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Changes in Water Chemistry and Biological Communities Associated with Metal Mining in Streams in the North Cascades

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Changes in Water Chemistry and Biological Communities Associated with Metal Mining in Streams in the North Cascades

By

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Accepted in Partial Completion

For the Degree of

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

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MASTER'S THESIS

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Changes in Water Chemistry and Biological Communities Associated with Metal Mining in Streams in the North Cascades

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Brooke G. Bannerman
January 2017

Abstract

Hard rock and placer mining have been occurring throughout the mountains in the northern portion of Washington State since the late-1800s. As a result, aquatic ecosystems in this region are susceptible to the physical, chemical and biological changes that result from mining activities. These alterations, which include changes in water chemistry, habitat modifications, and reduction or contamination of food sources, can adversely impact aquatic communities of periphyton, benthic macroinvertebrates and fish. To evaluate changes in water chemistry and biological communities in two regions with extensive mining histories, the Ruby Creek watershed and Upper Skagit River watershed, I analyzed metals in grab samples of surface water, on Stabilized Liquid Membrane Devices (SLMDs) which passively sample metals in surface waters over time, and in periphyton.

Metals were present in the water and benthos, and site-specific and temporal differences in the kinds and quantities of metals were linked to locations of hard rock and placer mining activities. Metal concentrations in surface waters differed between sites upstream and downstream of mining depending on different times when mining was or was not occurring. Metal concentrations in surface waters at some sites in the Ruby Creek watershed were high enough to be capable of adversely affecting aquatic organisms over time. Metals that were present in streams were not always detected in grab samples, but their presence was confirmed by SLMDs and periphyton. Clustering analyses of both SLMDs and periphyton each distinguished two different groups of samples, samples collected downstream of placer mining (SLMDs) and samples collected downstream of hard rock mining (periphyton). The accumulation of metals in periphyton indicated these communities could be a concentrated source of toxic metals to primary consumers, such as small aquatic insects, and may pass to other aquatic organisms at higher trophic levels through dietary exposures.

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Introduction

Mining, the process of extracting material from the environment to recover valuable minerals, has been officially permitted on public lands since 1872 when the General Mining Act was passed. The General Mining Act permits United States citizens to establish patented or unpatented claims on federal lands excluding national parks, national monuments, designated wilderness areas, and administrative areas such as campgrounds. If a claim is patented, the federal government releases the deed to the land to the claim owner. However, in 1984 a moratorium was placed on granting patents on claims. The Bureau of Land Management (BLM) is granted statutory authority for federal lands under the Mining Act (Woodhouse et al. 2006). Due to diverse statutory authority and management goals among state and Federal agencies responsible for managing public lands, a variety of recreational and commercial pursuits that alter the landscape may be permitted including road development, timber harvesting, hydroelectric installations, and recreational mining.

Since 1850 when gold was first discovered in the North Cascades, a portion of the Cascade Range located in northern Washington State, prospectors have searched for valuable metals using two methods: hard rock mining, which is underground mining, and placer mining, which entails the sorting of stream bed deposits (Woodhouse et al. 2006). Historically established hard rock mines on unpatented and patented claims are still operating, although the lack of commercial viability has caused a considerable reduction in activity. Since the 1950s placer mining through the use of suction dredges has become a popular recreational activity in mountain streams (Stern 1988). Both hard rock mining and suction dredging have been associated with a variety of long-term adverse physical, chemical, and biological effects on aquatic ecosystems (Hogsden and Harding 2012).

Acid mine drainage (AMD), the most well-documented environmental impact associated with hard rock mining, is toxic to aquatic systems, varies overtime, and persists even after mines are abandoned (Hogsden and Harding 2012). Pyrite is a ubiquitous sulfide-bearing mineral that is associated with nearly all metal ore deposits and is the most common cause of AMD (Gray 1997; Lottermoser 2003). AMD occurs by oxidation of sulfide minerals to generate sulfuric acid in water-filled mine tunnels or in water percolating through mine tailings. This results in the generation of both acidic surface water and ground water at a mine site (Lottermoser 2003). Typically, AMD water is enriched with metals because the acidity promotes metal dissolution from waste piles and underground mineral deposits (Lottermoser 2003; Bryne et al. 2012). The acidic metal-laden AMD water infiltrates streams through surface water runoff and ground water contributions to streamflow (Gray 1996; Bryne et al. 2012). The amount of AMD water entering streams varies with weather conditions, often increasing after heavy rains following long dry spells (OlÍas et al. 2004). AMD also occurs at abandoned mines because the groundwater table that is lowered by pumping during mining activities rebounds, flooding underground tunnels and exposing sulfide minerals to oxidation (Bryne et al. 2012).

AMD alters water chemistry in streams by reducing pH, elevating concentrations of metals, and forming metal hydroxide deposits. The amount of reduction in pH is determined by the buffering capacity of the stream, which then affects metal speciation and thus toxicity (Rand 1995; Gray 1997). In poorly buffered streams, metals are predominantly present in their dissolved form because the water is more acidic; in well-buffered streams, the water is less acidic causing metal hydroxides to precipitate and sink to the stream bed (Hogsden and Harding 2012).

The chemical and physical alterations associated with AMD reduce biological species richness and abundance (Hogsden and Harding 2012). In poorly buffered streams, acid-sensitive species of periphyton are replaced by more tolerant forms such as diatoms and filamentous green algae (Douglas et al. 1998, Verb and Vis 2000, DeNicola and Stapleton 2002, Bray et al. 2008, Hogsden and Harding 2012). This results in an increase in biomass because large-celled filamentous green algae become dominant (Verb and Vis 2000, Bray et al. 2008). In contrast, in well-buffered streams metal hydroxide deposits reduce colonization on those surfaces, cloud the water thus reducing light penetration and benthic photosynthesis, and smother algae by covering them (McKnight and Feder 1984; Niyogi et al. 1999); the overall effect is a reduction in periphyton biomass (Hogsden and Harding 2012). Benthic macroinvertebrates and fish are affected by AMD in similar ways. Osmoregulation is impaired as pH is reduced and metal concentrations are elevated (Letterman and Mitsch 1978; Baker and Schofield 1982; Herrmann et al. 1993; Schultheis et al. 1997; Wendelaar Bonga 1997; Rainbow 2002; Soucek et al. 2002a; Pane et al. 2004; Battaglia et al. 2005; MacCausland and McTammany 2007; Hogsden and Harding 2012). In less acidic conditions, metal hydroxide precipitates clog and damage gills causing respiratory distress and routes for secondary infection (Rosseland et al. 1992; Soucek et al. 2000b; Hogsden and Harding 2012). The coating of metal hydroxides on the stream bed reduces the amount of suitable habitat for periphyton, invertebrates, and incubating fish eggs (Rosseland et al. 1992; Soucek et al. 2000b; Hogsden and Harding 2012). Subsequently, AMD can reduce and contaminate food sources for invertebrates and juvenile fish (Letterman and Mitsch 1978; McKnight and Feder 1984; Woodward et al. 1995; Niyogi et al. 2002a; Hogsden and Harding 2012).

Suction dredging, as the most practiced form of placer mining, increases turbidity and mobilizes metals (Harvey and Lisle 1998; CDFG 2009). Turbidity increases downstream of suction dredging and may remain elevated above ambient levels up to 160 m downstream of mining activities (Harvey 1986; Thomas 1985; Harvey et al. 1982, 1986; Stern 1988; CDFW 2009). Increases in turbidity can alter growth and survival of aquatic animals by clogging and damaging gills, and reducing feeding efficiency (CDFW 2009). Also, suction dredging disturbs the substrate which can mobilize metals previously deposited on the stream bed. These metals, including arsenic, copper, silver, lead, chromium, nickel, antimony, cadmium, and selenium, can then be transported downstream, either in their particulate or dissolved forms (CDFW 2009). Royer and Prussian (1999) found elevated concentrations of copper and zinc in the water column detected up to 80 m downstream of dredging. Elevated metal concentrations from suction dredging can adversely impact aquatic organisms if concentrations exceed EPA established Aquatic Life Criteria, and exposure durations meet minimum requirements (Rand 1995; EPA 2016).

The impetus for my research was concern about the potential chemical and biological effects of mining on streams in the North Cascades. I focused my study on tributaries in the Ross Lake watershed. I selected a network of tributaries in the Ruby Creek basin for a watershed-scale analysis, and one stream in the Upper Skagit River watershed that has a history of mining activities. I selected these areas for my study because: 1) the Ruby Creek watershed has the highest concentration of mining and prospecting sites in the North Cascades, 2) these tributaries are the primary spawning grounds for adfluvial native trout, including federally threatened Bull Trout (*Salvelinus confluentus*) and Rainbow Trout (*Oncorhynchus mykiss*), 3) resident Bull Trout (*S. confluentus*), Dolly Varden (*Salvelinus malma*), and Rainbow Trout (*O. mykiss*) are present in both tributaries, and 4) the remoteness of these watersheds has previously impeded investigations of this kind.

Objectives

The goal of my research was to determine if changes in water chemistry and biological communities in streams in the Ross Lake watershed are associated with areas of historical and current metal mining; and if so, could these changes adversely affect resident and adfluvial trout populations. To achieve these objectives, the following questions were addressed:

1. Does water chemistry vary spatially and temporally? Are these variations attributable to mining activities or natural causes?
2. Do metal concentrations exceed established criteria for the protection of aquatic life?
3. Do metals accumulate in periphyton? Does metal accumulation in periphyton vary in relation to areas of mining?

Methods

1. Study Area

Located in northern Washington State, the North Cascades are topographically rugged and climatically wet and mild. The landscape is steep, jagged and rocky with elevations ranging from 185 to 3,286 m. The climate of the western side of the North Cascades is temperate and maritime, while the eastern side is more continental. Temperature variations are greater on the eastern side as there are more extreme seasonal fluctuations. Annual precipitation ranges from 100-250 cm on the western slopes and 25-130 cm on the eastern slopes. Snow accumulates in the early fall and persists until mid-summer at elevations above 900 m (Raymond et al. 2014).

The Ross Lake watershed is located in the northern portion of the North Cascades. Ross Lake, a hydropower reservoir, was created in 1947 when Ross Dam was constructed (Moen 1969). Nearly 35 km of the upper Skagit River valley flooded creating the reservoir which extends into Canada. The 8,250-km^2 Ross Lake watershed is extensive and encompasses a variety of public lands which have differing land management. These public lands include National Park, National Forest, National Recreation Area, and Designated Wilderness Area on the U.S. side, and two provincial parks in British Columbia (Figure 1); thus, the watershed is managed by several agencies including the National Park Service, U.S. Forest Service, and the Ministry of the Environment of British Columbia. My study areas specifically, the Ruby Creek and Upper Skagit River watersheds, are managed by the U.S. Forest Service and the Ministry of the Environment of British Columbia. The Ruby Creek watershed lies within the Mt Baker-Snoqualmie National Forest and is administered through Okanogan-Wenatchee National Forest; and the Upper Skagit River watershed is managed as part of the EC Manning and the Skagit Valley Provincial Parks.

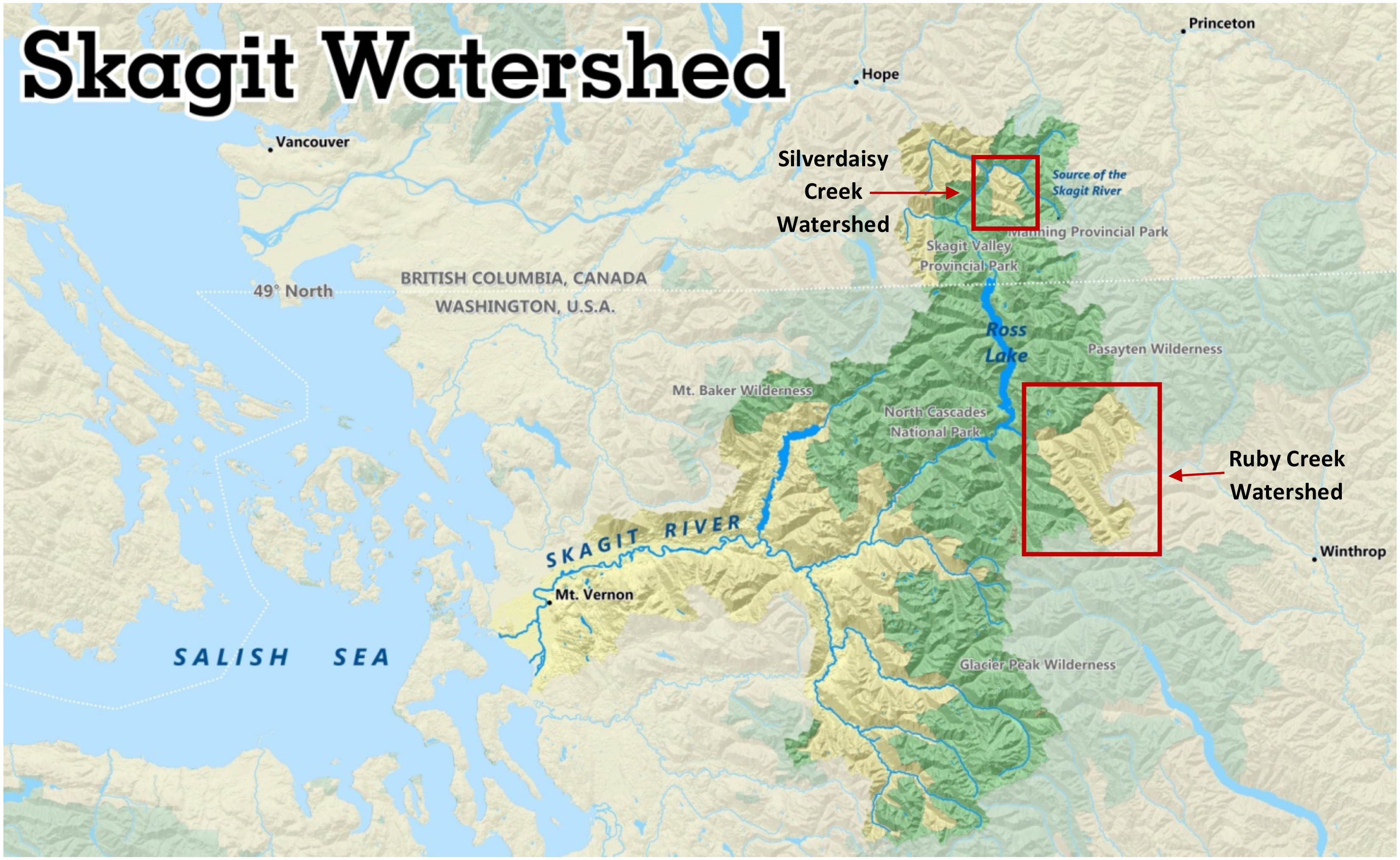

Figure 1. Public land designations in the Ross Lake watershed. Green shading shows protected lands including National Park Land, National Recreation Areas, or Designated Wilderness Areas. Yellow shading indicates the land is managed by the US Forest Service or is privately owned (Skagit Environmental Endowment Commission 2015).

I selected the Ruby Creek watershed for my research because of its extensive mining history. The Slate Creek Mining District, which extends from 121° W to the boundary between Whatcom and Okanogan counties, and south to north from the Skagit County line to the USA/Canada border, encompasses the Ruby Creek watershed (Moen 1969). The district was developed prior to passing the General Mining Act of 1872 as a means of settling claim disputes (Woodhouse et al. 2006). The amount of claims within the district is numerous, despite it being one of the most inaccessible regions in Whatcom County (Moen 1969). From 1894 to 1937, a total of 2,812 hard rock mining claims and 1,133 placer mining claims were filed in the Slate Creek Mining District (Moen 1969). Of the 2,812 hard rock mining claims, 1,628 were located on Slate Creek, 405 on Canyon Creek, 455 on Ruby Creek, 245 on Mill Creek, 68 on Granite Creek, and 11 on Panther Creek (Figure 2; Moen 1969). Fifty-two of the 2,812 hard rock claims were patented, but the two biggest producers of gold, the Azurite and the Eureka mines, were not patented. In total, 15 hard rock mines, 28 prospect sites, and numerous placer mining claims were developed (Moen 1969). Filing of hard rock and placer mining claims peaked in the late 1800s and again in the mid-1900s. Although mining activities are still evident at many of them, information about the specific locations and amounts of mineral extraction are not available.

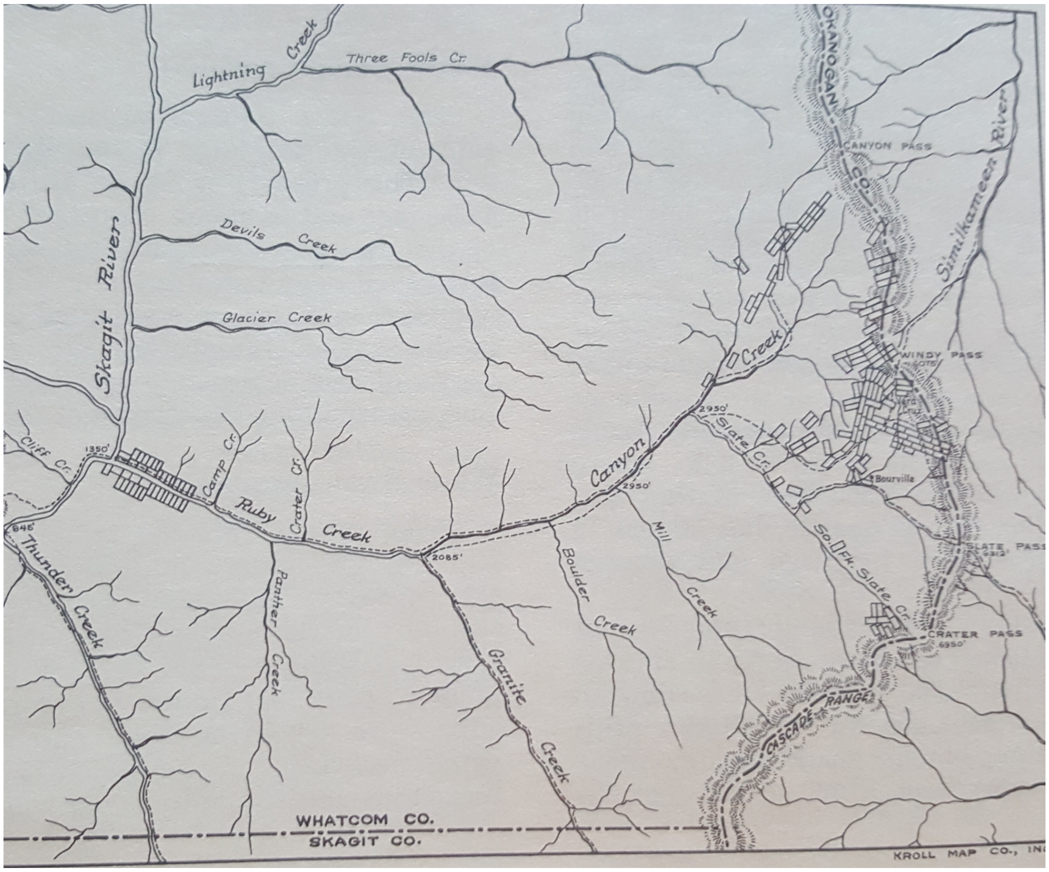

Figure 2. The Slate Creek Mining District, which includes the Ruby Creek watershed, showing established hard rock and placer claims as of 1899 from Woodhouse et al. (2006).

The watershed of Silverdaisy Creek, a tributary to the Upper Skagit River, also has over a century of mining history. In 1913, silver-lead ore was discovered at the Silverdaisy claim sites. In the following 20 years, considerable amounts of material were produced from the properties resulting in the construction of an aerial tram in 1934. In addition, between 1931 and 1932 a mining and smelting company attempted to placer mine by drilling test holes just north of the international boundary with the United States. As of 2007, the Silverdaisy mine has been very active as a result of high metal prices (Fraser-Cascade Mountain School and Hope Mountain Centre 2008).

