BIOLOGICAL INTEGRITY OF EAGLE CREEK BASED ON THE STRUCTURE AND COMPOSITION OF THE BENTHIC ALGAE COMMUNITY

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Summary

In June 2003, periphyton samples were collected from two sites on Eagle Creek in the Bullwhacker-Dog TMDL planning area in north central Montana for the purpose of assessing whether Eagle Creek is 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.

Since Eagle Creek begins in a mountain ecoregion and ends in a prairie ecoregion, diatom metrics generated from both samples were compared to criteria developed for both mountain and plains streams. The upper site (below Highway 236) had good biological integrity for a mountain stream and excellent biological integrity for a plains stream. The limiting factors at this site were sedimentation and organic loading. The lower site (near mouth) had fair biological integrity for a mountain stream and good biological integrity for a prairie stream. Here also, the limiting factors were sedimentation and organic loading.

The two sites had somewhat similar algal floras, indicating that only minor changes in environmental conditions occurred between them. Both sites had excellent diatom species richness, diversity, and equitability. The majority of diatoms at both sites indicated fresh, alkaline, and reasonably well oxygenated waters. Both the diatoms and the major non-diatom algae indicated a steady flow of cool water and elevated inorganic nutrients at both sites. There were no indicators (e.g., abnormal diatoms) of toxic chemicals at either site.

Introduction

This report evaluates the biological integrity[1], support of aquatic life uses, and probable causes of stress or impairment to aquatic communities in Eagle Creek in the Bullwhacker-Dog TMDL planning area of north central Montana. The purpose of this report is to provide information that will help the State of Montana determine whether Eagle Creek is 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 stream sites that were sampled in June 2003. 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, 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.

[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 in north central Montana in Chouteau County. Eagle Creek is a tributary of the Missouri River. It heads in the Bearpaw Mountains and flows southwesterly, entering the Missouri River downstream from Virgelle. Eagle Creek transects three ecoregions: the Northern Rockies, Montana Valley and Foothill Prairies, and the Northwestern Glaciated Plains (Woods et al. 1999). The upper sampling site is in the foothill ecoregion and the lower site is in the plains ecoregion.

Periphyton samples were collected at 2 sites on Eagle Creek (Table 1). Elevations at the sampling sites range from about 3200 feet above mean sea level at the upper site to about 2600 feet at the lower site. Vegetation in the study area is mainly mixed conifer forest in the upper reaches of Eagle Creek and mixed grassland along the middle and lower reaches (USDA 1976). Land use is primarily livestock grazing with some mining in the headwaters.

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, postassium dichromate, and 3% 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). Approximately 350 diatom cells (700 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 2001; Krammer 2002. Diatom naming conventions followed those adopted by the Academy of Natural Sciences for USGS NAWQA samples (Morales and Potapova 2000). 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 Mountains and Great Plains ecoregions of Montana (Tables 2 and 3). These criteria are based on the distribution of 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 Tables 2 and 3 are valid only for samples collected during the summer field season (June 21-September 21).

The criteria in Tables 2 and 3 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.

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., 2951-01. The first part of this number (2951) designates the sampling site (Eagle Creek near mouth) 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. Samples were collected at locations close to these sites in 2002 (Bahls 2003).

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 4, 5 and 6, which are located near the end of this report following the references section. Appendix A consists of a series of diatom reports, one for each sample. Each diatom report contains an alphabetical list of diatom species and their percent abundances, and values for 65 different diatom metrics and ecological attributes.

Sample Notes

Eagle Creek below Highway 236. The sample from this site was putrid but OK. Diatom epiphytes were extremely heavy on the filamentous green algae (e.g., *Cladophora*).

Eagle Creek near mouth. The sample from this site was putrid and soft algae were in poor condition. Fine through coarse sediment was very heavy.

Non-Diatom Algae

Only two genera of non-diatom algae were found in the sample from the upper site on Eagle Creek and both of these were filamentous green algae (Table 4). An abundance of *Cladophora* here indicates eutrophic conditions. Diatoms were dominant and ranked 1st in biovolume.

