainous and plains regions of the state. A basic overview of how to use the metrics is provided here, drawn primarily from Teply (2010b).

4.1 USE OF THE INCREASER TAXA METRICS

Step 1: <u>Classify a sample</u>

Classify a sample according to its stream group, using rules derived from the predominant fishery type (cold or warm) and Level III ecoregions, as presented in **Figure 4-1**. Use tables in **Appendix A (part A.1)** to determine which level III and level IV ecoregions comprise either the warm or cold fishery class. <u>Also, please carefully read the Note that follows **Figure 4-1**</u>.

[2] If interested in these earlier diatom-based metrics, see Bahls (1993).

Figure 4-1. Dichotomous key to stream groupings using MDEQ Fisheries Classification and Predominant Level III Ecoregion.

1. Site in a warm water fishery?
   a. Yes.....Warm-Water Stream Group (I.1a)
   b. No .....Go To 2
2. Site within the Middle Rockies Ecoregion?
   a. Yes.....Middle Rockies Stream Group (I.2a)
   b. No ..... Northern/Canadian Rockies Stream Group (I.2b)

Note: Samples collected in the Idaho Batholith (a level III ecoregion) are considered part of the Northern/Canadian Rockies Stream Group (I.2b)[3]. The following level IV ecoregions are also part of the Northern/Canadian Rockies Stream Group (I.2b): 42n, 42q. Samples collected in the following level IV ecoregions are part of the Middle Rockies Stream Group: 42l, 43s, 43t, 43u, 43v, and 43o (only the 43o polygon just south of Great Falls, MT). The level IV ecoregion "Foothill Grassland" (42r) has polygons associated with both the Middle Rockies and Northern/Canadian Rockies stream groups. 42r polygons are associated with the level III ecoregion (either Middle Rockies or Canadian Rockies) against which they abut and, in turn, the corresponding Cold Water Fishery Stream Group (**Figure 4-1**). Consult a current ecoregion map to determine where your diatom sample in question should be assigned.

Step 2: Calculate Assessment Metrics

Two metrics are required for interpretation of sample results. The number of taxa on the Increaser Taxa list is self-explanatory. Refer to Increaser Taxa lists in **Appendix A (part A.2)** for each stream group and impairment cause. Percent relative abundance (PRA) of taxa on the Increaser Taxa list is calculated as the sum of PRAs for each taxon on the Increaser Taxa list that is counted in the sample. PRAs are calculated by dividing the number of valves counted for each taxon by the total number of valves counted in the sample. These values must be calculated independently for each impairment cause with an Increaser Taxa list reported for the associated stream group. The Warm Water Fisheries and Northern/Canadian Rockies have two sets of Increaser Taxa – for sediment and nutrients – the Middle Rockies only has one – that is for sediment.

NOTE: When extracting data from EQuIS, two diatom species will download using nomenclature slightly different from that shown in the tables in **Appendix A (part A.2)**. *Surirella brebissonii kuetzingii* will be downloaded as *Surirella brebissonii* var. *kuetzingii*, and *Cocconeis pseudolineata* will be downloaded as *Cocconeis placentula* var. *pseudolineata*.

Step 3: Determine Probability of Impairment

The probability that the sample represents a stream impaired due to either sediment or nutrients can be determined via tables in **Appendix A (part A.3)**. These tables translate PRA values into an associated probability of impairment. To determine the probability of impairment, simply find the tabled PRA greater than and less than the PRA of Increaser Taxa (determined above) and interpret the associated probability as a range. For very low or very high PRA values, the probability would be interpreted to be less than 5% or greater than 95%, respectively. **As of this SOP, a diatom sample is indicating a nutrient or sediment problem when the probability of impairment, based on the tables in Appendix A, is > 51%.**

[3] The region of the Idaho Batholith ecoregion located in Montana is mainly the steep, high-elevation areas along the continental divide. Floristically and geographically, this region has more in common with the Canadian Rockies ecoregion and is therefore grouped with it.