2. Field Protocols

I. Site Establishment and Sampling Schedule

To evaluate the chemical and biological effects of mining in the Ruby Creek and Upper Skagit River watersheds, I established sampling sites upstream and downstream of hard rock and placer mines. To establish sampling sites, I first mapped historic hard rock mines using GPS coordinates from Woodhouse et al. (2006, Figure 3). This enabled me to identify stream segments upstream and downstream of areas of historic mining. During my first site visit I discovered locations of current placer mining. With this new information about additional mining activities, I attempted to establish sampling sites upstream and downstream of both types of mining (Figure 4). However, some downstream sites do not have paired upstream sites because of logistical constraints. The North Fork of Canyon site does not have an upstream site because the land is designated as Federal Wilderness Area, which prohibits installation of sampling devices (Figure 4). The Mill Creek site does not have a paired upstream site because the hiking distance (58 km roundtrip) was infeasible given the time limitations of my project. In total, I established 15 sampling sites, 13 in the Ruby Creek watershed and 2 in the Upper Skagit River watershed, with most of them being paired locations upstream and downstream of mining activities.

Figure 3. Locations of historic hard rock mines in the Ruby Creek watershed. GPS coordinates of historic hard rock mines from Woodhouse et al. (2006) were used to identify stream segments upstream and downstream of mines.

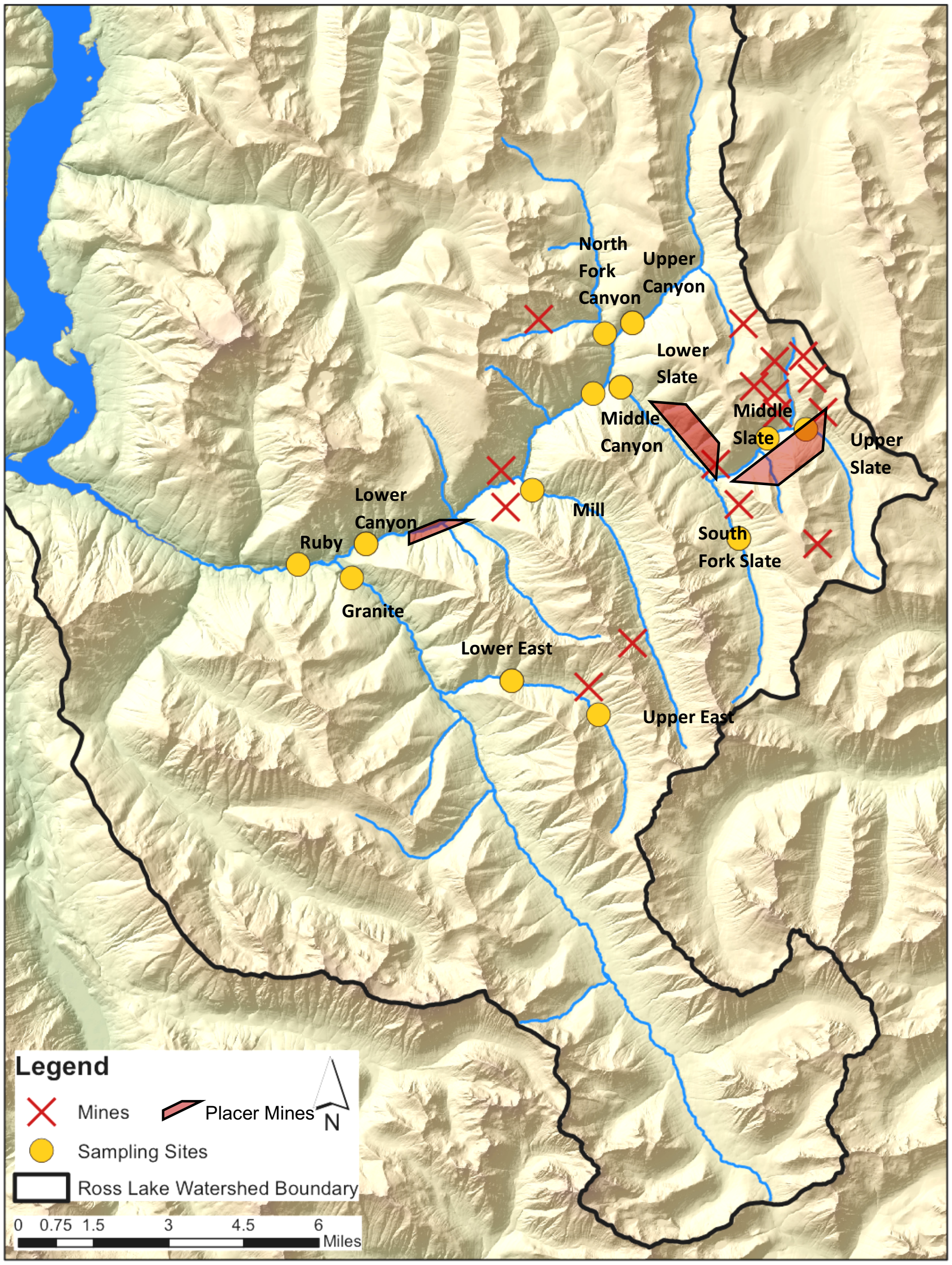

Figure 4. Historic hard rock mines, locations of placer mining, and sampling site locations in the Ruby Creek watershed. Thirteen sampling locations that are upstream and downstream of historic mining sites in the Ruby Creek watershed were selected for this project. Two additional sampling sites were established in the Upper Skagit River watershed upriver of Ross Lake.

My sampling schedule was constrained to May through September because of seasonal highway closures and research permit restrictions. Washington State Route 20, which provides the only automobile access to the Ruby Creek watershed, typically closes from mid-November through April due to heavy snow. Furthermore, Hart’s Pass Road provides the only automobile access to trails that intersect sampling sites in the eastern portion of the watershed, and it is usually closed from late-October to mid-June due to snow. Thus, I accessed the Ruby Creek watershed sites once highway 20 and Hart’s Pass Road opened and trails were clear of snow. In addition, my research permits allowed sampling through September, so I collected water and biological samples from 15 sites over a five-month period.

To collect water chemistry and biological samples throughout the Ruby Creek and Upper Skagit River watersheds, I installed two sampling stations at each of the 15 sampling sites. I installed all sampling stations during the first site visit in May or June (Table 1). To establish a sampling station, I wrapped a strand of 3/32” 7 x 19 stainless steel aircraft cable around a tree or root wad along the stream bank and attached an anchoring system. Anchors consisted of 30-cm segments of 10-cm diameter Schedule 40 gray-colored polyvinyl chloride (PVC) pipe filled with stream rocks that were held in place by stainless steel bolts at each end of the PVC segment. I attached water temperature and chemistry samplers, and biological samplers to each sampling station and placed the anchors, samplers, and cables in deeper pools to ensure the sampling stations would remain submerged throughout the summer (Section 2, I, i; Section 2, II, iii; Section 2, III, i). In total, I established 30 sampling stations in the Ruby Creek and Upper Skagit River watersheds.

Table 1. Equipment deployment and sampling schedule for 13 sites in the Ruby Creek watershed and 2 sites in the Upper Skagit River watershed during May through September, 2015.

Month	Site	Temperature, Dissolved Oxygen, Conductivity, pH	Grab Samples (Total & Dissolved)	SLMDs (6/sampling site)	Periphyton (2/sampling site)	HOBO Temp Logger	HOBO Water Level Logger	Discharge
May	Ruby, Lower Canyon, Granite, Mill, Lower East, Upper East	x	x	x	x	x		
June	Ruby, Lower Canyon, Granite, Mill, Lower East, Upper East, Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, Upper Skagit	x	x	x	x[1]	x[2]		
July	Ruby, Lower Canyon, Granite, Mill, Lower East, Upper East, Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, Upper Skagit	x[3]	x[3]	x[3]		x[3]	x[4]	x[5]
August	Ruby, Lower Canyon, Granite, Mill, Lower East, Upper East, Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, Upper Skagit	x	x	x	x[6]	x[6]	x[6]	x[5]
September	Ruby, Lower Canyon, Granite, Mill, Lower East, Upper East, Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, Upper Skagit	x	x	x	removed from all sites	removed from all sites	removed from all sites	x[5]

[1] Periphyton samplers were deployed at Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, and Upper Skagit.

[2] Temperature loggers were deployed at Middle Canyon, Upper Canyon, North Fork Canyon, Lower Slate, Middle Slate, Upper Slate, South Fork Slate, Silverdaisy, and Upper Skagit.

[3] Samples were not collected at the Middle Slate site. Sampling stations were removed.

[4] Water level loggers were deployed at Ruby, Granite, Lower East, Mill, Middle Canyon, South Fork Slate, North Fork Canyon, Silverdaisy, and in a tree at Middle Canyon for atmospheric compensation.

[5] Discharge was measured at Ruby, Granite, Lower East, Mill, Middle Canyon, South Fork Slate, North Fork Canyon, and Silverdaisy.

[6] Sampling devices were removed from Mill.

II. Water Chemistry

i. Physical and Chemical Measurements

Stream temperatures and chemical parameters affect habitat suitability for fish and other aquatic organisms and reflect the source of water. Thus, I measured temperature and dissolved oxygen (YSI Pro20 Dissolved Oxygen meter), conductivity (Oakton Ecotestr EC conductivity meter), and pH (Oakton pHtestr10 pH meter) at mid-depth in flowing water during each site visit. Also, I attached an Onset HOBO Water Temp Pro v2 Data logger, which measures stream temperatures with an accuracy of ±0.01°C, to the anchoring system of one sampling station per site to monitor stream temperatures hourly throughout the summer (Table 1).

ii. Surface Water Grab Samples

I adhered to standard method for collecting surface water grab samples to determine if mining activities were associated with changes in metal concentrations in streams in my study areas (AWWA et al. 2012). I followed method 1060B for sample collection, transport and preservation (AWWA et al. 2012). I collected water samples approximately 1 m from shore and 5 cm below the water surface. I used sterile, 20-mL Norm-Ject Leur Lock Syringes that were triple-rinsed with stream water prior to sample collection to collect two water samples. One sample was unfiltered (total metals) and dispensed directly into a 15-mL polypropylene centrifuge tube. The other sample (dissolved metals) was dispensed through a 0.45-µm polyvinylidene fluoride (PVDF) syringe filter into another centrifuge tube (AWWA et al. 2012). Samples containing total and dissolved metals were transported to Western Washington University (WWU), where they were acidified (1% v/v) with trace metal grade nitric acid (VWR International) for preservation. Preserved samples were stored at 4°C.

iii. Passive Integrative Water Samplers—Stabilized Liquid Membrane Devices

Metal concentrations in streams can vary over time in response to changes in water chemistry, flow, sources, and precipitation. Thus, frequent sampling is required to accurately depict the range of concentrations and duration of exposures to aquatic organisms. Grab samples are useful for determining metal concentrations at one point in time, but to evaluate metal concentrations in the long term, frequent grab samples must be obtained (Rand 1995).

The challenge of characterizing metal concentrations over time has led to the recent development of passive samplers that accumulate metals while deployed in streams. I elected to use a passive integrative water sampler called Stabilized Liquid Membrane Devices (SLMDs) for my research to evaluate them as an experimental technique in the context of my other sampling methods. SLMDs are novel passive samplers that have been used in three previous studies (Brumbaugh et al. 2000; Brumbaugh et al. 2008; Marshall and Era-Miller 2012). SLMDs accumulate metals as the internal mixture of a chelating agent and a fatty acid passes through a semi-permeable membrane and forms complexes with metals on the surface of the samplers (Brumbaugh et al. 2000). SLMDs may be particularly useful for capturing episodic pulses of metals or detecting metals present at very low concentrations that may be missed when grab samples alone are used. Their simple design, lightweight construction, and ability to be deployed for month-long intervals made them an attractive tool to test for this research.

I constructed SLMDs in the Plastic Engineering Technology Laboratory at WWU following the procedures of Brumbaugh et al. (2000). To assemble the SLMDs, I filled a semi-permeable membrane made of 15-cm x 2.5-cm strips of 4 mil thickness low-density polyethylene (LDPE) layflat tubing (Brentwood Plastics, MO), with a 1.5-mL 1:1 ratio mixture of oleic acid (cis-9-octadecenoic acid; VWR) and Kelex-100 (7-[4-ethyl-1-methyloctyl]-8-quinolinol; Allessa GmbH). I removed any air pockets and heat-sealed both ends. Once assembled, SLMDs were stored at 4°C in acid-washed 500-mL polyethylene sample bottles filled with NanoPure water and ~0.1 g Chelex resin (Sigma Aldrich).

SLMDs were deployed in a flat housing constructed of Vexar plastic netting (1-cm mesh), acryllic clips, stainless steel hardware and zip ties (Figure 5). This structure protected the membranes from floating debris, maintained SLMD placement, and allowed more water exchange than the typical deployment in a piece of perforated PVC pipe. One housing unit containing three SLMDs was attached to each sampling station using zip ties (six SLMDs per sampling site). SLMDs were deployed for approximately month-long intervals (28-41 days). Upon retrieval, individual SLMDs were placed in 20-mL pre-cleaned 2000 class scintillation vials with polytetrafluoroethylene (PTFE) lined lids. Three new SLMDs were placed in each housing unit and the sampling station was re-deployed. Retrieved SLMDs were returned to WWU and frozen until further processing. In total, 273 SLMDs were deployed and retrieved.

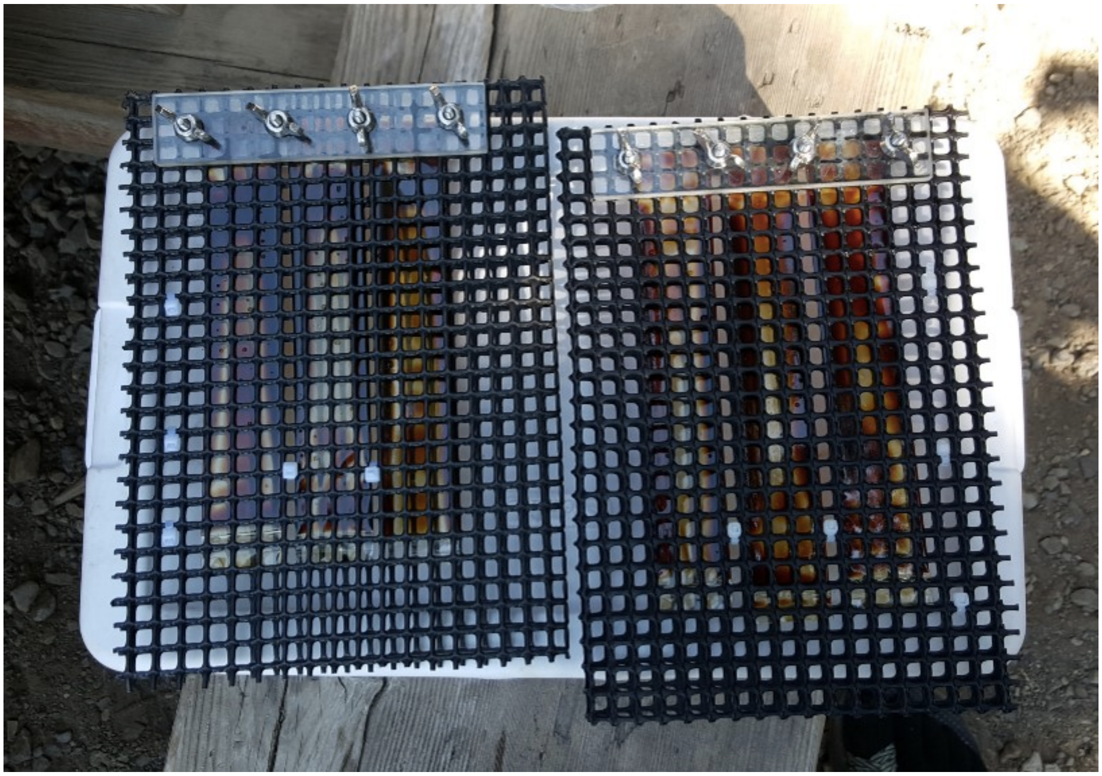

Figure 5. SLMDs and housing units constructed with Vexar plastic netting, acryllic clips, stainless steel hardware and zip ties. SLMDs were deployed at each sampling station (two per sampling site) for approximately month-long intervals in the Ruby Creek and Upper Skagit River watersheds from May through September, 2015.

III. Biological Samples

i. Periphyton

Periphyton, which consists of algae, bacteria, and detritus, attaches to submerged substrate in water bodies and can accumulate metals from surface waters and sediment (Farag et al. 1998). Thus, I analyzed metal concentrations in periphyton to determine if communities downstream of mining activities accumulated more metals than upstream communities. To collect periphyton I used Multiplate Hester-Dendy samplers instead of scraping natural substrate because the samplers have a consistent and quantifiable surface area (0.16 m^2) and colonization and metal accumulation period (88-121 days). Hester-Dendy samplers were attached to the anchors of each sampling station (two per sampling site) and left for the entire sampling season (88-121 days). In total, 30 periphyton samples (two per sampling site) were collected for this project. After I removed the Hester-Dendy samplers from the sampling stations, I stored them in pre-labeled Ziplock bags and transported them on ice to the Fish Ecology Laboratory at WWU. Periphyton was removed from the plates with a small, stiff, plastic brush that was acid washed between samples to prevent contamination. The periphyton was transferred into WHIRL-PAK® bags and frozen until further processing and analysis.

IV. Discharge

To quantify discharge at my sampling sites, I monitored water stage hourly and measured discharge during monthly site visits. In July I placed Onset HOBO Water Level Dataloggers at selected sampling sites throughout the watersheds to monitor water stage hourly throughout the summer (Table 1). I deployed each water level logger inside an open-ended section of gray-color Schedule 40 4.5-cm diameter polyvinyl chloride (PVC) pipe to protect the device. I used zip ties to secure the pipe to a rebar stake that was driven into the stream substrate of a deep pool near a sampling station. The water level loggers rely on changes in pressure to monitor water depth, so I also installed an additional water level logger in a tree adjacent to the Middle Canyon sampling site for atmospheric compensation. The Middle Canyon site was selected because it approximated the elevational mid-point of my sampling sites. During subsequent site visits I measured stream velocities and depths on cross-sectional transects to enable calculations of discharge. To do this I measured wetted width and divided the cross section into ten equally sized cells. In the middle of each cell I measured depth and velocity with a flow meter (Marsh McBirney Flo-Mate 2000) at 0.6 of the depth from the surface. I calculated discharge using the equation:

$$\boldsymbol{Q\ (\frac{m3}{s}) = \sum cell\ width(m)\ x\ cell\ depth\ (m)\ x\ velocity\ (\frac{m}{s})}.$$

Total pressures (water plus atmospheric) recorded by the data loggers were adjusted for changes in atmospheric pressure using the Middle Canyon Creek tree data logger and then converted to water stage relative to the depth of the data logger at the time of deployment. The stage-discharge relationship was modeled based on my measured discharges at each site. Using this model, I calculated discharges at 1-hour intervals from the water levels derived from the data loggers.

3. Laboratory Protocols

I. Metals Analysis

Depending on the desired detection level for specific metals, liquid samples can be analyzed with a variety of instruments. To compare metal concentrations in grab samples to contaminant concentrations which cause aquatic organisms to experience adverse effects from acute or chronic exposures, I required metal concentrations to be measured to parts per billion (µg/L, EPA 2016). To accomplish this I used an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at Advanced Materials Science and Engineering (AMSEC) at WWU which is capable of trace metal analysis as low as parts per trillion. I also used the ICP-MS to quantify metals accumulated on the SLMDs and in the periphyton samples. Before the SLMDs and periphyton samples could be analyzed for metals, each required processing to generate liquid samples.

i. Stabilized Liquid Membrane Devices (SLMDs)

To determine the quantity of metals that accumulated by binding to the chelating agent on the SLMDs, I extracted the metals off the surface of the membranes. To extract the accumulated metals off the SLMDs I followed the extraction procedures which involved acidification and sonication outlined by Brumbaugh et al. (2008). I analyzed the extraction solution using the ICP-MS.

Before processing the field samples, I performed a preliminary experiment to determine the efficiency of my extraction procedure. I identified the number of sequential extractions for each SLMD to consistently attain at least 90% of the total extractable metals. To do this I measured sequential amounts of each metal recovered over a series of six extractions. For the preliminary experiment, I analyzed 10 SLMDs that were collected from areas downstream of mining and two laboratory blanks. I selected these 10 SLMDs because I wanted to use SLMDs that might have accumulated more metals. I filled each scintillation vial containing a single SLMD with 15 mL of 20% (v/v) trace metal grade nitric acid (VWR). Each scintillation vial was placed in an ultrasonic water bath (Branson 2510) at 50 – 60 °C for 30 minutes to liberate the accumulated metals on the SLMD. After sonication, I poured the 15-mL extract into a pre-labeled 25-mL acid washed polypropylene storage bottle and repeated the procedure six times. To prepare the extraction solution for ICP-MS analysis, I generated a 1% (v/v) trace metal grade nitric acid (VWR) solution by diluting 0.5 mL of SLMD extract with 9.5 mL of NanoPure water in a 15-mL polypropylene centrifuge tube. Then I followed Method 3125 to analyze these samples (Section 3, I, iii; AWWA et al. 2012).