Vaucheria or "water felt" was the most abundant alga in the sample from the lower site on Eagle Creek (Table 4). *Vaucheria* requires a steady supply of cool, flowing water and is often found in springs and spring brooks. Diatoms were abundant here and ranked 2nd in biovolume. The filamentous green algae *Cladophora* and *Spirogyra* ranked 3rd and 4th, respectively. Both of these indicate eutrophic waters. These were followed in abundance by the cold-water chrysophyte *Tribonema* and the filamentous cyanophyte *Phormidium,* both of which were rare. The sample from the lower site contained a total of 5 genera of non-diatom algae.

Diatoms

Four of the major diatom species in Eagle Creek are sensitive to organic pollution. Three of these were abundant at both sites and one (*Staurosira construens*) was abundant only at the upper site (Table 5). Six of the major species are somewhat tolerant of organic pollution and these were present at both sites. *Nitzschia paleacea*, a somewhat tolerant species that is also highly motile, was abundant only at the lower site. None of the major diatom species in the 2003 samples from Eagle Creek are most tolerant of organic pollution (pollution tolerance class 1).

The **upper site** on Eagle Creek had good biological integrity for a mountain stream and excellent biological integrity for a prairie stream (Table 5). The limiting factors here were minor impairment from sedimentation and borderline minor impairment from organic loading. Diatom species richness, diversity, and equitability were excellent. The major diatom species here were either sensitive to or only somewhat tolerant of organic pollution. The most abundant species at the upper site were *Rhoicosphenia abbreviata* and *Staurosira construens*. Both are non-motile species. *R. abbreviata* is an epiphyte and *S. construens* is a free-living araphid diatom that

indicates stable flows of cool water and little physical disturbance. Teratological cells were not encountered during the diatom proportional count.

Diatom metrics at the **lower site** on Eagle Creek indicate fair biological integrity and partial support of aquatic life uses for a mountain stream and good biological integrity and full support of uses for a prairie stream (Table 5). The limiting factors here were sedimentation (moderate impairment for a mountain stream) and organic loading (minor impairment for a mountain stream). Diatom species richness and diversity were excellent and no teratological diatoms were encountered.

The lower site shared a little less than half of its diatom flora with the upper site on Eagle Creek. This indicates only a minor change in the diatom flora and a small difference in ecological conditions between the sites. Again, the dominant species was *Rhoicosphenia abbreviata*, a common epiphyte on *Cladophora.* The next most abundant species here were *Diatoma moniliformis*, which indicates somewhat elevated salt content, and *Nitzschia paleacea*, a motile diatom adapted to living on unstable and aggrading substrates.

Several ecological attributes were selected from the diatom reports in the appendix and modal categories of these attributes were extracted to characterize water quality tendencies at the two sites (Table 6). Modal categories for the selected attributes were identical for the two sites. The majority of diatoms at both sites were non-motile nitrogen autotrophs that exert a fairly high demand for dissolved oxygen. Nitrogen autotrophs require inorganic nitrogen (nitrates and ammonia) as nutrients. The majority of diatoms at both sites also indicate fresh, alkaline and eutrophic waters with only a small amount of BOD loading.

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Table 1. Location of MDEQ periphyton sampling stations on Eagle Creek near Big Sandy, Montana in 2003.

Station	Montana DEQ Station Code	*Hannaea* Sample Number	Latitude	Longitude	Sample Date
Eagle Creek below Highway 236	M23EAGLC02	2950-01	47 56 24	109 52 41	6/10/03
Eagle Creek near mouth	M23EAGLC06	2951-01	47 55 16	110 02 06	6/10/03

