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Recovery of a mining-damaged stream ecosystem

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Abstract

This paper presents a 30+ year record of changes in benthic macroinvertebrate communities and fish populations associated with improving water quality in mining-influenced streams. Panther Creek, a tributary to the Salmon River in central Idaho, USA suffered intensive damage from mining and milling operations at the Blackbird Mine that released copper (Cu), arsenic (As), and cobalt (Co) into tributaries. From the 1960s through the 1980s, no fish and few aquatic invertebrates could be found in 40 km of mine-affected reaches of Panther Creek downstream of the metals contaminated tributaries, Blackbird and Big Deer Creeks.

Efforts to restore water quality began in 1995, and by 2002 Cu levels had been reduced by about 90%, with incremental declines since. Rainbow Trout (*Oncorhynchus mykiss*) were early colonizers, quickly expanding their range as areas became habitable when Cu concentrations dropped below about 3X the U.S. Environmental Protection Agency's biotic ligand model (BLM) based chronic aquatic life criterion. Anadromous Chinook Salmon (*O. tshawytscha)* and steelhead (*O. mykiss*) have also reoccupied Panther Creek. Full recovery of salmonid populations occurred within about 12-years after the onset of restoration efforts and about 4-years after the Cu chronic criteria had mostly been met, with recovery interpreted as similarity in densities, biomass, year class strength, and condition factors between reference sites and mining-influenced sites. Shorthead Sculpin (*Cottus confusus*) were slower than salmonids to disperse and colonize. While benthic macroinvertebrate biomass has increased, species richness has plateaued at about 70 to 90% of reference despite the Cu criterion having been met for several years. Different invertebrate taxa had distinctly different recovery trajectories. Among the slowest taxa to recover were *Ephemerella*, *Cinygmula* and *Rhithrogena* mayflies, Enchytraeidae oligochaetes, and *Heterlimnius* aquatic beetles. Potential reasons for the failure of some invertebrate taxa to recover include competition, and high sensitivity to Co and Cu.

1. Introduction

The ecological impairment of lotic environments by metal mine contamination is a longstanding problem that has occurred in many areas (Woody et al., 2010; Byrne et al., 2012; Hogsden and Harding, 2012). In the USA, at least 156 hard-rock mining sites requiring restoration have been inventoried and could cost as much as $24 billion USD to address (Gustavson et al., 2007). Yet while river restoration has become a >$1 billion USD per year industry, its practice has been severely criticized for lacking scientific rigor and assessment (Bernhardt et al., 2005; Palmer et al., 2005; Lake et al., 2007; Palmer, 2009). While many of these criticisms are directed towards projects seeking to restore physical habitats, linking water quality restoration efforts to ecosystem responses has also been difficult (Harris, 2012). With some notable exceptions such as Adams et al. (2002) (Tennessee), Clements et al. (2010) (Colorado), and Murphy et al. (2014) (UK), the effectiveness of interventions designed to improve freshwater environments have been unclear because of projects that were based more on faith than science and with a lack of rigorous effectiveness monitoring (Bernhardt et al., 2005; Hilderbrand et al., 2005; Jähnig et al., 2011). A counterpart to this situation is that environmental assessment or cleanup projects in the USA tend to produce massive reports that may be data-rich, but difficult to access and in print formats that are difficult to extract data from. These factors may lead to a "data-rich and information-poor syndrome" (Ward et al., 1986; Gustavson et al., 2007).

The recovery of aquatic ecosystems can be controversial to even define (Ormerod, 2003). Interpreting recovery from metals pollution must draw upon the science of diverse practices including ecotoxicology, geochemistry, stream ecology, monitoring, and fisheries. The concept of recovery at least implicitly relies on underlying ecological concepts and assumptions relating to natural variability, disturbance, dispersal, and succession (Connell and Slatyer, 1977; Fisher, 1990; Palmer et al., 1997; Parker and Wiens, 2005; Lake et al., 2007). Many ecological questions arise when interpreting recovery in streams. For instance, after a long-term ("press") disturbance from metal contamination is relaxed, will the stream community reassemble itself similarly to nearby reference areas? With water quality restoration efforts driven by regulatory criteria, a key assumption is that numeric chemical criteria represent necessary and sufficient thresholds for ecological recovery. This leads to the question, as metals pollution declines, at what thresholds do organisms' internal limiting factors (physiological tolerance) give way to external limiting factors such as dispersal from colonist pools and biological tolerance or inhibition by early colonists of later arrivals? These concepts and questions give context to interpreting biological responses following water quality restoration efforts.

These factors are among the motivations for our present article. Over a 30+ year record, we examine changes in stream communities associated with declines in metals contamination from an inactive hard-rock mine in Idaho, USA. Our objectives include (1) assessing the effectiveness of water quality restoration efforts in reducing contamination in different stream media (water, sediment, periphyton, and macroinvertebrate tissues), (2) examining differing recovery trajectories for stream invertebrate and fish communities in response to improving water quality, (3) identifying apparent field thresholds for recovery of different taxa, and (4), considering whether the "recovering" stream ecosystems are "recovered."

1.1 Study area

The Blackbird Mine was a cobalt (Co) and copper (Cu) producer that operated from about 1948 to 1967. The mine is located on a high divide and flows south and north to the Blackbird Creek and Big Deer Creek drainages, respectively. To the south, mine drainage enters Blackbird Creek, a steep 2nd order stream which flows for about 10 km before reaching Panther Creek, a 4th order stream. To the north, the Blackbird Mine forms the headwaters of Bucktail Creek which flows to Panther Creek via the South Fork Big Deer Creek and Big Deer Creek (Figure 1, Figure S1). Blackbird and Big Deer Creeks each contribute about 12 to 13% of the streamflow in Panther Creek, as calculated immediately below their respective confluences (U.S. Geological Survey, 2012). Panther Creek is a tributary of the Salmon River, Idaho, USA and eventually drains to the Pacific Ocean about 1160 km downstream of the mouth of Panther Creek. Other ecologically important natural features include a series of waterfalls and cascades on Big Deer Creek situated about 1 km upstream of its mouth which prevents upstream passage of fish from Panther Creek, and several debris jams in the South Fork Big Deer Creek which impede upstream fish passage from Big Deer Creek.

The ore body consisted of the mineral cobaltite with equal portions of arsenic (As) and Co and lesser amounts of Cu. Ore was excavated from both open pit and underground operations and was processed into concentrate on-site. Mill effluents, which were highly enriched with Cu, Co, As, and iron (Fe) were run through a pipeline for about 5 km to a tailings dam and pond in the lower West Fork Blackbird Creek drainage where they were decanted. However, reagent spills, icing, pipeline breaks, and bypasses were frequent and the tailings frequently entered Blackbird Creek, and were transported downstream to Panther Creek. Acid mine drainage developed in both underground workings and waste rock dumps, which flowed to both the Big Deer Creek and Blackbird Creek drainages (Mebane, 1994; Gray and Eppinger, 2012).

Prior to mine development, Panther Creek supported abundant populations of anadromous Chinook Salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss)*. By the mid-1950s, the salmon were in decline in Panther Creek as water quality problems worsened, and no spawning redds were observed after 1962 during annual aerial surveys of index reaches. Electrofishing surveys in 1967 and 1980 found no fish in Panther Creek downstream of Blackbird and Big Deer Creeks, and close to 100% mortalities occurred during short-term caged fish tests conducted at both locations in 1985. Unsuccessful reintroduction efforts of steelhead and Chinook Salmon were made in the 1970s and 1980s (Mebane, 1994). In September 2001, about 1053 adult hatchery-origin Chinook Salmon were released in Panther Creek (Smith et al., 2012).

Concerted efforts to restore water quality began in 1995. The efforts included a variety of measures to divert clean water around disturbed areas, and to intercept, collect, and treat contaminated water. Measures included relocation and containment of mine waste and sediment, construction of reservoirs, water and sediment control structures, and tunneling through the mountain to re-purpose the mine workings to capture and convey mine water to a water treatment plant. (USEPA, 2003, 2013). In addition to the water quality restoration, interim losses of ecosystem services (natural resource damages) were compensated for by off-site habitat improvement projects (Chapman and Julius, 2005).

Other than chemical water quality, the watershed was largely free from disturbances that constrain recovery. The watershed is lightly populated, with anthropogenic disturbances other than mining mostly limited to a

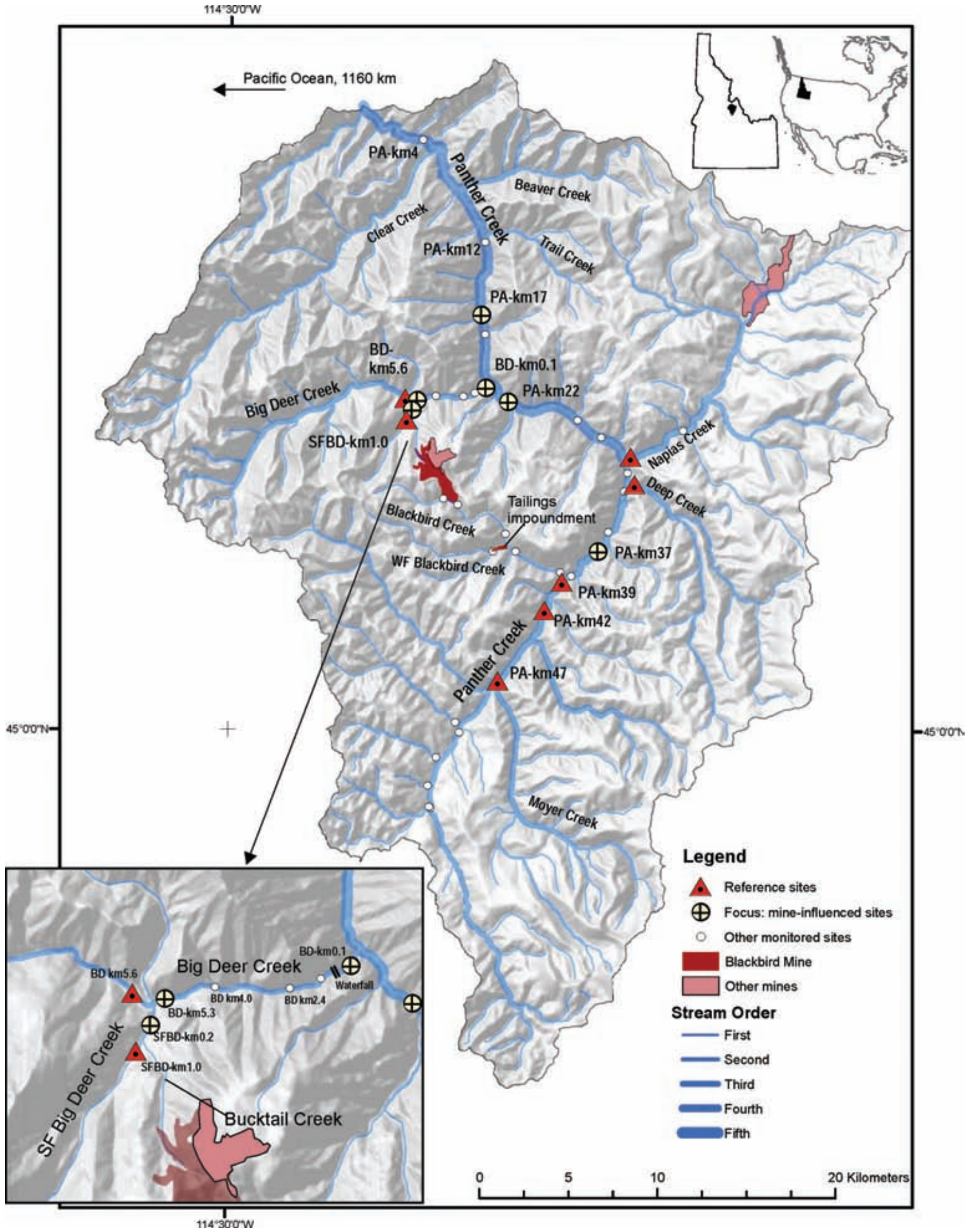

Figure 1

Panther Creek study area, Idaho.

"Focus: mine-influenced sites" are those sites on which we focus our analyses on here. The mining-affected areas are approximate and were traced from satellite imagery.

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network of forest roads and seasonal cattle grazing in the upper watershed. Thus stream hydrology, channel morphology and riparian zones are largely intact and upstream reaches and tributaries provide water quality refugia and colonizing sources of invertebrates and fish. The four mining-affected tributaries (Big Deer Creek, South Fork Big Deer Creek, Blackbird Creek, and the West Fork Blackbird Creek), have their headwaters in near pristine, roadless watersheds (Figure 1). Two other mines are located within the Panther Creek watershed (Figure 1), although as of 2013 they had not noticeably affected the water quality in Panther Creek (Text S2).

In 2000, a large forest fire burned about 83 km^2 in the watershed (Eppinger et al., 2003). Fire intensity was high in the Big Deer Creek drainage, with almost the entire riparian forest canopy burning at both reference and mining-influenced assessment sites. A large debris flow from the Clear Creek drainage near stream km 4, temporarily dammed Panther Creek, realigned the channel, and filled pools. More information on the fire related disturbances is given in Text S2 and Figure S3.

Previous investigations include a synthesis of chemical and biological surveys through the early 1990s (Mebane, 1994), geochemical studies (Mok and Wai,1989; MacRae et al., 1999; Gray and Eppinger, 2012), toxicity testing of Cu and Co in laboratory waters intended to reflect stream water characteristics (Marr et al., 1996, 1998, 1999), avoidance and olfactory toxicity testing of Cu and Co to salmonids (Hansen et al., 1999), toxicity testing of Co in Panther Creek water (Pacific EcoRisk, 2005), toxicity testing of sediments (Mebane, 1994), and field surveys of macroinvertebrate communities and salmonid populations (LeJeune et al., 1995; Beltman et al., 1999), in addition to many unpublished reports.

2. Methods

Evaluating "recovery" requires defining of the concepts of recovery and restoration in the context of stream ecology and water pollution. We consider a "recovered" ecosystem to be unconstrained by chemical disturbance and similar in community composition to what would be expected in natural settings, absent mine-drainage pollution.

In short, our assessment of recovery followed a multi-year, before-after-control-impact (BACI) design that incorporates spatial and temporal variability, where samples were collected concurrently at both the "impact" and "control" (reference) sites in an annual time-series before and after the perturbation (Stewart-Oaten et al., 1992; Parker and Wiens, 2005). In a turn from usual BACI designs, in our study the "impacts" are the imposition of pollution controls, and the "before" conditions are the degraded conditions that persisted for the prior ~50 years. Because no single measure of metals exposure or biological response is likely adequate to indicate recovery (e.g., Niemi et al., 1993; Adams et al., 2002), we collected a suite of exposure and response endpoints. We evaluated chemical recovery of the streams by comparing chemical measures against numeric guidelines, and biological recovery using various macroinvertebrate and fish condition, population, and community level biological metrics (Table 1).

Biological metrics from mining-influenced streams were evaluated relative to concurrently sampled reference sites. Expressing biological metrics as a proportion of concurrently sampled reference sites has two purposes. First, because both reference and mining-influenced sites were affected by the 2000 wildfire, and because regional factors such as weather and hydrologic pattern similarly affect both reference and mining-influenced sites, natural temporal variability should be dampened. Second, prior to the 2002–2013 period, datasets were collected with different sampling and analysis methods. While different sampling and taxonomic efforts prevent direct data comparisons across studies, normalizing samples from mining-influenced sites to values collected from concurrent reference sites puts all data on the same scale. Normalizing data from mining-influenced sites against concurrent reference sites allowed us to extend our record across the older datasets, which has often been a limitation in "long-term" aquatic ecology studies (Jackson and Füreder, 2006).

Project-specific biological recovery goals for Panther Creek were to "restore and maintain water quality and aquatic biota conditions capable of supporting all life stages of resident and anadromous salmonids and other fishes." The recovery goals for Big Deer Creek and South Fork Big Creek were similar, but for Blackbird Creek these goals were considered unattainable for the foreseeable future. Instead, a more limited recovery goal for Blackbird Creek was that water quality could be improved "such that cleanup levels are not exceeded in Panther Creek and to support some aquatic life in Blackbird Creek" (USEPA, 2003).