An example of using the tables is as follows. If the percent relative abundance of taxa from a sample on the Warm Water Fisheries, Sediment Increaser Taxa list is 23%, the probability that the sample represents a stream impaired by sediment can be interpreted to be about 60%. If a finer interpretation is desired, the probability of impairment can be interpolated accordingly; a straight-line interpolation is adequate for water quality assessments. If the PRA of the sample was 13%, it would correspond to a probability of impairment of 40%, while a sample PRA of 33% corresponds to a probability of impairment of nearly 80%. The 50% probability occurs at about 17.92 PRA; this is the threshold for sediment impairment reported by Teply (2010b).

The Department has developed an electronic spreadsheet tool (IPUPAS_v22.xlsm) that can, after you have uploaded a dataset of diatom data, be used to quickly calculate the metrics. Please contact staff of the Water Quality Standards Section to request a copy of the tool and its instructions for use.

Step 4: <u>Interpret the Sample Result</u>
Note: Diatom increaser metrics are one piece of data to be used in conjunction with other data when determining a stream's final assessment condition for a pollutant. Please consult the Sediment and Nutrient Assessment methodologies (MT DEQ, 2011b; MT DEQ, 2011c) to determine how diatom increaser metric results are incorporated into the particular pollutant assessment process.

The following is intended to help users of the diatom-increaser metrics with interpretation of results, within the context of empirical evidence presented by Teply (2010a). The following suggested language seeks to minimize inadvertent mis-interpretation or mis-representation of results using Increaser Taxa lists. First, all written interpretations using Increaser Taxa should begin with the following statement, clarifying the basis for the interpretation to follow:

> *"Sample diatom taxa counts were evaluated to determine the probability of [Impairment Cause] impairment using the [Impairment Cause] Increaser Taxa List for the [Stream Group]."*

The investigator would then describe the Increaser Taxa appearing in the sample and their autecological importance as indicators of stress due to the impairment cause:

> *"[Number of Increaser Taxa] of [Total Increaser Taxa on the List] diatom taxa on the [Impairment Cause] Increaser Taxa list were counted, representing a total percent relative abundance of [PRA of Increaser Taxa]. These taxa have autecological affinities that make them suitable indicators of [Impairment Cause]."*

Finally, statements regarding the probability of impairment, as determined above, are made as follows:

> *"This indicates that the sample represents a stream that has about a [Probability of Impairment] percent probability of being impaired due to [Impairment Cause] under 303(d) guidelines. This probability is based on past evidence of taxa associated with [Impairment Cause]-impaired streams in [Stream Group] streams. [Impairment Cause] Increaser Taxa do not discriminate other causes of impairment and this result does not indicate whether the stream may or may not be impaired due to other causes."*

The reader is urged to review the discussion of uncertainty associated with Increaser Taxa on Page 4 of Teply (2010b), and also the Results and Discussion sections of Teply (2010a). It is important to understand the probabilistic nature of these impairment determinations, and the associated error rates.

4.2 Other Periphyton Interpretation

Contractors must be able to calculate metrics other than those listed in **Section 4.1**, when requested in specific task orders. Examples might include earlier metrics provided in earlier SOPs. Contractors should also have the ability to work with MT DEQ in the development of new metrics as more ecological information is collected on Montana waterbodies.

4.3 Reports and Reporting

Contractors shall first provide a draft report (electronic submission is acceptable) for MT DEQ review prior to the submission of a final report. Contractors will provide a list of fully cited references used for species identification, ecological evaluation, and all other data interpretation. Citations should follow accepted scientific conventions. Electronic data for results data must conform to the specifications shown in **Section 4.4** below, or in a mutually agreed upon format. Final written reports must be delivered electronically on a CD rom using Office 2007.

Contractors must provide electronic copies of the bench sheets used for counting/identifying all submitted algae samples. Contractors should have the ability to travel to Montana to make presentations and attend meetings related to work completed under contract.

