Biological Integrity of Streams in the Ruby River TMDL Planning Area Based on the Structure and Composition of the Benthic Algae Community

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BIOLOGICAL INTEGRITY OF STREAMS IN THE RUBY RIVER TMDL PLANNING AREA BASED ON THE STRUCTURE AND COMPOSITION OF THE BENTHIC ALGAE COMMUNITY

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Summary

In July and September 2002, periphyton samples were collected from 20 sites on 14 streams in the Ruby River TMDL planning area in southwestern Montana for the purpose of assessing whether these streams are water-quality limited and in need of TMDLs. The samples were collected following MDEQ standard operating procedures, processed and analyzed using standard methods for periphyton, and evaluated following modified USEPA rapid bioassessment protocols for wadeable streams.

At the lower site on **Coal Creek**, a depressed pollution index and an elevated siltation index indicated minor impairment from organic loading and sedimentation. Other than slight organic loading at the upper site on **Indian Creek**, diatom metrics indicated excellent biological integrity, no impairment, and full support of aquatic life uses at both sites on this stream. An elevated siltation index and a depressed pollution index indicate *moderate impairment from sedimentation* and minor impairment from organic loading in lower **Ramshorn Creek**.

In addition to the natural stress evident in **Currant Creek**, this site exhibited minor impairment from sedimentation. Other metrics indicated no impairment and excellent biological integrity. The lower site on **California Creek** supported an exceptionally large number of motile diatoms for a mountain stream, which indicated *severe impairment from sedimentation*. A slightly depressed pollution index suggests minor impairment from organic loading at this site. Borderline values for the pollution index and siltation index suggest minor impairment from organic loading and sedimentation in **Harris Creek**.

Aside from a large number of *Achnanthidium minutissimum* and natural stress due to steep gradients, coldwater temperatures and low nutrients, diatom metrics indicate good biological integrity in **Shovel Creek**. **Hawkeye Creek** supported an exceptionally large number of motile diatoms for a mountain stream, suggesting *severe impairment from sedimentation*. This site also had a slightly depressed pollution index, indicating minor impairment from organic loading. An elevated number of motile diatoms indicate minor impairment from sedimentation in **Warm Springs Creek**. One teratological diatom valve was counted at this site. Otherwise, diatom metrics indicated excellent biological integrity.

Mill Gulch had excellent biological integrity for a mountain stream and diatom species richness was exceptional (70 species counted). The lower site on **Garden Creek** supported an elevated number of motile diatoms, indicating minor impairment from sedimentation. An elevated number of motile diatoms in **Mormon Creek** indicate *moderate impairment from sedimentation*. A depressed pollution index also indicates minor impairment from organic loading. A relatively large number of teratological diatoms suggest that this site may have elevated concentrations of heavy metals.

More than 13 percent of the diatoms in the **North Fork of Greenhorn Creek** were abnormal, which suggests *severe impairment from heavy metals*. The number of motile diatoms here was also elevated, indicating minor impairment from sedimentation. A large number of motile diatoms indicate *moderate impairment from sedimentation* in the **West Fork of the Ruby River**. The pollution index was also depressed here, indicating minor impairment from organic loading.

Introduction

This report evaluates the biological integrity[1], support of aquatic life uses, and probable causes of stress or impairment to aquatic communities in selected streams of the Ruby River TMDL planning area in southwestern Montana. The purpose of this report is to provide information that will help the State of Montana determine whether these streams are water-quality limited and in need of TMDLs.

The federal Clean Water Act directs states to develop water pollution control plans (Total Maximum Daily Loads or TMDLs) that set limits on pollution loading to water-quality limited waters. Water-quality limited waters are lakes and stream segments that do not meet water-quality standards, that is, that do not fully support their beneficial uses. The Clean Water Act and USEPA regulations require each state to (1) identify waters that are water-quality limited, (2) prioritize and target waters for TMDLs, and (3) develop TMDL plans to attain and maintain water-quality standards for all water-quality limited waters.

Evaluation of aquatic life use support in this report is based on the species composition and structure of periphyton (benthic algae, phytobenthos) communities at 20 sites on 14 streams that were sampled in July and September of 2002. Periphyton is a diverse assortment of simple photosynthetic organisms called algae that live attached to or in close proximity of the stream bottom. Some algae form long filaments or large colonies and are conspicuous to the unaided eye. But most algae, including the ubiquitous diatoms, can be seen and identified only with the aid of a microscope. The periphyton community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity in Montana streams (Bahls et al. 1992). Plafkin et al. (1989) and Barbour et al. (1999) list several advantages of using periphyton in biological assessments.

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[1] *Biological integrity* is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Project Area and Sampling Sites

The project area is located within the Middle Rockies Ecoregion (USEPA 2000) in Madison County, Montana. At higher elevations, vegetation is mainly mixed conifer forest, with alpine tundra on the highest peaks. At lower elevations, vegetation is mainly mixed grassland and sagebrush steppe (USDA 1976, Woods et al. 1999). The main land uses are livestock grazing and irrigated agriculture.

Periphyton samples were collected at 20 sites on 14 streams (Table 1). The study streams are in the Ruby River hydrologic unit (USGS HUC 10020003). The Ruby River, along with the Big Hole and the Beaverhead Rivers, are the three main tributaries of the Jefferson River. Streams in the project area are classified B-1 in the Montana Surface Water Quality Standards.