Table 1. Conceptual framework for monitoring recovering stream communities subject to metals stress

Endpoint	Expected response to reductions in metals stress
Benthic macroinvertebrate community	
Species richness	Increase. As concentrations decline, metals-sensitive taxa will expand their ranges and reoccupy habitats (Raddum and Fjellheim, 2003). Although simple and non-specific, species richness is often one of the most sensitive responses to many environmental stressors (Niemi et al., 1993)
Biomass	Variable. Under severe metals stress, species shifts to dominance by small-bodied taxa such as midges or aquatic mites would result in low biomass even though total abundance was high (Beltman et al., 1999; Hogsden and Harding,2012).
Mayfly and stonefly abundance	Increase. Most mayflies and some stoneflies are metals-sensitive (Clements et al., 2000; Hogsden and Harding, 2012).
Specific taxa	Variable. Within groups thought to be generally metals sensitive or resistant, specific taxa may differ in response (Courtney and Clements, 2002). Metals resistant taxa may decline with competition as metals-sensitive taxa recover.
Fish populations	
Species richness	Increase. In open-systems, large-bodied, more motile species such as salmonids will recolonize newly suitable habitats sooner than small-bodied fish with higher site fidelity (Gibbons et al., 1998; Milner et al., 2008).
Population size	Increase. In stressed systems, abundance may be limited by recruitment failure or indirectly from reduced prey base (Munkittrick and Dixon, 1989; Power, 1997; Milner et al., 2003).
Age structure	Shift toward younger. Natural fish populations are typically age-structured and numbers decrease with age in the total population. Selective mortality of sensitive fry may lead to recruitment failure in metal stressed populations, causing a shift to older fish (Schindler et al., 1985; Munkittrick and Dixon, 1989; Campbell et al., 2003; Milner et al., 2003).
Condition factor	Increase. Fish may have decreased growth or condition in metals-stressed systems from energy requirements of detoxification or food limitation/prey shifting. Condition factor and size are surrogates for energy reserves for overwinter survival and reproductive fitness (Munkittrick and Dixon, 1989; Farag et al., 1995; Cunjak et al., 1998; Campbell et al., 2003).

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We further explain our concepts for evaluating recovery in the context of the expected natural template for the affected streams, ecological assumptions, interpretations of chemical guidelines and project-specific goals in Text S2.

2.1 Sampling and analysis methods

A synthesis of data collected by different investigators over a 30+ period necessarily involves non-identical sampling and analyses, and details were sometimes sparse for older data. Our analyses center on the 2002–2013 time-series monitoring that repeatedly sampled the same locations at the same time of year (mid–September) using identical protocols, with continuity in field crews. Specific data sources and summaries are included in online supplemental datasets.

Site selection and sampling and analysis methods details are described in more detail in Text S2. In brief, all metals data in water are for "dissolved" fraction analyzed after 0.45 µm filtration. Water sampling effort varied over the years with effort biased toward the more variable spring runoff period. Since 2004, about 40–60 dissolved Co and Cu samples were collected per site per year, among other analytes (Table 2; Mebane et al., 2015). Metals in sediment were from the <2mm fraction from surficial samples, metals in periphyton (also referred to as biofilms or aufwuchs) were collected from rock scrapings, and metals in macroinvertebrate tissues were collected as a composite of species and sizes that were intended to represent the prevailing benthic community at each site. In 2012, additional collections targeted specific species. Hydrospychid caddisflies were targeted for Cu because they have been recommended as an indicator taxa for interpreting metals in tissues of aquatic insects (Rainbow et al., 2012). Hydrospychid caddisflies and two stonefly taxa at different trophic levels were also targeted for As speciation analyses in 2012 because of reports linking dietary exposure of inorganic As to reduced growth of Rainbow Trout (Erickson et al., 2010).

Benthic macroinvertebrate samples were all collected from riffle habitats using fixed area, substrate disturbance methods. In a survey undertaken in 1993 prior to water-quality restoration efforts, fish population counts were made by direct observation by snorkelers, but subsequent fish community surveys were conducted through electrofishing using blocknets and multiple-pass depletion. All fish were weighed and measured to enable calculation of fish condition, which is a measure of both individual and cohort (e.g., age- or size-group) wellness and is expected to decline under stress (Munkittrick and Dixon, 1989).

Methods, rationale, and study sites are described in more detail in Text S2.

2.2 Data analyses

Copper concentrations in water were evaluated across years in comparison to USEPA's (2007) biotic-ligand model (BLM) based aquatic life criteria. In this model, predicted toxicity is primarily a function of pH and dissolved organic carbon (DOC). Alkalinity, calcium (Ca), sodium (Na), magnesium (Mg), sulfate, and chloride are also included, but are less influential than DOC and pH. The BLM-based Cu criteria values tend to be higher during spring than baseflow conditions because streams tend to have elevated DOC during snowmelt runoff. For time periods without sufficient BLM-data being available (DOC, pH, etc.), we made conservative seasonal estimates of the BLM-based criteria of 5.3 and 2.3 µg/L for spring and near baseflow conditions respectively. No national or state criteria for Co have been established in the USA. We show Co concentrations in relation to both a site-specific chronic Co target of 86 µg/L and Environment Canada's (2013) guideline of 2.5 µg/L Co for freshwater habitats. Additional information on numeric, chemical-specific guidelines and calculations is given in the Text S2, and in the online data sets (Mebane et al., 2015).

To standardize comparisons across biological data, downstream measurements were generally calculated as a proportion of the concurrent reference value, which makes the reference condition always equal to 1. Where more than one reference site was concurrently monitored (e.g., Panther Creek from 2002–2013), reference site data were pooled for a single reference condition value for that point in time. To illustrate whether different biological measurements from the mine-influenced locations were similar to those from concurrently measured upstream reference comparison sites, some definition of "similar" is needed. If a biological measurement from the mine-influenced, downstream location was within the 95% confidence interval (CI) of the mean of the reference condition values, the values were considered similar to reference conditions (Di Stefano et al., 2005). Data used to calculate the confidence intervals were restricted to the periods with most consistent methods, which were 2002–2013 and 2003–2013, for the fish and macroinvertebrate data, respectively. The CIs ranged from 4% of reference for Panther Creek macroinvertebrate taxa richness to 35% of reference for Big Deer Creek benthic biomass. However, we did not interpret similarity to reference rigidly, as no two stream sites are identical and with sufficient sampling effort, the biology from any two sites can be statistically demonstrated to indeed be from two different sites (e.g., Martínez-Abraín, 2007). Further, classic inferential statistical tests for differences between groups are better suited for manipulative experiments than observational field studies. Samples in field studies are not independent of one another but are expected to occur in spatially and temporally autocorrelated gradients. For example, sample results from biological communities one year will be influenced by the community present at the location the year

Table 2. Summary of dissolved copper and cobalt concentrations in the Panther Creek watershed, pre- and post-water quality restoration effort[a]

Location	Dissolved Copper (μg/L)		Dissolved Cobalt (μg/L)	
	Average (range), n		Average (range), n	
	1993–1994	2013	1993–1994	2013
Panther Cr, PA-km39, above Blackbird Cr. (*Reference*)	1.1 (0.4–3.6), 12[b]	0.25 (<0.1–0.7), 48[c]	0.1 (0.05–0.2), 12[b]	0.85 (0.2–2.3), 48
Blackbird Cr., BB-km0.1	*380 (94–1525), 38*	*8.7 (2.–24), 16*	*845 (329–1880), 38*	*120 (50–251), 3*
Panther Cr, PA-km37, below Blackbird Cr.	51 (12–140), 91	1.0 (<0.1–2.9), 68[c]	89 (36–204), 19	22 (7.4–51), 65
Panther Cr, PA-km22, above Big Deer Cr	27 (7–73), 7	1.2 (<0.1–4.8) 50[c]	32 (17–69), 7	12 (4.3–20), 50
Big Deer Cr., BD-km5.6 (Reference)	*3.0 (0.9–4.7), 6*[d]	*0.26 (<0.1–0.7) 47*[c]	*<0.1 (<0.1), 6*	*0.54 (0.1–1.2) 48*
SF Big Deer Cr., SFBD-km0.1	*1611 (560–2740) 5*	*14 (7.6–17) 7*	*704 (480–912) 5*	*13 (5.7–15) 7*
Big Deer Cr., BD-km5.3	*139 (77–234), 3*	*2.6 (0.4–24), 66*	*63 (3 –80), 3*	*2.0 (0.6–5.3), 63*
Big Deer Cr., BD-km0.1	*105 (40–199), 6*	*4.7 (3.0–10.8), 49*	*58 (38–84), 6*	*2.0 (1–3), 49*
Panther Cr, PA-km17, below Big Deer Cr.	25 (12–59), 19	1.4 (<0.1–4.1) 58[c]	31 (13–63), 31	10 (4.2–19), 55

[a] Tributaries are indented and italicized.
[b] Values from 1994 only because of suspected Cu contamination in 1993 values, 1993 mean Cu at PA-km39 was 2.6 μg/L We consider the up to 10-fold "declines" in Cu concentrations at reference sites from 1993 to 2013 to be artifacts of better contemporary sample collection and processing, and improved laboratory practices, rather than environmental changes. However, with the higher concentrations present in mining-influenced sites in the 1990s, these low part-per-billion contamination artifacts would not affect interpretations.
[c] Averages estimated using the Kaplan-Meier procedure for datasets with censored (nondetected) values (Helsel, 2005). Nondetection rates in 2013 for Cu were 35% at PA-km39, 9% at PA-km37, 2% at PA-km22, 14% at PA-km17, and 14% at BD-km5.6.
[d] Data available from 1993 only, tablenote "b" is relevant.
Data sources: (Mebane et al., 2015), where 1993-1994 data were obtained by RCG/HaglerBailley, Boulder, CO, (unpublished) and 2013 data were obtained by Golder Associates, Redmond, WA, (unpublished).
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before, and by nearby upstream conditions. Thus in field studies of unplanned impacts, simple graphs and correlations may be more appropriate than inferential statistics. Impact of contaminants can be inferred if metals concentrations are correlated with biological metrics, and recovery can then be inferred as the disappearance of such a correlation through time (Wiens and Parker, 1995). Locally weighted (Loess) regression was used to smooth and visualize patterns in time series graphs (Cleveland and Devlin, 1988). We tested for bivariate and multivariate correlations between biological metrics, metals, and two potentially confounding factors, stream temperature, and streamflow. Details are given in Text S2.

3. Results

3.1 Metals in sediment, periphyton, and benthic macroinvertebrate tissues

At reference sites in both Panther Creek and Big Deer Creek, Cu, Co, and As were consistently low in sediment, periphyton, and macroinvertebrate tissues (Figure 2). In mining-influenced Panther Creek sites, Cu concentrations in sediment, periphyton, and tissue all declined by about an order of magnitude between 1993 and 2006, and remained relatively low and stable since. Panther Creek Co concentrations in sediment and tissue also declined over time, but were more variable and magnitudes of decline were less than with Cu. Cobalt in invertebrate tissues collected at PA-km17 downstream of Big Deer Creek were stable throughout the 1993–2013 period. Likewise, Co in periphyton in Panther Creek samples declined by less than a factor of two from 1993–2013. Arsenic concentrations in sediment, periphyton, and tissue were at least factor of 4 lower in 2013 than in 1993. Arsenic concentrations in Panther Creek sediment and periphyton increased in 2008 following a freshet in Blackbird Creek that overtopped banks and remobilized contaminated soils and sediment. Arsenic concentrations then steadily declined through 2013. However, arsenic in invertebrate tissues did not increase following the increases in sediment and periphyton arsenic, suggesting that the arsenic measured in sediment and periphyton had low bioavailability Since 2006, As concentrations in benthic invertebrate tissues have been near or <20 mg/kg dw (Figure 2).

In Big Deer Creek in 1993, sediments were exorbitantly high in Cu, at >11,000 mg/kg downstream of the confluence with South Fork Big Deer Creek and were still >6000 mg/kg in Big Deer Creek near its mouth. By 2013, Cu concentrations in sediments had declined 100-fold. No pre-restoration data for metals in periphyton and benthic macroinvertebrate tissues were collected in Big Deer Creek. From 2006 to 2013 Cu in periphyton declined by less than a factor of 2, and at site BD-km5.3, Cu in macroinvertebrate tissue

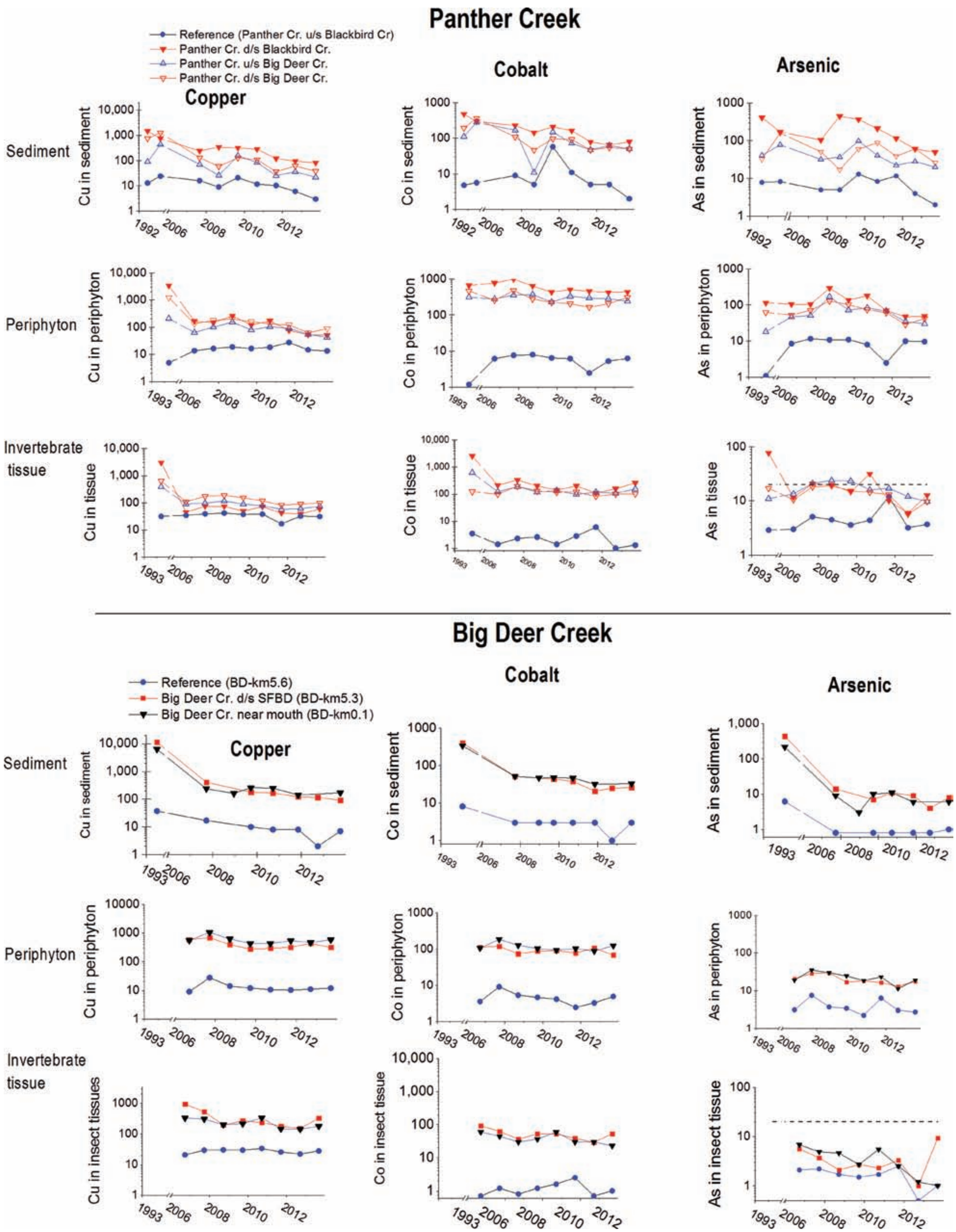

Figure 2

Changes in arsenic, cobalt, and copper residues in sediment, periphyton, and aquatic macroinvertebrate tissue.

All in mg/kg dry weight. Note differing vertical scales. Dashed line for arsenic in tissue is an approximate threshold for dietary effects of inorganic arsenic to salmonids (see text). Surveys with more than one sample per location were averaged.

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declined by a factor of 6. Co and As concentrations in periphyton and aquatic invertebrate tissues have been uniformly low since 2006 (Figure 2).

Despite the different patterns apparent in the individual time series graphs in Figure 2, from a broader view, when all matched samples were pooled across sites and years (n=47), concentrations of Cu, Co, and As in water, sediment, periphyton, and macroinvertebrate tissue residues were all strongly correlated with each other. The strongest correlations were between Co in water, periphyton, and tissue (Pearson's *r* coefficient values of 0.93 to 0.95), followed by Cu in water, periphyton, and tissue (*r* values of 0.89 to 0.93) (Text S2).

While the aquatic macroinvertebrate tissue samples in our study were pooled to reflect the general potential dietary exposure of fish to contaminants, in 2012, we also collected Hydropsychid caddisflies for tissue Cu analyses. In Big Deer Creek only *Arctopsyche* sp. were found, and in Panther Creek mostly *Hydropsyche* sp. were found. *Hydropsyche* and *Arctopsyche* have similar metal bioaccumulation patterns (Cain et al., 2004). Pooling both genera, Cu in Hydropsychidae caddisflies was highly correlated with, and slightly lower than Cu in the general community samples ($y = 1.45 \cdot x + 9.1$, $r^2 = 0.97$, $P = 0.002$, where x is Cu in Hydropsychidae tissues, and y is Cu in the community samples, as mg/kg dw). Both Cu in water and Cu in macroinvertebrate tissue were strongly correlated with benthic community metrics such as taxa richness or mayfly abundance (Text S2).

