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# Responses of benthic insect communities to effluent from the abandoned Ferris-Haggarty copper mine in southeast Wyoming, USA

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#### **Abstract**

Six criteria were used to evaluate 12 metrics for their sensitivity to effluent flowing from the Ferris-Haggarty copper mine into Haggarty Creek and then into Battle Creek West Fork. Through the evaluation process, we found that the Shannon-Wiener index, the random runs value, and Ephemeroptera taxa richness appeared to best reflect the impacts that have occurred in both Haggarty Creek and Battle Creek West Fork. In addition, Ephemeroptera/Plecoptera/Trichoptera taxa richness, total taxa richness, and Plecoptera taxa richness, were useful in reflecting those impacts. In contrast, we found that the abundance ratios, the Hilsenhoff Biotic Index, as well as Trichoptera taxa richness, did not reflect the impacts that occurred in Haggarty Creek and Battle Creek West Fork. Finally, this study provided information about the benthic insect communities that are present in the impacted reaches of Haggarty Creek. Such information is needed to assess the potential of those reaches as habitat for the Colorado River cutthroat trout, *Oncorhynchus clarki pleuriticus*, which is a species of special concern to the Wyoming Department of Game and Fish.

Key words: benthic insect communities; metric evaluation; evaluation criteria; Ferris-Haggarty copper mine

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# Introduction

Studies have shown that metal mining causes impacts to the quality of water in lotic systems (De Jonge et al., 2008; IIwasaki et al., 2009; Clements et al., 2010). Specific causes of such impacts are variable and therefore must be investigated separately for each mine-lotic system combination. Investigating the causes of impact for each mine provides the information that is needed for reclamation.

One lotic system that has been impacted by a mining operation is Haggarty Creek (Sierra Madre Range, southeast Wyoming), which is affected by effluent from the Ferris-Haggarty copper mine. This mine, which is situated near the headwaters of Haggarty Creek, was the most productive copper mine in North America at the turn of the 20th century (Rockwell, 2001). Operating procedures of the mine were such that untreated effluent was allowed to flow from the mine into Haggarty Creek. In the 1970s, the Wyoming Department of Environmental Quality instituted new environmental standards, which required treatment of the effluent before it was allowed to flow from the mine. Owners of the Ferris-Haggarty mine did not meet the costs for supplying this treatment, so the mine became inactive. The untreated effluent, however, continued to flow into

Haggarty Creek (Bell, 1996).

The affected reaches of Haggarty Creek begins where the effluent enters that creek and continues for approximately 18 km downstream through Battle Creek West Fork, and ends at the confluence of Battle Creek West Fork with Battle Creek. As officially indicated in the year 2006 listing of Wyoming 303(d) Impaired Waters and Impairments, copper, silver, and cadmium impact the affected reaches of Haggarty Creek while just copper impacts the affected reaches of Battle Creek West Fork (http://deq.state.wy.us/wqd/watershed/downloads/305b/2006/2006%20303d.pdf).

Discussions have begun for reclaiming the impacted reaches of Haggarty Creek and Battle Creek West Fork. More specifically, these have involved investigating the bioaccumulation of metals in the benthic fauna, and such investigations were previously conducted by Rockwell (2001). Discussions have also involved investigating the *in situ* response of benthic fauna to effluent from the Ferris-Haggarty mine. This, in part, may be investigated through the use of calculated metrics, which take into account the integrated composition of benthic insect communities (Karr, 1991). Such investigations have not yet been conducted on Haggarty Creek or Battle Creek West Fork.

Metrics that are commonly used to measure the extent

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of human disturbances (stressors) on benthic macroinvertebrates include various kinds of taxa richness, abundance ratios, and some diversity indices (Resh and Jackson, 1993; Wu and Legg, 2007, 2009). More specifically, the kinds of taxa richness that tend to be used are the Ephemeroptera taxa richness, Plecoptera taxa richness, Trichoptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, and total taxa richness. Measures of abundance ratios that tend to be used include percent Ephemeroptera, percent Plecoptera, percent Trichoptera, and percent Ephemeroptera/Plecoptera/Trichoptera. One diversity index that is generally used for ecological studies is the Shannon-Wiener Index. however, this has not been as widely used for monitoring benthic macroinvertebrate insect communities.

The effectiveness of some richness metrics may differ among various regions. For example, Clements and Kiffney (1994) found that, for a metal-polluted stream in Colorado, the Ephemeroptera/Plecoptera/Trichoptera taxa richness metric was relatively insensitive to moderate levels of metal contamination. In other studies, however, that metric was found to be reliable for assessing the impact of stressors (Lenat and Barbour, 1994). Different measures of richness should therefore be evaluated to see which best reflects the impacts being caused by each mine.