The ten experimental SLMDs required three extractions rounds to obtain 90% of the total extractable metals. To determine this I calculated the cumulative efficiency of metals extracted from each round. Total masses of accumulated metals were represented as the sum of each metal mass obtained from all six extractions. The cumulative proportion of the metal mass obtained after each extraction round compared to the total mass obtained was used to determine cumulative efficiency. After the third in the series of six consecutive extractions, at least 90% of the total extractable metals had been consistently removed from each SLMD (Table 2). Therefore, I processed all the remaining SLMDs using the same procedures as I used for the experimental extractions, with a series of three extractions performed for each SLMD. I composited the three extraction solutions for storage in a 50-mL polypropylene bottle for a total of 45 mL of extract per SLMD. Then I analyzed the 45-mL composited SLMD extraction solution for metals using the procedures described previously for the experimental SLMDs.

Table 2. Mean percent and standard deviation of metals recovered on ten SLMDs from six rounds of extractions. The SLMDs were deployed in the Ruby Creek and Upper Skagit River watersheds during May through September, 2015.

	Extraction Round					
Metal	**1**	**2**	**3**	**4**	**5**	**6**
Aluminum	73.8 (±8.8)	88.2 (±5.1)	**94.1 (±2.9)**	96.3 (±1.8)	98.8 (±0.8)	100.0 (±0.0)
Arsenic	88.2 (±9.6)	96.2 (±4.9)	**98.9 (±1.9)**	99.6 (±0.6)	100.0 (±0.2)	100.0 (±0.0)
Cadmium	98.3 (±4.5)	99.1 (±2.6)	**99.7 (±0.9)**	100.0 (±0.1)	100.0 (±0.0)	100.0 (±0.0)
Calcium	97.0 (±2.5)	99.9 (±0.3)	**100.0 (±0.0)**	100.0 (±0.0)	100.0 (±0.0)	100.0 (±0.0)
Chromium	79.6 (±15.2)	91.8 (±7.4)	**96.6 (±4.0)**	99.7 (±0.7)	99.9 (±0.4)	100.0 (±0.0)
Cobalt	89.4 (±9.3)	96.7 (±6.9)	**99.0 (±2.8)**	99.7 (±1.0)	99.9 (±0.2)	100.0 (±0.0)
Iron	64.7 (±10.3)	84.0 (±6.7)	**93.1 (±3.6)**	96.9 (±1.9)	98.8 (±0.8)	100.0 (±0.0)
Lead	78.9 (±39.6)	100.0 (±0.0)	**100.0 (±0.0)**	100.0 (±0.0)	100.0 (±40.0)	100.0 (±0.0)
Magnesium	68.3 (±13.2)	86.5 (±8.0)	**94.9 (±4.6)**	98.0 (±2.4)	99.3 (±0.8)	100.0 (±0.0)
Manganese	89.8 (±2.7)	96.9 (±1.1)	**98.6 (±0.6)**	99.3 (±0.3)	99.7 (±0.1)	100.0 (±0.0)
Nickel	92.4 (±6.7)	98.4 (±2.6)	**99.8 (±0.4)**	100.0 (±0.0)	100.0 (±0.0)	100.0 (±0.0)
Potassium	87.0 (±13.5)	96.9 (±5.6)	**99.7 (±0.8)**	100.0 (±0.0)	100.0 (±0.0)	100.0 (±0.0)
Uranium	97.2 (±3.1)	99.5 (±1.2)	**99.8 (±0.5)**	100.0 (±0.1)	100.0 (±0.0)	100.0 (±0.0)
Vanadium	71.9 (±12.4)	88.4 (±7.7)	**95.7 (±4.2)**	98.3 (±2.1)	99.4 (±0.7)	100.0 (±0.0)
Zinc	92.9 (±6.2)	96.3 (±7.0)	**96.4 (±7.0)**	96.4 (±7.0)	96.4 (±7.0)	100.0 (±0.0)

ii. Periphyton

The first step in the analysis of metal content of periphyton samples was to determine dry mass. To determine dry mass I dried the periphyton samples to a constant weight at 100°C in ceramic crucibles that had been pre-dried to a constant weight at 100°C. Then I used a 0.25- to 0.50-g subsample of each dried periphyton sample for analysis of metals.

The next step was to digest the dried periphyton subsamples. I used an Ethos E2 microwave digestion system for acid digestion. All vessels used for the acid digestion procedure were acid-washed and underwent a cleaning cycle prior to periphyton digestion. Periphyton subsamples were distributed into the pre-cleaned vessels and 10 mL (100% v/v) of trace metal grade nitric acid (VWR) was added to each vessel. The periphyton samples were digested at 180°C for 15 minutes using the pre-programmed Beech Leaves procedure. After cooling, I poured the digested periphyton solutions into 20-mL pre-cleaned 2000 class scintillation vials with polytetrafluoroethylene (PTFE)-lined lids. To prepare the digested periphyton for ICP-MS analysis, I generated a 1% (v/v) trace metal grade nitric acid (VWR) solution by diluting 0.1 mL of each of the periphyton digestion solutions with 9.9 mL of NanoPure water in a 15-mL polypropylene centrifuge tube.

iii. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

To determine metal concentrations in the grab samples, SLMDs extract solutions, and periphyton digestion solutions I used an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). I followed Method 3125 for my analyses and evaluated grab samples within six months of collection to comply with standard methods (AWWA et al. 2012). I generated standards with a Multi-Element Standard (Inorganic Ventures Corporation) for ICP-MS containing 25 elements (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr^3, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Th, Tl, U, V, Zn). I used the Advanced Materials Sciences and Engineering's (AMSEC) Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at WWU to analyze the samples. The ICP-MS was programmed to consecutively draw from and analyze each sample five times, and a mean concentration was calculated based on software-generated calibration curves.

4. Statistical Analyses

To determine watershed-scale effects, I evaluated metals in surface water grab samples, SLMDs and periphyton from the Ruby Creek watershed using nonparametric methods and clustering techniques. I omitted the Silverdaisy Creek and Upper Skagit River samples from the nonparametric tests and cluster analyses because those samples were collected from a different watershed. I first eliminated individual metals from each dataset (grab samples, SLMDs, and periphyton) if 50% of the sample concentrations fell below the detection level of a particular metal (Table 3). This was to ensure all metals in the dataset would be used for clustering analyses since variables with missing values (samples that fell below detection level values) are eliminated entirely from cluster development (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October 2016). For metals that met the 50% criteria but contained samples that fell below the detection level, I reported the concentration as half of the detection level. I selected this approach instead of replacing the below detection values with zero because certain clustering techniques (Hierarchical and Kmeans) use distance metrics to develop clusters. Thus values of zero influence cluster development with these variance-based clustering techniques.

Table 3. Metals included in statistical analyses for grab samples, SLMD samples and periphyton samples. Metals were included in statistical analyses if 50% of the samples were above the detection level as determined by the ICP-MS ("yes"= included, "no" = not included). SLMDs were analyzed on two different days (n=144, November 12th, 2015; n=167 on November 13th, 2015) so two detection levels are presented.

Metal	Grabs	DL[1] (µg/L)	SLMDs	DL[1,2] (µg/L)	DL[1,2] (µg/L)	Periphyton	DL[1] (µg/L)
Aluminum	yes	1.36	yes	0.91	1.08	yes	0.005
Antimony	yes	0.023	no	0.026	0.023	no	0.043
Arsenic	yes	0.033	yes	0.019	0.052	yes	0.15
Barium	yes	0.24	yes	0.28	0.42	yes	0.19
Beryllium	no	0.048	no	0.11	0.075	no	0.094
Cadmium	no	0.041	yes	0.030	0.036	yes	0.029
Calcium	yes	5.21	yes	7.95	2.29	yes	4.99
Chromium	yes	0.019	yes	0.032	0.021	yes	0.028
Cobalt	yes	0.018	yes	0.005	0.014	yes	0.037
Copper	no	0.13	no	0.15	0.16	yes	0.097
Iron	yes	1.46	yes	1.01	1.20	yes	0.92
Lead	no	0.050	yes	0.069	0.13	yes	0.092
Magnesium	yes	1.68	yes	1.03	1.16	yes	1.03
Manganese	yes	0.030	yes	0.021	0.060	yes	0.042
Molybdenum	yes	0.15	no	0.021	0.046	no	0.51
Nickel	no	0.021	yes	0.020	0.057	yes	0.070
Potassium	yes	5.63	yes	7.35	2.12	yes	6.40
Selenium	yes	0.50	no	0.69	1.11	yes	0.96
Silver	no	0.039	no	0.036	0.013	yes	0.004
Sodium	yes	7.77	no	7.04	5.34	yes	6.77
Thallium	no	0.20	no	0.007	0.27	no	0.023
Thorium	no	0.005	no	0.007	0.005	yes	0.021
Uranium	yes	0.003	yes	0.005	0.005	yes	0.031
Vanadium	yes	0.013	yes	0.009	0.012	yes	0.015
Zinc	no	0.49	yes	0.15	0.71	yes	0.26

[1] DL = Detection Level

[2] SLMDs had two separate detection levels because samples were analyzed on different day

Prior to running statistical analyses, metal concentrations of diluted SLMD extraction solutions and periphyton digestion solutions were converted to masses and normalized by days of deployment and, for periphyton specifically, the amount of periphyton that was acid digested. Prior to analyzing the SLMD extraction solutions and periphyton digestion solutions with the ICP-MS, each sample was diluted with NanoPure water. To account for the dilutions I used equations 1 and 2:

Equation 1: $[\mu g/L]_{\text{SLMD extraction solution}} = ([\mu g/L]_{\text{SLMD ICP}} \text{ X } (0.010 \text{ L})) \div (0.0005 \text{ L})$ and

Equation 2: $[\mu g/L]_{\text{periphyton digestion solution}} = ([\mu g/L]_{\text{Periphyton ICP}} \text{ X } (0.010 \text{ L})) \div (0.0001 \text{ L})$.

Next, I determined the mass of each metal accumulated on the SLMDs or in the periphyton using equations 3 and 4:

Equation 3: $(\mu g \text{ of metal})_{\text{SLMD extraction solution}} = ([\mu g/L]_{\text{SLMD extraction solution}} \text{ x } (0.045 \text{ L})_{\text{total SLMD extraction solution volume}})$, and

Equation 4: $(\mu g \text{ of metal})_{\text{periphyton digestion solution}} = ([\mu g/L]_{\text{periphyton digestion solution}} \text{X } (0.010 \text{ L})_{\text{total periphyton digestion solution volume}})$.

I accounted for differences in the mass of the periphyton subsample acid digested by dividing by the dry mass of the digested periphyton sample using equation 5:

Equation 5: $(\mu g \text{ of metal})_{\text{periphyton digestion solution}} \div$ mass of periphyton subsample

Additionally, I accounted for the variable number of days deployed for the SLMDs and periphyton using equations 6 and 7:

Equation 6: $(\mu g \text{ of metal})_{\text{SLMD}} \div$ deployment days

Equation 7: (μg of metal/g of periphyton) $\div$ deployment days

Metals on the SLMDs were reported as $(\mu g \text{ of metal})_{\text{SLMD}}$/day. Metals in periphyton were reported as (µg of metal)/(g of periphyton)/day. To account for duplicate SLMD measurements (two sampling stations per sampling site) I calculated monthly median values for each metal from the six SLMDs deployed per month. Also, I calculated the median value for each metal from the two periphyton samplers deployed for the entire season.

Next, I used rank-based correlation analyses (Kendall's tau) to determine the relationship between individual metals in each dataset, and for grab samples specifically, the relationship between total and dissolved metal concentrations within each sample (Table 4). This was to determine if metals or other variables were significantly autocorrelated before running any statistical analyses because correlated variables confound in multivariate statistical approaches (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October 2016). Total and dissolved metal concentrations in grab samples were correlated for all metals (Kendall's tau, $p \leq 0.05$; Table 4, e.g. Figure 6). I decided to retain the total metal concentrations for the grab samples for further statistical analyses because there were fewer samples that fell below detection level as compared to the dissolved metal concentrations. For grab samples with total metal concentrations that fell below the detection level of a particular metal, I reported the concentration as half of the detection level. This was to ensure all samples could be used in multivariate analyses (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October, 2016).

Table 4. Relation of total metal concentrations to dissolved metal concentrations in grab samples collected from 13 sites in the Ruby Creek watershed, May through September, 2015 (n=54), to evaluate autocorrelation (Kendall's tau, p≤0.05).

Metal	**Kendall's tau Coefficient**	**p-value**
Aluminum	0.425	≤0.0001*
Antimony	0.850	≤0.0001*
Arsenic	0.824	≤0.0001*
Barium	0.928	≤0.0001*
Calcium	0.958	≤0.0001*
Chromium	0.360	≤0.0001*
Cobalt	0.302	0.0005*
Iron	0.255	0.0034*
Manganese	0.476	≤0.0001*
Magnesium	0.962	≤0.0001*
Molybdenum	0.799	≤0.0001*
Potassium	0.900	≤0.0001*
Selenium	0.201	0.0207*
Sodium	0.927	≤0.0001*
Uranium	0.872	≤0.0001*
Vanadium	0.551	≤0.0001*

*Indicates a significant correlation (Kendall's tau, p≤0.05)

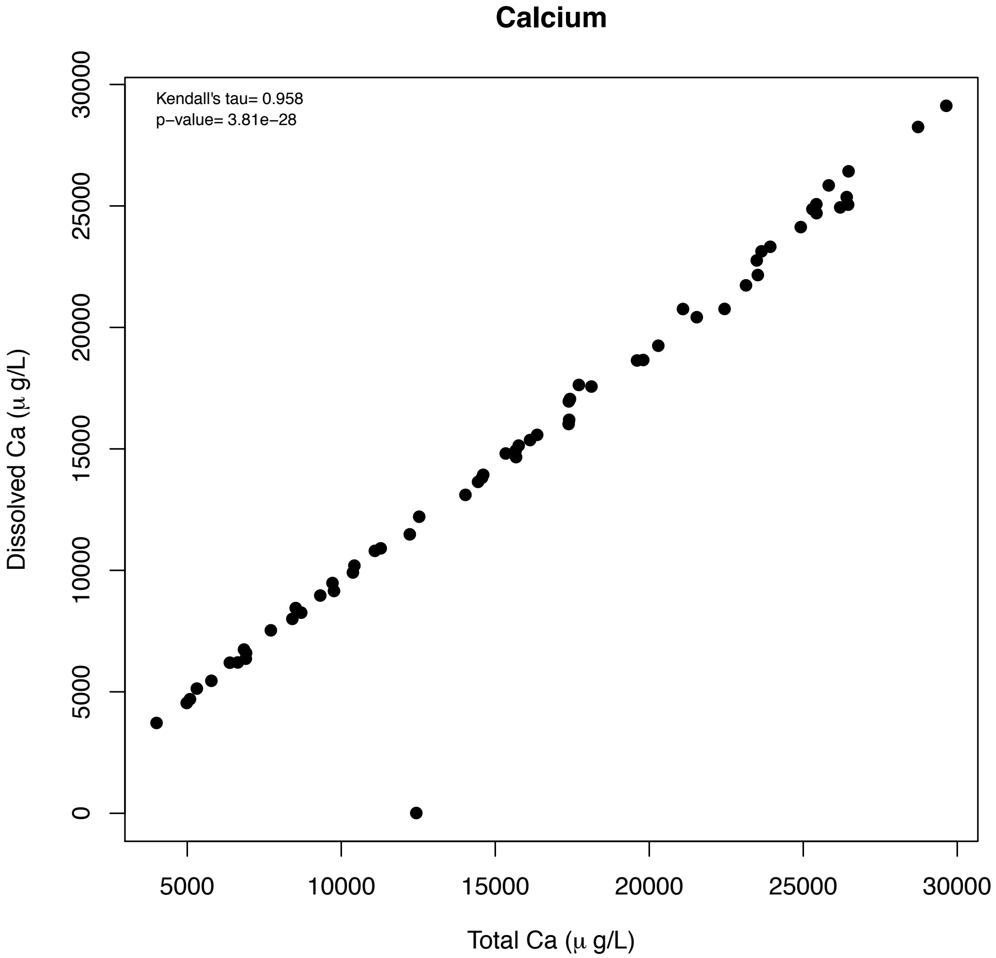

Figure 6. Total and dissolved calcium concentrations for all 54 grab samples collected from the 13 sampling sites in the Ruby Creek watershed, May through September, 2015 (Kendall's tau 0.958, P<0.0001).

Before proceeding with nonparametric and multivariate statistics, I first plotted total metal concentrations over time for each metal to evaluate seasonal trends and compared dissolved metal concentrations to Aquatic Life Criteria. Aquatic Life Criteria are contaminant concentrations in water below which aquatic organisms would be protected from adverse effects during acute or chronic exposures (EPA 2016). Aquatic Life Criteria include freshwater Criterion Continuous Concentrations (CCC) for metals, corrected for water hardness, which are concentrations of metals that freshwater organisms could be exposed to indefinitely and not be adversely impacted (EPA 2016). I corrected CCCs for metals for water hardness using equation 8, as indicated by the EPA (2016):

Equation 8: water hardness (mg/L) $=$ 2.497(calcium concentration (mg/L)) $+$ 4.118(concentration of magnesium (mg/L))

Then I compared the corrected freshwater CCCs to the dissolved metal concentrations in the surface water grab samples to determine if any criteria were exceeded.

For nonparametric and multivariate statistical approaches I categorized the Ruby Creek watershed grab samples based on the time and location of collection. Grab samples were divided into four sample groups: upstream of mining during non-mining periods (“before_nomining”); upstream of mining during placer mining activities (“during_nomining”); downstream of mining during non-mining periods (“before_mining”); and downstream of mining during placer mining activities (“during_mining”, Table 5). I defined the non-mining period (“before_nomining” or “before_mining”) as May and June because I did not observe any active placer mining during site visits. From July through September placer mining was occurring so these months are considered the mining season (“during_mining” or “during_nomining). The second component of the sample group was based on the location of the sampling site. Samples collected at a sampling site downstream of placer or hard rock mining claims were labeled “mining” and samples collected upstream of mining claims were labeled “nomining”. The exception was sites farther than 1.5 km downstream of a mining claim were labeled “nomining”. I based this approach on previous studies which indicated that water quality is impaired downstream of placer mining, specifically suction dredging, but the effects are localized and diluted as water flows downstream (Harvey 1986; Thomas 1985; Harvey et al. 1982, 1986; Stern 1988; CDFW 2009).

In addition, I separated the Ruby Creek watershed SLMDs and periphyton samples into four sample groups based on the collection location relative to mining and the specific type of mining they were associated with: upstream of mining (“none”); downstream of hard rock mining locations (“hardrock”); downstream of placer mining locations (“placer”); and downstream of both hard rock and placer mining locations (“both”, Table 5). As with the grab samples, if the mining claim was greater than 1.5 km upstream of the sampling site it did not influence the group classification. I did not assign SLMDs and periphyton a temporal component related to mining activity because of overlap in mining activity with deployment periods.

Table 5. Grab samples were classified by the location of collection relative to mining ("mining" = collected from sampling sites with placer mining claims upstream or "nomining" = no placer mining claims or claims farther than 1.5 km upstream) and collection time relative to mining activity ("before" = before placer mining in May or June or "during" = during placer mining in July, August, or September). Each listed site and month represents a single grab sample and the group it belongs to: "before_nomining" (n=13), "before_mining" (n=6), "during_nomining" (n=21), "during_mining" (n=14). SLMDs and periphyton were separated into sample groups based the specific type of mining claims upstream ("hard rock" = hard rock mining claims upstream, "placer" = placer mining claims upstream, "both" = both placer and hard rock mining claims upstream, "none" = no mining claims upstream). Group classifications based on sampling sites are listed for SLMDs: "hardrock" (n=9), "placer" (n=4), "both" (n=11), and "none" (n=17); and for periphyton: "hardrock" (n=3), "placer" (n=1), "both" (n=4), and "none" (n=5).