The three species individually targeted for As analyses did show differences: *Arctopsyche* sp., a net spinning caddisfly that feeds by collecting diatoms and animal matter, the stonefly *Pteronarcys californica* that feeds by shredding and consuming plant material, and the predatory stonefly *Hesperoperla pacifica,* all collected from station PA-km37 in September 2012. The range of As concentrations (n=3 for each organism) was about 1 to 1.5 mg/kg dw (20% organic As) for *Hesperoperla*, 3 to 7 mg/kg dw for *Arctopsyche* (50% organic As), and 7.5 to 11 mg/kg (80% organic As) for *Pteronarcys* (R.J. Erickson, U.S. Environmental Protection Agency, Duluth, MN, personal communication, 16Jul2013). The corresponding pooled community sample of 6 mg/kg dw was within the range of the *Arctopsyche* samples, slightly lower than the range for *Pteronarcys* range, and considerably higher than *Hesperoperla.* Since the predatory *Hesperoperla* stonefly had the lowest As residues, these results suggest bio-dilution through trophic transfer.

3.2 Metals in water

Natural background concentrations

In 2013 in Panther Creek upstream of Blackbird Creek, the average background Cu concentration was 0.25 (range < 0.1 to 0.7) µg/L, and in Big Deer Creek, upstream of South Fork Big Deer Creek, the average Cu concentration was 0.2 (range < 0.1 to 0.3) µg/L. Background Co concentrations were similar to Cu (Table 2). Background total As concentrations in Panther Creek were about 1 µg/L in the mid-1980s (Mok and Wai, 1989). However, because total As in Panther Creek downstream of Blackbird was usually only in the range of 2–6 µg/L (Mok and Wai, 1989), subsequent water sampling seldom included As.

Panther Creek downstream of Blackbird Creek

Prior to the onset of water quality restoration efforts, dissolved Cu concentrations ranged from 12 to 140 µg/L in Panther Creek downstream of Blackbird Creek, which exceeded the estimated Cu BLM-based chronic criterion (CCC) by greater than a factor of 10. In 2013, Cu concentrations ranged only from <0.1 to 2.9 µg/L, with a maximum Cu chronic criterion exceedance factor of 0.6 and 0.2 (Figure 3, Table 2). Cobalt declines have been proportionally less than Cu declines. From 1992 to 2013, the locally-weighted (Loess) average Co concentration declined by about a factor of 6.5, from 108 µg/L to 16 µg/L, whereas over the same period at this site, Loess smoothed Cu concentrations declined by about a factor of 25 (Figure 3). Because of the large number of Cu and Co samples (~750 each for PA-km37 alone), we emphasize the local Loess-smoothed values to simplify and visualize patterns. In most years at most sites, the maximum Cu and Co concentrations were about 3X and 2X greater, respectively (Figure 3; Table 1; Mebane et al. 2015).

Panther Creek downstream of Big Deer Creek

Patterns of decline with Cu and Co concentrations at this location were similar to those in Panther Creek downstream of Blackbird Creek, although pre-restoration, the absolute concentrations of both Cu and Co were lower in Panther Creek downstream of Big Deer Creek than downstream of Blackbird Creek. In 1993 and 1994, low flow Cu concentrations downstream of Big Deer Creek ranged from 12–15 µg/L, with high flow concentrations ranging from 25 to 97 µg/L. By 2004, nearly all Cu concentrations were below the BLM-based water quality chronic criterion, and as of 2013, Cu ranged only from 1.7 to 4.1 µg/L during high flows, and <0.1 to 1.1 during low flows.

Big Deer Creek

The only discrete source of metal loading to Big Deer Creek is via the South Fork of Big Deer Creek (Figure 1). In 1993–1994, dissolved Cu concentrations in Big Deer Creek immediately after mixing with the South Fork Big Deer Creek averaged 139 µg/L (range 71–234), compared with a 2013 average of 2.6 µg/L (range <0.1 to 24 µg/L, Table 2). The 2013 peak concentration of 24 µg/L occurred in response to a

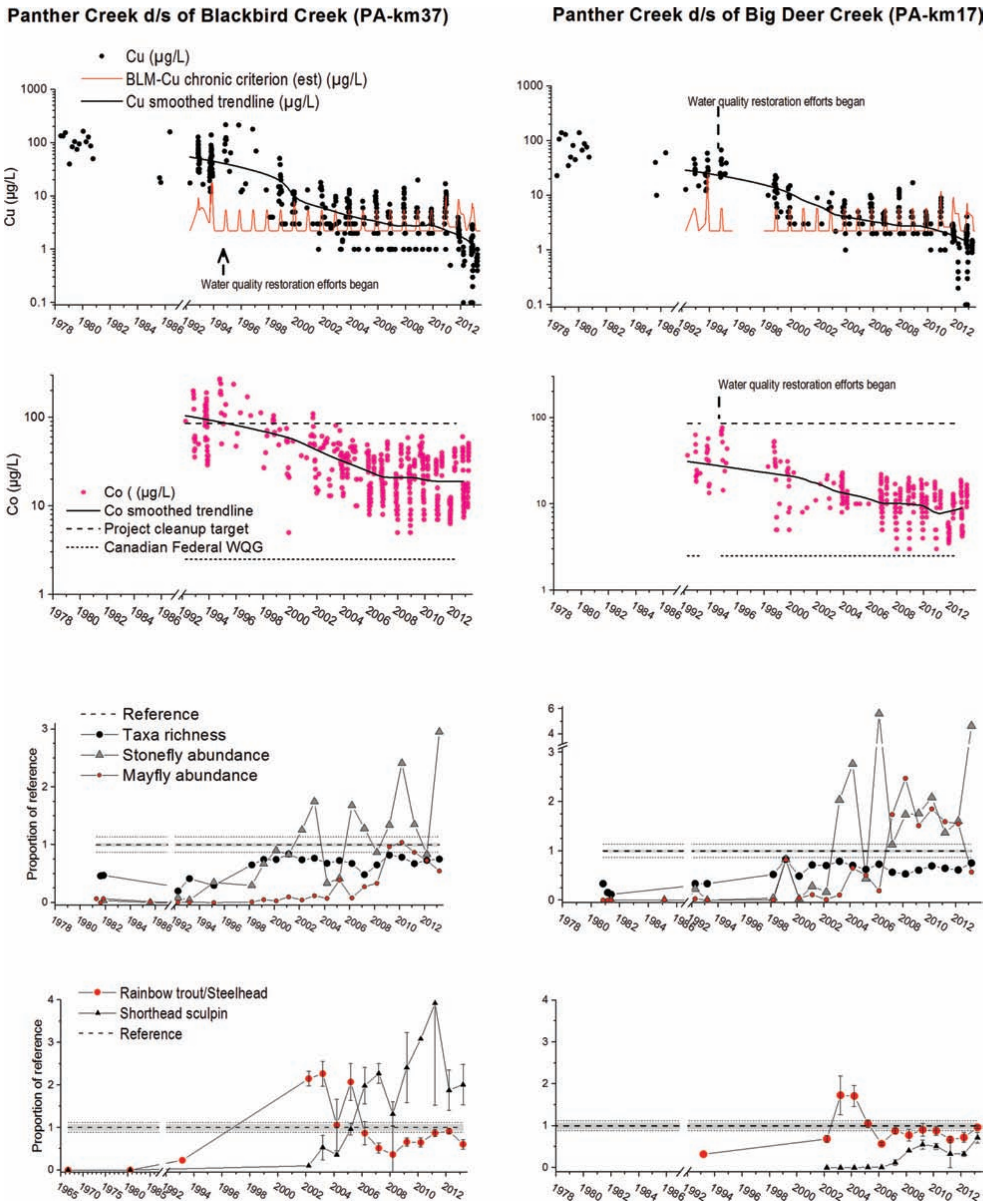

Figure 3

Declines in dissolved copper and cobalt and concurrent changes in aquatic communities in Panther Creek.

Comparisons are relative to pooled upstream reference conditions; d/s- downstream. Shading and lines bracketing the reference condition line indicate the 95% confidence interval (CI) on the mean reference condition values. Shading around the reference lines in their respective plots indicates the narrower CIs for inveterate taxa richness and Shorthead Sculpin densities; dotted lines bracketing the reference lines indicate CIs for mayfly and stonefly abundance (identical), and Rainbow Trout.

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thunderstorm on September 5, 2013, which was captured by an autosampler triggered by a stage increase in the headwaters source area (Bucktail Creek). Copper was elevated above background for about 12-hours, reaching 24 µg/L within 3-hours of the onset of the event. Eight-hours after the peak concentration, Cu had declined to 3 µg/L, similar to the base flow concentration. The same storm also caused an increase in stream stage on Blackbird Creek, triggering a downstream autosampler on Panther Creek, but no increase in Cu or Co was detected. This storm event was the only one detected since June 2008 when the stage triggered, telemetered autosamplers were installed.

In 1992–1994, Cu concentrations were lower at the mouth of Big Deer Creek (site BD-km0.1) than just downstream of the confluence of the South Fork (BD-km5.3) by a median factor of 0.67 (n=4 sample pairs). However as Cu loads from the Bucktail Creek drainage were reduced, this pattern switched, and Cu tended to increase in Big Deer Creek with distance away from the mine sources. As of 2013, Cu concentrations were higher at the mouth of Big Deer Creek by a median factor of 2.3 (n=43 sample pairs) than they were just downstream of the South Fork (site BD-km5.3). Cobalt declined in Big Deer Creek by about a factor of 30 over the same time frame. In the 1992–1994 sampling, Co at the Big Deer Creek sites averaged about 60 µg/L, and in 2013 averaged about 2 µg/L.

Blackbird Creek

From the 1993–1994 period to 2013, average Cu concentrations at the mouth of Blackbird Creek dropped from 380 to 8.7 µg/L and average Co concentrations declined from 845 to 120 µg/L (Table 2).

3.3 Correlations between biological and physiochemical covariates

During the early restoration period of 1993–2002, Cu was strongly, negatively correlated with macroinvertebrate taxa richness, mayfly abundance, stonefly abundance, and overall macroinvertebrate biomass. These metrics were also negatively correlated with Co, although not as strongly as with Cu. Macroinvertebrate biomass was also positively correlated with summer stream flows (Text S2).

During the latter restoration period of 2003–2013, correlations between Cu and Co and biological metrics had declined or disappeared relative to the earlier period. Mayfly and stonefly abundances and biomass were no longer correlated with Cu or Co, nor were trout or sculpin densities correlated with Cu or Co. For instance, from the 1993–2002 period, Cu and mayfly abundance had an r value of -0.90 (n=44 samples) whereas from the 2003–2013 period, the r value was only -0.17 (n=46 samples). Only taxa richness remained strongly correlated with metals (r = -0.80 with both Cu and Co). Trout and sculpin densities were negatively correlated (r -0.49, Text S2).

In Big Deer Creek, Rainbow Trout densities were not strongly correlated with any environmental variable analyzed, with the highest r value of only 0.37 between trout density and summertime flows. Macroinvertebrate taxa richness was negatively correlated with both Cu and Co (r values of -0.60 and -0.59, respectively), but the other biological metrics analyzed were not consistently correlated with metals. Invertebrate biomass was negatively correlated with temperature (r -0.68) and also Co and Cu (-0.66 and -0.62, respectively). Stonefly abundance was negatively correlated with summertime temperatures (r -0.72) (Text S2).

3.4 Aquatic community changes over time

Panther Creek downstream of Blackbird Creek (PA-km37)

Benthic macroinvertebrate taxa richness at this location increased from 20% of reference in the fall of 1992 to 75% of reference by 1999 (Figure 3). From 1999 through 2013, species richness had no consistent pattern over time, ranging from 60 to 90% of reference. Mayflies (Ephemeroptera) and stoneflies (Plecoptera) had been effectively extirpated from Panther Creek downstream of Blackbird and Big Deer Creeks prior to water quality restoration efforts. Stoneflies reappeared by 1998, and by 2002 reached the abundance of upstream reference sites. Subsequently, stonefly densities have been highly variable, but were usually abundant. Mayflies were sparse prior to 2008 and abundant since (Figure 3). The 2008 survey was about two years after the Loess smoothed Cu concentrations dropped below the chronic Cu criterion, and as of 2008 the low-flow Loess smoothed Co concentrations had dropped to about 20 µg/L.

No fish were found in Panther Creek downstream of Blackbird Creek in 1967 or 1980 (Figure 3). By 1993, Rainbow Trout had reappeared but were sparse, at 23% of reference. By 2002, Rainbow Trout abundance was 2X that of reference, at which time Cu concentrations during spring high-flow conditions had dropped to less than 2X the chronic criterion, and during low-flow concentrations were similar to the Cu chronic criterion. Shorthead sculpin (*Cottus confusus*) were detected in 2002, but were sparse. Sculpin abundance greatly increased after 2002, and trout abundances declined coincident with the sculpin increases (Figure 3).

Panther Creek downstream of Big Deer Creek
Benthic taxa richness at site PA-km17 increased from 33% of reference in 1993 to 71% in 2001. From 2001 through 2013, taxa richness had no obvious further increases, ranging from 63 to 90% of reference. Stoneflies were absent or sparse until 2003, with an irruption to 6X reference in 2006. Mayflies were also absent or sparse until 2007, but have mostly been abundant since. The increase in mayfly abundance was coincident with Loess smoothed Cu concentrations dropping below the Cu chronic criterion and Loess smoothed Co concentrations dropping to about 10 µg/L. Rainbow Trout were more abundant than at reference sites during 2003 and 2004, but then declined and were mostly similar to reference from 2005–2013. Shorthead Sculpin recovery lagged Rainbow Trout recovery by at least 6 years, only becoming common at this site in 2008 (Figure 3).

Big Deer and South Fork Big Deer Creeks
Recovery patterns of invertebrates and fish are contrasted in three locations with subtly differing Cu concentrations. Relative to Panther Creek, Co concentrations in Big Deer Creek are low (0.7 to 4 µg/L in 2013 at BD-km0.1. Figures 3 and 4). Prior to water quality restoration efforts, virtually all life had been extirpated in Big Deer Creek downstream of the mine-contaminated South Fork of Big Deer Creek.

Benthic invertebrate taxa richness in Big Deer Creek in 1993 was 18% of reference, when sampled at site BD-km5.3, located only 0.2 km downstream from the confluence of Cu and Co contaminated South Fork Big Deer Creek (Figure 4). Taxa richness steadily increased as Cu concentrations decreased, and by 2005 richness at BD-km5.3 was similar to reference, coincident with Loess smoothed Cu concentrations dropping to about 1.7X the CCC at BD-km5.3 (Figure 4). At the mouth of Big Deer Creek (BD-km0.1), 5.5km downstream of upstream sources of colonizing organisms, taxa richness was only 2% of reference in 1993. Taxa richness exceeded 50% of reference by 1999 as Cu declined to about 10X the CCC, and in 2012 coincident with Cu declining to about 1.3X the CCC during base flow, taxa richness approached 90% of reference. Taxa richness at sites BD-km0.1 and BD-km5.3 rose in parallel as Cu declined, yet richness was always lower at BD-km0.1, averaging 67% of that at BD-km5.3. Cu declines were roughly parallel between the two sites, but from 2002 to 2013, Cu concentrations at BD-km0.1 were typically about twice those of BD-km5.3. No invertebrate taxa were observed in South Fork Big Deer Creek prior to 2001. As Cu declined to about 8X the CCC in 2006, taxa richness in South Fork Big Deer Creek reached about 50% of reference, with little further increase by 2013 despite further Cu decreases to about 4X the CCC (Figure 4).

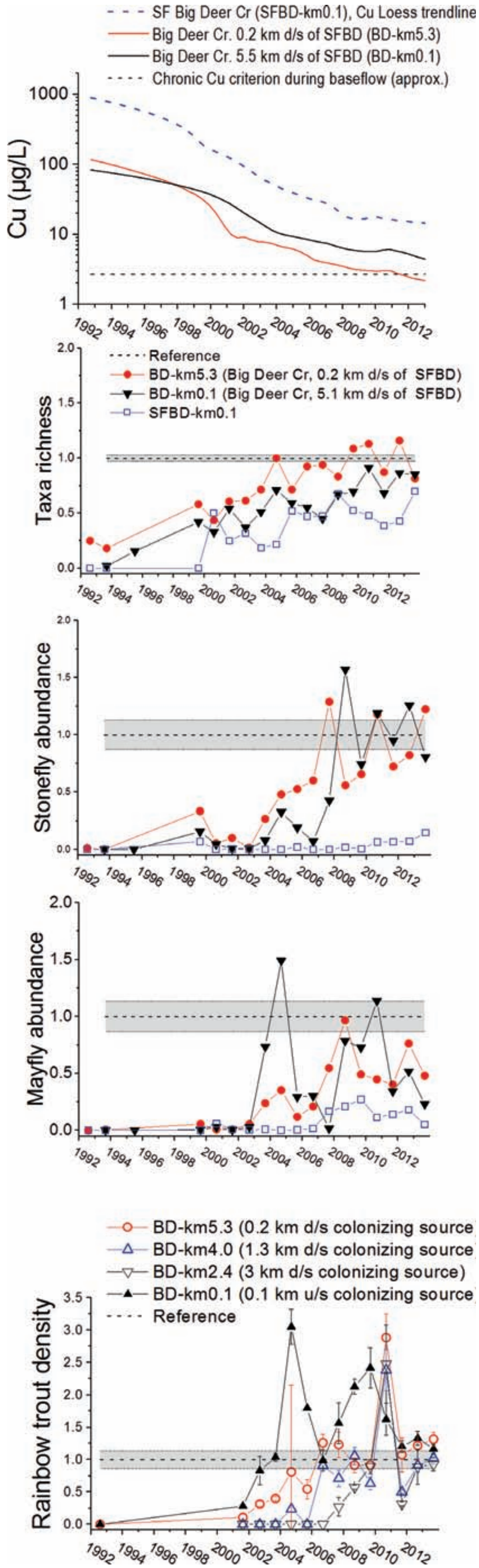

Figure 4

Differing recovery patterns at three locations with differing declines in dissolved copper.