The richness metrics, abundance ratio metrics, and the diversity metrics are relatively easy to calculate; however, any number of stressors can cause them to have unusual values. Further, some may not be as sensitive when detecting some forms of stress, particularly organic stressors. Recognizing this, Chutter (1972) developed a Biotic Index, which was modified by Hilsenhoff (1977), to assess the level of organic pollution in Wisconsin streams. The Hilsenhoff Biotic Index has since been used for assessing the influence of both point- and non-point sources of organic stress in many streams. Resh and Jackson (1993) suggested the Hilsenhoff Biotic Index could be used in other areas after modification of faunal tolerance values.

In this study, we evaluated several richness and abundance ratio metrics, the Shannon-Wiener Index, the Hilsenhoff Biotic Index, as well as a new metric, the random runs value, to see how well they reflect the impact that occurs from point-source effluent that flows from the Ferris-Haggarty mine. In addition, this study allowed us to record the composition of benthic insect communities that occur in Haggarty Creek, Battle Creek West Fork, as well as in Big Sandstone Creek. Big Sandstone Creek is a nearby stream that does not receive effluent from the Ferris-Haggarty mine and is considered to be a reference stream. Information about benthic insect communities in the affected reaches of Haggarty Creek will assist biologists when they study the potential of those reaches as habitat for the Colorado River cutthroat trout (Oncorhynchus clarki pleuriticus), which is a "species of special concern" to the Wyoming Department of Game and Fish. Finally, we have developed a set of criteria, which may be useful for evaluating metrics at other sites of pointsource metal contamination.

# 1 Materials and methods

### 1.1 Sampling site selection

Four sampling sites were established at riffles on Haggarty Creek (HC-1, HC-2, HC-3, and HC-4), and one sampling site (HC-5) was located on a riffle that occurs beyond the confluence of Haggarty Creek and Lost Creek (Fig. 1). The first sampling site, HC-1, was situated just upstream from where the effluent flows into Haggarty Creek while the others (HC-2, HC-3, HC-4, and HC-5) were located at various points downstream. Here we note that the site, HC-2, was the nearest site downstream from where effluent flows into and fully mixes with Haggarty creek, and the site, HC-5, was the farthest (located on Battle Creek West Fork). All sites, except the first were situated within the impacted reaches of Haggarty Creek and Battle Creek West Fork. Likewise, five sampling sites were established at riffles on Big Sandstone Creek (BSC-1, BSC-2, BSC-3, BSC-4, and BSC-5) (Fig. 1). These corresponded with identically-numbered sites located on Haggarty Creek and Battle Creek West Fork. All sites were deliberately selected so that the elevations of those on Big Sandstone Creek were similar to their identicallynumbered sites on Haggarty Creek and Battle Creek West Fork. The elevation ranges of the sampling sites on both streams were from 2236 to 2913 meter.

# 1.2 Benthic insect samples

A set of four samples was collected at each site, from each stream, during the summer (from 27 June to 2 July), and autumn (from 27 August to 7 September) of 2001 and 2002. Due to an early (and severe) snowstorm, which occurred in September 2001, samples were not collected from the second site on Big Sandstone Creek. At each site, four transects were visually decided in the field; a

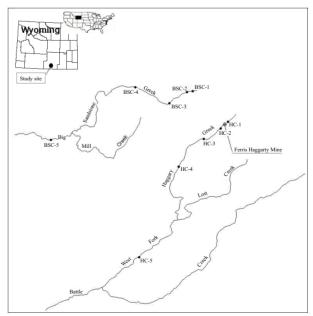


Fig. 1 Map of the sampling sites that were located on Haggarty Creek, Big Sandstone Creek, and Battle Creek West Fork; Sierra Madre Range, Southeast Wyoming.

sampling spot was randomly arranged at the right, middle, or left side in each transect; and one benthic sample was collected using a Surber sampler (Welch, 1948) fitted with a 280- $\mu$ m-mesh net. The samples were collected from downstream to upstream; the sampling sites were arranged from downstream to upstream in a sequence of sampling seasons and years.

Collected samples were washed through a 250-µm sieve at streamside, and then transported in 95% ethanol to the laboratory where they were sorted and preserved in 70% ethanol. Organisms were identified to genus except for those in the family, Chironomidae, which were identified just to family, and those in the order Collembola, which were identified just to order, using taxonomic keys provided by Merritt and Cummins (1996). Identified organisms were then counted and recorded.

#### 1.3 Description of the study sites

Physical and landscape characteristics within both Haggarty and Big Sandstone watersheds were investigated at relatively large spatial scales, ecoregion and Ecological Drainage Unit (EDU). Both Creeks are within the Southern Rockies ecoregion (http://www.pcds.org/share/ap\_envsc/classlabs/useco\_pg.pdf) and the Platte River Basin EUD developed by The Nature Conservancy (unpublished); they are similar in the physical and landscape characteristics based on which ecoregions and EDUs were developed at their individual spatial scales. Other than the Ferris-Haggarty Mine, which is the only known point-pollutant source, both creeks are considered typical for a relatively natural subalpine stream in the Sierra Madre Range of the Rocky Mountains.