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. Diatom association metrics used by the State of Montana to evaluate biological integrity in **prairie** 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]	Similarity Index[7]
Excellent/None Full Support	>39	>3.99	>2.25	<50.0	<25.0	<25.0	>59.9
Good/Minor Full Support	30-39	3.00-3.99	1.76-2.25	50.0-69.9	25.0-49.9	25.0-49.9	40.0-59.9
Fair/Moderate Partial Support	20-29	2.00-2.99	1.25-1.75	70.0-89.9	50.0-74.9	50.0-74.9	20.0-39.9
Poor/Severe Nonsupport	<20	<2.00	<1.25	>89.9	>74.9	>74.9	<20.0
References	Bahls 1979 Bahls 1993	Bahls 1979	Bahls 1993	Bahls 1993	Barbour et al. 1999	Barbour et al. 1999	Whittaker 1952
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	0.0-100.0
Expected Response	Decrease	Decrease	Decrease	Increase	Increase	Increase	Decrease

[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]Percent Community Similarity (Whittaker 1952)

Table 4. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from Eagle Creek in 2003.

Taxa	Eagle Creek **02** M23EAGLC02	Eagle Creek **06** M23EAGLC06
Cyanophyta (cyanobacteria)		
Phormidium		rare/6th
Chlorophyta (green algae)		
Cladophora	abundant/2nd	frequent/3rd
Oedogonium	occasional/3rd	
Spirogyra		common/4th
Chrysophyta (yellow-green algae)		
Tribonema		rare/5th
Vaucheria		abundant/1st
Bacillariophyta (diatoms)	dominant/1st	abundant/2nd
# Non-Diatom Genera	2	5

Table 5. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from Eagle Creek in 2003. 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 and plains streams in Table 3. Stress may be natural or anthropogenic (see text)

Species/Metric	PTC[2]	Eagle Creek **02** **Mountain** Criteria	Eagle Creek **02** **Prairie** Criteria	Eagle Creek **06** **Mountain** Criteria	Eagle Creek **06** **Prairie** Criteria
Achnanthidium minutissimum	3	0.61	0.61	3.35	3.35
Diatoma moniliformis	2	0.45	0.45	13.29	13.29
Encyonema cespitosum	2	4.09	4.09	0.24	0.24
Fragilaria vaucheriae	2	7.42	7.42	1.80	1.80
Navicula gregaria	2	3.94	3.94	1.92	1.92
Nitzschia dissipata	3	6.36	6.36	5.27	5.27
Nitzschia paleacea	2	2.42	2.42	10.06	10.06
Rhoicosphenia abbreviata	3	15.00	15.00	21.20	21.20
Staurosira construens	3	13.94	13.94	1.56	1.56
Surirella minuta	2	3.48	3.48	1.56	1.56
Number of Species Counted		72	72	68	68
Shannon Species Diversity		4.94	4.94	4.65	4.65
Pollution Index		2.51	2.51	2.29	2.29
Siltation Index		30.15	30.15	**44.43**	44.43
Disturbance Index		0.61	0.61	3.35	3.35
Percent Dominant Species		15.00	15.00	21.20	21.20
Percent Abnormal Cells		0.00	0.00	0.00	0.00
Similarity Index[3]				46.64	46.64

[1]A major diatom species accounts for 3.0% or more of the cells at one or more stations in a sample set.