Fish and invertebrates recovered slower at the sites with progressively less reduction in copper in water. Invertebrate data from Big Deer Creek and SF Big Deer Creek (SFBD) are shown relative to matched collections from their respective upstream (u/s) reference sites. Rainbow trout abundance increased later with increasing distance downstream of both copper and colonizing sources (d/s -downstream). Shading indicates 95% confidence interval on the reference condition mean value.

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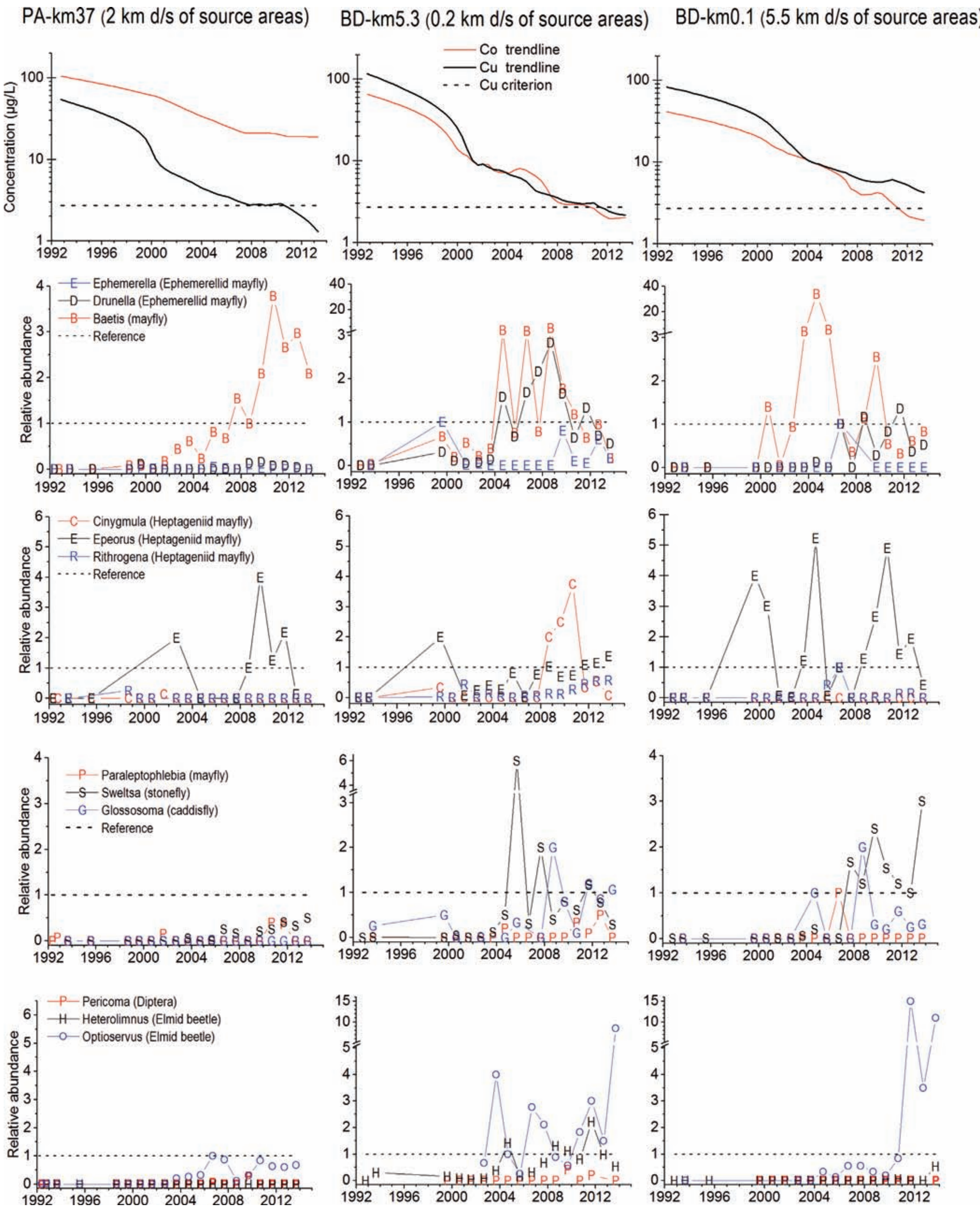

Figure 5

Differing taxa recovery trajectories with differing distance to colonists and differing Cu and Co declines.

Plotted as abundance relative to concurrent, upstream reference samples.

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No stoneflies or mayflies were found in Big Deer Creek in 1993 (Figure 4). As smoothed Cu concentrations dropped to about 2X the CCC at sites BD-km5.3 and BD-km0.1 in 2003 and 2004 respectively, stonefly abundance began increasing at both sites. By 2007, when smoothed Cu concentrations had dropped to about 1.3 to 1.6X the CCC, stoneflies at these two sites were similar in abundance to upstream reference. Stoneflies remain rare in South Fork Big Deer Creek. Mayflies, taken as a group, reoccupied the Big Deer Creek sites at about the same times as the stoneflies, but were much more variable. An irruption of mayflies (mostly *Epeorus grandi*s) occurred in 2004 in lower Big Deer Creek, followed by a collapse in 2007 (Figure 4). From 2008–2013, mayflies were always common, but still tended to be less abundant than reference, ranging from 45 to 113% of reference. Mayflies (mostly *Baetis tricaudatus*) have been detected in South Fork Big Deer Creek since 2008 (Figure 4), at which time Cu declined to about 5X CCC (~15 μg/L) during base flows.

Rainbow Trout had been extirpated from Big Deer Creek downstream of South Fork Big Deer Creek (Figure 4). By 2002, Rainbow Trout were present at BD-km5.3 and steadily increased in abundance until 2007. From 2007 through 2013, Rainbow Trout abundances at this site have usually been similar to upstream reference abundances. In 2002, when Rainbow Trout were present but sparse, Cu concentrations during low flows averaged 15 μg/L, about 4X higher than the CCC. About 3km downstream at station BD-km2.4, recovery of Rainbow Trout abundances to those similar with reference abundances lagged that at station BD-km5.3 by about 5 years. No fish were captured at site BD-km2.4 from 2002–2006, and when fish were first detected at this site in 2007, only age 2+ fish were observed. Young-of-year were captured the following year, indicating that reproduction was occurring in this formerly unsuitable and unoccupied habitat. By 2009, the year class strength and overall abundance was similar to reference. Fish in South Fork Big Deer Creek showed a similar temporal pattern, with first captures of pioneering adults in 2003, with increasing abundances of adult fish through 2008. From 2008–2012, fish were always present but did not generally increase in abundance, nor was there evidence of reproduction. The first evidence of successful reproduction was in 2013, with the capture of a single YOY Rainbow Trout. Smoothed Cu concentrations remained at about 3 to 4X the CCC as of 2013.

Blackbird Creek

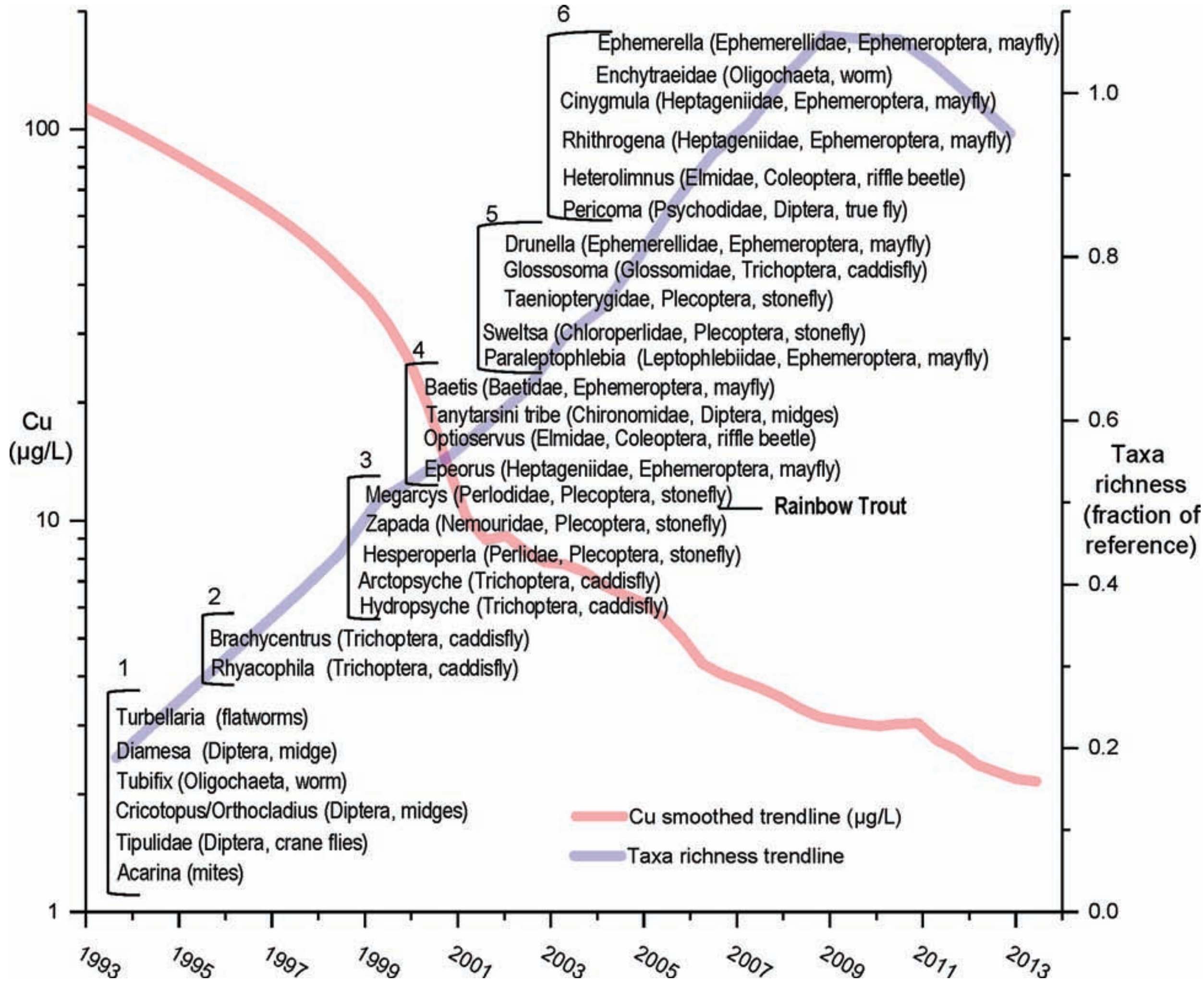
In 1993, Blackbird Creek just upstream of its mouth was virtually lifeless, with no more than 1 organism found per benthic sample. For fish, conditions in lower Blackbird Creek were acutely toxic in 1993, with 100% mortality of caged Rainbow Trout within 48-hours (Hansen et al. 1995). By 2002, conditions had improved to the point that Rainbow Trout, Chinook Salmon, and Bull Trout (*Salvelinus confluentus*) were found in Blackbird Creek near the mouth. As of 2013, fish occurrences downstream of mine sources remained limited to the lower 2 km of the creek. Further upstream, colonization by fish appears to be constrained by a combination of increasing substrate densities of oxyferrihydroxide floc and increasing Cu concentrations. Because of the limited recovery objectives for Blackbird Creek, it has received far less monitoring than the other streams, and the results are more qualitative. Aquatic community changes and factors limiting the recovery of Blackbird Creek are described in more detail in Text S2 and Figure S3.

3.5 Recovery trajectories of invertebrate taxa

As Cu and Co concentrations decreased, the recoveries of mayflies and stoneflies tended to lag the overall taxa increases (Figures 3 and 4). Recovery patterns among taxa had recurring patterns among the Panther Creek and Big Deer sites, and are illustrated for 12 genera at three sites which differ in the magnitude of Cu and Co declines, and in distance from colonizing sources (Figure 5).

Baetis was among the first mayfly to increase in abundance as Cu and Co concentrations declined. *Baetis* was similar in abundance to reference by 2002 in lower Big Deer Creek and by 2005 in Panther Creek. At Big Deer Creek at km 0.1, *Baetis* had a 3-year irruption from 2004–2006, reaching 34X reference before declining to abundances within a factor of 2 of reference. *Drunella* (Ephemerellidae) had recovered to reference densities at BD-km0.1 by 2008, but remained scarce at PA-km37. *Ephemerella* (Ephemerellidae) remain absent or scarce as of 2013. The recovery trajectories of three Heptageniid mayflies differed noticeably. *Epeorus* was the first mayfly to recover to reference abundances in lower Big Deer Creek, exceeding 4X of reference in 1999. Abundances have been highly variable since. For instance in 2007, no *Epeorus* (and few mayflies of any taxa) were found at BD-km0.1, but by 2008 had increased to 0.7X of reference, and then ranged from 0.4 to 4.9X through 2013. *Cinygmula* and *Rhithrogena* were seldom seen at PA-km37 or BD-km0.1 in any year surveyed, and the mayfly *Paraleptophlebia* (Paraleptophlebidae) was also scarce in most years. Only at site BD-km5.3 were the mayflies *Ephemerella, Cinygmula*, and *Rhithrogena* frequently collected, although their abundances were still less than those at the reference site only 0.3 km upstream (Figure 5).

The stonefly *Sweltsa* (Chloroperlidae) was absent or scarce in Big Deer Creek prior to 2007, but subsequently has remained abundant through 2013. This pattern is roughly similar to the overall pattern of recovery of stoneflies as a group at this location (Figure 4). In contrast, in Panther Creek stoneflies as a group were as abundant as at reference sites, but *Sweltsa* was seldom detected until 2006, and remained at 20–40% of reference abundance through 2013. The caddisfly *Glossosoma* (Trichoptera, Glossosomidae) was distinguished



by lagging behind caddisflies as a group before recovering to abundances similar to reference at BD-km5.3. The true fly *Pericoma* (Diptera, Psychodidae) remains scarce. Two beetles in the Elmidae family, *Heterlimnius* and *Optioservus* showed distinctly different recovery patterns. Whereas *Optioservus* was reasonably common most years, *Heterlimnius* has remained mostly absent, except at BD-km5.3 (Figure 5).

Oligochaetes in the family Enchytraeidae were common in reference samples across stream sizes (i.e., upper Panther Creek, Deep Creek, upper South Fork Big Creek and upper Big Deer Creek). However, enchytraeidids were never abundant at mining influenced sites downstream of the reference sites, with no obvious temporal or spatial patterns among the downstream sites. Average Enchytraeidae densities from pooled Panther Creek reference sites from 2003–13 were 43 per m^2 (CI 23–66), compared to 0.8 per m^2 (CI 0.2–1.4) from the pooled, downstream, mining-influenced sites. Tributary sites showed similar contrasts between the reference and downstream sites over the same time period, with Enchytraeidae densities averaging 38 per m^2 (CI 14–61) versus 0.9 per m^2 (CI 0.2–1.6), respectively. Aquatic insects were numerically dominant in 240 of 242 benthic macroinvertebrate samples collected from all sites between 2003 to 2013, accounting for 91% of all taxa. Molluscs were rare at both reference and mining-influenced sites.

Across sites, taxa repopulated the streams in a recurring progression. Out of the total taxa identified during the 2003–2013 sampling, which ranged from 127 to 211 taxa per event, about 26 commonly occurring taxa could be grouped among the earliest, intermediate, and later colonizers. We illustrate the progression at Big Deer Creek at km5.3 because of its extreme changes in Cu concentrations, low Co concentrations, and close proximity to upstream colonizing sources. In the initial surveys with smoothed Cu concentrations exceeding 125 µg/L, only six taxa persisted (Figure 6, bracket 1). These included Turbellaria flatworms, Acarina mites, Tubificidae oligochaete worms, and a few Diptera in the Chironomidae and Tipulidae families. Only in the

Figure 6

General order of appearance of commonly occurring taxa in study streams as copper concentrations declined.

Copper and taxa richness curves are from site BD-5.3km, but the progression was similar at other Big Deer sites. Brackets, numbered on their shoulders, group taxa with similar rank orders of appearance.