On-site observations were conducted to detect human disturbance sources existing within both watersheds. The general descriptions of vegetation and substrate were made at each site, on each stream at the outset of the study. Whenever samples were collected from a site on a stream, certain physical and chemical measures were taken. Including water temperature, pH, dissolved oxygen, conductivity, turbidity, ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, phosphate-phosphorus, total phosphorus, total organic carbon, and total suspended solids. Water temperature, conductivity, and pH were measured using a Yellow Springs Instrument meter, model 50, and nitrate, nitrite, ammonia, total organic carbon, total suspended solids, phosphate, total phosphorous were measured, using the procedures by Greenberg et al. (1992). Measures of heavy metals, including Cu, were reported elsewhere (Rockwell, 2001).

# 1.4 Metrics description

Considering the number of potential biological metrics that can be used in biological monitoring and assessment (Barbour et al., 1995), we chose to use those that are accurately computed, hierarchically sensitive, easily anticipatory, and integrative across stressor gradients (Cairns et al., 1993). We chose not to take functional feeding group metrics in this study due to difficulties in proper assignment of captured benthic insects to functional

feeding groups (Karr and Chu, 1997). Therefore, attention was focused on a set of 12 metrics: Ephemeroptera taxa richness, Plecoptera taxa richness, Trichoptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, total taxa richness, percent Ephemeroptera, percent Plecoptera, percent Trichoptera, percent Ephemeroptera/Plecoptera/Trichoptera, the Shannon-Wiener Index, the Hilsenhoff Biotic Index, and the random runs value. The four percent indicators were calculated based on abundance. The Shannon-Wiener Index was computed using the Equation of:

$$H' = -\sum P_i \times \ln(P_i)$$

where, H' is Index of species diversity,  $P_i$  is the proportional abundance of the *i*th taxon  $(n_i/N)$  (Shannon and Wiener, 1949).

The Hilsenhoff Biotic Index was computed as the average of tolerance values for captured taxa; tolerance values were obtained from the US Environmental Protection Agency (EPA) (http://www.epa.gov/owow/monitoring/rbp/wp61pdf/app\_b. pdf). Finally, the random runs value was regarded as a measure of diversity, which was indicated through preliminary investigations on the relationship between the random runs value and the Shannon-Wiener Index (Wu and Legg, unpublished); it was calculated using the computational procedures described by Wu and Legg (2007).

# 1.5 Criteria for evaluating the metrics

Rockwell (2001) assessed impacts of acid mine drainage on the Haggarty Creek aquatic ecosystem by investigating the bioaccumulation of metals in the benthic food web. His study showed spatial variations of Cu concentrations in water, sediment, aufwuchs, and aquatic vegetation. In addition, as was reported by Meyer et al. (2007), aqueous copper dominated this system and therefore the resulting changes in Haggarty Creek. It also indicated Cu accumulation in all insect trophic groups as well as in representative insect taxa: the closer a downstream sampling station was to the mine, the more Cu concentration and accumulation there were in the insects. Similar spatial gradients in Cu concentrations were observed in other studies (Cain et al., 1992).

Considering the spatial trends of Cu concentration in water and sediment, as well as the accumulation of Cu in benthic fauna, an ideal metric based on captures of benthic insects should be sensitive enough to detect effluent from the Ferris-Haggarty mine. Specifically, the ideal metric should have the following characteristics: (1) exhibit a statistically significant site × stream interaction term in an analysis of variance; (2) exhibit no statistically significant differences among the five sites of Big Sandstone Creek; (3) exhibit some statistically significant differences among some of the sites, HC-1, HC-2, HC-3, HC-4, and HC-5, with the average metric value for the site, HC-1, showing no impact from the effluent, and that site's value being significantly different from the average metric values of sites HC-2, HC-3, HC-4, and HC-5, with sites HC-2

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through HC-5 showing impact; (4) exhibit no statistically significant difference between the control site of Haggarty Creek (i.e., HC-1) and its respective site on the reference stream, Big Sandstone Creek (i.e., BSC-1); (5) exhibit a statistically significant difference between each of the impacted sites (i.e., HC-2, HC-3, HC-4, and HC-5) and their corresponding sites on Big Sandstone Creek (i.e., BSC-2, BSC-3, BSC-4, and BSC-5), and (6) exhibit consistency; i.e., have criteria 1–5 be met in each season of each year.

Metrics were evaluated by noting whether they met a criterion during each season in each year. A tally was then made of criteria that had been met, separately for each metric, to provide for a criterion fulfillment total. These were then used to rank the metrics, with those having the greatest values being most desirable and those having the least values being least desirable.