Methods

Periphyton samples were collected following standard operating procedures of the MDEQ Planning, Prevention, and Assistance Division. Using appropriate tools, microalgae were scraped, brushed, or sucked from natural substrates in proportion to the importance of those substrates at each study site. Macroalgae were picked by hand in proportion to their abundance at the site. All collections of microalgae and macroalgae were pooled into a common container and preserved with Lugol's (IKI) solution.

The samples were examined to estimate the relative abundance and rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described in Bahls (1993). Soft algae were identified using Smith (1950), Prescott (1962, 1978), John et al. (2002), and Wehr and Sheath (2003). These books also served as references on the ecology of the soft algae, along with Palmer (1969, 1977).

After the identification of soft algae, the raw periphyton samples were cleaned of organic matter using sulfuric acid, potassium dichromate, and hydrogen peroxide. Then permanent diatom slides were prepared using Naphrax, a high refractive index mounting medium, following

Standard Methods for the Examination of Water and Wastewater (APHA 1998). At least 400 diatom cells (800 valves) were counted at random and identified to species. The following were the main taxonomic references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Lange-Bertalot 1993, 2001; Krammer 1997a, 1997b, 2002; Reichardt 1997, 1999. Diatom naming conventions followed those adopted by the Academy of Natural Sciences for USGS NAWQA samples (Morales and Potapova 2000) as updated in 2003 (Dr. Eduardo Morales, Academy of Natural Sciences, digital communication). Van Dam et al. (1994) was the main ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics. A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999). Diatoms are particularly useful in generating metrics because there is a wealth of information available in the literature regarding the pollution tolerances and water quality preferences of common diatom species (e.g., Lowe 1974, Beaver 1981, Lange-Bertalot 1996, Van Dam et al. 1994).

Values for selected metrics were compared to biocriteria (numeric thresholds) developed for streams in the Rocky Mountain ecoregions of Montana (Table 2). These criteria are based on metric values measured in least-impaired reference streams (Bahls et al. 1992) and metric values measured in streams that are known to be impaired by various sources and causes of pollution (Bahls 1993). The biocriteria in Table 2 are valid only for samples collected during the summer field season (June 21-September 21).

The criteria in Table 2 distinguish among four levels of stress or impairment and three levels of aquatic life use support: (1) no impairment or only minor impairment (full support); (2) moderate impairment (partial support); and (3) severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor biological integrity, respectively. In cold, high-gradient mountain streams, natural stressors will often mimic the effects of man-caused impairment on some metric values.

Quality Assurance

Several steps were taken to assure that the study results are accurate and reproducible.

Upon receipt of the samples, station and sample attribute data were recorded in the Montana Diatom Database and the samples were assigned a unique number, e.g., 2626-01. The first part of this number (2626) designates the sampling site (Currant Creek) and the second part (01) designates the number of periphyton samples that that have been collected at this site for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with information on the sample label. A portion of the raw sample was used to make duplicate diatom slides. The slide used for the diatom proportional count will be deposited in the Montana Diatom Collection at the University of Montana Herbarium in Missoula. The duplicate slide will be retained by *Hannaea* in Helena. Diatom proportional counts have been entered into the Montana Diatom Database.

Results and Discussion

Results are presented in Tables 3, 4 and 5, which are located near the end of this report following the references section. Copies of aquatic plant field sheets are included as Appendix A. Appendix B contains a series of diatom reports, one for each sample. Each diatom report contains an alphabetical list of diatom species in that sample and their percent abundances, and values for 65 different diatom metrics and ecological attributes.

Sample Notes

Coal Creek. The coarse filamentous green alga found at the upper site was sparsely branched and therefore placed into *Rhizoclonium* rather than *Cladophora*. The *Cladophora, Oedogonium,* and *Tolypothrix* found at the lower site on 9/19/02 were senescent.

Indian Creek. Algae were sparse in the sample from the upper site, which consisted mostly of a few pebbles. The unknown cyanophyte at the lower site was a unicellular coccoid that sometimes formed small colonies.

Ramshorn Creek. The sample from the upper site contained moss. The sample from the lower site was very silty.

Currant Creek. The sample from Currant Creek contained macrophytes.

California Creek. The sample from the lower site was very silty; *Cladophora* in this sample consisted of one large unbranched filament.

Harris Creek. This sample was silty and contained moss and macrophytes.

Shovel Creek. This sample was silty and partly decomposed (smelled of hydrogen sulfide).

Hawkeye Creek. This sample was very silty and partly decomposed (smelled of hydrogen sulfide). The *Cladophora* in this sample was senescent.

Warm Springs Creek. This sample contained macrophytes and moss.

Mill Gulch. This sample was mostly moss.

Garden Creek. The samples from both sites were decomposing (smelled of hydrogen sulfide). The sample from the lower site was very silty and contained macrophytes.

Mormon Creek. This sample contained moss, lots of silt, and two large pebbles.

North Fork Greenhorn Creek. Teratological cells of *Synedra ulna* were common in a scan of the raw sample. The sample contained moss.

West Fork Ruby River. This sample was very silty an in the process of decomposing (smelled of hydrogen sulfide).