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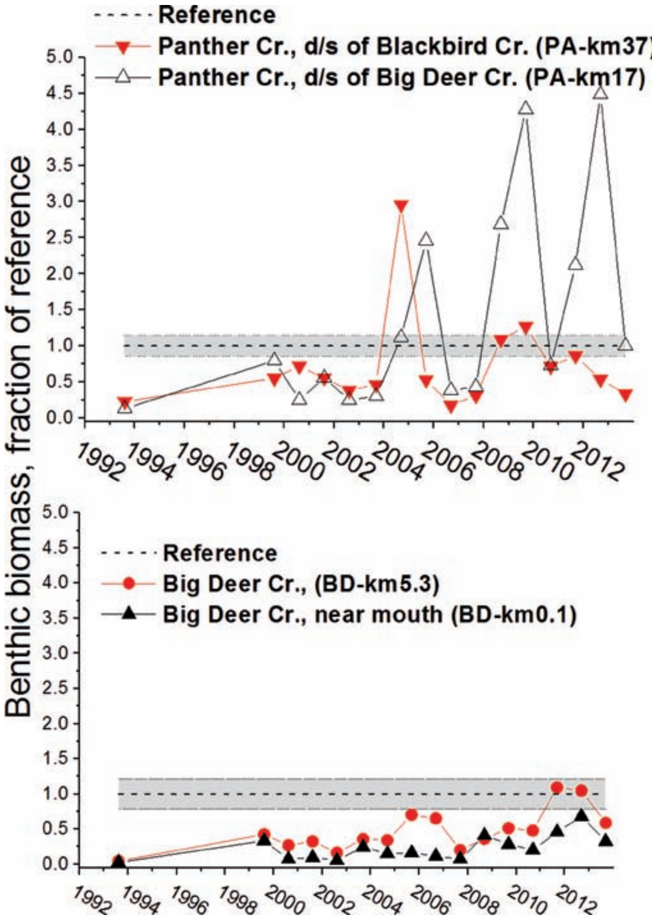

Figure 7

Changes in total biomass of benthic invertebrates in Panther Creek and in Big Deer Creek.

Shading indicates minimum detectable difference from reference.

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extreme conditions of South Fork Big Deer Creek with >1000 µg/L Cu were these (and all) taxa absent. The caddisflies *Brachycentrus* and *Rhyacophila* appeared in Big Deer Creek by 1995, and by 1998 Hydropsychid caddisflies and the stoneflies *Hesperoperla*, *Zapada* and *Megarcys* appeared. *Baetis* and *Epeorus* mayflies and the Elmid beetle *Optioservus* appeared the following year as smoothed Cu concentrations dropped to 30 µg/L. As Cu dropped below 6 µg/L in 2005, the stonefly *Sweltsa*, the caddisfly *Glossosoma*, and the Elmid beetle *Heterolimnus* appeared. The final arrivals, coincident with smoothed Cu concentrations dropping below 3 µg/L, were the mayflies *Cinygmula* and *Ephemerella* (Figure 6).

The progression of recovery at other locations in Big Deer Creek was similar, although the taxa in bracket 6 of Figure 6 diminish with distance downstream and as of 2013 remained absent or uncommon at station BD-km0.1. In Panther Creek, general patterns were similar with some noticeable taxa differences. The mayfly *Epeorus* appeared in lower Panther Creek (PA-km17 and PA-km2.7) by 2001 but not at PA-km37 until 2010. While upstream colonization sources were closer at PA-km37, Co is highest at PA-km37, at about 20 µg/L in 2010. *Sweltsa* is the only taxa grouped in Figure 6, brackets 5 and 6 that has been commonly found in Panther Creek downstream of the reference sites.

3.6 Benthic community biomass

We used total community biomass as an indicator of whether benthic invertebrates provided a sufficient prey base for fish. Because high counts of small organisms that contribute little to biomass (such as Acarina, aquatic mites) can be misleading in simple counts of total organisms, we considered biomass of benthic invertebrates a better indicator of the available prey base for fish. Because the vast majority of benthic invertebrate taxa collected were aquatic insects that were classified as being vulnerable to salmonid or sculpin predation (Suttle et al., 2004), we considered total biomass to reflect the available prey base for fish.

In mining-influenced reaches of Big Deer Creek prior to 2011, benthic biomass was less than half that of Big Deer Creek upstream of the mining-influenced segment (Figure 7). Biomass was consistently lower at the mouth of Big Deer Creek (BD-km0.1) than at site BD-km5.3. These differences correspond both with increased downstream Cu concentrations and increased distances from upstream colonizing sources. In Panther Creek, while benthic biomass was low prior to about 2003, the biomass in Panther Creek appears to track expected regional natural upstream to downstream patterns for mid-order streams with biomass increasing

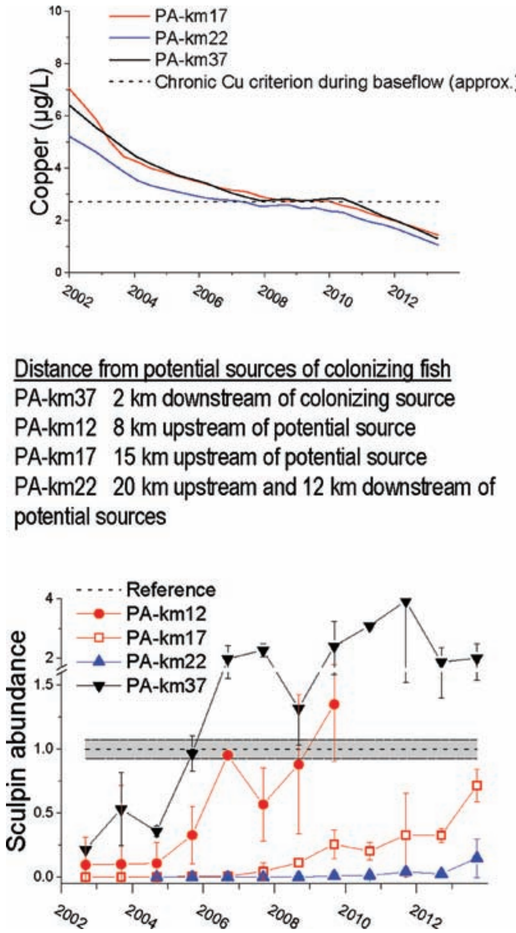

Figure 8

Recovery trajectories for Shorthead Sculpin relative to copper concentrations and distance from source populations.

At Panther Creek downstream of Blackbird Creek (PA-km37), it seems certain that the fish moved downstream from above Panther Creek. However, the source populations for the other sites are inferred from the downstream to upstream pattern in first detections and subsequent abundance increases.

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as the streams increased in size and temperature (Vannote et al., 1980). Prior to 2003, biomass was contrary to this expected natural pattern, with downstream biomass lower than upstream biomass, suggesting a more limited prey base for fish, relative to reference. Occurrences of high biomass in Panther Creek downstream of Big Deer Creek was driven by increased downstream abundance in large stoneflies, *Pteronarcys* and *Hesperoperla*, and the large caddisfly *Hydropsyche* which all do well in warmer water (Ott and Maret 2003). A 2005 spike in biomass downstream of Blackbird Creek was driven by a “bloom” of the oligochaete worm *Nais bicuspidalis,* which was rare in the reference sites.

In 2013 benthic biomass declined relative to reference across different Panther Creek and Big Deer Creek sites. The reasons for this decline in Panther Creek are not obvious. Measured metals concentrations did not increase in Panther Creek in 2013. Panther Creek fish densities in 2013 were the highest measured, about 150% of the long-term average, but the 2013 increase in fish density was similar at the reference sites. Streamflows in 2013 were unremarkable. Stream temperatures in 2013 were warmer than average (e.g., maximum summer temperatures of 20.8°C in 2013 vs. 19.6°C average maximum from 2003–2013 at PA-km37). Warm stream temperatures could plausibly be a factor, as some previous dips in benthic biomass occurred in previous warm years (2006 and 2007) and some higher biomasses occurred in cool years (2009, 2010, 2012) although these patterns were not apparent at all sites and all years. In Big Deer Creek, the plausible factors for decreased benthic biomass differ from those in Panther Creek. A 10X pulse in Cu concentrations 4 days prior to sampling site BD-km5.3 could have initiated drift, leaving lower biomass when sampled. Fish densities were also higher at BD-km5.3 in 2013 (150% of the long-term average for the site) whereas densities at the reference site, BD-km5.6 were only 109% of the long-term average density. Temperature patterns were similar between the streams.

3.7 Shorthead Sculpin

For the lower Panther Creek sites PA-km12, PA-km17, and PA-km 22, sculpin appeared to expand their range from downstream to upstream, based upon the later increases at the more upstream sites (Figure 8). The distances from potential source populations are given from Clear Creek, in which Shorthead Sculpin were the most abundant species in a 1998 survey by the Idaho Department of Environmental Quality, whereas no sculpin were found in two other potential refuge habitats, Beaver and Trail Creeks, (http://mapcase.deq.

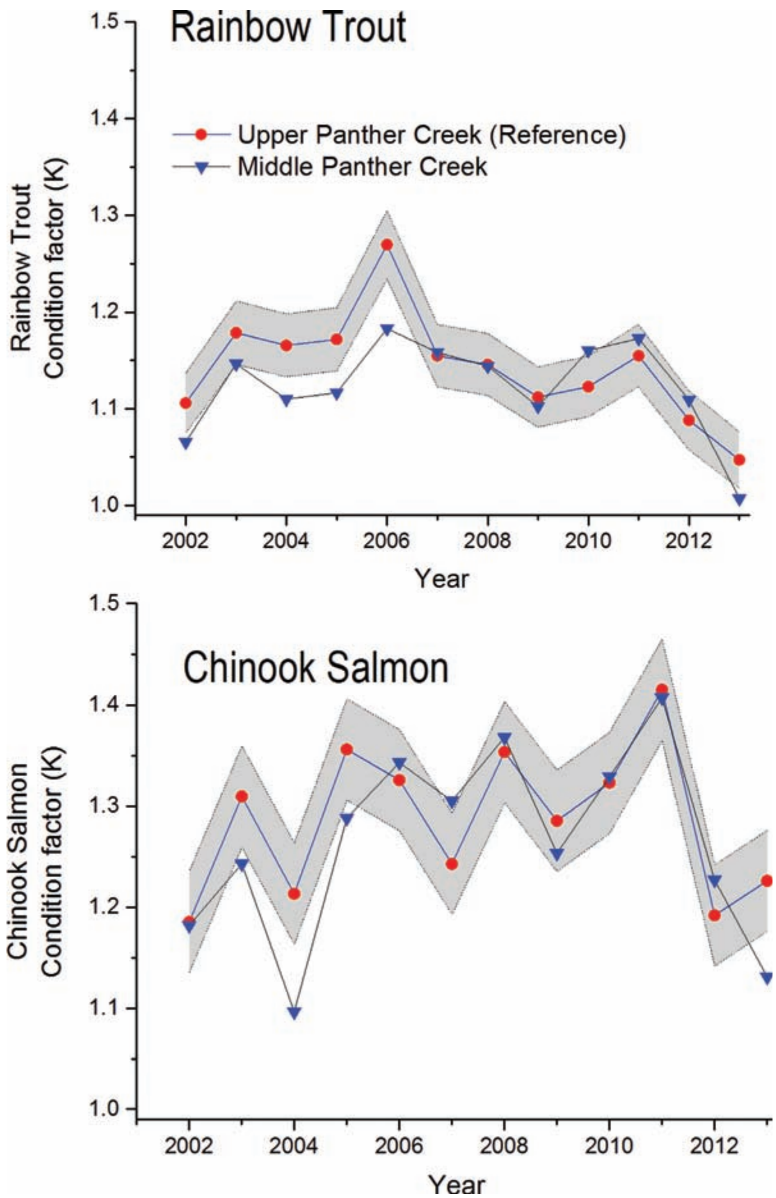

Figure 9

Rainbow Trout and Chinook Salmon body condition factor (*K*) in Panther Creek upstream and downstream of Blackbird Creek.

Prior to 2007, body condition was subtly, but consistently lower downstream of Blackbird Creek. ["Upper Panther Creek" pools data for all sites upstream of Blackbird Creek; "Middle Panther Creek" pools data for all sites in between Blackbird and Big Deer Creeks. Comparison was limited to these two adjacent reaches to minimize the influence of warmer temperatures in lower Panther Creek.]

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idaho.gov/wq2010/, accessed December 2014). Sculpin were present but sparse at site PA-km12 in lower Panther Creek at the time of our first survey, and thus their actual colonizing source is unknown. While no fish were found in Panther Creek just downstream of Big Deer Creek in 1980, not until 2002 was a quantitative electrofishing survey conducted in multiple locations in Panther Creek. Presumably Shorthead Sculpin began reoccupying lower Panther Creek by at least the early 1990s, by which time Cu had attenuated to below critical limits for Rainbow Trout to become re-established in Panther Creek. While the overall declining Cu concentrations are similar at the three different locations monitored, the site with the lowest Cu and greatest distance from source populations was last to be reoccupied by sculpin (Figure 8). By 2005, once smoothed Cu concentrations had declined to less than about 1.3X the chronic criterion, Cu did not appear to exert any constraint on sculpin recovery. Water chemistry was not monitored at site PA-km12, but Cu concentrations were likely similar to those monitored near PA-km17 since no large tributaries enter Panther Creek between these two sites.

As of our first survey (2002), sculpin were also abundant at the most downstream site on Panther Creek (PA-km4), but then disappeared following a massive debris flow entering Panther Creek from Clear Creek (1 km upstream) in June 2003. Sculpin did not return to their pre-landslide abundances at this location until 2012 (Text S2).

3.8 Fish population characteristics

Prior to 2007, average Rainbow Trout condition factors tended to be lower in mining-influenced sites, relative to reference sites, whereas from 2007 on, average Rainbow Trout condition factors were similar in upper (reference) and middle (mine-affected) reaches (Figure 9). While differences were subtle, the patterns were reasonably consistent during the early years of the water quality restoration efforts, and vanished in the later years as Cu concentrations continued to drop. Chinook Salmon showed roughly similar patterns. The timing of decreasing Cu concentrations and increasing mayfly abundances both corresponded with the timing of the disappearance of apparent growth differences (Figures 3 and 9). Upstream of direct mine-influences, Rainbow Trout condition was negatively correlated with Rainbow Trout density (r = -0.74), and Chinook Salmon condition was most strongly correlated with average summertime streamflow (r = 0.63) (Text S2).

The age-class structure of trout in Panther Creek in 1993 was skewed toward older fish, relative to reference reaches. Young-of-year (YOY) trout in particular were less common in Panther Creek relative to reference reaches, and trout of all ages were less abundant in mine-influenced reaches of Panther Creek than in reference reaches (Figure 10). For the 1993 data, the comparisons are made with all trout because most of the reference reaches were located in the Middle Fork Salmon River basin where Cutthroat Trout were the dominant species, while in Panther Creek, Rainbow Trout made up more than 95% of the total trout. By 2003, fish in mine-influenced reaches were as abundant as fish in upstream reference reaches. Also by 2003, a balanced age class structure was present in mine-influenced reaches, similar to reference reaches. In 2013, the densities and age class structure of Rainbow Trout in Panther Creek were similar to those in 2003, and also similar to expectations for salmonids in streams subject to natural population controls, discussed earlier.

Other native fish including Bull Trout, Mountain Whitefish, Longnose Dace (*Rhinichthys cataractae*) and Cutthroat Trout were regularly observed within the study area, but their distributions were variable and appeared unrelated to water quality, described in Text S2.

3.9 Chinook Salmon recolonization

The Panther Creek Chinook Salmon population appears to be self-sustaining as of 2013. The relatively high density of fry observed in 2002 followed a release of about 1053 hatchery-origin adults the previous year, and the cyclical density peaks in 2006 and 2011 indicate first and second generation progeny from this release (Figure 11A). Because about 99% of Chinook Salmon in the Salmon River, Idaho have either a 4- or 5-year life cycle (Mebane and Arthaud, 2010; Kennedy et al., 2013), the presence of YOY in off-cycle years from the 2001 release indicate natural-origin reproduction of wild salmon independent of the 2001 hatchery release. The "off-cycle" years with only natural-origin Chinook Salmon YOY present were 2003–2005, 2008–2009, and 2013 (Figure 11B).

4. Discussion

4.1 Attenuation of metals contamination

Streambed sediments can represent a persistent reserve of exchangeable metals that can be remobilized, released to the water column, and cause delays in biological recovery following pollution source controls (Hamilton, 2012). While only a fraction of the metal present in sediments is bioavailable at any one given time due to binding to particulate organic carbon and iron and manganese oxyhydroxides and sediment burial (Costello and Burton, 2014), trace contaminants associated with sediments will remain in the river-floodplain system and be subject to remobilization until stored deposits become depleted (Hamilton, 2012; Moore and Langner, 2012).