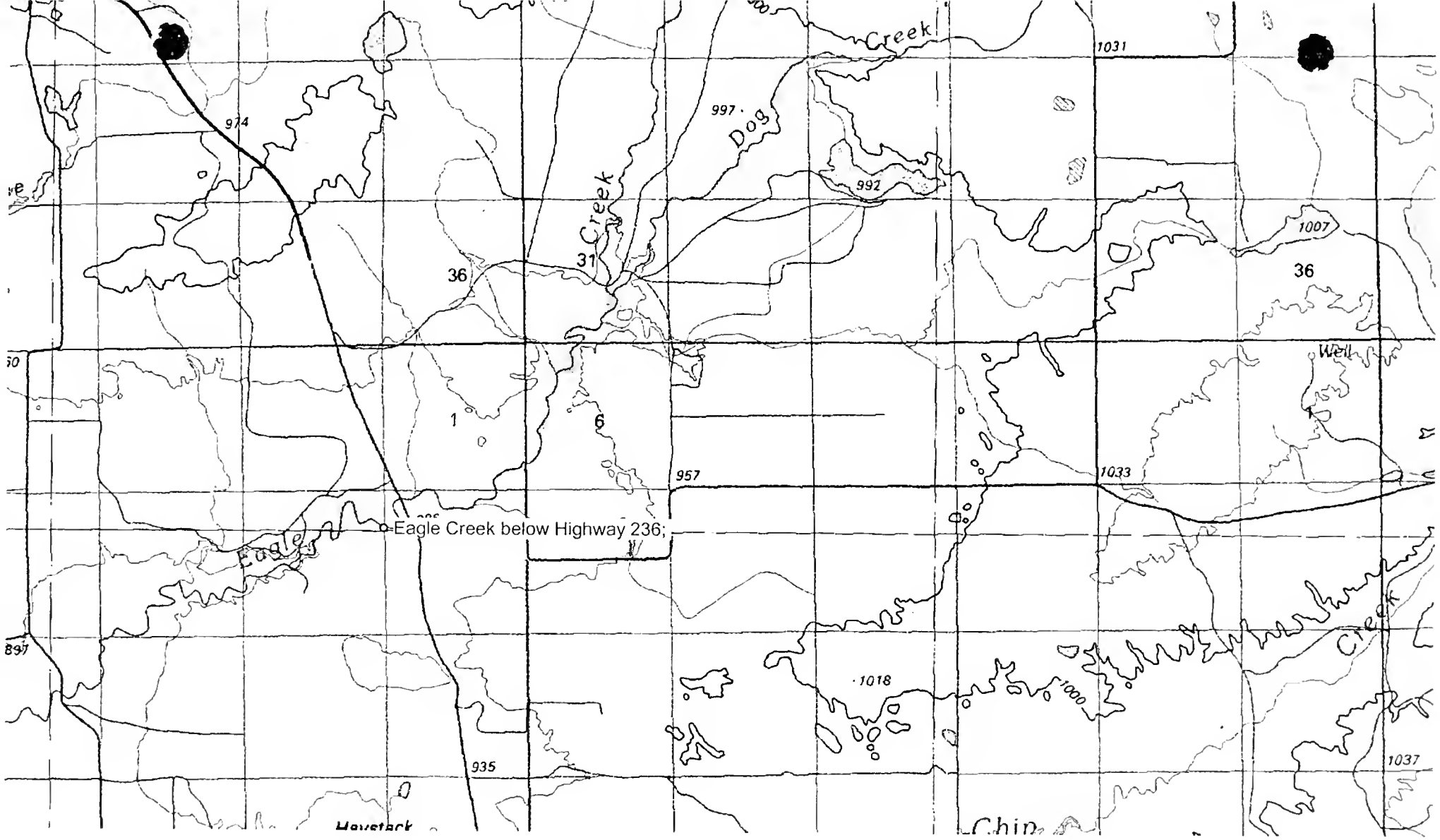
[2](Organic) Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive.