Non-Diatom Algae

Coal Creek. Coal Creek supported a diverse mixture of diatoms, green and blue-green algae (Table 3). The xanthophyte *Tribonema*, which indicates cool water of good quality, was also present at the upstream site. The nitrogen-fixing cyanophyte *Tolypothrix*, another indicator of cool, clean water, was the dominant alga at the upper site. Other nitrogen-fixing cyanophytes (*Anabaena, Nostoc, Nodularia, Rivularia*) were also common in Coal Creek, indicating that nitrogen may be the limiting nutrient here. Diatoms ranked first in biovolume at the mouth of Coal Creek in July and the filamentous green alga *Cladophora* ranked first here in September. Coal Creek supported more genera of non-diatom algae than any of the other streams in the study area (Table 3).

Indian Creek. The sample from the upper site on Indian Creek contained only diatoms, which were common. *Cladophora* was most abundant in the sample from the lower site, which also contained diatoms and three genera of cyanobacteria (Table 3).

Ramshorn Creek. Diatoms were the most abundant algae at both sites on Ramshorn Creek (Table 3). The upper site supported 9 genera of non-diatom algae, including the rare brown alga *Heribaudiella* and the red alga *Audouinella*. Both of these genera indicate cool flowing waters of good quality. Diatoms and *Cladophora* ranked first and second in biomass at the downstream site on Ramshorn Creek, followed by *Vaucheria*, a xanthophyte that indicates a steady supply of water at this site.

Currant Creek. Diatoms were the most common algae in the sample from Currant Creek, followed by three genera of cyanobacteria and the desmid *Closterium* (Table 3).

California Creek. *Ulothrix*, an unbranched filamentous green alga, was most abundant at the upper site, followed by the chrysophyte *Hydrurus foetidus* (Table 3). *H. foetidus* forms slimy, mucilaginous colonies in clear, cold streams in full sunlight. It is known for the offensive odor that it emits. Diatoms were common and ranked third in biovolume at the upper site. Near the mouth of California Creek, diatoms were abundant and ranked first in biovolume. *Ulothrix* and *Hydrurus* were absent at the lower site and were replaced by a more conventional algal flora. Here the cyanophyte *Osciallatoria* and the filamentous green alga *Cladophora* ranked second and third in biovolume, respectively.

Harris Creek. The most abundant alga here was the xanthophyte *Vaucheria*, which indicates a steady flow of water. Diatoms were frequent and ranked second. Common genera of green and blue-green algae were also found here (Table 3).

Shovel Creek. The filamentous cyanophyte *Oscillatoria* was the only non-diatom alga found at this site (Table 3). Diatoms were abundant and ranked first in biovolume.

Hawkeye Creek. *Cladophora* dominated the sample from Hawkeye Creek (Table 3). *Vaucheria* was frequent and ranked second in biovolume, while diatoms were also frequent and ranked third. Two other genera of green algae completed the periphyton community at this site.

Warm Springs Creek. The macrophytic green alga *Chara* dominated the sample from this site (Table 3). Dominance by this alga indicates a stream with hard waters, gentle gradient, slow current velocity, and a muddy bottom. The nitrogen-fixing cyanophyte *Nostoc* was also abundant here, indicating that nitrogen may be the limiting nutrient at this site. A total of 8 genera of green and blue-green algae comprised the non-algal flora here.

Mill Gulch. Diatoms and an occasional cell of the desmid *Closterium* were the only algae found in the sample from Mill Creek (Table 3).

Garden Creek. The upper and lower sites on Garden Creek had very similar algal floras (Table 3). Diatoms ranked first in biovolume at both sites, followed by the cyanophyte

Oscillatoria. *Amphithrix*, a cyanophyte that prefers cool waters, was common at the upper site but absent from the lower site.

Mormon Creek. An occasional diatom and filament of *Cladophora* were the only algae found in the sample from Mormon Creek (Table 3).

North Fork Greenhorn Creek. Diatoms ranked first in biovolume, followed by three genera of green algae and the red alga *Audouinella*, which indicates cool water temperatures.

West Fork Ruby River. Besides diatoms, which were abundant and ranked first in biovolume, this site supported 7 genera of algae, including green algae, blue-green algae, and the red alga *Audouinella* (Table 3).

Diatoms

All of the major diatom species from the Ruby River TMDL planning area are included in pollution tolerance classes 3 and 2, and are either sensitive to organic pollution or only somewhat tolerant of organic pollution (Table 4). None of the major diatom species are most tolerant of organic pollution (pollution tolerance class = 1).

Stresses indicated at some of the sites appear to be natural in origin. For example, high values for the disturbance index and percent dominant species in upper Ramshorn Creek and Shovel Creek indicate moderate stress related to steep gradients, fast currents, cold temperatures, and low nutrient concentrations. High values for the pollution index and low values for the siltation index and percent abnormal cells indicate that organic enrichment, sedimentation, and toxic metals did not have a significant effect on the benthic algae at these sites (Table 4). Lower but still elevated values for the disturbance index and percent dominant species indicate minor natural stress in Currant Creek, upper California Creek, Harris Creek, and upper Garden Creek.

Coal Creek. The dominant diatom species in upper Coal Creek was *Epithemia sorex* (Table 4). Cells of this diatom harbor nitrogen-fixing blue-green algae as endosymbionts. Along with the several genera of free-living nitrogen-fixing blue-green algae found at this site, the large number of *Epithemia sorex* indicates that nitrogen is likely the limiting nutrient in upper Coal Creek. Aside from a slightly depressed diversity index (due to dominance by *E. sorex*), there was no other indication of natural or anthropogenic stress or impairment in upper Coal Creek. At the lower site on Coal Creek, a depressed pollution index and an elevated siltation index indicated minor impairment from organic loading and sedimentation in July. In September, a very large number of *Diatoma moniliformis* indicated elevated dissolved solids in lower Coal Creek, possibly due to diminished stream flow. A depressed pollution index also indicated minor impairment from organic loading in September. The depressed diversity index was due mostly to the very large number of *D. moniliformis* that were present at this time. The upper and lower sites on Coal Creek shared less than 20 percent of their diatom assemblages in September, indicating that a major change in environmental conditions occurred between them.