Patterns with Big Deer Creek Cu concentrations are consistent with Hamilton's (2012) concept of streambed and alluvial sediments acting as a reserve of Cu that is slowly dissipated as it is conveyed downstream, released in trace amounts to the overlying water, and diluted with sediments from the upper, unaffected watershed. In Big Deer Creek prior to restoration efforts, Cu concentrations in both water and sediment were higher at the site nearest to the contaminated mine drainage (BD-km5.3) than at the farthest site (BD-km0.1). However, as source controls became effective and Cu concentrations in water dropped, this rank order was reversed, with consistently higher Cu concentrations in water and sediment at the more distant site from the mine source (Figure 2; Figure 4).

When examining changes in metals concentrations in different media through time-series, the graphs do show differences. For example, As concentrations in macroinvertebrate tissues did not track spikes in sediment or periphyton As concentrations in 2008. Copper in Panther Creek declined sharply during the first decade of recovery, whereas declines in Co and As concentrations have been slower and more variable (Figure 2). Yet when viewing the data broadly across all sites and years, Co and Cu were particularly strongly correlated within media, with Pearson r values ≥0.8 among water, sediment, periphyton, and invertebrate tissue (Text S2). The strength of these correlations suggests redundancy in the information provided by the exposure data from the different media, and possible monitoring efficiencies. For instance, with a correlation coefficient of

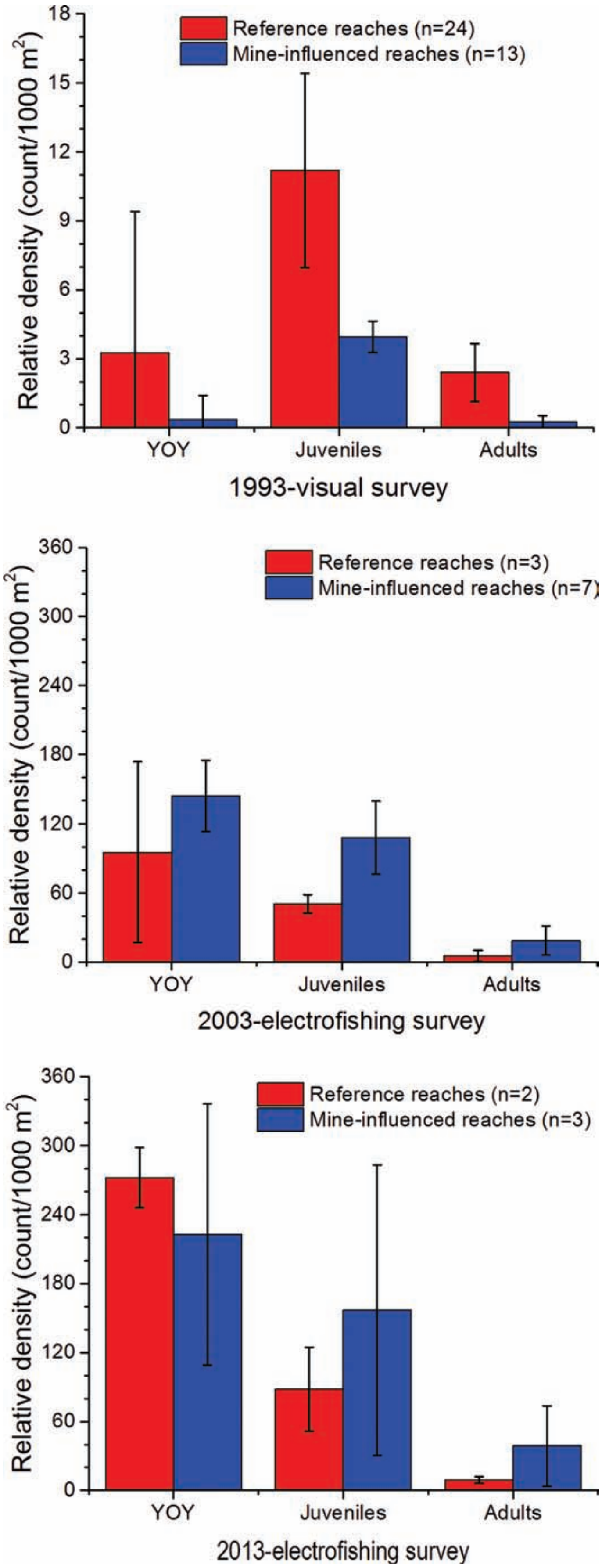

Figure 10

Mean relative densities of all trout by life stages in Panther Creek downstream of mine discharges, relative to reference reaches, at decade intervals.

Fish ≤75 mm in length were considered to be young-of-year (YOY), "Juveniles" are fish 76 to 179 mm in length and are probably 1 and 2 year old fish, and "Adults" are fish >180mm in length. Lengths in the 1993 snorkel survey were visually estimated, and fish lengths in the 2003 and 2013 surveys were measured fork lengths. Error bars show standard deviations. Contrasts between the 1993 visual, snorkel survey and the 2003 and 2013 electrofishing surveys need to be relative to the within-survey reference conditions because the different survey methods had markedly different detection efficiencies (note 20X difference in axis units).

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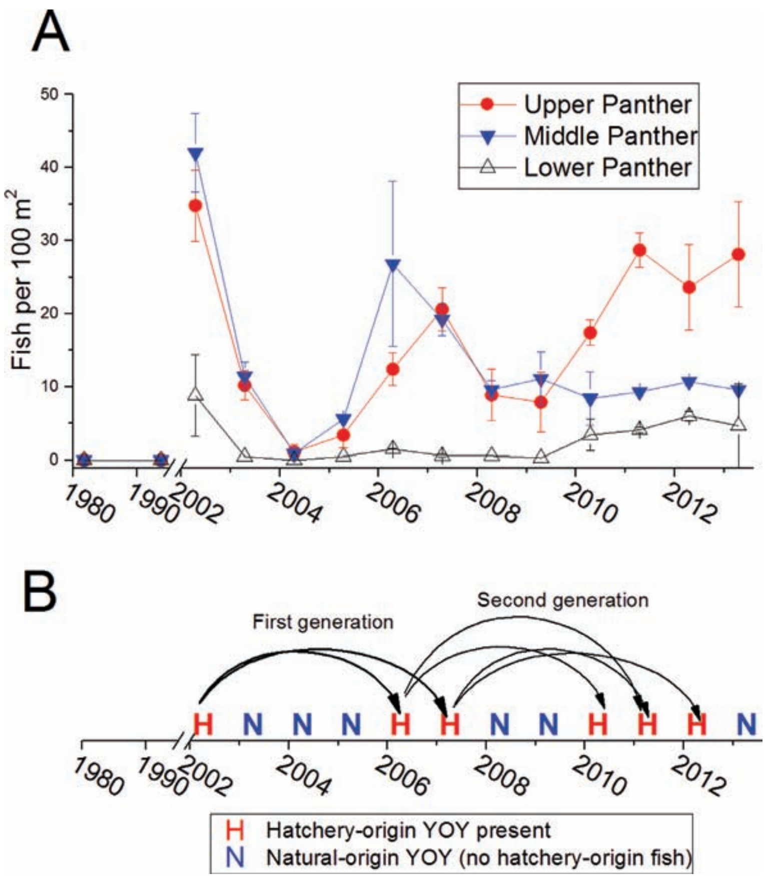

Figure 11

Chinook Salmon juvenile densities in Panther Creek, by water quality reach.

a. "Upper Panther" is upstream of all mine influence, average of 3 to 4 sites depending on year; "Middle Panther" is between Blackbird and Big Deer Creeks (average of 2 to 6 sites per year); and "Lower Panther" is downstream of Big Deer Creek (average of 2 to 4 sites).

b. Years in which the monitored densities of young-of-year (YOY) Chinook Salmon were likely influenced by hatchery-origin progeny from the 2001 release of hatchery adult fish, based on the 4- or 5-year life cycle of Chinook Salmon in the Salmon River, Idaho (see text).

doi: 10.12952/journal.elementa.000042.f011

0.95 between annual average Co in water and annual macroinvertebrate tissue sampling, monitoring one or the other might be sufficient to evaluate time trends. Strong but variable relations between metals concentrations in different media have been reported from other systems (Hornberger et al., 2009), suggesting that site-specific data would be needed before using concentrations in one media to predict another.

4.2 Benthic macroinvertebrates

The rank ordering of reoccurrences of common taxa as Cu declined formed a pattern reminiscent of species-sensitivity distributions from toxicity testing compilations (e.g., Brix et al., 2011). The patterns were also similar to recovery or sensitivity distributions developed from field studies of taxa recolonization streams recovering from acidification (Raddum and Fjellheim, 2003; Masters et al., 2007) and taxa occurrence in relation to freshwater ionic strength (Cormier et al., 2013). These similar distributions suggest ionoregulatory disruption as a common mode of toxicity, for Cu is known to block sodium transport. The colonization order as Cu declined in Big Deer Creek was also consistent with the susceptibility of insects to accumulate cadmium (Cd), which was suggested as being relevant to Cu iono-transport as well (Buchwalter et al., 2008). Taxa that were among the last to appear as Cu declined (*Ephemerella* and *Rhithrogena* mayflies, Figure 6) were the most susceptible to Cd accumulation. *Drunella* mayflies with intermediate Cd susceptibility appeared earlier than *Ephemerella* and *Rhithrogena* mayflies, and the caddisfly *Rhyacophila*, which had low susceptibility to Cd accumulation was an early colonist as Cu declined (Buchwalter et al., 2008; Figure 6).

The similarity between recovery patterns of benthic taxa in our study area expands and generalizes the observations of metals sensitive taxa from Colorado streams that were enriched with Cd, Cu, and Zn mixtures (Clements et al., 2000; Courtney and Clements, 2002; Clements et al., 2010). We are not aware of any previous reports of oligochaetes in the family Enchytraeidae appearing to be sensitive to metals in lotic systems. However, terrestrial enchytraeids (potworms) are a standard soil toxicity test organism and are sensitive to elevated Cu and other metals in moist soil. Severe declines in taxa richness have been noted by about 200 mg/kg dw Cu in soil, with low-effects thresholds occurring at less than 100 mg/kg dw Cu (Maraldo et al., 2006; Cedergreen et al., 2013). We were unable to evaluate whether enchytraeids were sensitive to Cu or Co in other stream studies, because most studies we reviewed on effects of metals in lotic systems did not report actual taxonomic data, and of those that did, oligochaetes were not identified beyond "Oligochaeta."

This in turn may reflect a prevalent but incorrect perception that aquatic oligochaetes are 'tolerant' to pollution, and thus not appropriately sensitive for use in ecological assessments (Chapman, 2001).

Two other exceptions to previous work were the mayfly *Epeorus* and the caddisfly *Rhyacophila*, both of which appeared to be sensitive to metals in Colorado field studies (Clements et al., 2000) but not here. *Rhyacophila* was one of the early colonizers in Big Deer Creek and was one of the most Cu tolerant species, while *Epeorus* appeared less sensitive to Cu than other mayflies. *Rhyacophila* was more prevalent in cooler Big Deer Creek, where it was common in both reference and high-Cu downstream areas, than in Panther Creek. Ott and Maret (2003) showed that in reference streams *Rhyacophila* and *Epeorus* were obligate coldwater taxa that declined with increasing stream temperatures. This suggests that the apparent sensitivity of *Rhyacophila* and *Epeorus* to metals in some surveys could actually have reflected an upstream/downstream temperature gradient, since reference streams are often located upstream of disturbed areas.

A conundrum in the literature on the effects of metals on stream insects has been the dichotomy between laboratory and field-based studies. Conventional short-term toxicity tests with field-collected stream insects have produced exorbitantly-high effect values relative to apparent effects from field studies, longer-term community microcosm tests, or to short-term tests with fish or cultured, small crustaceans such as amphipods or daphnids (Brinkman and Johnston, 2008; Brix et al., 2011; Mebane et al., 2012; Clements et al., 2013; Poteat and Buchwalter, 2014). For example, 137 μg/L Cu was required to reduce the survival of the mayfly *Rhithrogena hageni* by 50% in a 96-hour laboratory toxicity test (Brinkman and Johnston, 2008), yet recovery of *Rhithrogena* sp. in Big Deer Creek was apparently prevented by about 5 μg/L Cu or less (Figure 5, BD-km0.1). In our results, the insect-dominated benthic macroinvertebrate community was apparently very responsive to Cu and possibly Co at concentrations that did not produce discernable effects in fish populations. We think the present results support the views that (1) benthic macroinvertebrate communities may be susceptible to metals in the environment at concentrations far lower than effects concentrations produced from conventional short-term toxicity tests with aquatic insects, and (2) relative sensitivity rankings of aquatic insects to metals based on the latter may be misleading (e.g., von der Ohe and Liess, 2004; Malaj et al., 2012).

Tissue-residues have been advanced as a more meaningful measure of metals exposure and risk than metals in water because by definition, metals in tissues have already been taken up by the organism and reflect contributions from both dietary and waterborne exposures (Adams et al., 2011). Most of our macroinvertebrate tissue data were from community samples pooled from multiple species. The initial rationale for this community sample approach was to relate metals concentrations in benthic invertebrates to potential dietary toxicity in fish (Woodward et al., 1994; Beltman et al., 1999). While mixed taxa samples are less than ideal for trends monitoring, we have retained the original method over the years for continuity in the long-term monitoring record. In contrast, because of interspecies differences in metals bioaccumulation, others have related metals in tissues of targeted taxa such as the cosmopolitan, metals tolerant Hydropsychid caddisflies to predict alteration of sensitive stream benthos due to metals (Cain et al., 2004; Rainbow et al., 2012; Balistrieri et al., 2015). Consistent with Rainbow et al.'s (2012) prediction, Cu in Hydropsychidae was correlated with mayfly abundance (r^2 =0.63) and when limited to Big Deer Creek with its minimal Co concentrations, Hydropsychidae was very strongly correlated with mayfly abundance (r^2 =0.96).

Copper in both Hydropsychidae and the macroinvertebrate community tissue samples was strongly correlated with both Cu in water and periphyton, e.g., r^2 of 0.99 between Cu in periphyton and Hydropsychidae and r^2 of 0.97 for Cu in water and Hydropsychidae. Overall, the correlations between Cu and Co in water, periphyton, and tissue residues support the idea of using metals tissue residues as a predictor of effects to benthic communities. Metals in the diet of aquatic insects have been shown to be the predominant route of total tissue accumulation in controlled laboratory studies (Poteat and Buchwalter, 2014). Yet, the present results indicate that water chemistry also exerts a strong control on the levels of metals accumulated by periphyton and aquatic macroinvertebrates, which in turn has implications for toxicity.

4.3 Fish populations

Sampling issues

While bias, representativeness, and comparability of data are key concerns in all components of long-term monitoring efforts, the explicit emphasis of recovery goals on fish populations and controversies in the literature give these issues focus with fish population data. Although we did not perform any capture efficiency experiments, Meyer and High (2011) evaluated capture efficiency and bias in population estimates from electrofishing surveys that used methods very similar to our 2002–2013 collections. On the average their methods overestimated capture efficiency and underestimated absolute population size by about 22 to 27% (Meyer and High, 2011).

However, because we are evaluating recovery from disturbance rather than stock assessment, we are not as interested in absolute abundances as we are in relative abundances between reference and exposure locations. For comparisons with concurrently (within a few days) sampled reference sites, this requires an assumption of similar detection efficiency at reference and exposure sites. Our study sites were selected to have similar habitat features (Text S2 and Figure S3), and we believe this assumption is reasonable. However, the pre-restoration fish surveys used visual snorkel count methods that were not directly comparable to our post-restoration methods. Densities at reference sites estimated from snorkel surveys were about 20X lower than those we obtained by electrofishing at reference sites, with YOY fish underrepresented (Figure 10). Thus it is again necessary to assume that the snorkel data are internally valid, that is, the fish detection efficiency in the snorkel survey was similar between reference and mine-influenced sites, and that when snorkel counts are standardized as fractions of the reference condition, the standardized fractions are comparable between the snorkel and electrofishing data. Because the factors that likely influence the ability of snorkelers to detect fish (such as water clarity, depth, substrate, observer spacing, and lighting) were generally similar between the reference and mining-influenced sites (LeJeune et al., 1995), we assume similar detection efficiency at reference and mining-influenced sites.

The use of upstream reference sites presumes that in flowing waters, upstream conditions are little affected by downstream conditions. This assumption is not always true, especially with anadromous or other migratory species which need to transit downstream conditions in order to complete their life cycles. Anadromous salmon and steelhead use olfactory cues to return to their natal streams to spawn (Quinn, 2005), and Cu can disrupt chemoreception at sublethal concentrations (Hansen et al., 1999; Meyer and Adams, 2010). Declines of returning adult Pacific salmon also led to decreased delivery of marine-derived nutrients to streams, and locations with no or few salmon carcasses may in turn have lower productivity than streams with more abundant carcasses (Ebel et al., 2014). Thus if anadromous fish migration were impeded by water quality conditions, upstream sites would make unsuitable reference sites. For this reason, LeJeune et al. (1995) used geomorphically similar reaches from regional wilderness streams that were uninfluenced by downstream water pollution as reference sites for mining-influenced Panther Creek reaches. Because expeditionary wilderness sampling was infeasible for an annual monitoring strategy, we assumed that with water quality improvements, anadromous fish would not be impeded from reaching upper Panther Creek. While this assumption was met (Figure 10), regional salmon populations remain influenced by dams and other factors that likely limit adult returns and subsequent marine-derived nutrient delivery (Ebel et al., 2014).