4.4 Database Compatibility: MT eWQX Reporting Requirements

All contractors must submit all taxonomic abundance results to MT DEQ in a Montana EQuIS Water Quality Exchange (MT-eWQX) electronic data deliverable (EDD) format. The MT-eWQX EDD and associated guidance is available on the Water Quality Planning Bureau's (WQPB) MT-eWQX Support web page: http://deq.mt.gov/wqinfo/datamgmt/MTEWQX.mcpx. Contractors will load the result data into the EQuIS Data Processor (EDP) for review prior to submission to MT DEQ. The EDP application is made available for download at the MT-eWQX Support web page noted above and assists in identifying errors in the EDD and correcting them.

Part of the MT-eWQX EDD requirements is that all taxonomic names reported correspond exactly to a MT-eWQX reference value. The current Biological Taxonomic Name reference value list is available for download at the MT-eWQX Support web page noted above. If a taxonomic name does not exist in the Biological Taxonomic Name reference value list, the contractor shall notify the MT-eWQX Manager by submitting a new taxonomic name request that includes the taxonomic name and rank. The Biological Taxonomic Name reference value list is constrained to taxonomic names entered in the Integrated Taxonomic Information System (ITIS) or the Catalogue of Life (http://www.catalogueoflife.org/search/all). If the MT-eWQX Manager finds the requested name in ITIS or the Catalogue of Life, it will be added to the reference value list. If the requested name is not found, either a more course taxonomic ID will have to be used (e.g., report to genus instead of species) or, if that is unavailable, that particular taxonomic name will need to be removed from the MT-eWQX EDD.

4.5 VOUCHERING OF SPECIMENS

The Contractor shall provide to MT DEQ a voucher slide of each permanent strewn-mount sample examined. Vouchers must be delivered within 90 days of the completion of the analysis. Ultimately, voucher slides will be housed at the University of Montana Herbarium (UM) in Missoula, MT. UM maintains a permanent research collection for use by researchers within the University and the scientific community at large. Each slide will be labeled a unique identification number provided by MT DEQ or generated by the Contractor, which will be either scribed directly on the slide with a diamond stylus or written in ink on an adhesive paper label. Contractors working under this SOP will be instructed as to the protocol for properly labeling voucher slides. A general description of the UM, the collection, its labeling protocol and other relevant information can be found in **Appendix B**.

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APPENDIX A. STREAM CLASSIFICATIONS FOR APPLICATION OF THE DIATOM INCREASER TAXA METRICS, TAXA LISTS, AND IMPAIRMENT PROBABILITIES