#### 1.6 Statistical analyses

Values for each metric were analyzed using analysis of variance (ANOVA). Specifically, the linear statistical model had streams as a fixed effect factor, with sites within stream (i.e., the site  $\times$  stream interaction) also being fixed. However, samples within site at a stream were a random effects factor; this was used as the error term for the site  $\times$  stream interaction (P < 0.05). Separate ANOVAs were conducted for each season of each year. Averages for each combination of site and stream were separated using Tukey's HSD (mean separations) (Steel and Torrie, 1980). Statistical calculations were facilitated by use of the GLM

procedure of the Statistical Analysis System (SAS version 8.3, SAS Inc., USA).

#### 2 Results

#### 2.1 Description of the study sites

Haggarty and Big Sandstone Creeks are within mixed conifer/aspen forest and are bordered with riparian and wetland vegetation. Such streams are rather narrow and shallow, and have relatively cool temperatures and low discharge rates. The substrate at all sites on both streams was principally covered by gravels (0.1–25 cm) with some sands (< 0.1 cm) and a few cobbles (> 25 cm). The physical and chemical characteristics of streams are summarized in Tables 1 and 2.

#### 2.2 Insect taxa collected

A total of 74 taxa were collected over both seasons and years from these two streams. These represented 72 genera, 38 families, and 7 orders. Of the 74 taxa, 46 were present in both streams, while 23 were present in Big Sandstone Creek, and 5 were present in Haggarty Creek.

#### 2.3 Assessing the criteria

With regards to the first criterion, P values for the site  $\times$  stream interaction term indicated that all metrics had at least two ANOVAs for which this term was statistically significant. However, five metrics had this term be statis-

 Table 1
 Range of physical and chemical parameters for streams in Haggarty Creek (HC) and Big Sandstone Creek (BSC), Sierra Madre Range,

 Southeast Wyoming

Parameter	Summer 2001		Autumn 2001		Summer 2002		Autumn 2002	
	НС	BSC	НС	BSC	HC	BSC	НС	BSC
Temperature (°C)	8.0–14.5	6.6–14.7	3.4–13.4	6.6–8.9	12.8–15.3	9.2–15.0	7.3–10.3	5.7–10.7
pH	7.11-7.36	7.28-7.67	7.50-8.25	7.86-8.17	7.50-8.12	7.46-7.90	7.32-8.37	7.22-7.79
Dissolved oxygen (mg/L)	8.50-9.10	6.10-8.90	4.65-5.98	4.83-5.60	5.28-5.88	5.48-6.52	6.88-7.33	6.16-7.62
Conductivity (µS/m)	24.3-50.9	30.6-58.1	37.8-274.0	59.3-96.9	30.0-153.0	35.0-70.0	10.3-46.0	41.0-112.0
Turbidity (NTU)	0.43 - 0.90	0.31 - 2.01	0.22 - 0.38	0.46-0.91	0.25 - 0.85	0.48 - 1.45	0.32 - 0.49	0.30-1.38
NH <sub>4</sub> -N (mg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.4	< 0.2	< 0.1	< 0.1
NO <sub>2</sub> -N (mg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
NO <sub>3</sub> -N (mg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
$PO_4$ -P (mg/L)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total P (mg/L)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total suspended solids (mg/L)	< 0.002	< 0.002	< 0.001	< 0.002	< 0.003	< 0.003	< 0.001	< 0.003
Total organic carbon (µg/L)	1.18-2.02	0.62-2.09	0.62-1.23	0.90-1.28	0.80-1.55	0.49-3.02	0.93-1.56	0.72-1.97

Table 2 Physiochemical characteristics of sampling sites located on Haggarty Creek (HC) and Big Sandstone Creek (BSC)

Stream	Site	Stream order	Vegetation	Substrate	Pollution status <sup>a</sup>	Aqueous copperb (ppb)
Haggarty Creek	HC-1 1	1	Forest	Gravel	Non-polluted	< 1 (less than detection limits)
	HC-2	1	Forest	Gravel	Polluted	229
	HC-3	1	Forest	Gravel	Polluted	78
	HC-4	1	Forest	Gravel	Polluted	3
	HC-5	2	Forest/shrub	Gravel	Polluted	
	BSC-1	1	Forest	Gravel	Non-polluted	
Big Sandstone Creek	BSC-2	1	Forest	Gravel	Non-polluted	
	BSC-3	1	Forest	Gravel	Non-polluted	
	BSC-4	1	Forest	Gravel	Non-polluted	
	BSC-5	2	Forest/shrub	Gravel	Non-polluted	6

<sup>&</sup>lt;sup>a</sup> URL: http://deq.state.wy.us/wqd/watershed/downloads/305b/2006/2006%20303d.pdf; <sup>b</sup> Rockwell, 2001.