Grab samples

before_nomining	**before_mining**	**during_nomining**	**during_mining**
Ruby/May	Lower Canyon/May	Ruby/July	Lower Canyon/July
Ruby/June	Lower Canyon/June	Ruby/August	Lower Canyon/August
Granite/May	Middle Canyon/June	Ruby/September	Lower Canyon/September
Granite/June	Lower Slate/June	Granite/July	Middle Canyon/July
Lower East/May	Middle Slate/June	Granite/August	Middle Canyon/August
Lower East/June	Upper Slate/June	Granite/September	Middle Canyon/September
Upper East/May		Lower East/July	Lower Slate/July
Upper East/June		Lower East/September	Lower Slate/August
Mill/May		Upper East/July	Lower Slate/September
Mill/June		Upper East/September	Middle Slate/August
Upper Canyon/June		Mill/July	Middle Slate/September
North Fork Canyon/June		Mill/August	Upper Slate/July
South Fork Slate/June		Upper Canyon/July	Upper Slate/August
		Upper Canyon/August	Upper Slate/September
		Upper Canyon/September	
		North Fork Canyon/July	
		North Fork Canyon/August	
		North Fork Canyon/September	
		South Fork Slate/July	
		South Fork Slate/August	
		South Fork Slate/September	

SLMDs and Periphyton

	Hardrock	**Placer**	**Both**	**None**
	Lower East	Lower Canyon	Lower Slate	South Fork Slate
	Mill	Middle Canyon	Middle Slate	Upper East
	North Fork Canyon		Upper Slate	Upper Canyon
				Ruby
				Granite

I performed pairwise comparisons to address whether individual metals differed among sample groups for each of the three sampling methods. Distributions were not normal and variances were not homogeneous based on Shapiro-Wilk's test for normality and Fligner-Killeen test for homogeneity of variances ($\alpha=0.05$), so I used the nonparametric Kruskal-Wallis Rank Sum test to identify metals that differed among sample groups. If significant differences were found among sample groups, I used the Pairwise Wilcoxon Signed-Rank test post hoc to identify specific differences between sample groups. The Wilcoxon test controls for Type I error associated with performing multiple comparisons using Holm's correction. For the periphyton dataset specifically, I omitted the placer group from the Kruskal-Wallis Rank Sum test and the pairwise comparisons because this group contained one value ($n=1$) so variances and distributions could not be analyzed.

I determined whether I could differentiate between the sample groups assigned to each sampling type based on the suite of kinds and quantities of metals detected. I accomplished this by applying multivariate clustering techniques. To assess the robustness of patterns in the data, I used three different clustering methods for my analysis: hierarchical, Kmeans and nonmetric (RIFFLE) clustering. For hierarchical clustering, I used Wards method because it preserves groups with small internal variance. I tested for an association between cluster membership and sample groups using a chi-squared test; if the association analysis for hierarchical clustering was significant, I proceeded with Kmeans cluster analysis. Both hierarchical and Kmeans clustering use distance metrics to determine cluster association, but Kmeans clustering provides cluster centers so the variables that contribute to the greatest cluster separation can be identified by calculating relative distance (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October 2016). RIFFLE clustering is nonparametric and nonmetric; therefore, it uses ranks and medians instead of distance metrics to define similarity (Matthews and Hearne 1991). Also, RIFFLE eliminates variables that can obscure patterns between other variables. RIFFLE, like

Kmeans, also provides proportional reduction of error (PRE) scores so the variables that make the greatest contribution to the cluster separation are identified (Matthews and Hearne 1991).

Because both Kmeans and RIFFLE clustering are stochastic, they produce a different result every run. Therefore, I performed 20 iterations of Kmeans and RIFFLE to evaluate the reliability of the cluster solutions based on the frequency of occurrence of the same solutions. I identified significant cluster solutions using a chi-squared test and reported significant cluster solutions and best cluster separators based on either a relative distance ≥50% or a PRE score ≥0.5 (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October 2016).

To analyze the Silverdaisy Creek and upper Skagit River watershed samples, I compared metal quantities in the SLMD and periphyton samples at the Silverdaisy Creek site to the Upper Skagit River site. I generated boxplots to perform these comparisons. I did not compare metal concentrations in surface water grab samples between sites or to Aquatic Life Criteria because concentrations were below the detection level of the ICP-MS.

5. Quality Assurance and Quality Control

For quality assurance and quality control I developed a plan to account for potential sources of error in the field and in the laboratory. My focus was on obtaining the most precise and accurate information I could for metals. Most of my plan involved duplication of sampling and measurement efforts and assessment of consistency between duplicates.

In the field I replicated sampling efforts and collected field-blanks to identify potential sources of contamination. I accounted for spatial variability at a stream sampling site by establishing two sampling stations at every sampling site, thus duplicating SLMD and periphyton sampling devices. To account for potential variation in uptake of metals by the SLMD sampling devices, I deployed SLMDs in triplicate for a total of six per site per month. During every trip, I collected field-blank grab samples (NanoPure water transported onsite in an acid washed 500-mL polypropylene sample bottle) using the procedures described previously (Section 2, II, ii) to account for potential sources of metals in the environment outside the stream. Also, I brought an SLMD on every sampling trip which underwent the same process as the other SLMDs except for deployment (field blank). I analyzed both the field-blank grab samples and SLMDs for metals (Section 3, I, iii) which enabled me to determine if there were potential sources of metal contamination in the transport process.

In the laboratory I generated replicates to evaluate the repeatability of my procedures and used lab-blanks to determine additional sources of metals. I set aside 12 SLMDs (lab blanks; 4% of the total number deployed) which were not deployed and underwent the same extraction procedures as the deployed SLMDs and field blanks. These SLMDs helped identify if there were sources of metals in the materials used to assemble the SLMDs, or additional contamination during the assembly or extraction processes. I generated 25 duplicate solutions using two 0.5 mL aliquots of the 45-mL composited SLMD extraction solution (9% of the total number of solutions) that were analyzed by the ICP-MS. I did this to determine how representative each aliquot was of the metals in the 45-mL composited SLMD extraction solutions. I calculated relative percent difference between the SLMD extraction solution replicates using equation 9:

Equation 9: Relative percent difference (%) = (maximum value–minimum value/mean) x 100%. For the seven periphyton samples with the highest dry masses, I homogenized the dried material and then split each sample into two or three portions of 0.25 to 0.5 g each and acid digested each portion separately; I did this to determine how representative each subsample was of the metal content in the entire dried periphyton sample. In addition, I generated five duplicate or triplicate solutions using two or three 0.1 mL aliquots of periphyton digestion solutions that were analyzed by the ICP-MS. This information helped me determine how representative each aliquot was of the metal content in the periphyton digestion solutions. I calculated relative percent difference between the periphyton replicates and the periphyton digestion solution replicates using equation 10:

Equation 10: Relative percent difference (%) = (maximum value–minimum value/mean) x 100%.

To determine the accuracy of the drying or acid digestion processes, I used Apple Leaves standard reference material (SRM NIST:1515) which contains a known amount of metals. I dried and acid digested the reference material in the same manner as the periphyton samples to compare the observed with the expected values. I compared the observed and expected values by calculating percent deviation with equation 11:

Equation 11: Percent deviation (%) = (observed – expected/expected) x 100%.

Lastly, I programmed the IPC-MS to perform five repetitions per sample analyzed. If the five replicate measurements for a sample were above the detection level but the relative standard deviation was greater than 15%, then the sample was flagged. For flagged samples, I removed the highest and lowest count values and calculated a new mean counts value. Then I used the mean counts values to calculate concentration using the calibration curves generated by the instrument software. For a sample with a relative standard deviation less than or equal to 15%, I used the software generated concentration which uses the same method to calculate concentration using the mean from all five repetitions.

Results

1. Ruby Creek Watershed

I. Physical and Chemical Parameters

Streams in the Ruby Creek watershed during summer 2015 had cool temperatures, similar dissolved oxygen concentrations, alkaline pH, and variable conductivity (Table 6). Temperatures ranged from 3.38-17.01°C, and mean temperatures were greater at lower elevation sites (Table 6). Dissolved oxygen ranged from 9.58-12.93 mg/L (Table 6). Mean conductivity was highest at five sites which were downstream of active placer mining: Lower Canyon, Middle Canyon, Lower Slate, Middle Slate, and Upper Slate (Table 6). Additionally, mean conductivity at Upper Canyon was similar to or greater than mean conductivity at Lower Canyon, Lower Slate, Middle Slate, and Upper Slate, all of which were downstream of active placer mining (Table 6).

Streams in the Ruby Creek watershed exhibited baseflow conditions following water level logger deployment. The contribution of snow pack and rain to spring runoff ended prior to water level logger deployment in July, shown by little daily variation in water stage or stream temperature (Figure 7). Water stage decreased gradually from early July to early September while water temperatures remained fairly constant (±0.4°C), indicating subsurface flow was entering streams and gradually depleting (Figure 7). The hydrographs indicated four precipitation events: two which caused large runoff events at the beginning and end of September, and two smaller events which occurred at the end of July and the middle of August (Figure 7).

Table 6. Minimum, maximum and mean temperature, dissolved oxygen, pH, and conductivity. Measurements of dissolved oxygen, pH and conductivity were obtained approximately monthly and temperature was recorded hourly at 13 sites in the Ruby Creek watershed during May through September, 2015. For dissolved oxygen, pH, and conductivity measurements were collected at gran, ruby, and lcan five times (n=5), at mill, lslate, midcan, upcan, nfcan, least, sfslate, upeast, and upslate four times (n=4), and at midslate three times (n=3). Bolded sites were sites downstream of placer mining.

		Temperature (°C)			**Dissolved Oxygen (mg/L)**			**pH**			**Conductivity (µS)**		
Site[1]	**Elevation (m)**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
gran	536	7.19	16.44	12.20	9.90	12.24	11.26	8.0	8.4	8.2	30	60	45
ruby	546	5.05	17.01	11.22	9.76	12.01	10.88	8.1	8.4	8.2	60	110	90
lcan	550	5.23	16.07	10.98	9.97	12.18	11.30	7.9	8.4	8.3	100	170	143
mill	817	5.05	15.49	10.09	10.40	11.21	10.69	8.1	8.4	8.2	70	100	87
lslate	873	5.02	14.41	10.33	10.28	11.56	10.94	8.2	8.5	8.4	90	190	143
midcan	883	5.05	13.57	9.72	10.59	11.86	11.24	8.3	8.4	8.4	80	180	138
upcan	885	5.02	14.41	10.33	10.38	11.31	10.94	8.1	8.3	8.2	90	200	158
nfcan	928	4.79	15.34	10.64	9.64	11.23	10.66	8.2	8.4	8.3	50	130	95
midslate[2]	932	6.20	10.40	8.47	10.65	10.92	10.81	8.2	8.4	8.3	90	160	133
least	1209	4.06	11.20	8.06	9.66	11.16	10.52	7.9	8.4	8.2	40	90	68
sfslate	1220	3.67	12.56	8.41	10.49	12.93	11.47	8.1	8.3	8.2	70	120	98
upeast	1340	3.38	10.83	7.36	9.58	11.69	10.77	8.0	8.3	8.1	30	70	55
upslate	1365	3.54	13.55	8.77	10.02	10.90	10.62	8.3	8.5	8.4	90	160	133

[1] Site abbreviations: gran = Granite, ruby = Ruby, lcan= Lower Canyon, mill = Mill, lslate = Lower Slate, midcan = Middle Canyon, upcan = Upper Canyon, nfcan = North Fork Canyon, midslate = Middle Slate, least = Lower East, sfslate = South Fork Slate, upeast = Upper East, upslate = Upper Slate

[2] HOBO temperature logger was removed from the Middle Slate site in July. Minimum, maximum and mean temperatures are based on temperatures measured with a YSI Pro20 during monthly site visits.

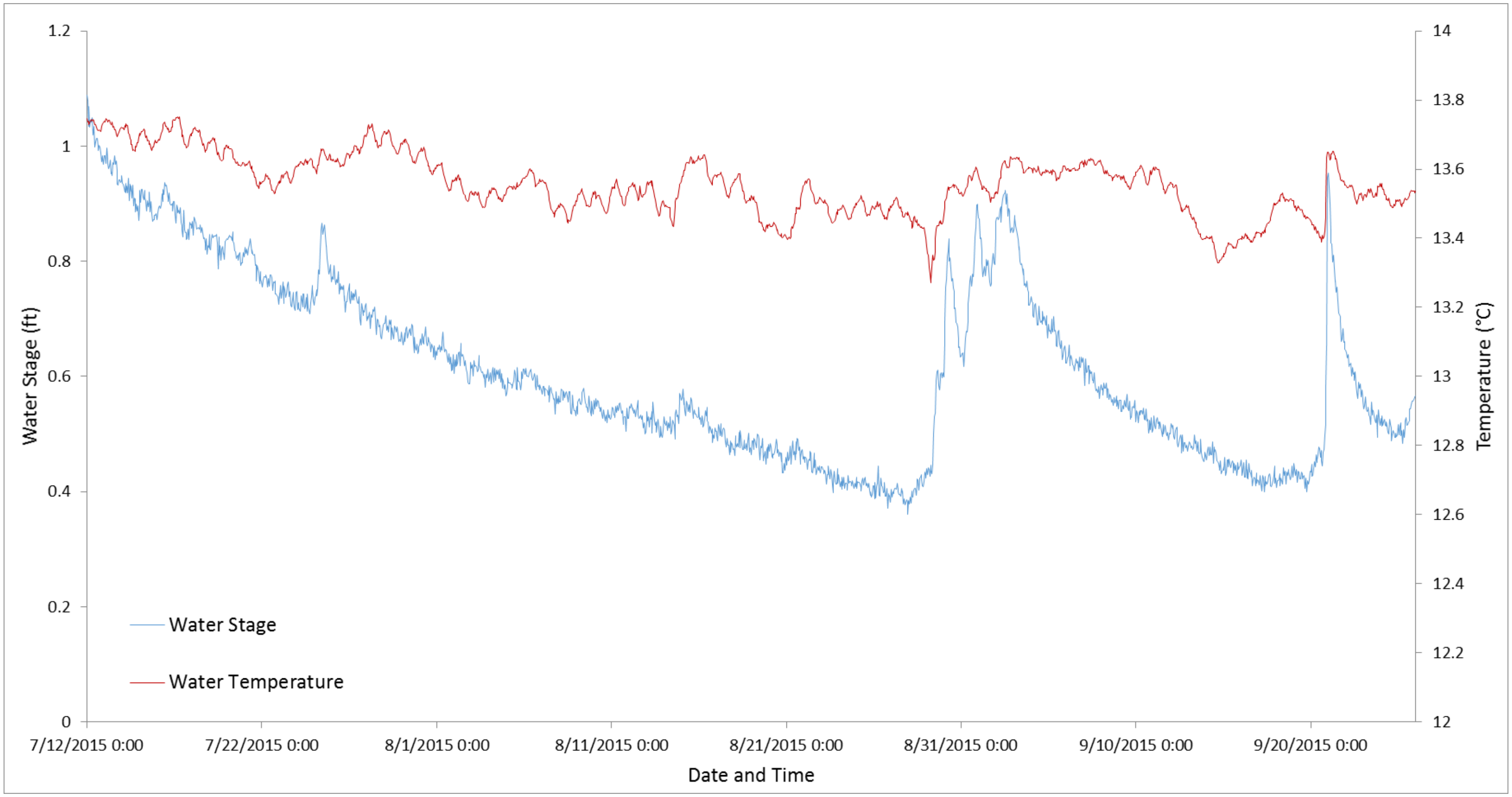

Figure 7. Water stages relative to gauge depth and stream temperatures at the Middle Canyon site in the Ruby Creek watershed. Water stage and water temperatures were recorded hourly from the date of deployment (July 12th, 2015) through the final sampling day (September 26th, 2015). The Middle Canyon site was at the elevational mid-point of the Ruby Creek watershed study area and reflected the patterns in the hydrographs and thermographs obtained from other sites where water level loggers were deployed (Granite, Mill, Lower East, North Fork of Canyon, and South Fork of Slate).

II. Surface Water Grab Samples

Temporal trends

Two seasonal patterns of metal concentrations were evident in surface water grab samples collected monthly from all sites in the Ruby Creek watershed. Total aluminum concentrations decreased at 12 of 13 sites in the Ruby Creek watershed throughout the summer (Figure 8); no obvious trend could be discerned at the 13th site (Middle Slate) because I was unable to collect a sample in July at this location. Concentrations of total sodium, magnesium, potassium, calcium, molybdenum, and barium increased at all sites throughout the summer (Figure 9).

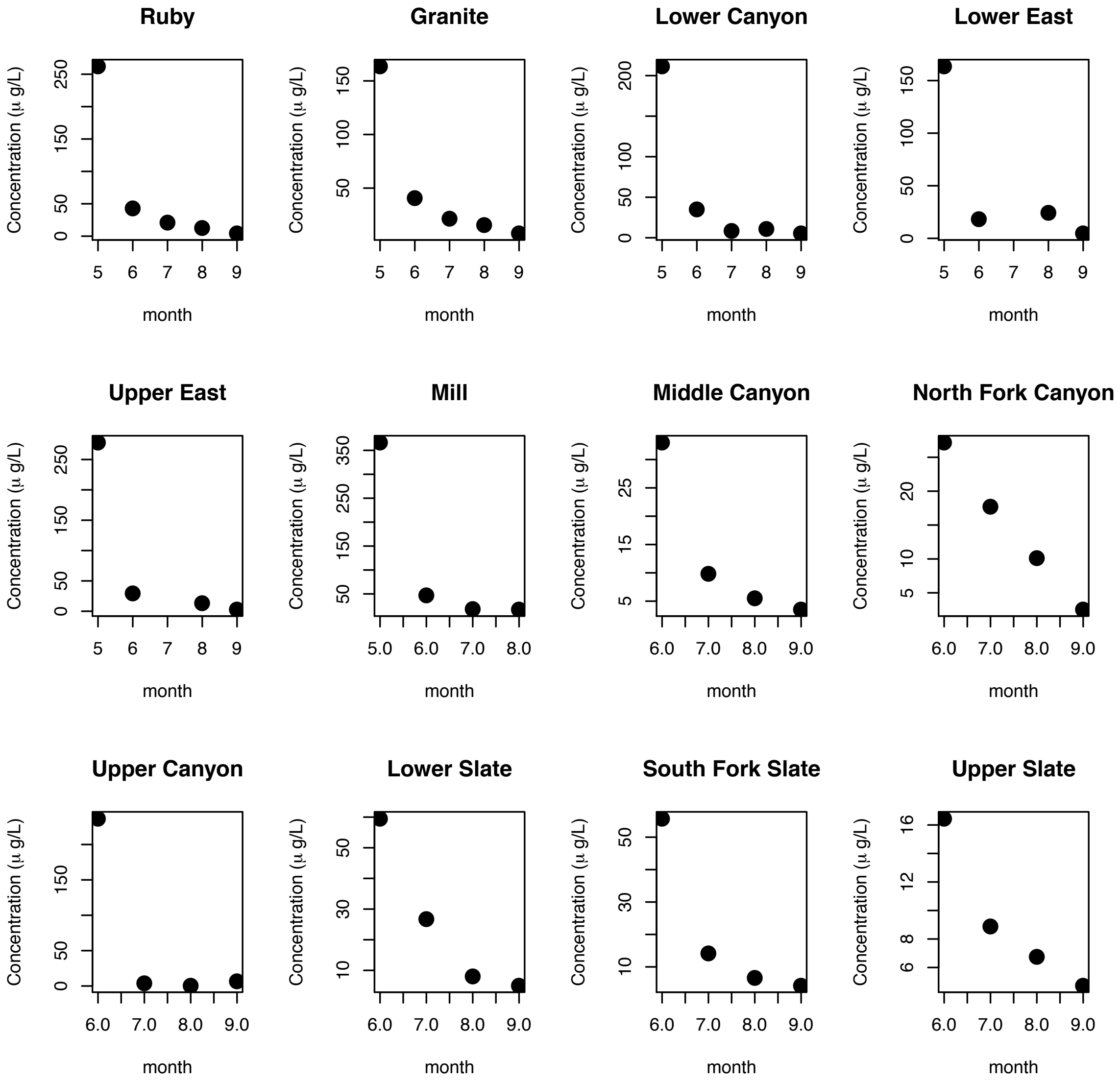

Figure 8. Total aluminum concentrations in surface water grab samples at 12 of the 13 sampling sites in the Ruby Creek watershed collected May through September, 2015. Total aluminum concentrations decreased throughout the summer. Months are indicated by numbers (e.g. 6=June) and the vertical scale varies by site.