Indian Creek. The upper site on Indian Creek had a slightly depressed pollution index that indicated minor impairment from organic loading. This site supported large numbers of *Fragilaria vaucheriae* and *Synedra ulna*, both of which tolerate small amounts of organic matter. *Hannaea* arcus, an indicator of clean, cold waters, was also a major species here. Other than slight organic loading at the upper site, diatom metrics indicated excellent biological integrity, no impairment, and full support of aquatic life uses at both sites (Table 4).

Ramshorn Creek. Aside from the natural stress evident in upper Ramshorn Creek, this site had excellent biological integrity and no impairment (Table 4). An elevated siltation index and a depressed pollution index indicate moderate impairment from sedimentation and minor impairment from organic loading in lower Ramshorn Creek. The lower site shared only 17 percent of its diatom assemblage with the upper site, indicating that major environmental changes occurred between the two sites.

Currant Creek. In addition to the natural stress evident in Currant Creek, this site exhibited minor impairment from sedimentation (Table 4). Other metrics indicated no impairment and excellent biological integrity.

California Creek. The upper site on California Creek supported a few teratological diatom cells and the lower site supported a few more abnormal cells, indicating possible minor impairment from heavy metals at both sites (Table 4). The lower site supported an exceptionally large number of motile diatoms for a mountain stream, which indicated **severe impairment from sedimentation**. A slightly depressed pollution index suggests minor impairment from organic loading at the lower site. The two sites shared only 18 percent of their diatom floras, indicating that a major change in environmental conditions occurred between these sites.

Harris Creek. Borderline values for the pollution index and siltation index suggest minor impairment from organic loading and sedimentation in Harris Creek. A somewhat elevated percentage of *Achnanthidium minutissimum* suggests minor stress from natural environmental conditions (Table 4).

Shovel Creek. Aside from a large number of *Achnanthidium minutissimum* and natural stress due to steep gradients, coldwater temperatures and low nutrients, diatom metrics indicate good biological integrity in Shovel Creek. One abnormal diatom valve was counted at this site.

Hawkeye Creek. Hawkeye Creek supported an exceptionally large number of motile diatoms for a mountain stream, suggesting **severe impairment from sedimentation** (Table 4). This site also had a slightly depressed pollution index, indicating minor impairment from organic loading. A single teratological diatom valve was counted at this site.

Warm Springs Creek. An elevated number of motile diatoms here indicate minor impairment from sedimentation (Table 4). One teratological diatom valve was counted at this site. Otherwise, diatom metrics indicated excellent biological integrity in Warm Springs Creek.

Mill Gulch. Other than a single abnormal diatom valve and a slightly elevated siltation index, Mill Gulch had excellent biological integrity for a mountain stream (Table 4). Diatom species richness in Mill Gulch was exceptional (70 species counted).

Garden Creek. Aside from minor natural stress and a single abnormal diatom valve, the upper site on Garden Creek had excellent biological integrity (Table 4). The lower site on Garden Creek supported an elevated number of motile diatoms for a mountain stream, indicating minor impairment from sedimentation. A few teratological diatom valves were also counted at this site. The two sites on Garden Creek shared over 40 percent of their diatom assemblages, which indicates that only minor environmental change occurred between them.

Mormon Creek. An elevated number of motile diatoms indicate **moderate impairment from sedimentation** in Mormon Creek. A depressed pollution index also indicates minor impairment from organic loading. A relatively large number of teratological diatoms suggests that this site may have elevated concentrations of heavy metals.

North Fork Greenhorn Creek. Over 13 percent of the diatoms at this site were abnormal (Table 4), which suggests **severe impairment from heavy metals**. The number of motile diatoms here was also elevated, indicating minor impairment from sedimentation. The pollution index was near the threshold for minor impairment and the dominant species here (*Synedra ulna*) is tolerant of organic loading (PTC = 2).

West Fork Ruby River. A large number of motile diatoms indicate **moderate impairment from sedimentation** at this site. The pollution index was also depressed, indicating minor impairment from organic loading. No teratological diatom cells were counted at this site.

Modal Categories

Several ecological attributes assigned by Stevenson and Van Dam et al. (1994) were selected from the diatom reports in the appendix and modal categories of these attributes were extracted to characterize water quality tendencies in streams of the Ruby River TMDL planning area (Table 5). The largest category of diatoms at most sites was "not motile", but highly motile diatoms comprised the largest category in California Creek near mouth, Hawkeye Creek, and the West Fork of the Ruby River. These three sites also had the highest sedimentation indexes.

The modal category for pH at all of the sites was either circumneutral or alkaliphilous, except at the upper site on Coal Creek, where the modal category was alkalibiontic. While alkaliphilous diatoms prefer pH values greater than 7, alkalibiontic diatoms *require* pH values greater than 7. The higher pH in upper Coal Creek may have been transient and due, in part, to a large standing crop of algae undergoing photosynthesis. All of the sites were rated as fresh-brackish (<900 mg/L dissolved solids) except the lower site on Coal Creek in September, where the modal category was brackish-fresh (900-1800 mg/L dissolved solids). Elevated dissolved solids at this site were also indicated by a large number of salt-tolerant diatoms in the species *Diatoma moniliformis* (Table 5).