Table 3 The P values for the site × stream interaction term of the analysis of variance (ANOVA), which was conducted separately for each metric, each season, and each year in Sierra Madre Range, Southeast Wyoming

Metric	Summer 2001	Autumn 2001	Summer 2002	Autumn 2002
ETR	0.0155*	0.0203*	0.0127*	0.0001*
PTR	0.0382*	0.0001*	0.0097*	0.0004*
TTR	0.1121	0.0261*	0.8589	0.0392*
EPTTR	0.0185*	0.0001*	0.0004*	0.0010*
TotTR	0.0857	0.0001*	0.0132*	0.0001*
SWI	0.0110*	0.0042*	0.0138*	0.0001*
Ephemeroptera (%)	0.1447	0.0001*	0.0027*	0.0009*
Plecoptera (%)	0.1165	0.0067*	0.0201*	0.2336
Trichoptera (%)	0.4078	0.0007*	0.0501	0.0003*
EPT (%)	0.0559	0.0024*	0.1601	0.0025*
HBI	0.0272*	0.0005*	0.0857	0.0069*
RRV	0.0100*	0.0335*	0.0023*	0.0027*

ETR: Ephemeroptera taxa richness, PTR: Plecoptera taxa richness, TTR: Trichoptera taxa richness, EPTTR: Ephemeroptera/Plecoptera/Trichoptera taxa richness, TotTR: total taxa richness, SWI: Shannon-Wiener Index, HBI: Hilsenhoff Biotic Index, RRV: random runs value. \* Indicates statistical significance ( $\alpha = 0.05$ ).

tically significant throughout the study (Table 3).

Assessments of criteria 2 through 5 were made using the mean separations depicted in Figs. 2a-d (Random Runs Value, Shannon-Wiener Index, Trichoptera taxa richness, and Ephemeroptera taxa richness were selected). From these, we constructed Table 4, which identifies as well as summarizes which criteria were met for each metric in each season of each year. From this table we determined that the first two criteria (having a significant site × stream interaction term and there being no differences among the sites of Big Sandstone Creek) were somewhat useful for helping us discriminate among the metrics, while the 3rd and 5th criteria (having statistically significant differences among some sites of Haggarty Creek and having differences occur between each of the impacted sites on Haggarty Creek and Battle Creek West Fork and their corresponding sites on Big Sandstone Creek) were very useful for us to discriminate among the metrics. Conversely, the 4th criterion (that the control site of Haggarty Creek is the same as and its respective site on Big Sandstone Creek) was met by each metric in at least three of four season/year combinations, and the 6th criterion (that all criteria were met over all seasons and years) was met by none of the metrics. These were not useful for helping us discriminate among the metrics. They were, however, useful for evaluating each metric's overall sensitivity to effluent from Haggarty Creek.

# 2.4 Evaluating the metrics

When interpreting the criterion fulfillment totals and metric ranks (Table 4), we created some evaluation categories. Specifically, we considered metrics to be 'sensitive' to a criterion if it met that criterion in at least three of the season/year combinations. Metrics were considered to be moderately sensitive if they met that criterion in just two of the season/year combinations. Metrics were considered to be insensitive to a criterion if they met that criterion just once, or not at all.

Metric rankings indicated that both the Shannon-Wiener Index and the random runs value were most desirable, with the Shannon-Wiener Index being sensitive to all criteria, and the random runs value being sensitive to all criteria except the 3rd, to which it was moderately sensitive. These rankings were followed closely by the ranking of Ephemeroptera taxa richness, which was sensitive to all criteria except those of the third and fifth, to which it was moderately sensitive.

Over the metrics for Ephemeroptera/Plecoptera/Trichoptera taxa richness. total taxa richness, and Plecoptera taxa richness

Table 4 Assessment criteria that each metric met, the total criteria that were met, and each metric's rank (1 means the highest)\*

Metric		Total number of	Rank			
	Summer 2001	Autumn 2001	Summer 2002	Autumn 2002	criteria met	
SWI	1, 2, 4	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 3, 5	16	1
RRV	1, 2, 4	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 5	16	1
ETR	1, 2, 4	1, 2, 4	1, 2, 3, 4, 5	1, 3, 4, 5	15	3
EPTTR	1, 2, 4	1, 3, 4, 5	1, 3, 5	1, 4, 5	13	4
TotTR	2, 4	1, 4, 5,	1, 2, 4, 5	1, 4, 5	12	5
PTR	1, 2, 4	1, 3, 5	1, 4	1, 3, 4	11	6
HBI	1, 2, 4	1, 2, 4	2, 4	1, 2, 4	11	6
EPT%	2, 4	1, 2, 4	2, 4	1, 2, 4	10	8
Plecoptera%	2, 4	1, 3, 4	1, 2, 4	2, 4	10	8
Ephemeroptera%	2, 4	1, 4	1, 2, 4	1, 4	9	10
Trichoptera%	2, 4	1, 2, 4	2, 4	1, 4	9	10
TTR	2	1, 4	2, 4	1, 4, 5	8	12

SWI, ETR, EPTTR, TotTR, PTR, HBI, TTR are the same as those in Table 3.