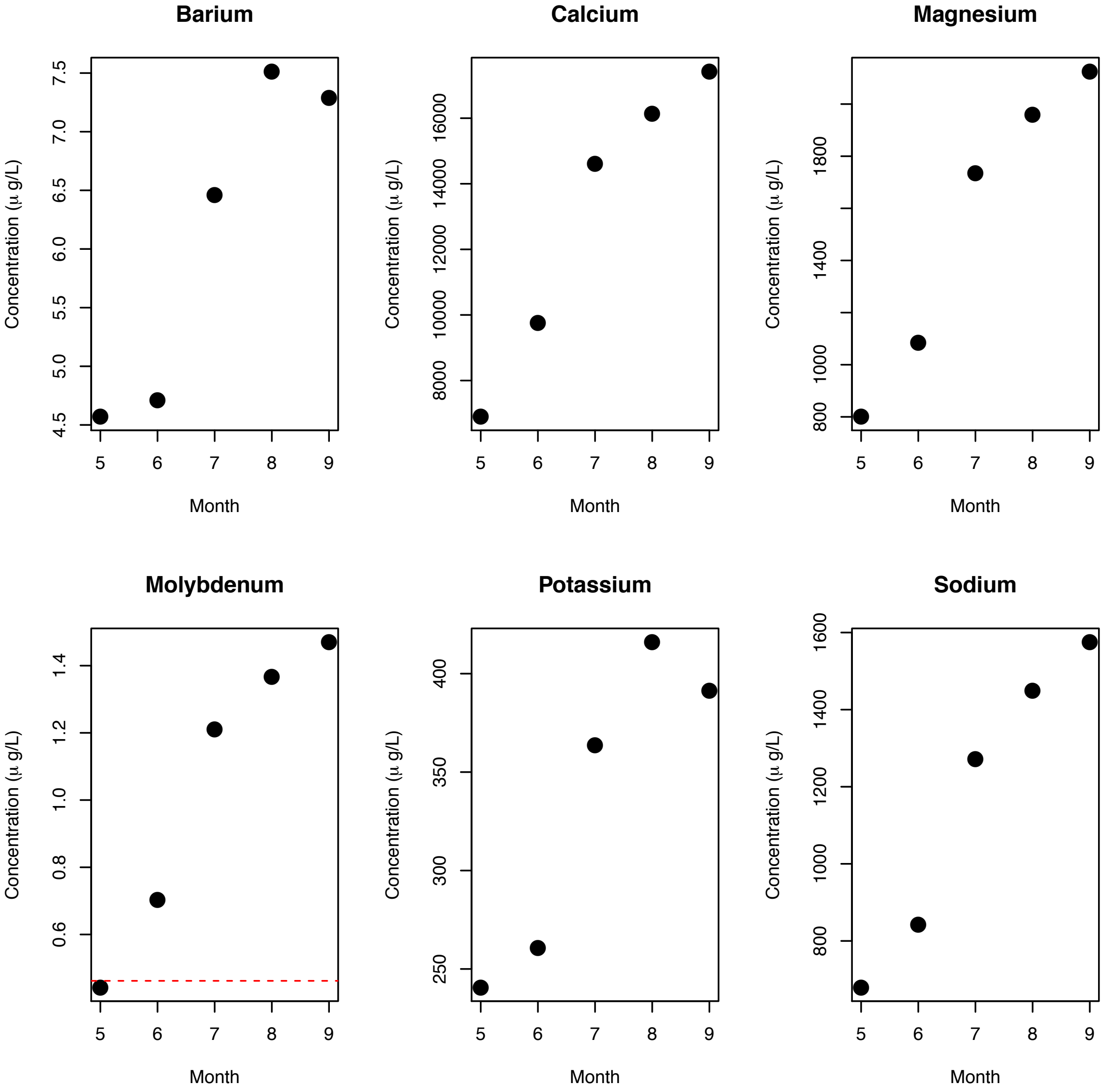

Figure 9. Total concentrations of six metals detected in surface water grab samples at the Ruby Creek site, the most downstream of the 13 sites in the Ruby Creek watershed, during May through September, 2015. Total concentrations of these six metals decreased throughout the summer at all 13 sites. Months are indicated by numbers (e.g. 6=June) and the vertical scale varies by site. The dashed red line on the molybdenum plot vertical axis indicate the detection level.

Analysis of variance and pairwise comparisons of grab sample groups

Concentrations of 13 metals differed among grab sample groups, and differences reflected places and periods of mining activity (Table 7). These 13 metals include aluminum, barium, calcium, chromium, cobalt, iron, manganese, magnesium, molybdenum, potassium, selenium, sodium, and vanadium (Table 7). Of the 13 metals, molybdenum was the only metal where pairwise comparisons did not show significant differences between grab sample groups (Figure 10).

Seasonal effects were evident at active mine sites. Grab samples collected before the mining season (May and June) downstream of mining were different from those collected during the mining season (July-September; “before_mining” vs. “during_mining”; Table 7). This was the case for six metals: aluminum, calcium, manganese, magnesium, potassium and sodium (Figure 10). Of these six metals, concentrations of calcium, magnesium, potassium and sodium were higher once the mining season began (Figure 10). Aluminum and manganese were lower after the mining season started (Figure 10).

Spatial patterns were apparent for metal concentrations in grab samples. Five metals were significantly different between “during_mining” and “during_nomining” groups, indicating there are differences in metal concentrations from samples collected during the mining season (July-September) from sites downstream of mining compared to upstream of mining (Figure 10). Barium, calcium, iron, magnesium, and sodium were higher at locations downstream of mining compared to locations upstream of mining (Figure 10). Calcium and magnesium concentrations were different in grab samples collected before the mining season above and below mines (“before_mining” vs “before_nomining”; Figure 10). Calcium and magnesium concentrations were higher downstream of mines (Figure 10).

Table 7. Median concentrations of total metals among the four groups of surface water grab samples. Samples were collected from 13 sites in the Ruby Creek watershed, approximately monthly from May through September, 2015. Groups include "before_mining" (n=6), "during_mining" (n=14), "before_nomining" (n=13), and "during_nomining" (n=21). Before or during distinctions indicate whether the sample was collected during the placer mining season ("during_mining" and "during_nomining") from July to September or before ("before_mining" and "before_nomining") during May or June. Mining or no mining distinctions indicate whether the sample was collected downstream of hard rock and placer mining activities ("before_mining" and "during_mining") or from sites upstream of mining or greater than 1.5 km downstream of active placer mining ("before_nomining" and "during_nomining").

Metal	**before_mining (µg/L)**	**during_mining (µg/L)**	**before_nomining (µg/L)**	**during_nomining (µg/L)**	**H**	**p-value**
Aluminum	34.17	7.39	55.75	10.15	30.00	≤0.0001*
Barium	5.75	9.58	4.05	5.74	24.64	≤0.0001*
Calcium	15115.20	25361.17	8700.85	15761.28	33.08	≤0.0001*
Chromium	0.16	0.078	0.27	0.081	13.69	0.0034*
Cobalt	0.039	0.020	0.083	0.018	22.28	≤0.0001*
Iron	85.07	73.74	103.70	54.00	18.09	0.0004*
Manganese	2.97	0.34	4.52	0.95	28.73	≤0.0001*
Magnesium	1678.61	3215.53	936.99	1734.16	32.00	≤0.0001*
Molybdenum	0.76	2.11	0.54	0.91	9.58	0.0224*
Potassium	198.66	324.74	260.60	363.56	9.27	0.0258*
Selenium	0.66	0.75	0.45	0.70	11.84	0.0079*
Sodium	810.67	1658.81	667.87	1178.30	35.62	≤0.0001*
Vanadium	0.28	0.21	0.36	0.21	8.28	0.0406*
Antimony	0.12	0.19	0.047	0.096	3.66	0.3002
Arsenic	1.57	1.63	0.76	1.01	5.11	0.1642
Uranium	0.036	0.089	0.11	0.086	6.66	0.0837

*Significant differences based on Kruskal-Wallis Rank Sum test (p ≤0.05)

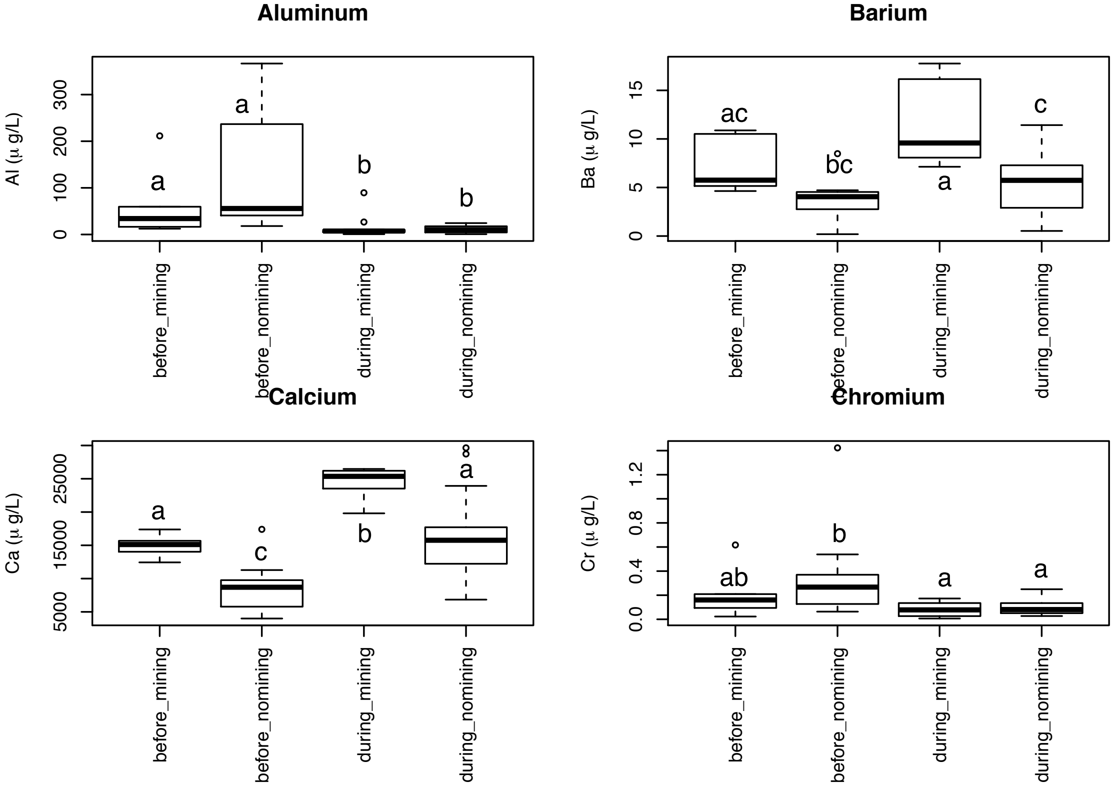

Figure 10. Concentrations of total metals among the four groups of surface water grab samples for the 12 metals that indicated a significant difference among groups (Kruskal-Wallis p ≤0.05). Pairwise comparisons confirmed significant differences between groups as indicated by different letters (Wilcoxon Rank Sum Test p ≤0.05). Pairwise comparisons for molybdenum were not significant so it was omitted. Samples were collected from 13 sites in the Ruby Creek watershed, approximately monthly from May through September, 2015. Groups include "before_mining" (n=6), "during_mining" (n=14), "before_nomining" (n=13), and "during_nomining" (n=21). Before or during distinctions indicate whether the sample was collected during the placer mining season ("during_mining" and "during_nomining") from July to September or before ("before_mining" and "before_nomining") during May or June. Mining or no mining distinctions indicate whether the sample was collected downstream of hard rock and placer mining activities ("before_mining" and "during_mining") or from sites upstream of mining or greater than 1.5 km downstream of active placer mining ("before_nomining" and "during_nomining").

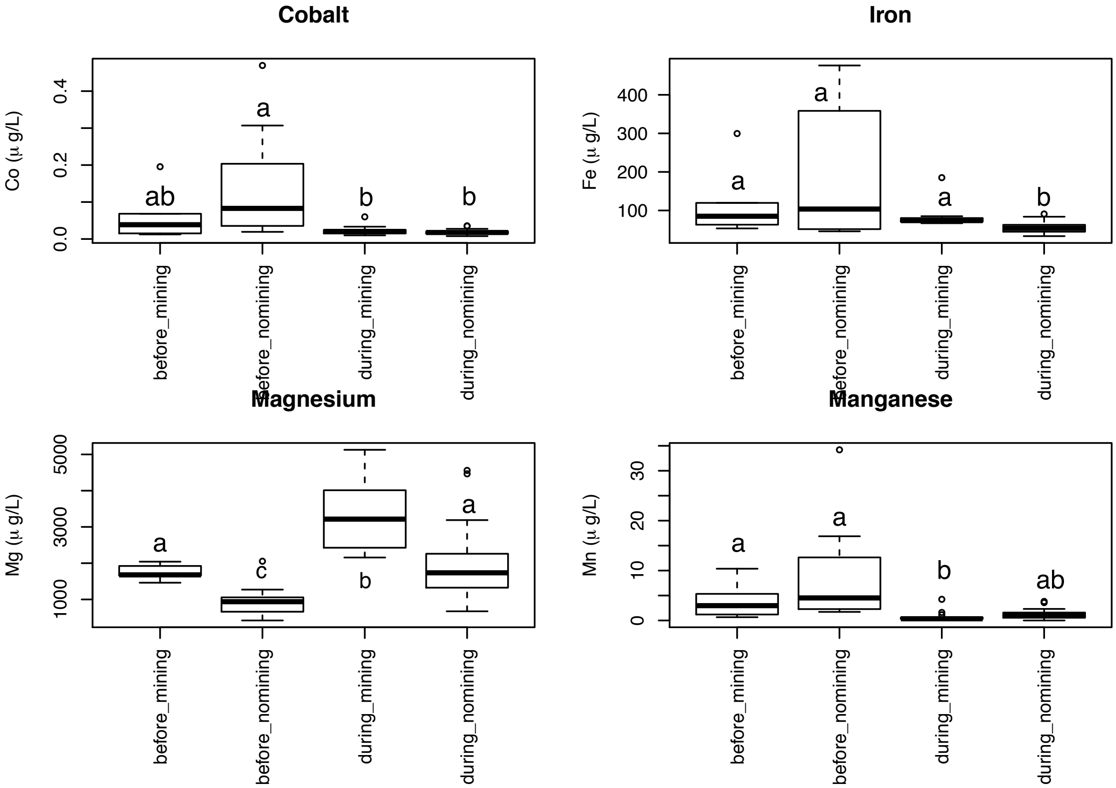

Figure 10. Continued

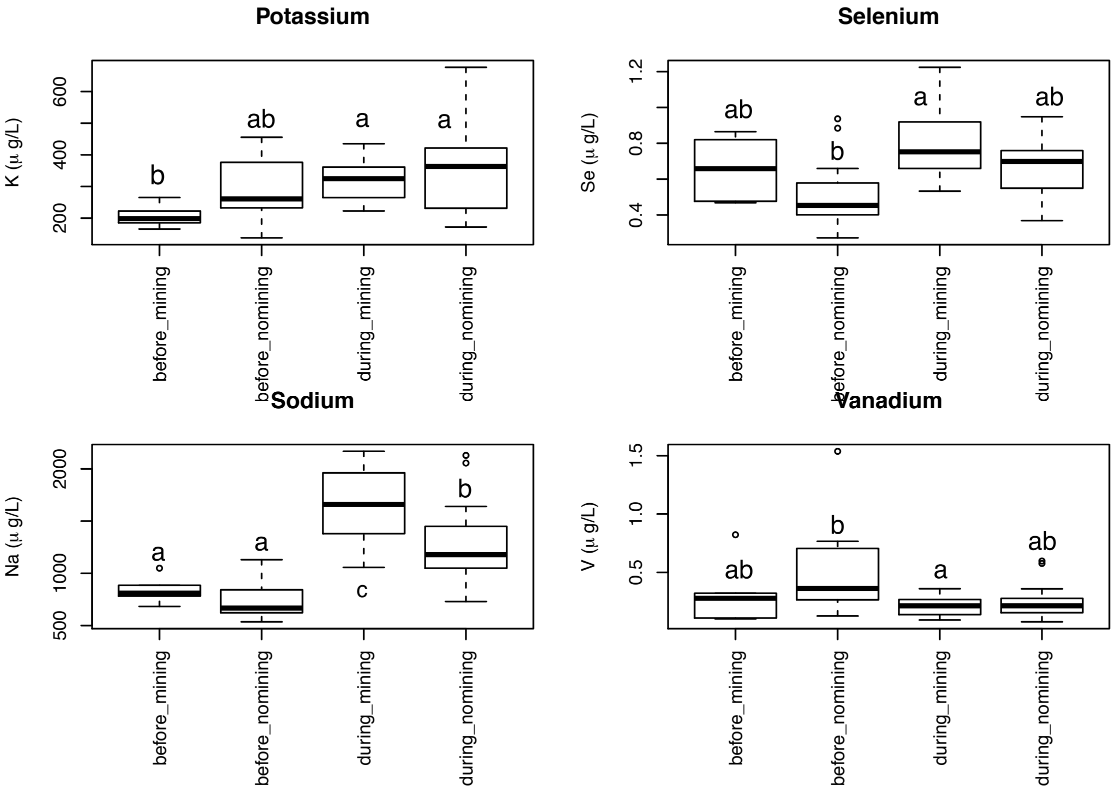

Figure 10. Continued

Relation of all metals to grab sample groups—Hierarchical cluster analysis

Unconstrained cluster analysis separated nearly all surface water grab samples influenced by mining into one of two distinct clusters. Grab samples collected during the mining season downstream of mining (“during_mining”) formed a distinct cluster (Figure 11); the cluster composed of “during_mining” samples also included three samples collected from during the mining season from the Upper Canyon site (“during_nomining”, Figure 11). Samples collected from Upper Canyon before the mining season (“before_nomining”) were separated into the other cluster containing all other sample groups (“before_mining”, “before_nomining”, and “during_nomining”; Figure 11). A significant association between cluster groups and grab sample groups indicated that sample groups were distinguishable based on the kinds and concentrations of metals within the samples (Table 8).

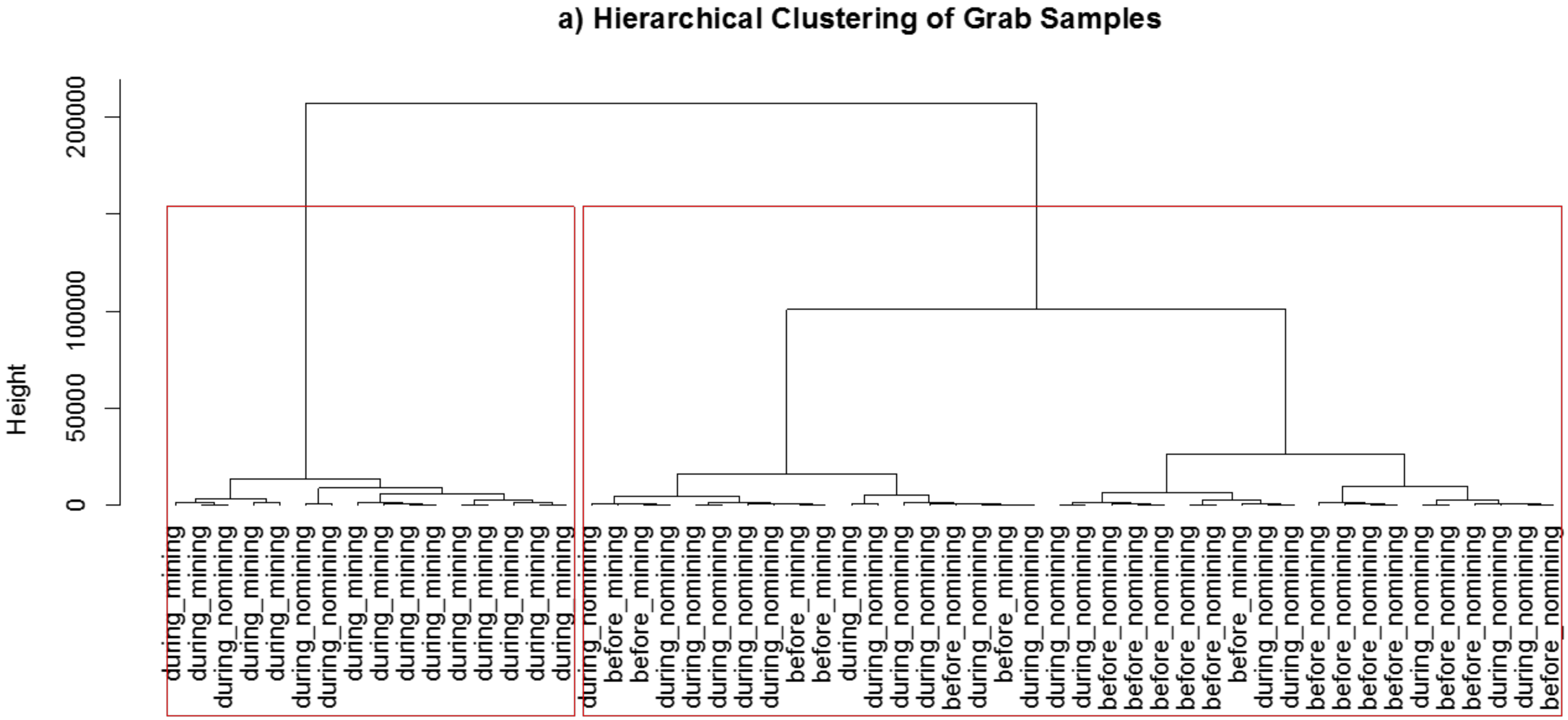

Figure 11. Hierarchical clustering of grab samples collected in the Ruby Creek watershed from May-September, 2015. Figures a) and b) are the same cluster solutions with different labels. a) Labels indicate the four sample groups: “before_mining” (n=6), “during_mining” (n=14), “before_nomining” (n=13), and “during_nomining” (n=21). Before or during distinctions indicate whether the sample was collected during the placer mining season (“during_mining” and “during_nomining”) from July to September or before (“before_mining” and “before_nomining”) during May or June. Mining or no mining distinctions indicate whether the sample was collected downstream of hard rock and placer mining activities (“before_mining” and “during_mining”) or from sites upstream of mining or greater than 1.5 km downstream of active placer mining (“before_nomining” and “during_nomining”).