Resident fish

Rainbow Trout rapidly occupied new habitats as they became marginally habitable. About 4 years elapsed from the first detections to when densities were similar to reference in Big Deer Creek. In Big Deer Creek, the trout population advanced downstream at about 0.5 km/year (Figure 4). Abundances of Rainbow Trout in Panther Creek appeared to initially overshoot reference densities, and then declined. This nonlinear recovery trajectory of bloom and decline of Rainbow Trout densities was similar to patterns observed in the recovery of an experimentally acidified lake. The first species to recover was White Sucker (*Catostomus commersonii*), and as the pH of the lake recovered to circumneutral levels, abundance greatly overshot pre-disturbance abundance but then declined as other species recovered (Mills et al., 2000). With Panther Creek Rainbow Trout, the decline following the initial rebound was coincident with increases in Shorthead Sculpin densities. In Panther Creek downstream of Blackbird Creek, trout outnumbered sculpin by 3 to 1 in 2002, but since 2006, sculpin have outnumbered trout by as much as 10 to 1. Because both sculpin and juvenile salmonids tend to preferentially feed on baetid mayflies, chironomids, and simuliids (Brocksen et al., 1968; Johnson et al., 1983; Boag, 1987; Riehle and Griffith, 1993), this suggests potential competition for food resources between Shorthead Sculpin and Rainbow Trout.

The age structure of Rainbow Trout in Panther Creek shifted toward younger fish contributing a greater proportion of the total number. In 1993, young-of-year were less abundant in mine-influenced sites in Panther Creek than in reference sites, yet by 2003 YOY were similarly abundant in mine-influenced and reference sites. In the quantitative electrofishing surveys, YOY were more abundant than older fish (Figure 10). While for space, we only showed plots at decadal intervals, YOY were the most abundant age group in all Panther Creek surveys from 2002–2013. This shift toward younger fish in the population age structure is consistent with expectations for recovering populations (Table 1).

Condition factors of Rainbow Trout in the upper Panther Creek reference sites and in the middle Panther Creek sites clearly rose and fell in synchrony (Figure 9). However prior to 2007, rainbow trout condition factors were subtly lower than those from the upstream reference reaches. Within the range of mean summer temperatures occurring in Panther Creek (about 10-15°C), we would expect higher growth in lower, warmer reaches (Railsback and Rose, 1999). Thus, the lower condition in Rainbow Trout collected in the mining-influenced middle reach of Panther Creek was counter to expected natural patterns. While co-occurrence alone does not indicate cause, the lower condition factors in the middle reaches of Panther Creek prior to 2007 and subsequent disappearance of condition factor differences as Cu declined and mayfly abundance increased is congruent with a scenario of metals stress and recovery (Figure 3; Figure 9; Table 1). While patterns were subtle, small differences in relative condition or size may be important to survival of juvenile salmonids in streams (Mebane and Arthaud, 2010). The subtleness of differences in fish condition in mine-influenced and reference streams may be inherent to the measure, as differences in condition factor reported even from areas with well documented metals stress were usually on the order of 10% or less (Schindler et al., 1985; Gauthier et al., 2009).

The interannual differences in Rainbow Trout condition upstream of the mine-influenced reaches, appeared to be density dependent, with a strong negative correlation between condition and Rainbow Trout density (r = -0.77, Text S2). Contrary to expectations that growth would be related to prey abundance, neither total invertebrate biomass or mayfly density were correlated with Rainbow Trout condition with r values of 0.00 and -0.11 respectively. Unlike Rainbow Trout, Chinook Salmon condition factor in Panther Creek upstream of mine-influences was not strongly correlated with either Chinook Salmon or Rainbow Trout density (r = 0.05 and -0.18 respectively, Text S2). This suggests that Chinook Salmon densities remain too low for density dependent growth limitation, and limited competition occurs between Rainbow Trout and Chinook Salmon relative to intraspecific Rainbow Trout competition.

Shorthead Sculpins have been slower to return than Rainbow Trout, presumably because of their limited home ranges and movements. In Panther Creek downstream of Blackbird Creek, about 2 km downstream from upstream source areas, Shorthead sculpin increases in abundance lagged Rainbow Trout by about 3-years. Yet at the sites downstream and upstream of Big Deer Creek, about 9 to 12 km from presumed source areas respectively, sculpin recovery lagged Rainbow Trout recovery by over 10 years (Figures 3 and 8). Shorthead Sculpin and Mottled Sculpin (*Cottus bairdii*) are closely related, and Mottled Sculpin and Rainbow Trout have overlapping sensitivities to prolonged Cu exposures (Besser et al., 2007). Thus differences in the intrinsic sensitivities to metals doubtfully explain the slower recovery of Shorthead Sculpin than Rainbow Trout. Sculpin usually have very restricted home ranges with typical annual movements on the order of 10 m or less, and their maximum measured annual movements have only been up to about 500 m, whereas stream-resident trout may move tens of kilometers or more per year (Brown and Downhower,1982; Schmetterling and Adams, 2004; Breen et al., 2009). Sculpin have been observed to recover more slowly than more motile fish elsewhere. Milner et al. (2008, 2011) reported salmonids colonized newly accessible stream habitat much faster than sculpin. In the recovery of an experimentally acidified lake, sculpin had not recolonized the lake 13 years after chemical recovery even though it was connected to a seed population by an inlet stream (Mills et al., 2000). Niemi et al. (1990) found that after relatively small stream habitat areas were decimated, such as by experimental pesticide pulses to small streams where the pollutant would be quickly flushed out and refugia was nearby, the median time for the first appearance of salmonids was 10X shorter than for cottids (0.17 years for salmonids vs. 2.0 years for cottids). However, for less severe disturbances that did not completely extirpate local populations, recovery times were similar between salmonids and cottids (Niemi et al., 1990), likely because recovery times were limited by maximum reproductive rates and resource availability, rather than fish movements and distance from refugia.

Anadromous fish

Natural recolonization of Chinook Salmon into Panther Creek was occurring by 2002, as evidenced by observations of spawning adults during September of that year. As of 2013, the Chinook Salmon population was naturally reproducing and self-sustaining with abundances within the range of unpolluted streams in the region. In quantitative surveys using sodium cyanide to obtain a complete kill of fish within block netted sections of tributary streams to the mid-Columbia River, the median and 90th percentile Chinook Salmon densities at 69 stream sites were 8 and 36 fish/100 m^2 (Mullan et al., 1992), whereas we measured 10-30 fish per 100 m^2 in the middle and upper reaches of Panther Creek in 2010–2013 (Figure 11). Higher densities of Chinook Salmon in Upper Panther Creek above Blackbird Creek than in lower reaches are likely related to proximity to hatching sites. About 80% of the suitable habitat for Chinook Salmon spawning in the Panther Creek watershed occurs upstream of Blackbird Creek, which is mostly due to lower channel gradients found in upper Panther Creek and in Moyer Creek (Reiser, 1986). Young-of-year salmon do not disperse widely from their hatching location, and their distribution in streams generally reflects redd distribution (Richards and Cernera, 1989).

The recolonization of Chinook Salmon in Panther Creek was complicated by a large release of hatchery-origin adult salmon in the fall of 2001. Because Chinook Salmon from the Salmon River have either a 4- or 5-year life cycle (Mebane and Arthaud, 2010; Kennedy et al., 2013), YOY found in Panther Creek in 2002, 2006–2007, and 2010–2012 can be attributed back to the 2001 transplants of South Fork Salmon River fish. Genetic analyses of Chinook Salmon tissue samples collected in 2010 and 2011 showed that 85 to 90% of the fish could be linked to hatchery origins and the remainder resembled fish from the nearby Middle Fork Salmon River and Upper Salmon River populations as well as a small component from an uncharacterized population (Smith et al., 2012). This implies that in 2010–2011, the descendants of the hatchery salmon swamped any natural origin salmon. However because of their rigid life cycle timing, adult Chinook Salmon observed in 2002, 2004, 2007–2008 and 2012, and YOY observed in 2003–2005, 2008–2009 and 2013 could not have resulted from the 2001 hatchery releases. The original source(s) of the naturally colonizing fish are uncertain. Possible source populations for the natural-origin fish in Panther Creek may include early recolonizers from Clear Creek. Clear Creek, located low in the Panther Creek watershed, may have served as a clean water refuge with limited exposure of migrants to elevated Cu concentrations. At least the surveyed lower 6 km of Clear Creek had suitable steelhead and Chinook Salmon spawning and rearing habitat (Reiser, 1986). During the 1980s and early 1990s when Cu concentrations in middle Panther Creek upstream of Clear Creek were still likely too high to support fish, repeated anadromous fish sightings were reported from lower Panther Creek near Clear Creek. (Reiser, 1986; Mebane, 1994).

Anadromous steelhead are difficult to assess because the pre-smolt juveniles are physically indistinguishable from resident Rainbow Trout. The historical steelhead population was presumably extirpated or at least greatly diminished along with Chinook Salmon in the 1960s, but began to return to the lower reaches of Panther Creek near Clear Creek by the mid-1980s (Mebane, 1994). Wild and hatchery steelhead populations in the Salmon River basin, including Panther Creek, have been evaluated through tagging out-migrants with PIT tags and genetic stock identification. As of 2011, the modeled abundance of steelhead spawners returning to Panther Creek was 485 adults (Copeland et al., 2013). Because of plasticity between the resident and anadromous forms of Rainbow Trout (Sloat et al., 2014) it is possible that a portion of the present Panther Creek steelhead population was contributed by straying adults from other drainages or from the existing resident Rainbow Trout that persisted in upstream or tributary refugia such as Clear or Beaver Creeks.

4.4 Factors affecting recovery

Copper

Particularly in Big Deer Creek, declining Cu concentrations clearly corresponded with progressive range expansions and increasing abundances of invertebrates and fish. At the outset of our study, Big Deer Creek downstream of the South Fork Big Deer Creek was nearly lifeless and because of the waterfall in the lower reaches, colonization could only occur from upstream to downstream. As of 2007 and later, the biological communities in Big Deer Creek immediately after mixing with the South Fork Big Deer Creek (site BD-km5.3) were comprised of mostly similar taxa as those present upstream. Cobalt is low in Big Deer Creek relative to Panther Creek, averaging 2.0 µg/L at BD-km5.3 vs. 22 µg/L at PA-km37 in 2013 (Table 2), suggesting that colonization in Big Deer Creek was limited more by Cu and distance from colonizing sources than Co.

The timing of first appearances of different taxa at the sites close to upstream colonizing source areas suggests thresholds above which Cu appeared to prevent occupancy. With Rainbow Trout in Big Deer Creek and South Fork Big Deer Creek (BD-km5.3 and SFBD-km0.2), the pioneering adults first appeared when Cu dropped to below about 4X the chronic criterion during baseflow conditions (Figure 4). When Cu dropped below about 3X the baseflow chronic criterion, the presence of YOY fish indicated some reproduction and survival of early life stages was occurring. By the time (2006) that the Rainbow Trout population characteristics at BD-km5.3 were indistinguishable from reference, smoothed Cu concentrations had dropped to 1.2X the baseflow chronic criterion. Benthic macroinvertebrate taxa richness at BD-km5.3 first reached that of reference in 2004, at which time smoothed Cu had dropped to about 1.8X the baseflow chronic criterion. However, while few taxa present at the reference site (BD-km5.6) were absent from the samples at BD-km5.3, abundances of some mayfly taxa that were common upstream at BD-km5.6 remained consistently fewer at BD-km5.3. As of 2013, abundances of the common mayflies *Caudatella hystrix, Ephemerella, Cinygmula,* and *Rhithrogena* were 50 to 60% that of reference, at which time smoothed Cu concentrations were about 0.6X the chronic criterion. Reductions in total mayfly abundance on the order of 50% have been observed with 10-day aquatic insect microcosm exposures to Cu at only 0.8X the mean BLM-based chronic criterion (Clements et al., 2013), which suggests that Cu concentrations at BD-km5.3 averaging about 0.6X the Cu criterion could plausibly have caused reduced abundances of particularly Cu sensitive mayflies.

Shorthead Sculpin in Panther Creek downstream of Blackbird Creek were present in low numbers during our initial surveys in 2002 when smoothed Cu was at about 1.5X the chronic criterion. Sculpin densities greatly increased after 2004, which corresponded to smoothed Cu concentrations only 1.2X higher than the Cu chronic criteria (Figure 7). However, it is not possible to detangle whether this increase in sculpin densities was related to relaxed Cu stress, increased aquatic macroinvertebrate prey base, or simply reflects the exponential phase of a population growth curve in newly colonized habitats.

Pulsed vs. stable Copper exposures

Copper concentrations fluctuate seasonally in the study area, which has implications for interpreting Cu concentrations in relation to apparent sensitivities with co-occurring aquatic organisms. Prior to 2012, Cu was elevated for about a week to a month during the spring snowmelt runoff period with concentrations 5X or more higher than the mostly stable concentrations occurring during the other ~11 months of the year. In 2012–13, average concentrations during the runoff were about 2X higher than baseflow concentrations (Figure 2). While concentrations are mostly stable outside the spring runoff season, isolated summer thunderstorms may cause brief, pulsed Cu exposures, such as the September 5, 2013 event with a several hour pulse of increased Cu that peaked at 10X pre-storm baseline. This situation raises the question, are effects attributable to Cu more likely from the higher, brief pulse exposures or from the much lower and longer press exposures to Cu during low flow periods? Because our biological sampling was annual, organisms were exposed to both the annual short-term pulse and to long-term, less variable and lower concentrations. These are difficult influences to separate even in controlled experiments (Johnston and Keough, 2002), and our data alone are insufficient to untangle these influences. However, three lines of reasoning suggest that the lower, long-term Cu exposures had the greater influence. First, the timing of the snowmelt-driven Cu pulses occurs at times that larger and less metals-sensitive life stages of aquatic insects would be expected to be present (Clark and Clements, 2006). The sensitivity of fish to metals toxicity is also size-dependent. Egg and alevin stages are particularly resistant to metals, and the most sensitive sizes appear to be YOY salmonids of about 6 to 16 weeks post-hatch, and newly emerged YOY sculpin (Chapman, 1978; Besser et al., 2007; Mebane et al., 2008, 2012). In nearby streams of similar elevation, Chinook Salmon, Rainbow Trout, and Shorthead Sculpin tend emerge from the gravels in June to August, after peak runoff (Bailey, 1952; Orcutt et al., 1968; Richards and Cernera, 1989). During the annual April to early June Cu pulses, less sensitive eggs and alevins would be present. Second, DOC mitigates Cu toxicity and because DOC also tended to increase during the spring runoff, the DOC-influenced Cu criteria tends to rise and fall in synchrony with the ambient Cu concentrations (Figure 3). Third, correlations between biological endpoints and average Cu concentrations tended to be stronger than with peak annual Cu concentrations (Text S2).

The September 2013 storm-pulse of Cu in Big Deer Creek appeared to influence the benthic community structure at BD-km5.3, sampled 4-days later. Taxa richness (presence of taxa) was subtly lower and some mayfly abundances declined in Big Deer Creek in 2013 relative to 2012. Most notable was *Baetis* with the lowest relative abundance since 1993 (Figure 5). Massive drift of mayflies and *Baetis* in particular has been reported within minutes of a chemical disturbance in streams (Ormerod et al., 1987), and drift appears to be an avoidance response of some aquatic insects to short-term, novel metals exposures (Clements, 2004). Because recovery times for benthic abundance following non-catastrophic drift episodes in streams with nearby upstream colonization sources have been on the order of two to six weeks (Wallace, 1990; Clearwater et al., 2011), it is likely that our collections were affected by the storm pulse 4-days earlier. A pulse of Cu from storm runoff would also be expected to be accompanied by an increase in DOC which could mitigate Cu toxicity. For instance, in Silver Bow Creek, Montana, Balistrieri et al. (2012) captured an increase in dissolved Cu from about 6 to 27 μg/L and an increase in DOC from about 4 to 13 mg/L during the first 1.5h of a rain storm. The extent to which an increase in DOC would mitigate Cu toxicity would likely be influenced by whether the pulses were in synchrony, their relative concentrations, and contact time between the DOC and Cu.

Cobalt

While the risks of Co in aquatic ecosystems are less well known than those of Cu, Co in the Blackbird Creek drainage is elevated more than two orders of magnitude above background concentrations. In 2013 concentrations in Panther Creek at site PA-km37 exceeded 40 μg/L during September stable flow periods (Figure 3). In contrast with Cu, Co concentrations are lowest during the brief spring snowmelt period, and highest and fairly stable during the low flow periods.