A.1. CLASSIFICATION FOR APPLICATION OF DIATOM-INCREASER TAXA METRICS

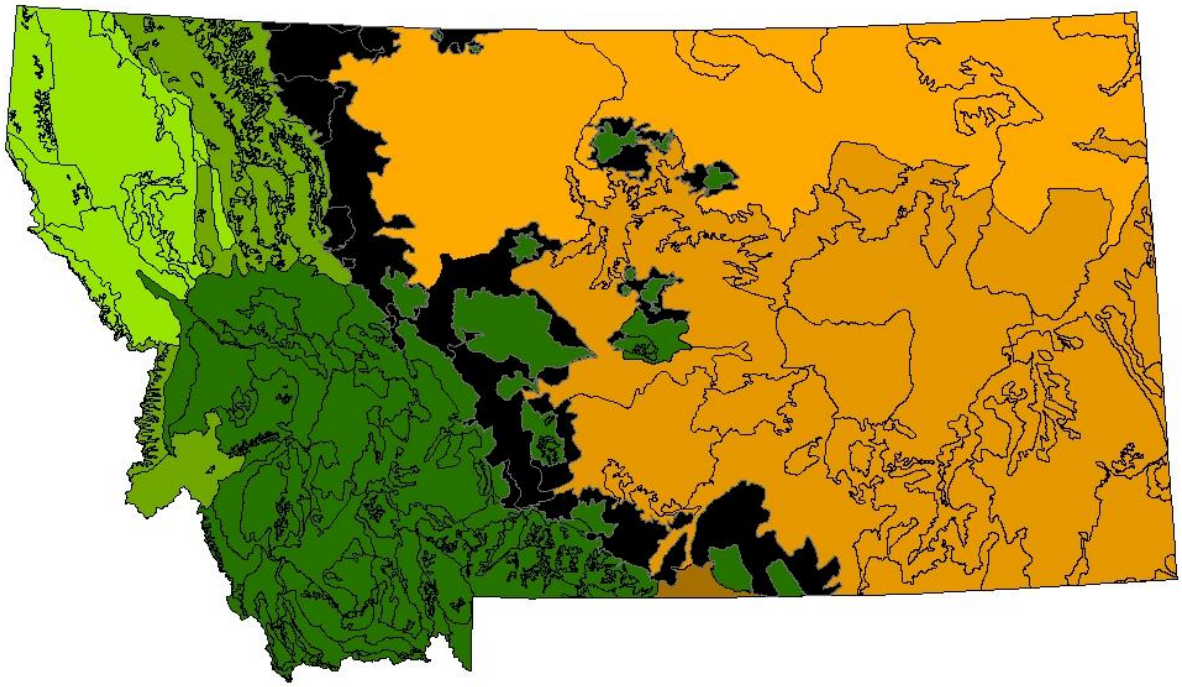

Figure A.1. Montana map showing location of the different classes.
The Cold Water Fishery Class includes the areas shown in shades of green, and black. Black area comprises level IV ecoregions; consult the Tables and Notes below to determine which level IVs are associated with which Cold Water Fishery class subgroup. Warm Water Fishery Class is represented by the areas in shades of brown.

Cold Water Fishery Class

Ecoregions (levels III and IV) Corresponding to the Cold Water Fishery Class.

Ecoregion Scale	Ecoregion Name	Ecoregion Number
Level III	Northern Rockies	15
Level III	Idaho Batholith	16
Level III	Middle Rockies	17
Level III	Canadian Rockies	41
Level IV	Sweetgrass Uplands	42l
Level IV	Milk River Pothole Upland	42n
Level IV	Rocky Mountain Front Foothill Potholes	42q
Level IV	Foothill Grassland	42r
Level IV	Unglaciated Montana High Plains	43o
Level IV	Non-calcareous Foothill Grassland	43s
Level IV	Shields-Smith Valleys	43t
Level IV	Limy Foothill Grassland	43u
Level IV	Pryor-Bighorn Foothills	43v

Note: For purposes of using the diatom metrics in this SOP, the Idaho Batholith (a level III ecoregion) is considered part of the Northern/Canadian Rockies Stream Group (I.2b), and is therefore included with the Northern and Canadian Rockies ecoregions.

Also, the level IV ecoregion "Unglaciated Montana High Plains" (43o) has more than one polygon in Montana. Only the polygon located just south of Great Falls, MT in associated with the Cold Water Fishery Class. Also, the level IV ecoregion "Foothill Grassland" (42r) has polygons associated with both the Middle Rockies *and* Canadian Rockies level III ecoregions. 42r polygons are associated with the level III ecoregion (either Middle Rockies or Canadian Rockies) against which they abut and, in turn, the corresponding Cold Water Fishery Stream Group.

Warm Water Fishery Class

Ecoregions (level III) Corresponding to the Warm Water Fishery Class. Note the level IV ecoregions that are *excluded from* the Warm Water Fishery Class.

Ecoregion Scale	Ecoregion Name	Ecoregion Number
Level III	Northwestern Glaciated Plains	42
Level IV ecoregions of the Northwestern Glaciated Plains not in the Warm Water Fishery Class		
Level IV	Sweetgrass Uplands	42l
Level IV	Milk River Pothole Upland	42n
Level IV	Rocky Mountain Front Foothill Potholes	42q
Level IV	Foothill Grassland	42r
Level III	Northwestern Great Plains	43
Level IV ecoregions of the Northwestern Great Plains not in the Warm Water Fishery Class		
Level IV	Unglaciated Montana High Plains	43o
Level IV	Non-calcareous Foothill Grassland	43s
Level IV	Shields-Smith Valleys	43t
Level IV	Limy Foothill Grassland	43u
Level IV	Pryor-Bighorn Foothills	43v

Note: The level IV ecoregion "Unglaciated Montana High Plains" (43o) has more than one polygon in Montana. Only the polygon located just south of Great Falls, MT is excluded from the Warm Water Fishery Class.