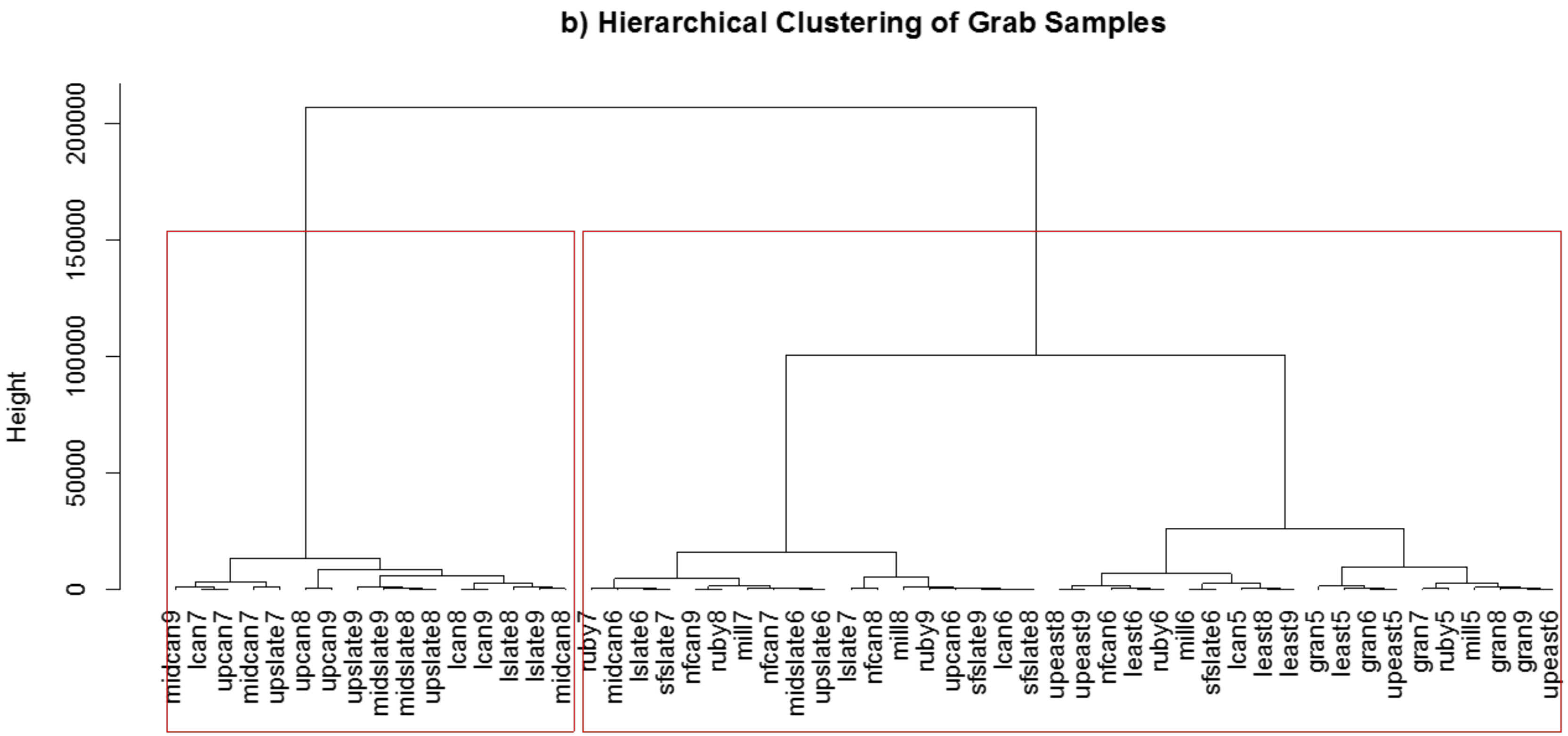

Figure 11. Continued.

Table 8. A significant association (chi-squared test, $p \leq 0.05$) between hierarchical cluster groups and grab sample groups: “before_mining” (n=6), “during_mining” (n=14), “before_nomining” (n=13), and “during_nomining” (n=21). The ”mining” sites were downstream of active hard rock or placer mining; “nomining” sites were upstream of mining or greater than 1.5 km downstream of active placer mine claims. The “before” and “during” distinctions indicate whether the sample was collected during the mining season (“during”=July-September) or before (“before”=May-June).

	Cluster			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	6	0	37.21	≤0.0001*
During_mining	1	13		
Before_nomining	13	0		
During_nomining	18	3		

*Significant association based on chi-squared test ($p \leq 0.05$)

Relation of all metals to grab sample groups—Kmeans cluster analysis

Consistent patterns among concentrations of metals in surface water grab samples were evident using Kmeans cluster analysis. Kmeans clustering uses a random starting point to develop clusters, so repetitive iterations are used to identify the reliability of the cluster solutions. After 20 Kmeans iterations, two significant (chi-squared test, $p \leq 0.05$) cluster solutions were developed. Solution A resulted for 40% of the iterations, and solution B resulted for 60% of the iterations. Both cluster solutions had a significant association between cluster group and grab sample group (Table 9). Solution A and B produced similar results as hierarchical clustering because all samples collected during the mining season downstream of mining ("during_mining") belonged to the same cluster group, cluster two for solution A and cluster one for solution B (Table 9). Solution A also included four samples collected during the mining season from sites upstream or far downstream (>1.5 km) of mining into cluster 2 ("during_nomining"; Table 9). Ten additional grab samples were included into cluster 1 for solution B: 8 of the 10 samples were collected during the mining season at sites upstream or far downstream (>1.5 km) of mining, and 2 samples were collected before the mining season, 1 from a site downstream of mining and the other from a site upstream or far downstream (>1.5 km) of mining ("before_mining" and "before_nomining"; Table 9). Based on relative distance between cluster centers, aluminum, calcium, chromium, and manganese were four of the best cluster separators for both cluster solutions (Table 9).

Table 9. Contingency tables of the grab sample groups and significant Kmeans cluster solutions (chi-squared test, p≤0.05) based on surface water grab samples collected at 13 sites in the Ruby Creek watershed from May through September, 2015. Grab sample groups are “before_mining” (n=6), “during_mining” (n=14), “before_nomining” (n=13), and “during_nomining” (n=21). Before or during distinctions indicate whether the sample was collected during the placer mining season (“during_mining” and “during_nomining”) from July to September or before (“before_mining” and “before_nomining”) during May or June. Mining or no mining distinctions indicate whether the sample was collected downstream of hard rock and placer mining activities (“before_mining” and “during_mining”) or from sites upstream of mining or greater than 1.5 km downstream of active placer mining (“before_nomining” and “during_nomining”).

Solution A	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	6	0	39.42	≤0.0001*
During_mining	0	14		
Before_nomining	13	0		
During_nomining	17	4		

Metal	***Relative distance from cluster centers (%)***
Co	97.1
Mn	86.5
Al	81.3
Mo	65.8
Cr	61.9
Mg	60.5
Ba	59.3
Ca	51.3

Solution B	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	1	5	26.82	≤0.0001*
During_mining	14	0		
Before_nomining	1	12		
During_nomining	8	13		

Metal	***Relative distance from cluster centers (%)***
Ca	73.8
Al	67.5
As	61.0
Mn	61.0
U	57.1
Cr	52.3
Fe	51.2

*Significant association based on chi-squared test (p≤0.05)

Relation of all metals to grab sample groups—Nonmetric (RIFFLE) cluster analysis

Nonmetric clustering was not as reliable as hierarchical or Kmeans clustering for recognizing consistent patterns with total metal concentrations in the surface water grab samples. RIFFLE clustering, like Kmeans, begins at a random starting point for cluster development, so multiple iterations are performed to evaluate the consistency of the cluster solutions. Out of 20 RIFFLE iterations, 12 significant cluster solutions (A-L) and one nonsignificant cluster solution resulted (Table 10). Cluster solutions A, C, and D occurred three times, solution B occurred twice, and solutions E-L occurred once. Despite inconsistent cluster solutions, RIFFLE clustered all samples collected during the mining season from sites downstream of mines together in solutions A and B, like the Kmeans and hierarchical clustering solutions (Table 10). All other cluster solutions misclassified at least one of the samples collected during the mining season downstream of mining ("during_mining"; Table 10). Based on high PRE scores, aluminum was one of the best cluster separators for 7 of the 12 clustering solutions (Table 10).

Table 10. Contingency tables of the grab sample groups and significant RIFFLE cluster solutions (chi-squared test, $p \leq 0.05$) based on surface water grab samples collected at 13 sites in the Ruby Creek watershed from May through September, 2015. Grab sample groups are "before_mining" (n=6), "during_mining" (n=14), "before_nomining" (n=13), and "during_nomining" (n=21). Before or during distinctions indicate whether the sample was collected during the placer mining season ("during_mining" and "during_nomining") from July to September or before ("before_mining" and "before_nomining") during May or June. Mining or no mining distinctions indicate whether the sample was collected downstream of hard rock and placer mining activities ("before_mining" and "during_mining") or from sites upstream of mining or greater than 1.5 km downstream of active placer mining ("before_nomining" and "during_nomining").

Solution A	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	6	0	29.74	≤0.0001*
During_mining	0	14		
Before_nomining	12	1		
During_nomining	9	12		

Metal	***PRE score***
Ca	0.85
Mg	0.85
Ba	0.78
Na	0.78
Al	0.63
Mn	0.63

Solution B	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	5	1	26.02	≤0.0001*
During_mining	0	14		
Before_nomining	12	1		
During_nomining	10	11		

Metal	***PRE score***
Ca	0.93
Mg	0.93
Ba	0.70
Na	0.70
Al	0.63
Mn	0.63

Table 10. Continued.

Solution C	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	4	2	14.97	0.0018*
During_mining	5	9		
Before_nomining	12	1		
During_nomining	6	15		

Metal	***PRE score***
Cr	0.78
Co	0.78
Al	0.70
V	0.70

Solution D	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	2	4	10.37	0.0156*
During_mining	5	9		
Before_nomining	11	2		
During_nomining	7	14		

Metal	***PRE score***
Cr	0.85
V	0.78
Co	0.70
Al	0.63

Solution E	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	4	2	24.38	≤0.0001*
During_mining	1	13		
Before_nomining	13	0		
During_nomining	9	12		

Metal	***PRE score***
Ca	0.85
Mn	0.78
Mg	0.78
Al	0.70
Ba	0.63
Na	0.56

Table 10. Continued

Solution F	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	5	1	22.69	≤0.0001*
During_mining	1	13		
Before_nomining	12	1		
During_nomining	9	12		

Metal	***PRE score***
Ca	0.85
Mn	0.78
Mg	0.78
Al	0.70
Ba	0.63
Na	0.56

Solution G	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	4	2	22.00	≤0.0001*
During_mining	2	12		
Before_nomining	13	0		
During_nomining	8	13		

Metal	***PRE score***
Al	0.78
Mn	0.78
Ba	0.70
Co	0.63
Ca	0.56
Mg	0.56

Solution H	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	6	0	20.56	0.0001*
During_mining	2	12		
Before_nomining	11	2		
During_nomining	8	13		

Metal	***PRE score***
K	0.63
Mo	0.63
Na	0.63

Table 10. Continued

Solution I	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	5	1	13.80	0.0032*
During_mining	4	10		
Before_nomining	11	2		
During_nomining	7	14		

Metal	***PRE score***
K	0.63
Mo	0.63

Solution J	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	2	4	9.44	0.0240*
During_mining	3	11		
Before_nomining	10	3		
During_nomining	12	9		

Metal	***PRE score***
As	0.93
Sb	0.85
U	0.56

Solution K	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	3	3	8.39	0.0386*
During_mining	3	11		
Before_nomining	10	3		
During_nomining	11	10		

Metal	***PRE score***
As	0.85
Sb	0.78
Ca	0.56
Mg	0.56

Solution L	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Before_mining	2	4	8.35	0.0393*
During_mining	3	11		
Before_nomining	9	4		
During_nomining	13	8		

Metal	***PRE score***
Sb	0.78
As	0.70

*Significant association based on chi-squared test ($p \leq 0.05$)

Predicted Toxicity

Metal concentrations in surface water grab samples were compared to established EPA Aquatic Life Criteria to evaluate toxicity. Metal concentrations in grab samples exceeded freshwater CCCs for two different metals (Table 11). The lead freshwater CCC was exceeded in a grab sample collected in June at the Middle Slate site (Table 11). The cadmium freshwater CCC was exceeded in a grab sample collected in May at the Granite Creek site (Table 11). Both results indicate that aquatic organisms would be adversely impacted by long-term exposures to concentrations of lead or cadmium at Granite or Slate Creeks.

Table 11. The maximum dissolved concentration of metals detected in surface water grab samples, the collection site and month, the freshwater criterion continuous concentration (CCC) from Aquatic Life Criteria (EPA 2016), and whether the sample exceeded the CCC shown. Bold indicates the CCC was exceeded.

Metal	Max (µg/L)	site[1]/month	Freshwater CCC (µg/L)	exceeded?
Aluminum	31.33	gran/may	87	no
Arsenic	2.63	upcan/june	150	no
Cadmium*	**0.137**	**gran/may**	**0.136**	**yes**
Copper	0.93	upeast/may	1.63	no
Chromium*	0.96	midslate/june	37.38	no
Iron	103.07	upcan/sept	1000	no
Lead*	**3.07**	**midslate/june**	**1.00**	**yes**
Nickel*	0.86	upcan/july	39.23	no
Zinc*	32.97	nfcan/sept	61.22	no

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[1] Site abbreviations: gran = Granite, ruby = Ruby, lcan= Lower Canyon, mill = Mill, lslate = Lower Slate, midcan = Middle Canyon, upcan = Upper Canyon, nfcan = North Fork Canyon, midslate = Middle Slate, least = Lower East, sfslate = South Fork Slate, upeast = Upper East, upslate = Upper Slate

*Freshwater CCCs were hardness corrected (EPA 2016).

III. Metal Analysis Using Passive Samplers - Stabilized Liquid Membrane Devices (SLMDs)

Metal detection in SLMDs vs. grab samples

The SLMDs were more effective than grab samples at detecting metals analyzed by the ICP-MS. Based on the difference between the number of samples above the detection level in SLMDs and grab samples, 19 of the 25 metals analyzed were more frequently detected in SLMD samples (Figure 12). Although these 19 metals were not present at detectable levels in grab samples on particular sampling dates at particular sampling sites, the fact that they were present on an SLMD indicates that the metal was present at the sampling sites at some point during the sampling period from May through September.

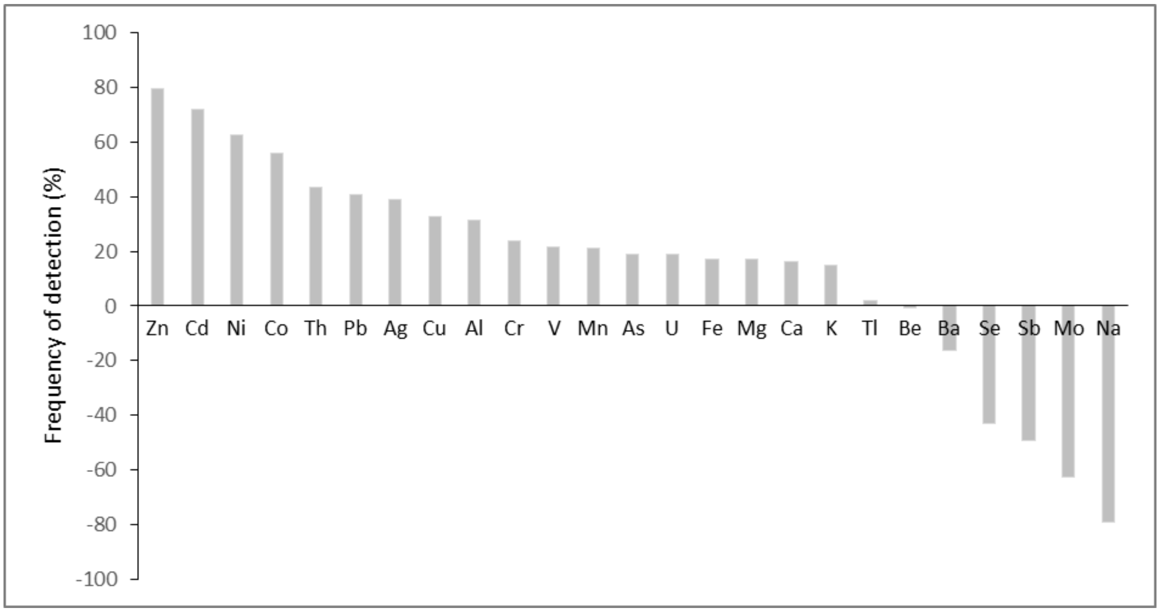


Figure 12. Frequency of detection of metals on SLMDs compared to grab samples. Values were calculated by subtracting the percentage of samples above the detection level in grab samples from SLMD samples for each metal. Positive values indicate greater frequency of detection in SLMD samples, and negative values indicate greater frequency of detection in grab samples.

Analysis of variance and pairwise comparisons of SLMD sample groups

Metal accumulation on SLMDs downstream of mining differed based on mining type. Seven metals: cobalt, chromium, manganese, magnesium, nickel, and vanadium, differed among SLMD groups (Table 12). Cobalt, chromium, nickel and magnesium accumulations were higher at sites downstream of placer mining compared to sites without mining (Figure 13). SLMDs downstream of both placer mining and hard rock mining accumulated less cobalt, chromium, and manganese than sites with no mining upstream (Figure 13). Less iron and vanadium collected on SLMDs downstream of both types of mining compared to only placer mining (Figure 13).

Table 12. Median mass of metals accumulated among the four groups of SLMDs. SLMDs were deployed at 13 sites in the Ruby Creek watershed for approximately month-long intervals from May through September, 2015. SLMD sample groups include hardrock (n=9), placer (n=4), both (n=11), and none (n=17). The sample groups indicate if the SLMDs were deployed at a site that is downstream of hard rock mining, placer mining, both types of mining, or no mining.