<sup>\*</sup> Groups of numbers for a season/year/metric combination indicate which specific objectives were met; metrics associated with the greatest number of objectives met were the most desired for bio-monitoring purposes.

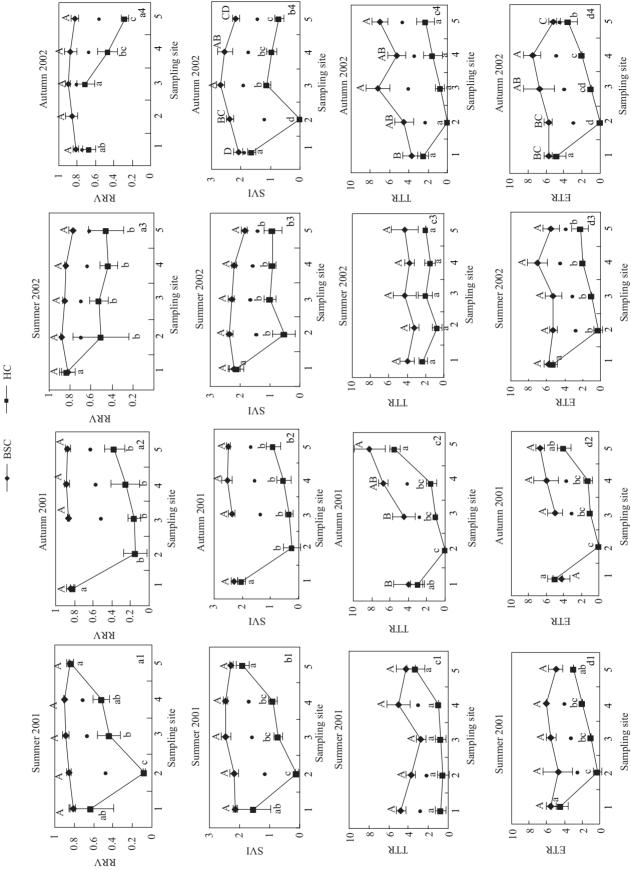


Fig. 2 Comparison of average values with standard deviation for the random runs value (RRV) (a), the Shannon-Wiener index (SWI) (b), Trichoptera taxa richness (TTR) (c), and Ephemeroptera taxa richness (ETR) (d) among sites on Haggarty Creek (HC) and among sites on Big Sandstone Creek (BSC), during the summer and autumn of 2001 and 2002. Average values for Big Sandstone Creek with the same upper case letters were not significantly different. Average values for Haggarty Creek with the same lower case letters were not significantly different. Dots indicate significant differences between the two streams at a site, Tukey's HSD ( $\alpha = 0.05$ ).

appeared to be somewhat less sensitive to the first five criteria. More specifically, these metrics were sensitive to the first criterion, with both the Ephemeroptera/Plecoptera/Trichoptera taxa richness and total taxa richness metrics being sensitive to the 5th criterion. However, these metrics were either moderately sensitive, or insensitive to the 2nd and 3rd criteria (Table 4).

The remaining metrics, which included the Hilsenhoff Biotic Index, the abundance ratios, and Trichoptera taxa richness, were less desirable as they were sensitive to some criteria and insensitive to others (e.g., the Hilsenhoff Biotic Index) or generally insensitive to criteria throughout the study (e.g., Trichoptera taxa richness). These outcomes were reflected in their mediocre to low rankings (Table 4).

#### 3 Discussion

#### 3.1 Criteria and metrics

Criteria used in this study were established based on three central concepts (Butcher et al., 2003). First, they represented appropriate regionalization (US EPA, 2007). Second, they represented a multi-metric approach to assess lotic system health (Kenney et al., 2009). Third, they made use of reference conditions (Stoddard et al., 2006).

Outcomes of this study indicated that none of the metrics were ideal; that is to say, each was insensitive to at least one of the criteria. This was anticipated as it is generally thought that there is no single metric that is perfect; hence the need for multiple metrics. In addition, the outcomes showed that the performance of some metrics varied considerably at the temporal and spatial scale, which again justifies the need for using multiple metrics to assess benthic insect communities.

The inconsistency of metrics among seasons and years can rise from temporal differences in the physical and chemical factors of stream ecosystems (Šporka et al., 2006; Clements et al., 2010) and/or climate change (Lawrence et al., 2010). Meyer et al. (2007) indicated that aqueous copper from all three sites on Haggarty Creek demonstrated seasonal trends in which aqueous Cu decreased with time from July to September. In addition, temporal variation of macroinvertebrate life-history characteristics may also affect abundance, diversity and biomass of benthic fauna. Hutchens et al. (1998) indicated that temporal variation of both macroinvertebrate abundance and biomass increased with longer juvenile development times. More recently, Resh et al. (2005) explained temporal rarity or commonness of some taxa using species traits, and Resh and Rosenberg (2010) summarized the relationship between life-history characteristics of benthic macroinvertebrates and bioassessment. Comparing the temporal inconsistency, some metrics indicated considerable spatial gradients among the sampling sites.