A.2. FINAL INCREASER TAXA LISTS (AFTER TEPLY 2010)

Sediment Increaser Taxa

Warm Water Fisheries - Sediment
Amphora pediculus
Caloneis bacillum
Cocconeis placentula
Diatoma moniliformis
Epithemia sorex
Gomphonema minutum
Navicula capitatoradiata
Navicula cryptotenella
Navicula gregaria
Navicula reichardtiana
Nitzschia inconspicua
Nitzschia liebethruthii
Nitzschia linearis
Reimeria sinuata
Surirella brebissonii kuetzingii

Middle Rockies - Sediment
Amphora inariensis
Cocconeis pediculus
Cocconeis pseudolineata
Eolimna minima
Geissleria acceptata
Gomphonema drutelingense
Meridion circulare
Navicula gregaria
Navicula lanceolata
Navicula tripunctata
Nitzschia recta
Planothidium frequentissimum
Planothidium lanceolatum
Reimeria sinuata
Sellaphora pupula
Staurosirella leptostauron

Northern Rockies - Sediment
Achnanthidium deflexum
Aulacoseira italica
Eolimna minima
Gomphonema minutum
Gomphonema pumilum
Gomphonema rhombicum
Gomphosphenia sp.
Melosira varians
Meridion circulare
Navicula cryptocephala
Navicula radiosa
Nitzschia archibaldii
Nitzschia palea
Nitzschia perminuta
Planothidium frequentissimum
Pseudostaurosira brevistriata
Reimeria sinuata
Rhopalodia gibba
Staurosira construens

Nutrient Increaser Taxa

Warm Water Fisheries - Nutrients
Amphora pediculus
Gomphonema parvulum
Navicula cryptotenella
Navicula libonensis
Navicula tripunctata
Nitzschia acicularis
Nitzschia amphibia
Nitzschia archibaldii
Nitzschia fonticola
Nitzschia gracilis
Nitzschia inconspicua
Nitzschia linearis

Northern Rockies - Nutrients
Achnanthes nodosa
Achnanthidium deflexum
Adlafia minuscula
Eolimna minima
Geissleria acceptata
Gomphonema minutum
Gomphonema pumilum
Gomphosphenia sp.
Meridion circulare
Navicula cryptocephala
Nitzschia fonticola
Nitzschia inconspicua
Nitzschia perminuta
Planothidium frequentissimum
Synedra rumpens

NOTE: When extracting data from EQuIS, two diatom species will download using nomenclature slightly different from that shown in the tables above. *Surirella brebissonii kuetzingii* will be downloaded as *Surirella brebissonii* var. *kuetzingii*, and *Cocconeis pseudolineata* will be downloaded as *Cocconeis placentula* var. *pseudolineata*.

A.3. IMPAIRMENT PROBABILITIES (AFTER TEPLY 2010)

Sediment Increaser Taxa

Warm Water Fisheries - Sediment	
Percent Relative Abundance	Probability of Impairment
0.00	5%
0.00	10%
0.00	15%
2.14	20%
5.28	25%
8.09	30%
10.70	35%
13.17	40%
15.56	45%
17.92	50%
20.28	55%
22.67	60%
25.14	65%
27.75	70%
30.56	75%
33.70	80%
37.35	85%
41.94	90%
48.75	95%

Middle Rockies - Sediment	
Percent Relative Abundance	Probability of Impairment
0.00	5%
0.00	10%
2.21	15%
4.67	20%
6.79	25%
8.69	30%
10.46	35%
12.13	40%
13.75	45%
15.34	50%
16.93	55%
18.55	60%
20.22	65%
21.99	70%
23.89	75%
26.01	80%
28.47	85%
31.58	90%
36.18	95%

Northern/Canadian Rockies - Sediment	
Percent Relative Abundance	Probability of Impairment
0.00	5%
0.00	10%
0.73	15%
4.07	20%
6.93	25%
9.50	30%
11.88	35%
14.14	40%
16.33	45%
18.48	50%
20.63	55%
22.82	60%
25.08	65%
27.46	70%
30.03	75%
32.89	80%
36.23	85%
40.43	90%
46.65	95%