The modal category for nitrogen uptake at most of the sites was of nitrogen autotrophic taxa that tolerate elevated concentrations of organically bound nitrogen (Van Dam et al. 1994). At the upper site on Coal Creek the modal category was also of nitrogen autotrophs, but those that tolerate only very small concentrations of organically bound nitrogen (Table 5). Most sites had fairly high or continuously high requirements for dissolved oxygen, but the modal category at four sites was only moderate: Coal Creek at mouth (September), upper Indian Creek, Mormon Creek, and North Fork Greenhorn Creek. A "moderate" rating is equivalent to 50% saturation by dissolved oxygen, whereas fairly high and continuously high ratings are equivalent to 75% and 100% saturation, respectively (Van Dam et al. 1994).

Beta-mesosaprobous was the modal category for saprobity at most sites. This indicates water that is relatively free of organic matter, oxygen saturation levels ranging from 70-85%, and BOD concentrations ranging from 2-4 milligrams per liter (Van Dam et al. 1994). The modal category at two sites was alpha-mesosaprobous, which indicates elevated organic matter, 25-70% oxygen saturation, and BOD concentrations ranging from 4-13 mg/L. These two sites--lower Coal Creek (September) and lower Ramshorn Creek—also had the lowest pollution index values (Table 4).

Except for two sites where the modal category for trophic status was "not classified", all of the sites supported mostly eutraphentic diatoms or diatoms that tolerate a wide range of trophic conditions from oligotrophic to hypereutrophic (Van Dam et al. 1994). Eutraphentic diatoms require large concentrations of macronutrients (N, P, and C) for optimum growth.

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Table 1. Location of periphyton sampling stations in the Ruby River TMDL planning area, 2002.

Station	MDEQ Station Code	*Hannaea* Sample Number	Latitude	Longitude	Sample Date
Coal Creek (upper)	MO4COALC02	2620-01	44-49-36	111-58-43	9/19/02
Coal Creek near mouth	MO4COALC01	2621-01	44-51-00	111-59-56	7/11/02
Coal Creek near mouth	MO4COALC01	2621-02	44-51-00	111-59-56	9/19/02
Indian Creek (upper)	MO4INDC01	2622-01	45-29-57	112-07-54	7/12/02
Indian Creek near mouth	MO4INDC02	2623-01	45-27-59	112-12-12	7/12/02
Ramshorn Creek (upper)	MO4RAMHC01	2624-01	45-26-22	112-03-09	9/17/02
Ramshorn Creek near mouth	MO4RAMHC02	2625-01	45-23-37	112-08-44	9/17/02
Currant Creek	MO4CURRC01	2626-01	45-26-17	112-04-01	9/17/02
California Creek (upper)	MO4CALC02	2627-01	45-24-46	112-00-05	9/18/02
California Creek near mouth	MO4CALC01	2628-01	45-22-07	112-06-39	9/17/02
Harris Creek	MO4HARRC01	2629-01	45-24-54	112-01-09	9/18/02
Shovel Creek	MO4SHOVC01	2630-01	44-50-38	111-59-43	9/18/02
Hawkeye Creek	MO4HWKEC01	2631-01	44-52-11	112-00-02	9/18/02
Warm Springs Creek	MO4WARMSC01	2632-01	45-02-16	111-57-12	9/19/02
Mill Gulch	MO4MILLG01	2633-01	45-25-11	111-57-16	9/24/02
Garden Creek (upper)	MO4GARDC01	2634-01	45-14-42	112-13-00	9/24/02
Garden Creek near mouth	MO4GARDC02	2635-01	45-13-27	112-08-30	9/24/02
Mormon Creek	MO4MORMC01	2636-01	45-11-26	112-10-22	9/25/02
Greenhorn Creek (North Fork)	MO4GHCNF01	2637-01	45-07-19	112-02-21	9/25/02
Ruby River (West Fork)	MO4RURWF01	2628-01	44-54-24	112-00-23	9/26/02

Table 2. Diatom association metrics used by the State of Montana to evaluate biological integrity in mountain streams: references, range of values, expected response to increasing impairment or natural stress, and criteria for rating levels of biological integrity. The lowest rating for any one metric is the rating for that site.

Biological Integrity/ Impairment or Stress/ Use Support	No. of Species Counted[1]	Diversity Index[2] (Shannon)	Pollution Index[3]	Siltation Index[4]	Disturbance Index[5]	% Dominant Species[6]	% Abnormal Cells[7]
Excellent/None Full Support	>29	>2.99	>2.50	<20.0	<25.0	<25.0	0
Good/Minor Full Support	20-29	2.00-2.99	2.01-2.50	20.0-39.9	25.0-49.9	25.0-49.9	>0.0, <3.0
Fair/Moderate Partial Support	19-10	1.00-1.99	1.50-2.00	40.0-59.9	50.0-74.9	50.0-74.9	3.0-9.9
Poor/Severe Nonsupport	<10	<1.00	<1.50	>59.9	>74.9	>74.9	>9.9
References	Bahls 1979 Bahls 1993	Bahls 1979	Bahls 1993	Bahls 1993	Barbour et al. 1999	Barbour et al. 1999	McFarland et al. 1997
Range of Values	0-100+	0.00-5.00+	1.00-3.00	0.0-90.0+	0.0-100.0	~5.0-100.0	0.0-30.0+
Expected Response	Decrease[8]	Decrease[8]	Decrease	Increase	Increase	Increase	Increase