Through the evaluation process, the Shannon-Wiener index, the random runs value, and Ephemeroptera taxa richness, appeared to be quite useful for reflecting the impacts that have occurred in Haggarty Creek and Battle

Creek West Fork due to effluent from the Ferris-Haggarty mine. In addition, Ephemeroptera/Plecoptera/Trichoptera taxa richness, total taxa richness, and Plecoptera taxa richness, while not being as strong as the previous three but highly correlated with each other (unpresented result), were nonetheless deemed useful for reflecting the impacts that have occurred in Haggarty Creek and Battle Creek West Fork.

Use of these six metrics among between streams generally showed that the sites, HC-2, HC-3, HC-4, and HC-5 were impacted. This is consistent with each of those reaches being identified in the year 2006 listing Wyoming 303d Impaired Waters and Impairments (http://deq.state.wy.us/wqd/watershed/downloads/305b/ 2006/2006%20303d.pdf). In addition, use of these six metrics generally showed that the control site for Haggarty Creek (HC-1) was the same as its companion site on Big Sandstone Creek (BSC-1). These results, however, were not consistent. For example, some of these metrics had significant differences between HC-1 and BSC-1 (e.g., Fig. 2a, autumn of 2002, the random runs value), and some did not show significant differences between HC-5 and BSC-5 (e.g., Fig. 2b, summer and autumn of 2001, the Shannon-Wiener Index). Further, others at times did not show a distinct difference between the control site on Haggarty Creek (i.e., HC-1) and each of the impacted sites on the same stream (e.g., Fig. 2c, summer 2001, summer and autumn 2002, Trichoptera taxa richness).

The outcome of no significant differences occurring at times between HC-5 and BSC-5 may be explained by lower aqueous and sediment copper concentrations occurring at HC-5, as compared with values that have been reported from the sites, HC-4, HC-3, and HC-2 by Rockwell (2001). Similar observations of 'graded' copper values occurring further downstream from a point source have been observed in other studies (Cain et al., 1992), with the highest concentrations occurring at locations closest to the source of contamination. Clements et al. (2000) demonstrated that none of the community measures and none of the 16 dominant taxa they used were significantly lower at low-metal sites, even though some appeared to respond to low levels of metal contamination. Evidently, therefore, some organisms at the site, HC-5, which was furthest from the point where effluent enters Haggarty Creek, were indeed affected by effluent from the Ferris-Haggarty mine, but they were not as affected as those that inhabited the other impacted sites on Haggarty Creek. This phenomenon can be seen in the 'graded' results that often occurred in mean separations of sites HC-2 through HC-5 (e.g., Fig. 2d, autumn of 2002, Ephemeroptera taxa richness).

# 3.2 Responses of benthic insects to effluent from the Ferris-Haggarty mine

Many metrics of benthic macroinvertebrates have been used as biological indicators of stressors. However, due to the presence of different benthic insects in different lotic systems over various regions, the usefulness of each can

vary in its appropriateness for biological investigations and monitoring. Therefore, it is prudent to evaluate a suite of metrics to determine their usefulness as biological indicators in each region or, when appropriate, in given

Some studies have shown that four of the six metrics that we have found suitable for assessing the impacts of effluent from the Ferris-Haggarty mine, i.e., Ephemeroptera taxa richness, Plecoptera taxa richness, Ephemeroptera/Plecoptera/Trichoptera taxa richness, and total taxa richness, were quite sensitive to disturbances in other aquatic ecosystems (Kiffney and Clements, 1996; IIwasaki et al., 2009). Other studies, however, have not. For example, Clements and Kiffney (1994), who studied a metal-impacted stream in Colorado, showed that Ephemeroptera/Plecoptera/Trichoptera taxa richness was relatively insensitive to moderate levels of metal contamination because metal-sensitive mayflies were replaced by metal-tolerant caddisflies at the polluted sites. In addition, taxa of Plecoptera and Trichoptera that were previously known to be relatively intolerant to heavy metals (Kiffney and Clements, 1996) were observed being unaffected by heavy metals (Clements, 1999). It appears that such contradictory findings may occur either from the replacement of intolerant taxa by metal-tolerant species (Millward and Grant, 1995) or from taxa adaptation to specific pollutants if such taxa reside in chronic levels of contaminants for generations.