Nutrient Increaser Taxa

Warm Water Fisheries - Nutrients	
Percent Relative Abundance	Probability of Impairment
0.00	5%
0.00	10%
0.00	15%
0.44	20%
2.58	25%
4.50	30%
6.28	35%
7.97	40%
9.60	45%
11.21	50%
12.82	55%
14.45	60%
16.14	65%
17.92	70%
19.84	75%
21.98	80%
24.48	85%
27.61	90%
32.26	95%

Northern/Canadian Rockies - Nutrients	
Percent Relative Abundance	Probability of Impairment
0.00	5%
0.00	10%
0.00	15%
2.01	20%
4.63	25%
6.98	30%
9.15	35%
11.22	40%
13.21	45%
15.18	50%
17.15	55%
19.14	60%
21.21	65%
23.38	70%
25.73	75%
28.35	80%
31.39	85%
35.23	90%
40.91	95%

APPENDIX B. THE MONTANA DIATOM COLLECTION (MDC)

The Montana Diatom Collection (MDC) is a collection of permanent strewn mounts of acid-cleaned diatoms on glass microscope slides representing a variety of habitats in the northern Great Plains, the northern Rocky Mountains, and the Pacific Northwest. The MDC functions as a repository of diatom biodiversity for the region. It also provides a historical record of diatom assemblages and ecological conditions in water bodies of the region. Although the MDC contains few type specimens at this time, it does contain voucher slides for many water quality assessments, biological criteria development projects, and long-term water quality monitoring networks. Presently the MDC is located in Helena, MT at the residence of Dr. Loren Bahls, but will eventually be housed at the University of Montana Herbarium in Missoula, MT.

Accompanying the MDC is an electronic database in Microsoft Access 2000. The complete database consists of eight tables: Diatoms (a working list of species and varieties), Algae (a working list of non-diatom genera), Stations (site attribute data), Samples (sample attribute data), Diatom Counts (species level), Algae Counts (genus level), Chemistry (values for selected water quality variables), and Diatom Metrics (species richness, diversity, etc.). The Samples table contains two fields for slide numbers, one for the number of the primary slide and one for the number of a duplicate slide if one was made. Slide identification numbers are assigned as explained below.

B.1 ORGANIZATION OF THE MDC

Microscope slides in the MDC are housed in three Eberbach oak storage cabinets and a series of wood frame, 100-slide capacity slide boxes. Each of the oak cabinets is divided into four sections, and each section contains 25 numbered and stamped aluminum slide trays. Each tray has compartments for 20 slides lying flat, thus each cabinet has a nominal capacity of 2,000 slides. In addition, each cabinet has two drawers at the bottom and each drawer (sections 5 and 6) will hold three small (25-slide) plastic slide boxes. This brings the nominal capacity of each cabinet to 2,150 slides. Duplicate slides are sometimes stacked in the aluminum trays, thus increasing the total capacity.

Slides made from samples collected through 2002 are housed in one of the three oak storage cabinets. The cabinets are named Basin File, Project File, and Taxon File. Each tray in the Basin File represents one of the 100 hydrologic cataloging units in Montana and contains miscellaneous collections from that cataloging unit. In some trays, compartments that would otherwise be empty contain overflow slides from nearby cataloging units in which more than 20 samples have been collected. Each slide is identified according to the file name (Basin), the section number (1-6), the tray or box number, and the compartment or slot number (1-20 for the trays in sections 1-4 and 1-25 for the boxes in sections 5 and 6). Hence a slide in compartment 17 of tray 23 of section 3 of the Basin File would be numbered B3-23-17 and a slide in slot 24 of box 3 in section 5 would be numbered B5-3-24.

Slides representing samples collected between 1968 and 2002 as part of 20 major monitoring and assessment projects are housed in the Project File. These projects include the ongoing Clark Fork River Biological Monitoring Project. Project slides are organized and numbered in the same manner as they are in the Basin File, except that the letter P (for Project File) is used instead of the letter B. Hence a slide in compartment 9 of tray 6 in section 1 of the Project File is labeled P1-6-9.