[1]Based on a proportional count of 400 cells (800 valves)
[2]Base 2 [bits] (Weber 1973)
[3]Composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species
[4]Sum of the percent abundances of all species in the genera *Navicula, Nitzschia* and *Surirella*
[5]Percent abundance of Achnanthidium minutissimum (synonym: *Achnanthes minutissima*)
[6]Percent abundance of the species with the largest number of cells in the proportional count
[7]Cells with an irregular outline or with abnormal ornamentation, or both
[8]Species richness and diversity may increase somewhat in mountain streams in response to slight to moderate increases in nutrients or sediment

Table 3. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from tributaries of the Ruby River in 2002: d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare.

Taxa	Coal (upper) 9/19/02	Coal (mouth) 7/11/02	Coal (mouth) 9/19/02	Indian (upper)	Indian (mouth)	Ramshorn (upper)	Ramshorn (mouth)	Currant	California (upper)	California (mouth)
Cyanophyta										
Amphithrix	o/12	c/8	c/5			o/9				
Anabaena	o/11	o/10								
Calothrix		c/5	c/6							
Chamaesiphon					o/4					
Nodularia	f/5									
Nostoc	c/6									
Oscillatoria		r/13			o/5			c/3	c/4	c/2
Phormidium						r/10		f/2		
Rivularia	c/7	o/6								
Schizothrix						o/8		c/4	o/5	o/5
Tolypothrix	d/1	o/9	f/3							
unknown bluegreen					c/3					
Rhodophyta										
Audouinella						c/5				
Chlorophyta										
Ankistrodesmus	o/14	o/12	o/12							r/6
Cladophora		f/2	d/1		f/1		a/2			o/3
Closterium	o/9	c/4	o/7			f/3	o/5	r/5		o/4
Cosmarium	o/10	o/7	o/9							
Cylindrocapsa		o/11								
Mougeotia	c/3		c/4			o/6				
Oedogonium			o/10				c/4			
Rhizoclonium	o/8									
Scenedesmus	r/13		r/13							
Stigeoclonium	r/15	f/3	o/11	o/2		o/7				
Tetraspora			o/8							
Ulothrix						c/4			a/1	

Table 3. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from tributaries of the Ruby River in 2002: d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare. Continued...

Taxa	Coal (upper) 9/19/02	Coal (mouth) 7/11/02	Coal (mouth) 9/19/02	Indian (upper)	Indian (mouth)	Ramshorn (upper)	Ramshorn (mouth)	Currant	California (upper)	California (mouth)
Xanthophyta										
Tribonema	f/4									
Vaucheria							a/3			
Chrysophyta										
Hydrurus foetidus									a/2	
Bacillariophyta	a/2	d/1	a/2	c/1	c/2	a/1	d/1	f/1	c/3	a/1
Phaeophyta										
Heribaudiella						f/2				
# Non-Diatom Genera	14	12	12	1	4	9	4	4	4	5

Table 3. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from tributaries of the Ruby River in 2002: d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare. Continued...

Taxa	Harris	Shovel	Hawkeye	Warm Springs	Mill	Garden (upper)	Garden (mouth)	Mormon	North Fork Greenhorn	West Fork Ruby
Cyanophyta										
Amphithrix						c/3				
Anabaena										o/8
Nostoc				a/2						
Oscillatoria	c/5	c/2		c/5		f/2	c/2			
Phormidium	c/3									
Schizothrix				o/7						
Symploca						o/4	o/3			o/6
Rhodophyta										
Audouinella									o/5	f/3
Chlorophyta										
Chara				d/1						
Cladophora			d/1	o/6				o/1	o/4	c/4
Closterium	c/4			c/4	o/2	r/5	r/4		c/2	c/2
Cosmarium				o/8						
Microspora									o/3	
Mougeotia				r/9						
Oedogonium										o/5
Spirogyra	o/6		o/5							o/7
Ulothrix			c/4							
Xanthophyta										
Vaucheria	a/1		f/2							
Bacillariophyta	f/2	a/1	f/3	f/3	f/1	a/1	f/1	o/2	a/1	a/1
# Non-Diatom Genera	5	1	4	8	1	4	3	1	4	7

Table 4. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from tributaries of the Ruby River in 2002. Underlined values indicate minor stress; **bold values** indicate moderate stress; **underlined and bold** values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2. Stress may be natural or anthropogenic (see text).