Metal	**Hardrock (μg/day)**	**Placer (μg/day)**	**Both (μg/day)**	**None (μg/day)**	**H**	**p-value**
Aluminum	137.91	204.28	55.44	103.60	9.43	0.0241*
Chromium	0.39	0.73	0.10	0.20	19.00	0.0003*
Cobalt	0.19	0.22	0.047	0.10	18.48	0.0004*
Iron	166.61	263.37	70.81	126.55	12.69	0.0054*
Manganese	11.93	7.94	3.33	7.53	9.10	0.0280*
Magnesium	70.09	117.41	21.36	40.41	15.05	0.0018*
Nickel	0.68	1.64	0.40	0.45	15.31	0.0016*
Vanadium	0.60	0.95	0.20	0.32	13.41	0.0038*
Arsenic	0.39	0.38	0.18	0.19	6.53	0.0885
Barium	0.49	0.73	0.28	0.49	1.67	0.6430
Cadmium	0.087	0.081	0.049	0.058	1.71	0.6342
Calcium	108.49	191.14	162.46	87.94	5.10	0.1644
Lead	0.17	0.025	0.022	0.093	3.37	0.3382
Potassium	29.80	38.70	11.11	20.36	8.04	0.4052
Uranium	0.057	0.033	0.044	0.40	5.88	0.1175
Zinc	10.66	6.15	6.76	10.92	1.61	0.6564

*Significant differences based on Kruskal-Wallis Rank Sum test ($p \leq 0.05$)

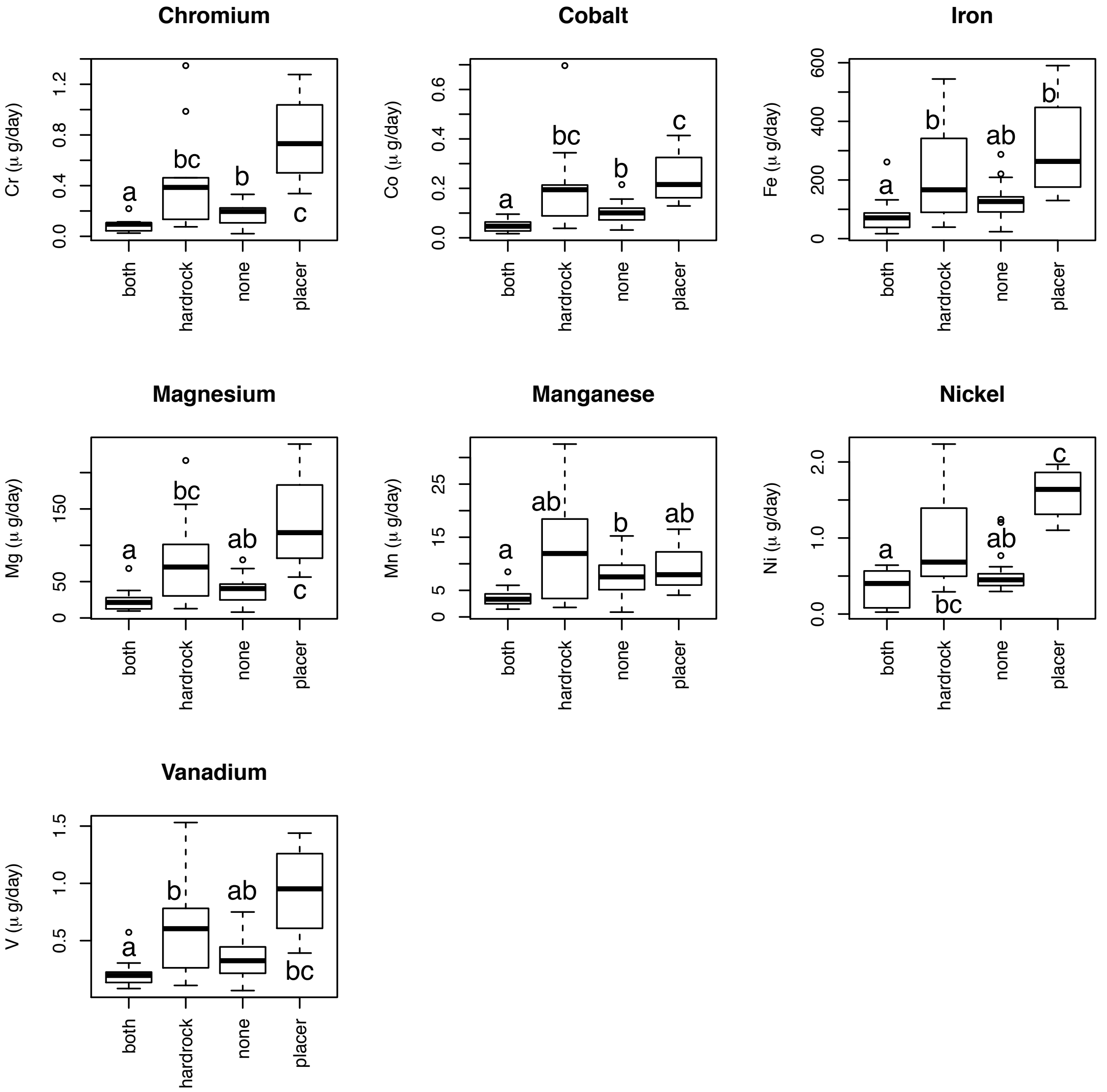
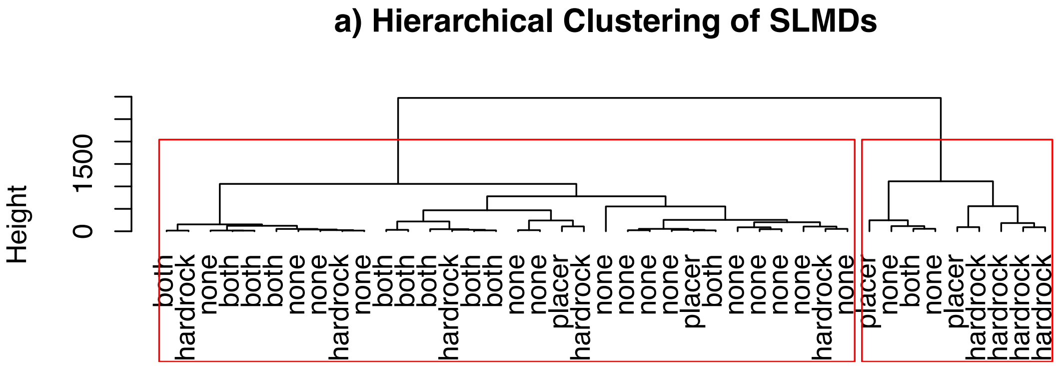


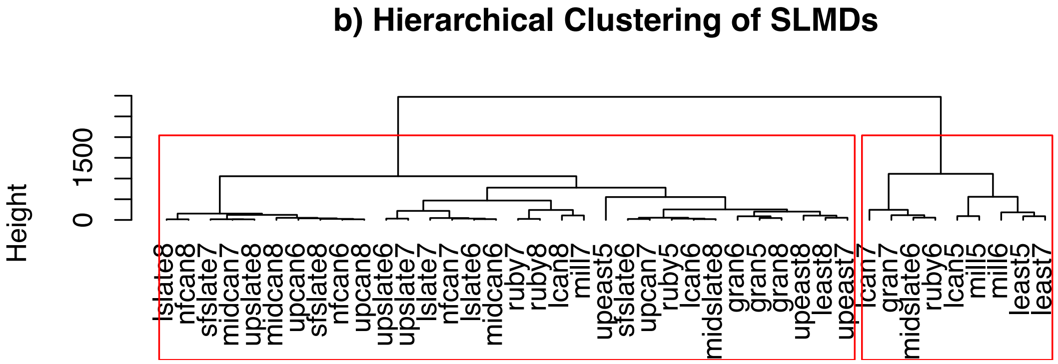
Figure 13. Metals accumulated among the four groups of SLMDs for the eight metals that indicated a significant difference among sample groups (Kruskal-Wallis, $p \leq 0.05$). Pairwise comparisons confirmed significant differences between sample groups as indicated by different letters (Wilcoxon Rank Sum Test, $p \leq 0.05$). Pairwise comparisons for aluminum were not significant so it was omitted. SLMDs were deployed at 13 sites in the Ruby Creek watershed for approximately month-long intervals from May through September, 2015. Sample groups are hardrock (n=9), placer (n=4), both (n=11), and none (n=17) and indicate if the SLMDs were deployed at a site that is downstream of hard rock mining, placer mining, both types of mining, or no mining.

Relation of all metals to SLMD sample groups—Hierarchical cluster analysis

The four SLMD sample groups based on locations of hard rock mining, placer mining, both types of mining, or no mining, were not associated into distinguishable clusters using hierarchical clustering (Figure 14). A nonsignificant association (chi-squared test, $p>0.05$) between cluster groups and SLMD sample groups indicates that sample groups were not distinguishable based on the kinds and quantities of metals in the samples (Table 13). Hierarchical clustering relies on distance metrics for cluster development, and no relationship between metal concentrations and sample group could be established using this technique. Thus, I did not present Kmeans clustering because it also uses distance metrics for cluster development and also produced nonsignificant cluster solutions.



a) Hierarchical Clustering of SLMDs



b) Hierarchical Clustering of SLMDs

Figure 14. Hierarchical clustering of SLMDs deployed at 13 sites in the Ruby Creek watershed May through September, 2015. Figure 14a) and 14b) are the same cluster solutions with different labels. Figure 14a) labels indicate the four SLMD sample groups: hardrock (n=9), placer (n=4), both (n=11), and none (n=17). The group indicates if the SLMD was deployed at a site downstream of hard rock mining, placer mining, both, or no mining. Figure 14b) labels indicate the site and month the SLMD was deployed (e.g. gran6: site=Granite and Month=June). The SLMD retrieval date was approximately a month after deployment (e.g. gran6: month deployed=June, month retrieved= July). Site abbreviations are gran = Granite, ruby = Ruby, lcan= Lower Canyon, mill = Mill, lslate = Lower Slate, midcan = Middle Canyon, upcan = Upper Canyon, nfcan = North Fork Canyon, midslate = Middle Slate, least = Lower East, sfslate = South Fork Slate, upeast = Upper East, upslate = Upper Slate.

Table 13. The nonsignificant association (chi-squared test, p>0.05) between hierarchical cluster groups and SLMD sample groups: hardrock (n=9), placer (n=4), both (n=11), and none (n=17). The sample group indicates if the SLMD was deployed at a site downstream of hard rock mining, placer mining, both, or no mining.

	Cluster			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Hardrock	5	4	6.59	0.0863*
Placer	2	2		
Both	10	1		
None	15	2		

*Significant association based on chi-squared test (p≤0.05)

Relation of all metals to SLMD sample groups—Nonmetric (RIFFLE) cluster analysis

Nonmetric clustering recognized consistent patterns with metal quantities in SLMD samples that distinguished sample groups. Out of 20 RIFFLE iterations, 70% produced three significant cluster solutions (chi-squared test, $p \leq 0.05$), 30% produced two nonsignificant cluster solutions. Of the significant cluster solutions, A resulted 12 times and B and C each occurred once (Table 14). The same eleven metals had high (≥ 0.5) PRE scores in cluster solutions A and B (Table 14); aluminum, chromium and magnesium were the top three separators based on PRE scores for cluster solutions A and B (Table 14). Cluster solution C had three different metals, lead, zinc and cadmium, with high PRE scores compared to the other solutions (Table 14). Based on the frequency of occurrence of cluster solution A in the 20 iterations, the similarity of results with the association analysis and of PRE scores for cluster solutions A and B, and the low misclassification rates of sample sites into sample groups in cluster solutions A and B, these are the more reliable cluster solutions.

Both Solution A and Solution B show distinct patterns of metal accumulation on SLMDs at sample sites in relation to locations of mining activity. Solutions A and B grouped all four of the SLMDs collected downstream of placer mining into the same cluster (cluster 1; Table 14); SLMDs collected downstream of both hard rock mining and placer mining ($n=11$) clustered into the opposite cluster (cluster 2) aside from 2 misclassifications in solution A and 1 misclassification in solution B (Table 14). SLMDs collected downstream of hard rock mining or from sites without mining were split between clusters for all three solutions (Table 14).

Table 14. Contingency tables of the SLMD sample groups and significant RIFFLE cluster solutions (chi-squared test, $p \leq 0.05$) based on SLMDs deployed at 13 sites in the Ruby Creek watershed from May through September, 2015. SLMD sample groups include hardrock (n=9), placer (n=4), both (n=11), and none (n=17). The sample groups indicate if the SLMDs were deployed at a site that is downstream of hard rock mining, placer mining, both types of mining, or no mining.

Solution A	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
hardrock	5	4	8.60	0.0350*
placer	4	0		
both	2	9		
none	9	8		
Metal	***PRE score***			
Al	0.9			
Cr	0.9			
Mg	0.9			
V	0.9			
Co	0.8			
Fe	0.8			
Ba	0.5			
K	0.5			
Mn	0.5			
Ni	0.5			
U	0.5			

Solution B	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
hardrock	6	3	12.40	0.0061*
placer	4	0		
both	1	10		
none	9	8		
Metal	***PRE score***			
Cr	1.0			
Mg	1.0			
Al	0.8			
Co	0.8			
Fe	0.8			
V	0.8			
K	0.6			
Mn	0.6			
Ni	0.6			
U	0.5			

Table 14. Continued.

Solution C	**Cluster**			
Sample Group	**1**	**2**	**χ^2**	**p-value**
hardrock	5	4	8.43	0.0379*
placer	1	3		
both	2	9		
none	12	5		
Metal	***PRE score***			
U	0.8			
Mn	0.7			
Pb	0.7			
Zn	0.7			
Co	0.6			
Mg	0.5			
Al	0.5			
Ba	0.5			
Cd	0.5			
Cr	0.5			
Fe	0.5			
K	0.5			
V	0.5			

*Significant association based on chi-squared test ($p \leq 0.05$)

IV. Periphyton

Analysis of variance and pairwise comparisons of periphyton sample groups

Metal accumulation in periphyton differed for one metal between sample groups (Table 15). Periphyton collected downstream of hard rock and placer mining (“both”) accumulated greater quantities of calcium than periphyton collected upstream of mining or far downstream (1.5 km) of mining (Table 15).

Table 15. Median mass of metals accumulated per gram of periphyton acid digested per day of sampler deployment among the four groups of periphyton. Periphyton samplers were deployed at 13 sites in the Ruby Creek watershed for approximately five months from May through September. Periphyton sample groups include hardrock (n=3), placer (n=1), both (n=4), and none (n=5). The sample groups indicate if the periphyton was collected at a site downstream of hard rock mining, placer mining, both types of mining, or no mining.

Metal	**Hardrock (µg/g/day)**	**Placer (µg/g/day)**	**Both (µg/g/day)**	**None (µg/g/day)[1]**	**H**	**p-value**
Calcium	64.25	102.40	95.58	69.26	6.49	0.0389
Aluminum	160.90	166.50	136.35	114.80	0.73	0.6953
Arsenic	0.72	0.35	0.64	0.54	0.83	0.6601
Barium	0.64	0.48	0.81	0.69	2.52	0.2834
Cadmium	0.028	0.001	0.005	0.002	0.43	0.8067
Chromium	0.53	0.73	0.44	0.35	3.25	0.1963
Cobalt	0.15	0.13	0.11	0.09	1.48	0.4754
Copper	2.25	2.06	1.15	0.83	0.83	0.6601
Iron	554.90	341.90	363.40	375.30	4.23	0.1204
Lead	0.16	0.08	0.22	0.15	0.06	0.9703
Manganese	9.13	5.30	8.10	7.43	0.24	0.8865
Magnesium	72.36	100.60	59.60	44.57	2.73	0.2545
Nickel	0.47	0.59	0.26	0.22	2.83	0.2430
Potassium	15.92	25.11	40.13	27.86	1.94	0.3794
Selenium	0.063	0.05	0.079	0.07	3.19	0.2024
Silver	0.019	0.004	0.010	0.005	2.11	0.3479
Sodium	0.89	8.01	3.17	2.48	0.74	0.6917
Thorium	0.005	0.004	0.005	0.008	0.18	0.9142
Uranium	0.024	0.005	0.13	0.013	0.03	0.9854
Vanadium	0.60	0.69	0.43	0.38	1.93	0.3794
Zinc	24.20	19.69	28.80	16.10	2.39	0.3020

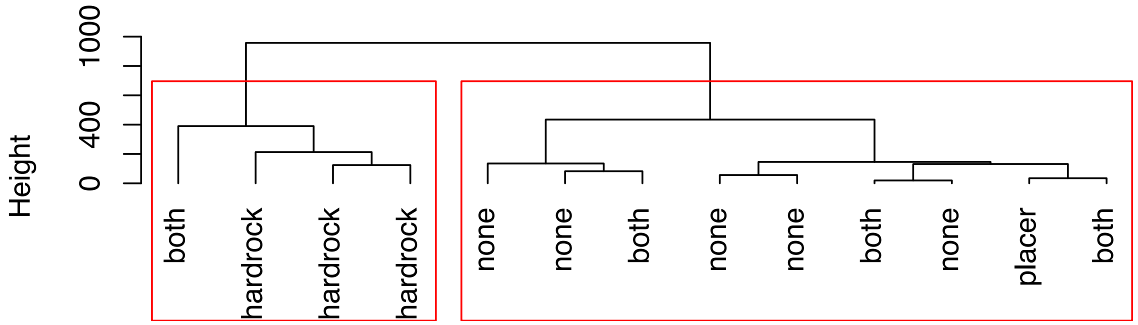
*Significant differences based on Kruskal-Wallis Rank Sum test ($p \leq 0.05$)

[1] The placer group (n=1) was not included in the Kruskal-Wallis Rank Sum test

[2] Pairwise comparisons between groups with Wilcoxon Rank Sum test show a significant differences between the “both” and “none” groups ($p \leq 0.05$)

Relation of all metals to periphyton sample groups—Hierarchical cluster analysis

Periphyton samples were separated into two distinct clusters using hierarchical clustering. A significant association (chi-squared test, $p \leq 0.05$) between cluster groups and periphyton sample groups indicates that sample groups were identifiable based on the kinds and quantities of metals within the samples (Table 16). Based on metal quantities, periphyton collected at sample sites downstream of hard rock mining clustered separately and almost exclusively from the other sample groups (Figure 16). The exception in this cluster was periphyton collected at the Middle Slate site, which is downstream of both placer and hard rock mining (Figure 16).


a) Hierarchical Clustering of Periphyton

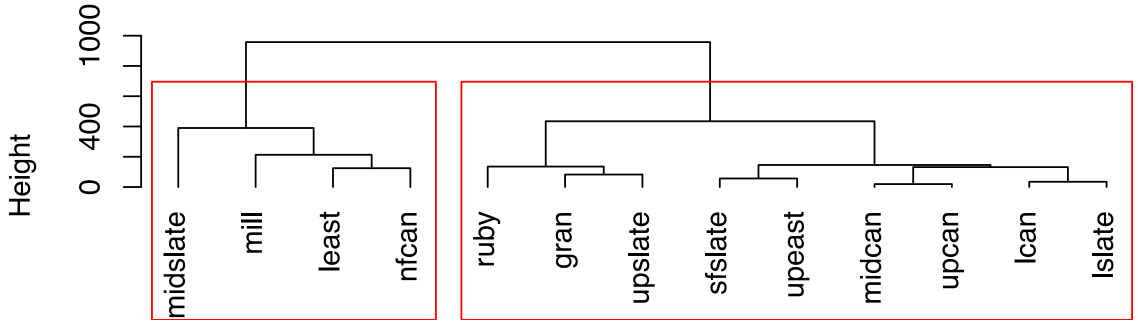

b) Hierarchical Clustering of Periphyton

Figure 15. Hierarchical clustering of periphyton samples collected from 13 sites in the Ruby Creek watershed. Clusters are established based on quantities of metals in periphyton, which accumulated during an approximately five-month sampler deployment from May through September, 2015. Figures 16a) and 16b) are the same cluster solutions with different labels. a) Labels indicate the periphyton sample groups: hardrock (n=3), placer (n=1), both (n=4), and none (n=5). The sample groups indicate if the periphyton samplers were deployed at a site that is downstream of hard rock mining, placer mining, both types of mining, or no mining. b) Labels indicate the site the periphyton were collected from. Site abbreviations are gran = Granite, ruby = Ruby, lcan= Lower Canyon, mill = Mill, lslate = Lower Slate, midcan = Middle Canyon, upcan = Upper Canyon, nfcan = North Fork Canyon, midslate = Middle Slate, least = Lower East, sfslate = South Fork Slate, upeast = Upper East, upslate = Upper Slate.

Table 16. A significant association (chi-squared test, p≤0.05) between hierarchical cluster groups and periphyton sample groups: hardrock (n=3), placer (n=1), both (n=4), and none (n=5). The sample groups indicate if the periphyton samplers were deployed at a site that is downstream of hard rock mining, placer mining, both types of mining, or no mining.