The Taxon File was originally intended as the repository for voucher specimens of Montana diatom taxa, but has been used primarily to store duplicates of slides made from 1999 through 2002. (Slides containing voucher specimens for this catalogue of Northwest diatoms may be found in any of the cabinets or slide boxes that compose the MDC.) Slides in the Taxon File are designated by the letter T, but are otherwise numbered in the same manner as those in the Basin and Project Files.

Beginning in 1999, duplicate slides have been deposited in the University of Montana Herbarium (UM) in Missoula. These slides are stored in wooden slide boxes covered with heavy black embossed paper. These boxes have plastic inserts with numbered slots that hold up to 100 slides. The boxes at UM are numbered 1, 2, 3, etc., hence slide 33 in box 16 is designated as slide 16-33. The other set of duplicate slides is stored in Helena. To date, these slides have been stored in the Taxon File, which is nearly full, so beginning with samples collected in 2003, duplicates of new slides will be stored wooden boxes similar to those at UM. These boxes will be numbered 101, 102, 103, etc. Hence, slide 16 in box 103 will be numbered 103-16.

B.2 THE SLIDES

Slides in the MDC are all randomly strewn mounts of natural diatom assemblages. About three-quarters of the sites represented in the MDC are flowing waters, that is, dashed or solid blue lines labeled “river” or “creek” on United States Geological Survey topographic maps. Other habitats represented in the MDC include lakes and reservoirs, springs, freshwater seeps, saline seeps, mine seeps and pits, wetlands of all sorts, stock ponds, industrial ponds, sewage lagoons, fossil deposits, and soils. About 95 percent of the samples in the MDC were collected from benthic habitats, almost all of them from natural substrates. Most represent composite samples collected from multiple substrates, but a few are substrate specific. The remaining five percent of the samples are plankton samples, either grab samples or net tows.

Each slide in the MDC is labeled with its locator number (e.g. P2-3-19), which is either scribed directly on the slide with a diamond stylus or written in ink on an adhesive paper label. Slides made before 1980 were made with Carmount-165 Medium™; slides made from 1980 through 1998 were made with Hyrax™; slides made since 1998 have been prepared with Naphrax™. Up until 2003, diatom mounts in the MDC were prepared using #1 cover slips and standard microscope slides that are 1 mm thick. Beginning in 2003, some mounts are being prepared with #1.5 cover slips and slides that are 1.2 mm thick in order to optimize performance of my 100X lens and oil condenser.

B.3 UNIVERSITY OF MONTANA HERBARIUM (UM)

Duplicate slides currently on deposit at the University of Montana Herbarium (UM) in Missoula are available for examination by researchers. These slides may be identified by their box numbers and they currently occupy boxes 1 through 20 of the MDC. Inquiries regarding the loan or on-site examination of slides at UM should be addressed to:

David L. Dyer
Collections Manager
University of Montana Herbarium
Division of Biological Sciences
The University of Montana
Missoula, MT 59812-4824

E-mail: dave.dyer@mso.umt.edu
Phone: (406) 243-4743

The University of Montana Herbarium (UM), a unit of the Division of Biological Sciences of the University of Montana, is committed to the collection and preservation of botanical specimens for the purposes of research, teaching, and community outreach.

UM maintains a permanent research collection for use by researchers within the University and the scientific community at large. Through the loan and exchange programs, specimens are acquired from and loaned for research purposes to herbaria throughout North America and other parts of the world. Also, a teaching collection is maintained for use in courses at the University. Community outreach is conducted in part through cooperation with the Montana Native Plant Society.

UM specializes in the flora of Montana and the Northern Rocky Mountains, particularly the montane and alpine regions of Western Montana. Other areas emphasized are the states and provinces adjacent to Montana, the rest of the western cordillera, and the Great Plains. UM is the primary herbarium in North America for the Northern Rocky Mountain region and an important herbarium of the Pacific Northwest. As such, it is the major repository of botanical specimens for researchers at the University of Montana, independent researchers, The Nature Conservancy, Montana Natural Heritage Program, and the United States Forest Service. In addition, UM acts as the primary reference and data source in the region for these agencies.