Species/Metric	PTC[2]	Coal (upper) 9/19/02	Coal (mouth) 7/11/02	Coal (mouth) 9/19/02	Indian (upper)	Indian (mouth)	Ramshorn (upper)	Ramshorn (mouth)	Currant	California (upper)	California (mouth)
Achnanthidium minutissimum	3	0.96	2.62	1.92	20.44	2.11	69.24	1.35	38.08	49.02	1.64
Amphora pediculus	3	0.38	1.25		0.25	0.22	1.74	0.25	1.97	0.73	6.69
Cocconeis placentula	3	0.10			1.48	10.11	3.61	6.15	3.93	5.01	7.75
Cyclotella meneghiniana	2							11.69			
Cymbella affinis	3	0.67	6.62	1.20		0.56		0.12			
Diatoma moniliformis	2	6.89	15.75	61.46							
Diatoma vulgaris	3	1.15		8.76		22.00					0.23
Encyonema minutum	2	0.10						2.09	10.69	2.69	0.12
Encyonema silesiacum	2				3.82	5.89	1.49	2.58	5.90	11.49	
Epithemia sorex	3	61.44									
Fragilaria vaucheriae	2	0.19	3.25	0.72	15.64	3.33	0.37		1.35	1.47	0.59
Gomphonema minutum	3					23.22					
Gomphonema sp.	3	2.01	5.75	4.68	6.40	9.78	1.37	1.72		0.61	2.82
Hannaea arcus	3				7.88	0.33	0.50			0.73	
Navicula capitatoradiata	2	2.78	1.25	1.44				8.86			3.17
Navicula reichardtiana	2	1.34	4.88	0.60	0.25	0.22		10.95			2.58
Nitzschia dissipata	3	1.05	0.50	0.24				0.62	0.12	0.12	11.50
Nitzschia sociabilis	2							0.12			20.07
Nitzschia sp.	2	7.94	5.25	3.12	0.12		0.75	5.54	3.56	0.12	3.99
Planothidium lanceolatum	2	0.38	1.12		0.49	0.56	0.75	5.90	1.23	0.73	1.64
Reimeria sinuata	3	0.19	1.12		3.82	5.00	1.99	0.86	1.35	2.57	0.12
Rhoicosphenia abbreviata	3					3.22	0.12	4.06	0.37	0.61	5.16
Synedra ulna	2	0.57	9.00		19.09	1.78	7.22	1.35	1.60	1.96	1.41
unknown cymbelloid	3	0.57	4.50	0.12	3.94	2.22	1.87	1.85	3.32	10.39	0.94

Table 4. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from tributaries of the Ruby River in 2002. Underlined values indicate minor stress; **bold values** indicate moderate stress; **underlined and bold** values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2. Stress may be natural or anthropogenic (see text). Continued...

Species/Metric	PTC[2]	Harris	Shovel	Hawkeye	Warm Springs	Mill	Garden (upper)	Garden (mouth)	Mormon	North Fork Greenhorn	West Fork Ruby
Achnanthes sp.	3	0.49	2.57	7.71	0.24	0.63		0.40	0.51	3.94	0.85
Achnanthidium minutissimum	3	26.59	52.75		5.09	18.91	45.18	18.10	9.11	11.37	2.54
Amphora pediculus	3	1.22	0.23	3.06	2.18	2.21	1.53	6.73	5.73	0.93	1.57
Cocconeis pediculus	3			10.57	3.88						0.48
Cocconeis placentula	3	2.44		1.28	20.61	1.89	3.06	9.89	13.61	13.81	1.94
Cymbella affinis	3	4.63	5.85		10.42			2.77		0.93	1.21
Diatoma vulgaris	3				1.58					0.35	6.66
Encyonema minutum	2	5.85	1.29				20.12	1.38		0.93	0.36
Encyonema silesiacum	2	8.90	1.87		0.12	1.68		3.96		1.39	0.24
Gomphonema sp.	3		0.23	6.23	4.61	0.32	0.24	4.25	0.72	2.09	4.96
Navicula gregaria	2	0.37		5.53		1.79	0.94	3.86	13.72		1.94
Navicula margalithii	3	6.71		5.63	18.79	0.53	4.35	4.45	2.05	8.70	
Navicula menisculus	2	0.73	1.05	1.09	0.85			0.40	0.10	0.12	5.08
Navicula reichardtiana	2	0.73	0.70	6.03	2.67	0.53	0.24	2.37	1.84		1.57
Nitzschia amphibia	2								2.46		7.63
Nitzschia linearis	2	2.32	0.58	3.85		5.88	0.12	1.29	2.35	2.78	
Nitzschia sp.	2	1.34	1.29	6.13	2.30	3.36	0.47	0.99	2.15	0.23	13.44
Planothidium lanceolatum	2	5.24	1.17	0.49	0.61	15.76	0.24	2.37	9.62	0.93	1.69
Synedra ulna	2	4.63	0.35		3.15	0.32	0.35	0.40		25.64	7.87
unknown cymbelloid	3	5.49	2.69		2.06					0.70	

Table 4. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from tributaries of the Ruby River in 2002. Underlined values indicate minor stress; **bold values** indicate moderate stress; **underlined and bold** values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2. Stress may be natural or anthropogenic (see text). Continued...

Species/Metric	PTC[2]	Coal (upper) 9/19/02	Coal (mouth) 7/11/02	Coal (mouth) 9/19/02	Indian (upper)	Indian (mouth)	Ramshorn (upper)	Ramshorn (mouth)	Currant	California (upper)	California (mouth)
Number of Species Counted		39	43	30	42	33	30	58	46	43	52
Shannon Species Diversity		2.59	4.57	2.51	3.73	3.55	2.11	4.80	3.77	3.04	4.52
Pollution Index		2.71	2.24	2.21	2.49	2.80	2.82	2.20	2.55	2.62	2.42
Siltation Index		18.95	31.37	11.64	0.99	1.89	3.11	**49.08**	23.59	6.36	**66.67**
Disturbance Index		0.96	2.62	1.92	20.44	2.11	**69.24**	1.35	38.08	49.02	1.64
Percent Dominant Species		**61.44**	15.75	**61.46**	20.44	23.22	**69.24**	11.69	38.08	49.02	20.07
Percent Abnormal Cells		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.61	1.17
Similarity Index[3]				19.71		24.28		16.98			18.09

[1]A major diatom species accounts for 5.0% or more of the cells at one or more stations in a sample set.