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

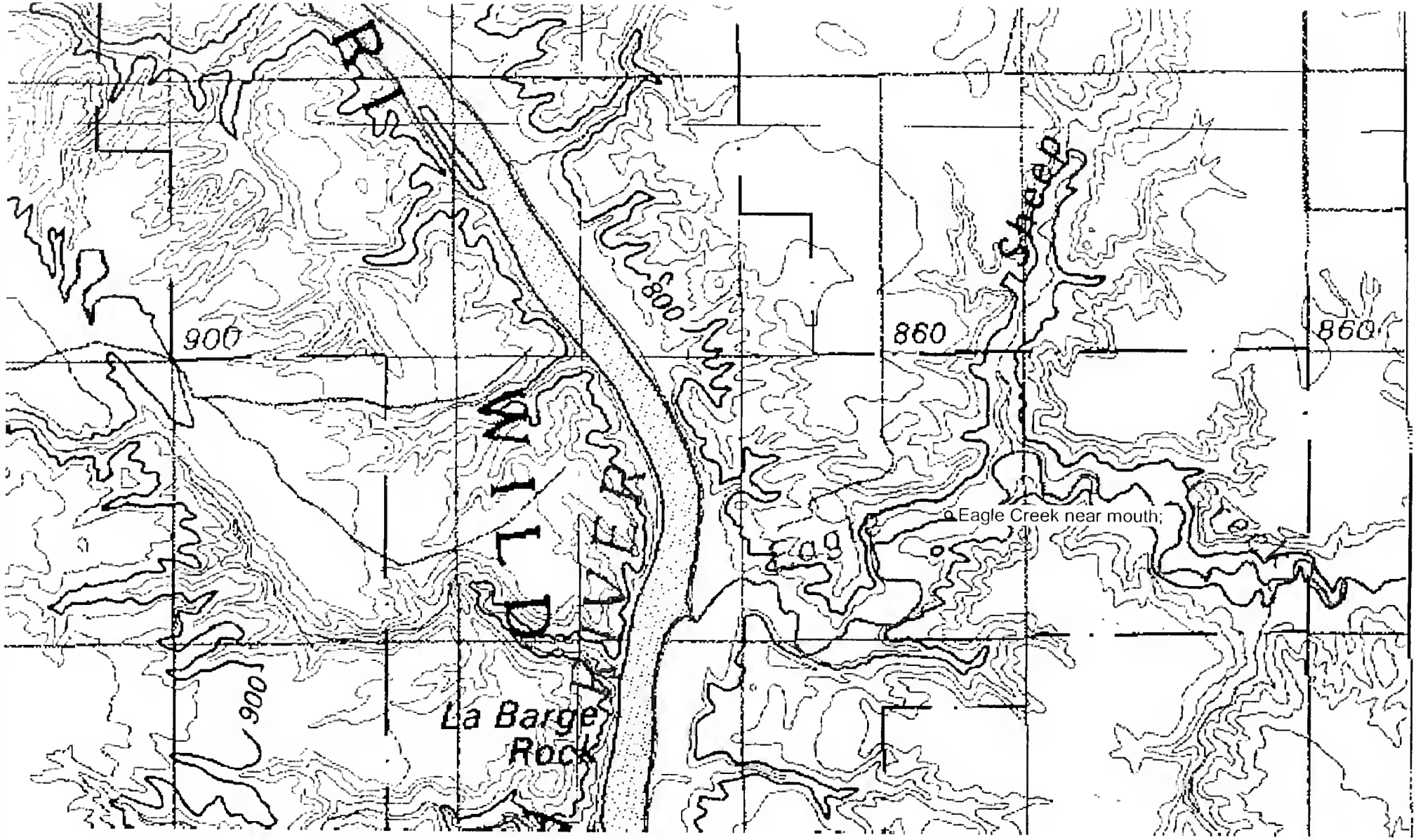
Table 6. Modal categories for selected ecological attributes of diatom species in Eagle Creek.

Ecological Attribute	Eagle Creek **02** M23EAGLC02	Eagle Creek **06** M23EAGLC06
Motility[1]	Not Motile	Not Motile
pH[2]	Alkaliphilous	Alkaliphilous
Salinity[2]	Fresh	Fresh
Nitrogen Uptake[2]	Autotrophs (tolerate high organics)	Autotrophs (tolerate high organics)
Oxygen Demand[2]	Fairly High	Fairly High
Saprobity[2]	beta-Mesosaprobous	beta-Mesosaprobous
Trophic State[2]	Eutraphentic	Eutraphentic

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



'Winifred 100K; MT'; Scale: 1" = 0.953Mi 1,534Mt 5,031Ft, 1 Mi = 1.049" , 1 cm = 604Mt



'Fort Benton 100K; MT'; Scale: 1" = 0.476Mi 767Mt 2,516Ft, 1 Mi = 2.099" , 1 cm = 302Mt