With regards to the abundance ratios, some studies have found that certain abundance ratios were sensitive to aquatic ecosystem disturbances (Hannaford and Resh, 1995). For example, percent Ephemeroptera abundance was one of the most sensitive composition metrics in an integrative assessment of a watershed impacted by acid mine drainage (Soucek et al., 2000). Further, Butcher et al. (2003) indicated that percent Trichoptera abundance was one of ten metrics that satisfied the metric selection criteria in one study for streams in the Northern Lakes and Forest Ecoregion. However, such was not the case for effluent flowing from the Ferris-Haggarty mine into Haggarty Creek. This was so because the abundance ratios were quite variable among the five sites of both Haggarty and Big Sandstone Creeks, suggesting the occurrence of localized phenomena. Such variability was most evident for percent Ephemeroptera at the sites, HC-3 and HC-4 with most captured Ephemeroptera being of the genus, Beatis. Another localized phenomenon occurred for percent Trichoptera, which was caused by a large number being captured at the sites, HC-5 and BSC-5. Given these localized phenomena, it is not clear how effective these abundance ratio metrics might be for detecting the impact that is caused by effluent from the Ferris-Haggarty mine.

The Shannon-Wiener Index (Shannon and Wiener, 1949) is a diversity index that was adopted from information theory. Its use is increasingly more common for measuring the effect of stressors on community structure. The Shannon-Wiener Index does not just take into account both the number of taxa present as well as the number of individuals in each taxa. Theoretically, when aquatic insects are stressed, the number of taxa is reduced and the number of individuals is changed, resulting in a lower value for the index. In general, the Shannon-Wiener Index has not been commonly used for assessing the effects of stressors in aquatic ecosystems. This is so because several studies, like that of Pontasch and Brusven (1988), have indicated that the Shannon-Wiener Index failed to reflect the major differences that occurred, in absolute species abundances, between the reference and impacted sites when assessing macroinvertebrate recovery following a gasoline spill. Others have found the Shannon-Wiener Index to be unacceptable in quantifying benthic macroinvertebrate response to pollution (Murphy, 1978; Perkins, 1983). In this study, however, we found the Shannon-Wiener Index to be sensitive to impacts caused by effluent flowing from the Ferris-Haggarty mine.

The Hilsenhoff Biotic Index has been used to determine the effects of organic pollution on benthic macroinvertebrates in many lotic systems. In our study, however, the Hilsenhoff Biotic Index was inconsistent, particularly with respect to the first criterion (i.e., the site × stream interaction being statistically significant). Therefore, the Hilsenhoff Biotic Index may be inappropriate for detecting impacts caused by effluent from the Ferris-Haggarty mine.

Failure of this metric to reflect those impacts indicates that use of the current EPA-Idaho tolerance values, which were assigned to each of the taxa for the purpose of detecting organic forms of pollution, may not be appropriate for detecting aqueous Cu pollution in Haggarty Creek. In the future, if some form of the Hilsenhoff Biotic Index should be used at Haggarty Creek, modified tolerance values must be employed. The creation of these values may require verification through an experimental approach (Clements et al., 1992). Doing so would avoid subjective assignment of tolerance value scores (Hickey and Clements, 1998).

The random runs value is, in one way, similar to the sequential comparison index of Cairns et al. (1968), which was based on a sign test and founded on the theory of runs (Dixon and Massey, 1951). However, it differs from the sequential comparison index in many ways, the most important of which is that it has a known level of taxonomic identification.

When calculating the random runs value, we noticed that when there was just one insect in the sample, we obtained a spurious value of 1.0; this falsely suggested a high level of diversity. Therefore, we did not calculate a random runs value for samples that contained just one insect. This happened most frequently at the heavily impacted site, HC-2. Likewise, we did not calculate a random runs value for samples containing no insects. Over all, the number of samples containing no or just one insect was small, occurring in just 6 of 156 total samples.

In summary, we observed different levels of sensitivity among 12 metrics for their abilities to detect impacts that are known to occur in the upper reaches of Haggarty Creek and Battle Creek West Fork. Specifically, six of those metrics: (1) the Shannon-Wiener index, (2) the random runs value, (3) Ephemeroptera taxa richness, (4) Ephemeroptera/Plecoptera/Trichoptera taxa richness,

(5) total taxa richness, and (6) Plecoptera taxa richness demonstrated enough consistency, with respect to our selection criteria, to be considered in a Haggarty Creek/Battle Creek West Fork monitoring program. In contrast, all abundance ratios, the Hilsenhoff Biotic Index, and Trichoptera taxa richness were too variable to be considered for such a program. Finally, this study provided information on benthic insect communities that occur in the impacted reaches of Haggarty Creek. Such information will be useful for evaluating the potential of those reaches as habitat for the Colorado River cutthroat trout, which occupies less than 1% of its former range (Behnke and Zarn, 1976).

As effluent that flows from various mines affects streams in different ways, depending on the chemical/physical properties of the effluent, the chemical/physical/biological properties of the stream that is being influenced, and other geological/hydrological characteristics of the streams in question, these findings should be used for assessing the influenced reaches of Haggarty Creek, Wyoming. A similar approach may be used for developing criteria to assess mine-influenced reaches of other streams both in the USA and elsewhere, but the metrics will not necessarily be the same owing to differing characteristics of mine effluent, water chemistry, and species that are present in those affected reaches.

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