[2]Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive to organic pollution

[3]Percent Community Similarity (Whittaker 1952) when compared to the diatom assemblage at the adjacent upstream station

Table 5. Modal categories for selected ecological attributes of diatom species in tributaries of the Ruby River.

Ecological Attribute	Coal (upper) 9/19/02	Coal (mouth) 7/11/02	Coal (mouth) 9/19/02	Indian (upper)	Indian (mouth)	Ramshorn (upper)	Ramshorn (mouth)	Currant	California (upper)	California (mouth)
Motility[1]	Moderately Motile	Not Motile	Not Motile	Not Motile	Not Motile	Not Motile	Not Motile	Not Motile	Not Motile	**Highly Motile**
pH[2]	**Alkali-biontic**	Alkali-philous	Alkali-philous	Alkali-philous	Circum-neutral	Circum-neutral	Alkali-philous	Circum-neutral	Circum-neutral	Alkali-philous
Salinity[2]	Fresh-brackish	Fresh-brackish	**Brackish-fresh**	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish
Nitrogen Uptake[2]	Autotroph **Low Organics**	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics
Oxygen Demand[2]	Fairly High	Not Classified	**Moderate**	**Moderate**	Not Classified	Continuous High	Not Classified	Continuous High	Continuous High	Fairly High
Saprobity[2]	beta-Mesosap.	Not Classified	**alpha-Mesosap.**	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	**alpha-Mesosap.**	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.
Trophic State[2]	Eutrophic	Eutrophic	Eutrophic	Variable	Eutrophic	Variable	Eutrophic	Variable	Variable	Eutrophic

[1]Dr. R. Jan Stevenson, Michigan State University, digital communication.
[2]Van Dam et al. 1994

Table 4. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from tributaries of the Ruby River in 2002. <u>Underlined values</u> indicate minor stress; **bold values** indicate moderate stress; <u>**underlined and bold**</u> values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2. Stress may be natural or anthropogenic (see text). Continued...

Species/Metric	PTC[2]	Harris	Shovel	Hawkeye	Warm Springs	Mill	Garden (upper)	Garden (mouth)	Mormon	North Fork Greenhorn	West Fork Ruby
Number of Species Counted		54	40	51	49	70	40	62	57	38	61
Shannon Species Diversity		4.28	3.16	4.77	4.16	4.82	<u>2.97</u>	4.74	4.50	3.91	4.86
Pollution Index		<u>2.46</u>	2.72	<u>2.46</u>	2.76	2.41	2.65	2.55	<u>2.35</u>	2.53	<u>2.34</u>
Siltation Index		<u>20.12</u>	15.91	<u>**63.24**</u>	<u>37.33</u>	<u>31.41</u>	13.88	<u>37.09</u>	**40.23**	<u>22.51</u>	**58.47**
Disturbance Index		<u>26.59</u>	**52.75**	0.00	5.09	18.91	<u>45.18</u>	18.10	9.11	11.37	2.54
Percent Dominant Species		<u>26.59</u>	**52.75**	10.57	20.61	18.91	<u>45.18</u>	18.10	13.72	<u>25.64</u>	13.44
Percent Abnormal Cells		0.00	<u>0.12</u>	<u>0.10</u>	<u>0.12</u>	<u>0.11</u>	<u>0.12</u>	<u>0.30</u>	<u>2.97</u>	<u>**13.46**</u>	0.00
Similarity Index[3]								42.16			

[1]A major diatom species accounts for 5.0% or more of the cells at one or more stations in a sample set.
[2]Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive to organic pollution
[3]Percent Community Similarity (Whittaker 1952) when compared to the diatom assemblage at the adjacent upstream station

Table 5. Modal categories for selected ecological attributes of diatom species in tributaries of the Ruby River. Continued...

Ecological Attribute	Harris	Shovel	Hawkeye	Warm Springs	Mill	Garden (upper)	Garden (mouth)	Mormon	North Fork Greenhorn	West Fork Ruby
Motility[1]	Not Motile	Not Motile	**Highly Motile**	Not Motile	Not Motile	Not Motile	Not Motile	Moderately Motile	Not Motile	**Highly Motile**
pH[2]	Circum-neutral	Circum-neutral	Alkali-philous	Alkali-philous	Alkali-philous	Circum-neutral	Alkali-philous	Alkali-philous	Alkali-philous	Alkali-philous
Salinity[2]	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish	Fresh-brackish
Nitrogen Uptake[2]	Autotroph High Organics	Autotroph High Organics	Not Classified	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Autotroph High Organics	Not Classified
Oxygen Demand[2]	Continuous High	Continuous High	Not Classified	Fairly High	Continuous High	Continuous High	Not Classified	**Moderate**	**Moderate**	Not Classified
Saprobity[2]	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	beta-Mesosap.	Not Classified
Trophic State[2]	Variable	Variable	Not Classified	Eutrophic	Eutrophic	Variable	Eutrophic	Eutrophic	Variable	Not Classified

[1]Dr. R. Jan Stevenson, Michigan State University, digital communication.
[2]Van Dam et al. 1994