Ecotoxicology data for Co are sparse relative to Cu, but what there are suggest very different aquatic toxicity profiles for Co and Cu. Whereas both fish and invertebrates may be sensitive to low Cu concentrations that were elevated only 3 to 5X above background concentrations (e.g., Besser et al., 2007; Mebane and Arthaud, 2010; Clements et al., 2013), we found no reports of Co having any direct adverse effects to fish at environmentally relevant concentrations. In a 60-day growth and survival test of Rainbow Trout fry using dilution water from upper Panther Creek, the lowest observed effect was a 5% reduction in growth at 242 μg/L (Pacific EcoRisk, 2005). Marr et al. (1998) compared the relative toxicity of Cu and Co to Rainbow Trout in 14-day toxicity tests (test pH 7.6, DOC 0.2 mg/L, hardness 25 mg/L). Copper was both a more potent and faster acting toxicant with incipient lethal levels >20X lower than those for Co (14 vs. 346 μg/L respectively). In mixture tests with Cu and Co with Rainbow Trout, the acute toxicity of Cu was not consistently increased by the presence of Co or vice versa (Marr et al. 1998).

In contrast to the apparent indifference of fish to Co, adverse effects to freshwater invertebrates from Co have been observed at concentrations almost three orders of magnitude lower than those adverse to fish. Norwood et al. (2007) obtained a 28-day LC25 of only 4 μg/L with the amphipod *Hyalella azteca* (test pH 8.2, DOC 1.1 mg/L, water hardness 122 mg/L). Similar low effects concentrations with Co have been obtained with daphnids and snails (Environment Canada, 2013). However, we are only aware of two long-term exposures of stream-resident aquatic insect species with Co. A 20-day exposure of *Chironomus dilutus* larvae to Co in Panther Creek water only produced a 20% reduction in survival at 216 μg/L (Pacific EcoRisk, 2005). These insensitive results were congruent with field observations in Panther Creek. Prior to restoration efforts, Chironomids were abundant in Panther Creek at PA-km37 with >6,000 individuals/m^2 and average Co concentrations of about 90 μg/L (Beltman et al. 1999; Table 2). In contrast to the insensitive *Chironomus* results, exposure of mayfly *Ephemerella ignita* nymphs for 28-days to 33 μg/L Co in the Rickleå

River, Sweden, resulted in only 26% survival compared to 77% survival in the controls, and a 48% reduction in growth (as wet weight) relative to controls. Low effects (7% reduction in weight; a 4-day delay in median emergence times) were observed in the lowest 5.2 μg/L Co treatment (Södergren, 1976). These results suggest that Co concentrations in the range of 10–50 μg/L in Panther Creek could contribute to the scarcity of *Ephemerella* and other taxa (Figure 3; Figure 5). Cobalt toxicity is moderated by Ca and DOC (Richards and Playle, 1998), and Co potency in Panther Creek is probably roughly comparable to that in the Rickleå River tests. Compared to the Rickleå River water, Panther Creek has higher Ca which would make Co relatively less toxic that in Södergren's (1976) tests, but lower DOC which would have the opposite effects (Ca about 12 mg/L vs. 3.5 mg/L, and DOC about 2 mg/L vs. about 10 mg/L during base flows for Panther Creek and the Rickleå River respectively, with DOC estimated from Hoppe et al. (2015)).

Arsenic

Arsenic has been persistently elevated in sediment and periphyton in Panther Creek, and in some years has been elevated in invertebrate tissues. However, the high arsenic concentrations in sediment and periphyton did not appear to pass through to aquatic macroinvertebrate tissues (Figure 2), suggesting that the arsenic sorbed to sediments or periphyton might have low bioavailability. In toxicity testing of Panther Creek sediments with the benthic invertebrate *Hyalella azteca*, correlations between reduced biomass were much weaker with arsenic than with Cu ($r = -0.27$ and -0.84, respectively) suggesting arsenic had less influence on benthic communities than Cu or Co (Mebane, 1994). Arsenic and Fe have been shown to be strongly correlated in Panther Creek sediments ($r > 0.9$), suggesting sequestration in Fe oxyferrihydroxides in stream sediment (Mok and Wai, 1989; Mebane, 1994; Gray and Eppinger, 2012). Erickson et al. (2010), noted that inorganic arsenic in the diet of trout at about 20 mg/kg dry weight (dw) or higher has been correlated with reduced growth, and in their feeding study with live invertebrate diets enriched with arsenic, 26 mg/kg dw or higher arsenic in the diet was directly demonstrated to impair growth in Rainbow Trout. Arsenic residues in Panther Creek invertebrate tissue were slightly above 20 mg/kg dw in some years, although the years with lower condition factors in fish did not match years with elevated arsenic in invertebrate tissues (Figures 2 and 10). Our 2012 targeting of specific taxa showed decreasing concentrations with increasing trophic level, suggesting bio-dilution through trophic transfer.

Biological factors

The recoveries of benthic macroinvertebrate communities have been uneven as Cu and Co stress have lessened over time. The community at one site, BD-km5.3, has become mostly similar to that of the nearby upstream reference site. However, species richness at other sites remains lower than that at reference sites, even though major groups such as the mayflies and stoneflies have become well represented throughout Big Deer and Panther Creeks.

Distance to source populations and interspecific competition could influence aquatic insect recolonization. Dispersal abilities by air or drift vary greatly among freshwater invertebrates, but are usually reported as <1 km per generation, and dispersal is often from downstream to upstream (Mackay, 1992; Elliott, 2003; MacNeale et al., 2005). Perennial tributaries with clean-water refugia areas are present along Panther Creek (Figure 1), suggesting dispersal is unlikely a persistent limiting factor in recovery of stream insects in our study area, unlike some areas (Masters et al., 2007; Milner et al., 2008; Brederveld et al., 2011). Still, sites in lower Panther Creek (PA-km17 and km22) could be distant enough from large clean water tributaries that dispersal distances contribute to a lag in the recovery of benthic communities following water quality improvements.

Taxa richness is greater in mine-influenced sections of Big Deer Creek than those in Panther Creek (Figure 3, Figure 4). Colonization began on nearly bare substrates in Big Deer Creek, whereas in Panther Creek prior to water-quality restoration, benthic macroinvertebrates were about as abundant as at upstream reference sites, but were dominated by a few taxa, usually chironomids and *Brachycentrus* caddisflies (Mebane, 1994; Beltman et al., 1999). *Brachycentrus* is a strong competitor that once established can exclude other insects (Peterson et al., 1993). This suggests the possibility of biotic resistance from extant or early colonizing taxa that could compete with later arrivals. Competition and trophic changes may lead to indirect biological effects that can either mask or amplify direct effects through modifying competition for limited food or space resources (Fleeger et al., 2003; Johnston and Keough, 2003). After following the colonization and succession of macroinvertebrates for several years after re-watering of a river channel, Minshall et al. (1983) suggested that the final structure of the benthic community may be determined to a large extent by which species become established first. Conceptually, succession and resilience dynamics in macroinvertebrate communities could result in new stable states, and resist return to reference conditions (McAuliffe, 1984; Fisher, 1990).

Benthic macroinvertebrates and fish populations

Fish populations in mining-influenced streams may be indirectly constrained by reduced macroinvertebrate prey availability (Hogsden and Harding, 2012), which could be reflected in reduced abundances or reduced condition factor (Munkittrick and Dixon, 1989). However, a few benthic macroinvertebrate taxa probably are of disproportionate importance to fish populations. Chinook Salmon, other stream-resident salmonids, and sculpin have been shown to feed primarily on *Baetis* mayflies, chironomids, simuliids, as well as other bite-sized, abundant taxa such as the stonefly *Zapada* (Bailey, 1952; Allan, 1983; Esteban and Marchetti, 2004). These taxa were early colonizers in Panther Creek and remain abundant. However, salmonids are opportunistic and will eat any invertebrates that are about the right size for their gape and are palatable. Conceptually, increased benthic diversity might decrease the reliance of any particular taxa and reduce the likelihood of seasonal shortages following hatches. While as of 2013 benthic macroinvertebrate diversities downstream of mine-influenced tributaries remain about 10 to 30% lower than those at the reference sites, juvenile fish growth, as inferred from body condition and length vs. weight regressions in salmonids, increased from the headwaters sites downstream (not shown), as would be expected with increasing temperatures in least-disturbed natural streams. Panther Creek in 2013 had the highest overall fish densities measured during this project, and a decline in invertebrate biomass relative to 2012. Whether salmonids can effectively depress benthos is uncertain (Allan, 1983), but sculpin certainly can, at least for some vulnerable taxa such as chironomids and *Baetis* mayflies (Brocksen et al., 1968; Flecker, 1984). In sum, fish populations do not appear to be any more limited by prey availability in mine-influenced downstream reaches than upstream reference reaches.

4.5 Natural ecological variability and recovery

Natural stream communities are predicted to change along longitudinal gradient as the physical features of streams change from headwaters to mid-order streams to large rivers (Vannote et al., 1980). This presents a challenge in stream pollution ecology studies because reference sites may need to be located upstream of anthropogenic disturbances in river basins, resulting in overlying longitudinal natural and anthropogenic gradients. For instance, fish species richness increases with increasing stream size, and steep gradient, headwater streams are commonly only inhabited by a single salmonid species. As the streams become less steep and larger and riffle habitats become more common, sculpin will appear and become numerically dominant in mid (3rd and 4th order streams). As streams transition to rivers, shallow riffles give way to deeper run and pool habitats, and the numerical dominance of sculpins and salmonids will decline as minnows and suckers become abundant (Platts, 1979; Mebane et al., 2003).

Several lines of evidence allow us to attribute biological changes to water quality changes rather than co-occurring streamflow or temperature differences. First, even between our most distant sites (PA-km4, ~40 km downstream from reference sites), summertime high temperatures are about 2°C warmer but habitats are otherwise fundamentally similar. Shorthead Sculpin and Rainbow Trout remain abundant, and temperature sensitive genera such as *Epeorus* and *Zapada* and metals sensitive taxa such as *Cinygmula*, and *Ephemerella* were common at both PA-km4 and at upstream reference sites (Mebane et al., 2015). Second, the tributary sites had particularly well matched reference and assessment sites. In Big Deer Creek upstream of the waterfall, the physical stream characteristics were very similar between the reference and mining-influenced sites.

Changing correlation patterns over time between physicochemical and biological metrics give clues to relative influences of mining and natural factors. Wiens and Parker (1995) suggest "impact" can be defined as a statistically significant correlation between injury and exposure, and "recovery" can then be defined as the disappearance of such a correlation through time. During the 1993–2002 period which bracketed the onset of restoration activities, there were strong, negative correlations between mayfly and stonefly densities with Cu (r values of -0.89 and -0.76 respectively), whereas during the latter 2003–2013 recovery period, the correlations were much weaker or reversed, with r values of -0.17 and 0.25, respectively. In contrast, taxa richness remained strongly correlated with Cu (r -0.80) but not with temperature (r -0.15). The weak correlation between taxa richness and temperature in Panther Creek is consistent with natural patterns from least-disturbed streams elsewhere in the Salmon River basin. Species richness in 33 streams selected for their minimal anthropogenic disturbances was only weakly correlated with annual maximum weekly maximum temperatures (r -0.38) despite a span of 12°C (Ott and Maret, 2003). In contrast, the difference between the matched furthest upstream and furthest downstream sites in Panther Creek was only about 2°C.

Interpretations of perceived changes may always be open to some debate in ecological field studies and inferences of effects and recovery rely on coherence of lines of evidence rather than strict causality (Parker and Wiens, 2005; Munkittrick, 2009). Prior to the restoration efforts that began in 1995, there were clear differences between the reference and mine-influenced sites in metals exposures and associated biological measures. Throughout the post-restoration recovery period, declines in metals concentrations were accompanied by or followed by declines in apparent biological effects attributable to metals, and correlations between exposure and effects have weakened or disappeared.

5. Conclusions

The changes that we observed in the Panther Creek watershed gave insights on ecosystem recovery patterns that would not necessarily have been predicted from chemical monitoring or small-scale toxicity testing alone. These include:

1. Fish populations recovered rapidly once the limiting water quality constraint was relaxed, on the order of about three generations to reach reference densities;
2. Speed of recovery differed greatly for species with different traits. The larger bodied, comparatively pelagic, salmonid species recovered much faster than the less motile smaller-bodied, benthic sculpin species;
3. Recovery needed to be viewed on a landscape scale. Recovery of anadromous fish was limited by factors outside of the watershed, in addition to any within-watershed constraints such as water-quality, and the influences of disturbances other than those that were the focus of the study (e.g., wildfire) had to be considered;
4. When investigating the effects of disturbances in streams such as mine pollution from discrete tributary sources, conditions upstream of the affected environment are an obvious point of reference. Yet in flowing waters, a study design relying on upstream-downstream, reference-comparison monitoring sites introduces the complication that potential effects of pollution could be masked by or mistaken for natural longitudinal changes in the stream ecology;
5. Water quality suitability was not a binary, yes/no question hinging on criteria being met or not. Fish began to move into marginally suitable habitats while Cu concentrations were still suboptimal (~3X criterion), yet it is possible that even at <1X criterion, Cu continued to influence the insect community;
6. The benthic macroinvertebrate community gained species as Cu and Co declined in patterns that were congruent with concepts of sensitivities of insects to ionoregulatory disturbance and critical body residues of metals;
7. Cu and Co concentrations in water, sediment, periphyton, and macroinvertebrate tissues were all correlated, yet taxa richness was most strongly correlated with Co in water followed by Cu in water;
8. Lower taxa richness and scarcity of some invertebrate taxa relative to nearby reference sites suggests that Co and/or Cu may be toxic to a minority of taxa at concentrations lower than numeric recovery targets. There was little evidence that this reduced taxa richness measurably affected recovery of fish populations, which in turn probably depend more on overall abundance of common taxa such as baetid mayflies, simuliids, and chironomids;
9. Distance and direction to source areas of colonists both appeared to influence recovery times with at least Shorthead Sculpin appearing to extend their range mostly from downstream to upstream; and
10. For both fish and invertebrates, the presence and abundance of species are probably nonexclusively influenced by both internal factors (intrinsic physiological sensitivity to metals), and external factors (competition from early colonists inhibits later arrivals).

Reviews of recovery of freshwater ecosystems have emphasized times to recovery (Niemi et al., 1990; Detenbeck et al., 1992; Jones and Schmitz, 2009). In some pulse disturbances (such as accidental spills or deliberate fish poisonings, experimental insecticide treatments, or re-watering a de-watered channel), there is a discrete point in time in which the direct disturbance ended, and the time to recovery began. However, water quality improvements from longstanding impairments on the physical scale of the Blackbird Mine remediation are progressive and adaptive (Gustavson et al., 2007; USEPA, 2013). Without discrete starting or finish lines to measure time to recovery, recovery times are decidedly ambiguous.

The Panther Creek restoration largely meets Palmer et al.'s (2005) five criteria for ecologically successful river restoration. These criteria were 1) the project design should be based on a specified guiding image of a more dynamic, healthy river that could be attained [most germane to projects with physical or hydrological manipulations]; 2) the river's ecological condition must be measurably improved; 3), the river system must be more self-sustaining and resilient to external perturbations so that only minimal follow-up maintenance is needed; 4) during the construction phase, no lasting harm should be inflicted on the ecosystem, and 5) both pre- and post-assessments must be completed and data made publicly available (Palmer et al., 2005). We think criteria 1, 2, 4, and 5 have been met. For the criterion no. 3, while the river's ecology is self-sustaining so long as active pollution controls are maintained, the costs of pollution controls are nontrivial. The water treatment plant must continue to operate for the foreseeable future, and the various runoff controls require annual upkeep. Finally, the past environmental costs and subsequent financial costs of the Panther Creek watershed restoration were substantial.

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Contributions

- Contributed to conception and design: RJE, BGF, CAM
- Contributed to acquisition of data: RJE, BGF, CAM
- Contributed to analysis and interpretation of data: CAM, RJE
- Drafted and/or revised the article: CAM, RJE, BGF, WJA
- Approved the submitted version for publication: CAM, RJE, BGF, WJA

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Competing interests

CAM, RJE, and BGF have no competing interests. WJA is employed by Rio Tinto, a metals and mining corporation with partial financial responsibility for the Panther Creek restoration efforts.

Supplementary material

- **Figure S1. Interactive study area detail map, viewable with Internet-based, two-dimensional map and three-dimensional Earth browsers such as the Google Earth application (http://www.google.com/intl/en/earth/index.html).** doi: 10.12952/journal.elementa.000042.s001
- **Text S2. More information on: (1) Methods, including concepts for evaluating recovery, and (2) Supplemental findings relating to statistical analyses, Blackbird Creek, native fishes, and wildfire influences.** doi: 10.12952/journal.elementa.000042.s002
- **Figure S3. Photographs of habitat features of the study sites, including substrates and organisms.** doi: 10.12952/journal.elementa.000042.s003

Data accessibility statement

Major datasets generated for this study are provided via the Dryad Digital Repository http://doi.org/10.5061/dryad.67n20. These include stream chemistry, temperature, and streamflow data, metals concentrations in tissues and sediments, and fish and benthic macroinvertebrate community data.

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