	Cluster			
Sample Group	**1**	**2**	**χ^2**	**p-value**
Hardrock	0	3	9.48	0.0235*
Placer	1	0		
Both	3	1		
None	5	0		

*Significant association based on chi-squared test (p≤0.05)

2. Upper Skagit River and Silverdaisy Creek watersheds

Stabilized Liquid Membrane Devices (SLMDs)

The SLMDs in Silverdaisy Creek accumulated higher quantities of five metals compared to the SLMDs in the Upper Skagit River. These five metals are arsenic, cadmium, nickel, lead and zinc (Figure 17).

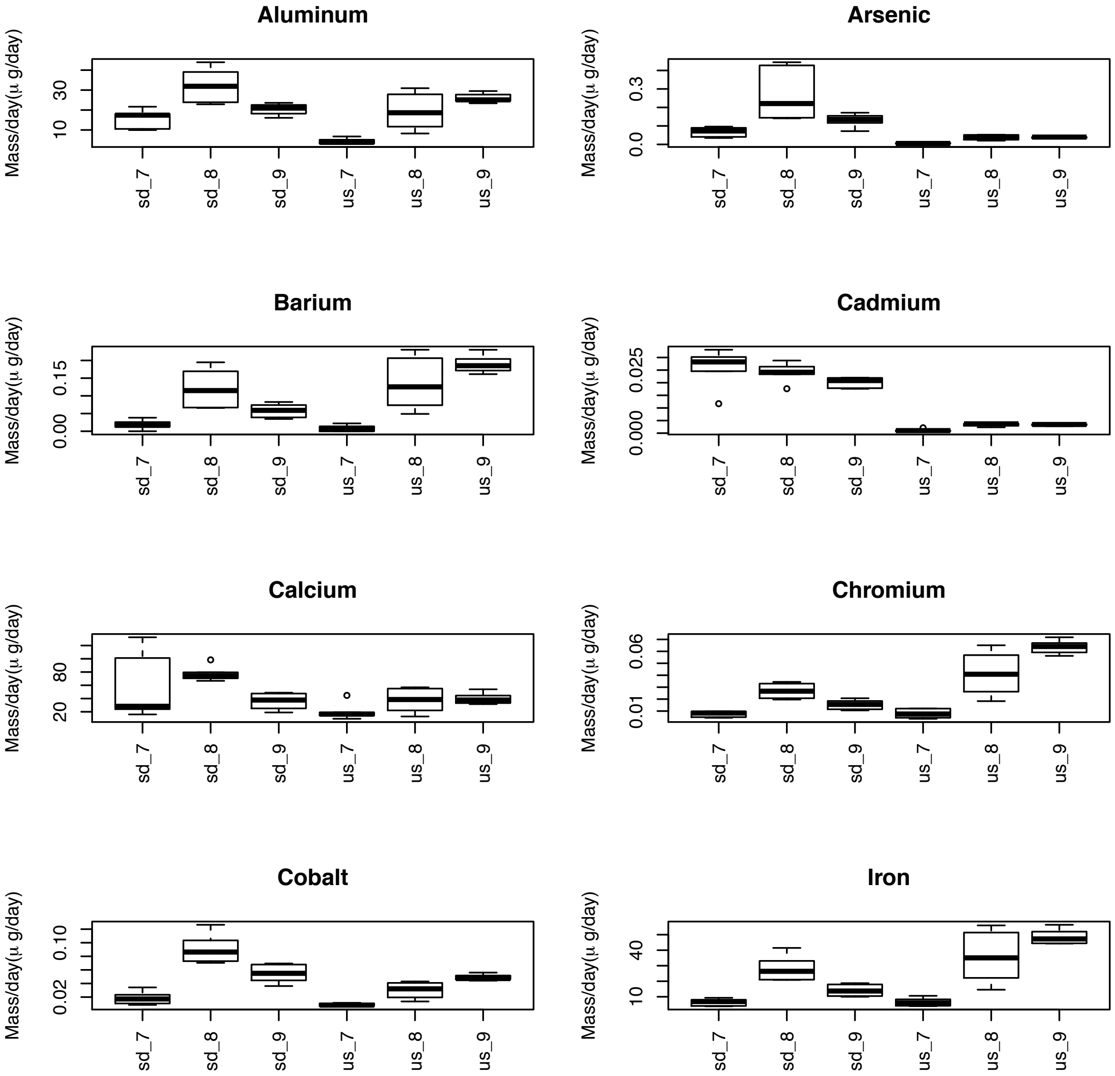


Figure 16. Metals accumulated on SLMDs deployed at approximately monthly intervals in Silverdaisy Creek (n=18) and the Upper Skagit River (n=18), June through September, 2015. Labels show the site ("sd"= Silverdaisy Creek and "us" = Upper Skagit River) where the SLMDs were deployed and the month ("7" = July, "8" = August and "9" = September) the SLMDs were retrieved.

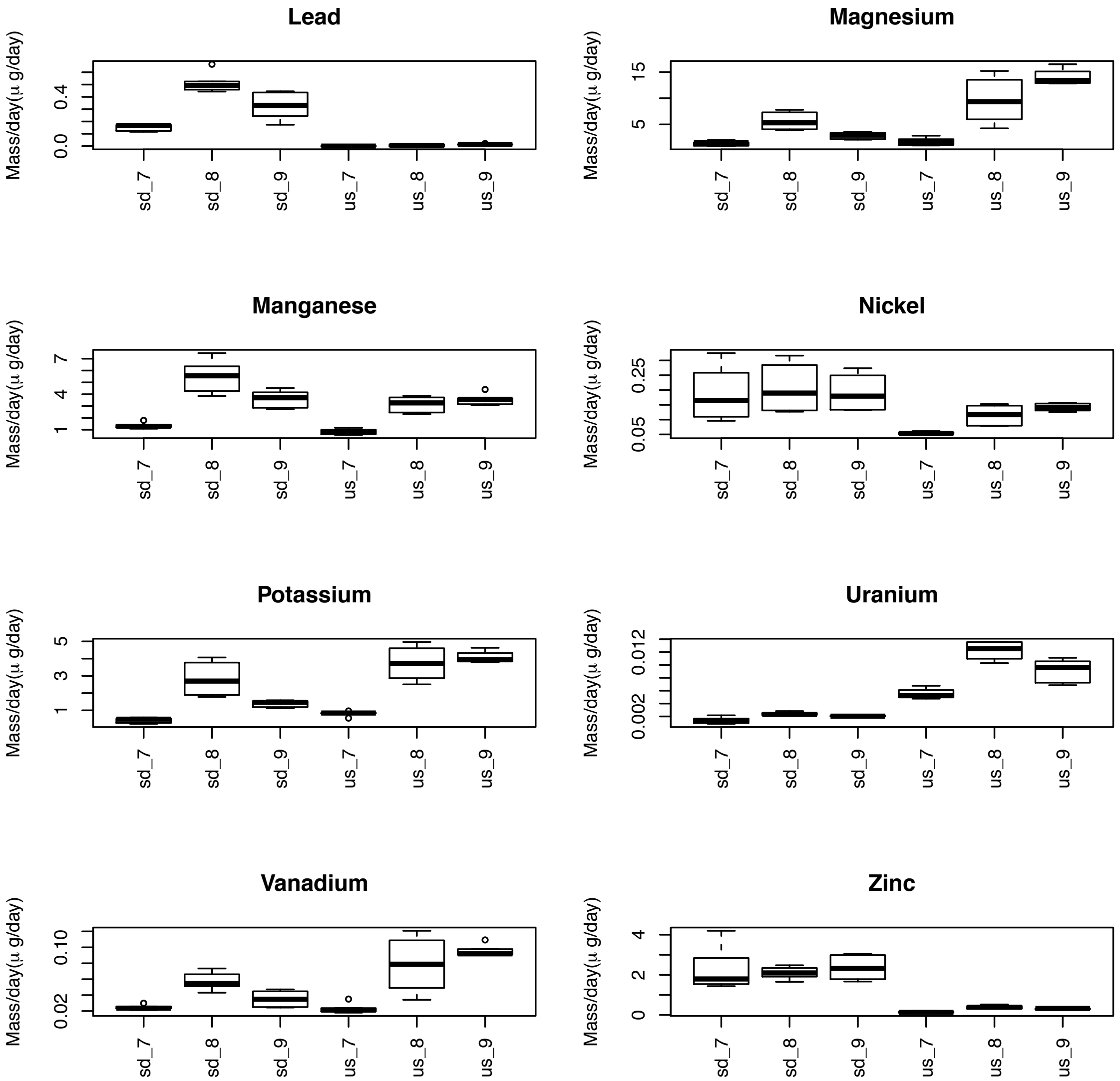


Figure 16. Continued.

Periphyton

Five metals were present in greater quantities in the Silverdaisy Creek periphyton compared to the Upper Skagit River periphyton. These five metals were arsenic, cadmium, lead, selenium and zinc (Figure 18). Four of these metals—arsenic, cadmium, lead, and zinc—also accumulated in greater quantities on the SLMDs deployed in Silverdaisy Creek than those in the Upper Skagit River (Figure 18).

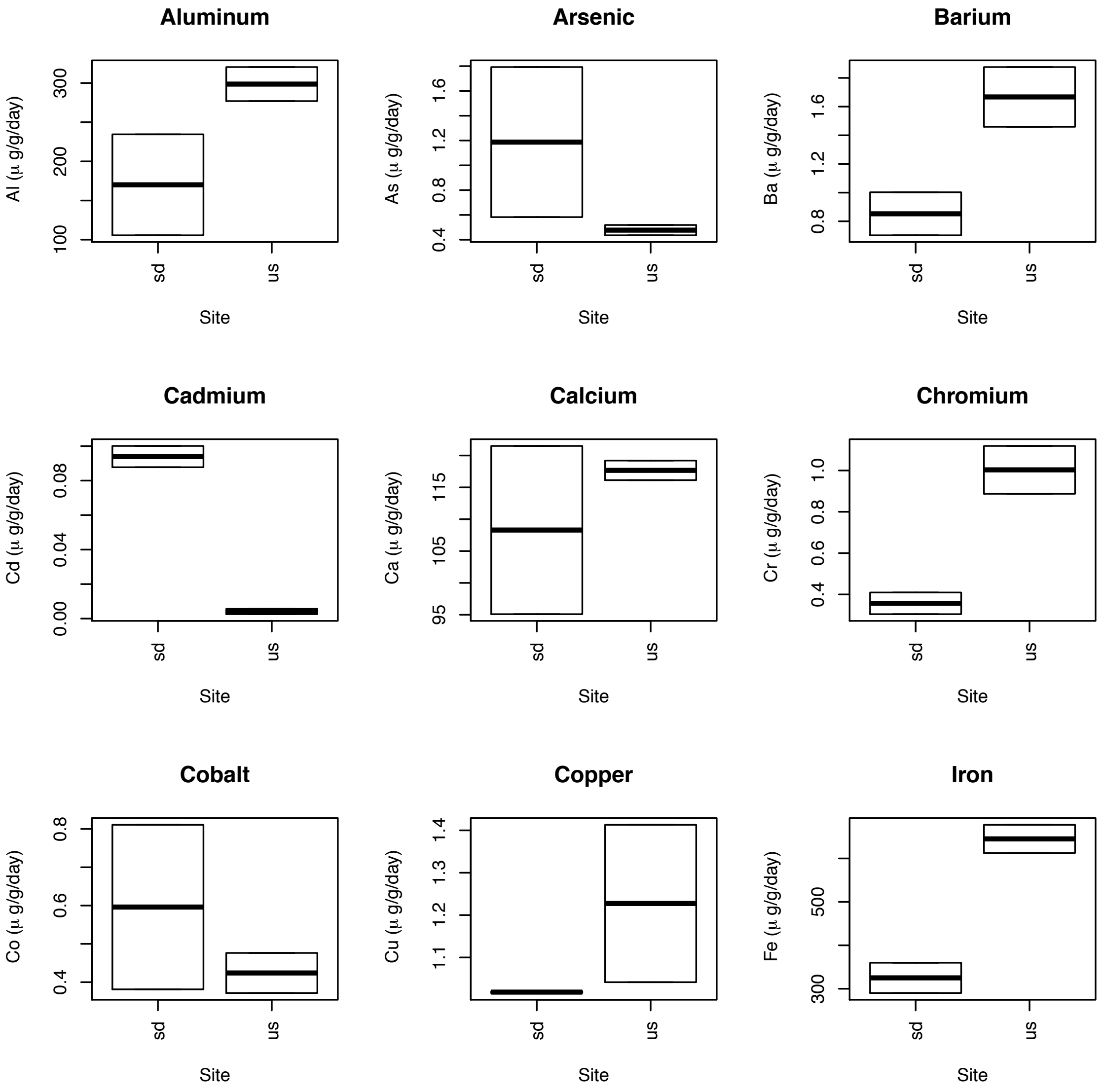


Figure 17. Metals accumulated in periphyton collected from Silverdaisy Creek (n=2) and the Upper Skagit River (n=2). Two periphyton samplers per site were deployed from June through September, 2015 allowing for periphyton colonization and metal accumulation. Labels indicate the site (“sd”= Silverdaisy Creek and “us” = Upper Skagit River) where the periphyton samplers were deployed.

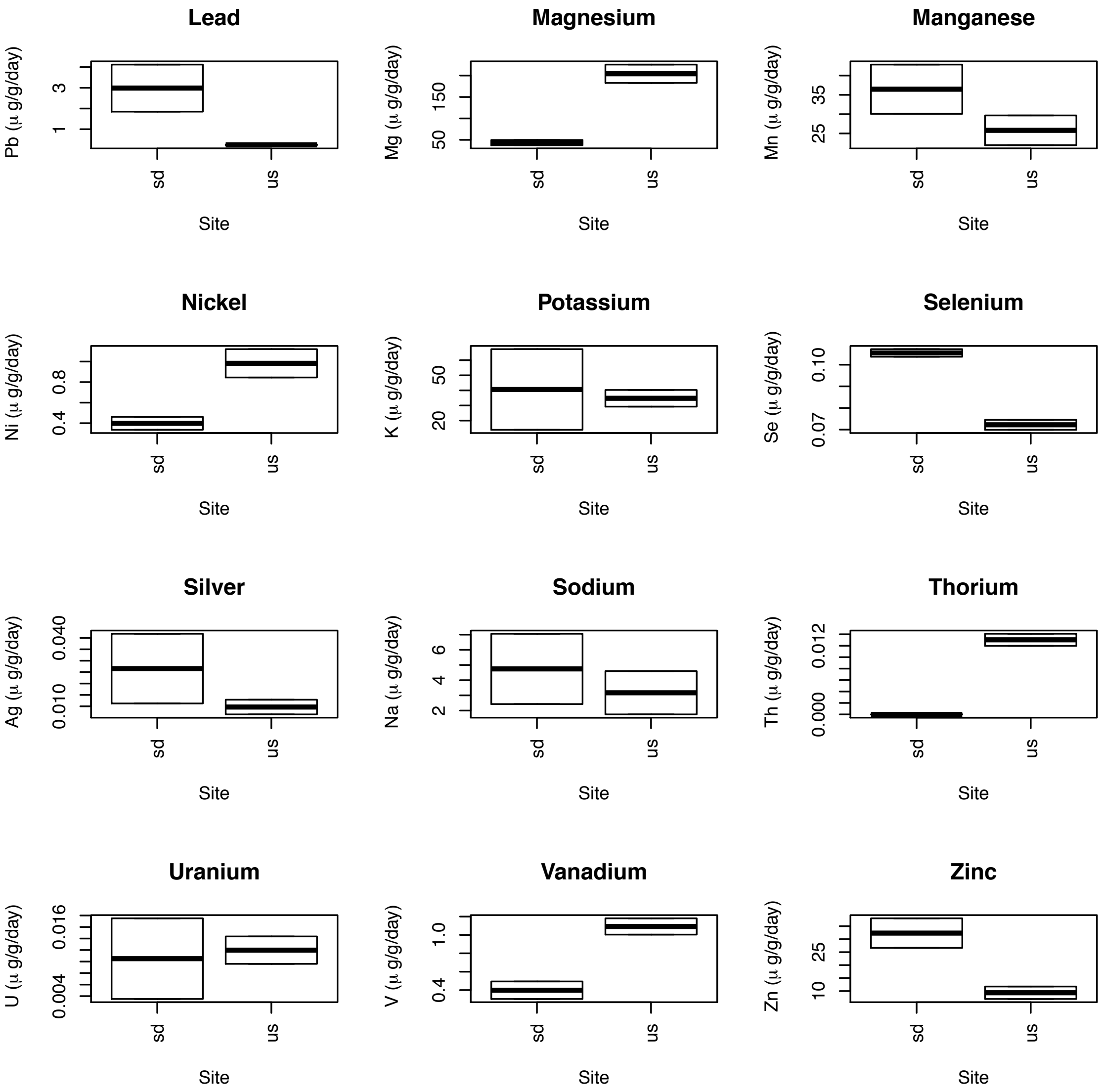


Figure 17. Continued.

3. Quality Assurance and Quality Control

Several quality assurance and quality control techniques were used to ensure that samples that were analyzed for metal content were precise and accurate, samples were representative of site conditions, and samples were not contaminated from external sources. Two sampling stations were established per sampling site, which SLMD and periphyton samplers were deployed on, to account for habitat variability. To quantify contamination sources in the field for water sampling techniques, I used field blanks for grab samples (NanoPure water) and SLMDs (SLMDs that were transported to sampling sites but not deployed). I checked for contamination sources in the laboratory for water sampling techniques using laboratory blanks for grab samples (NanoPure water) and SLMDs (SLMDs that were stored in the laboratory and underwent the same extraction process as the deployed SLMDs). To determine if metal quantities in periphyton subsamples that were acid digested were representative of the metal quantities in the entire periphyton sample collected, I generated laboratory replicates. Thus, I homogenized and divided periphyton samples with large enough masses to be split into two or three subsamples, and acid digested each separately and analyzed them for metals. In addition, I generated replicate solutions with SLMD extraction solutions and periphyton digestion solutions to assess how representative the aliquots were of the metal content in the SLMD extraction solutions and periphyton digestion solutions; also, assuming each aliquot from the same SLMD extraction solution and periphyton digestion solution would produce the same results for metal quantities, this was a measure of the variability with the instrumentation used for analysis. Finally, I used a standard reference material with a known metal content to check for contamination sources in the periphyton drying and acid digesting process.

Field Blanks and Laboratory Blanks:

No contamination in grab sample field (n=14) or laboratory blanks (n=4) was detected. None of the total or dissolved grab samples had concentrations of any metal above the detection level of the ICP-MS (Table 19 and Table 20).

Approximately two out of thirteen SLMD field blanks per metal had concentrations above the detection level, indicating there may have be a potential source of contamination in the field on two sampling days (Table 21). Laboratory blanks indicate that the SLMDs had background levels of aluminum, zinc, calcium, manganese and chromium (Table 22).

Table 17. Total (n=14) and dissolved (n=14) metal concentrations in grab sample field blanks and the detection level for each metal from the ICP-MS. Field blanks were collected during site visits from an acid washed polypropylene sample bottle filled with NanoPure water. NanoPure water was collected from the sample bottle using the same equipment and techniques as the surface water grab samples.

Sample	Type	Metal	Total Metal Concentrations (µg/L)	Dissolved Metal Concentrations (µg/L)	Detection level (µg/L)
Grab Samples	**Field Blanks**				
		Aluminum	<dl*	<dl*	1.36
		Antimony	<dl*	<dl*	0.023
		Arsenic	<dl*	<dl*	0.033
		Barium	<dl*	<dl*	0.24
		Beryllium	<dl*	<dl*	0.048
		Cadmium	<dl*	<dl*	0.041
		Calcium	<dl*	<dl*	5.21
		Chromium	<dl*	<dl*	0.019
		Cobalt	<dl*	<dl*	0.018
		Copper	<dl*	<dl*	0.13
		Iron	<dl*	<dl*	1.46
		Lead	<dl*	<dl*	0.050
		Manganese	<dl*	<dl*	0.030
		Magnesium	<dl*	<dl*	1.68
		Molybdenum	<dl*	<dl*	0.15
		Nickel	<dl*	<dl*	0.021
		Potassium	<dl*	<dl*	5.63
		Selenium	<dl*	<dl*	0.50
		Silver	<dl*	<dl*	0.039
		Sodium	<dl*	<dl*	7.77
		Thallium	<dl*	<dl*	0.20
		Thorium	<dl*	<dl*	0.005
		Uranium	<dl*	<dl*	0.003
		Vanadium	<dl*	<dl*	0.013
		Zinc	<dl*	<dl*	0.49

*<dl= below detection level

Table 18. Total (n=3) and dissolved (n=3) metal concentrations in grab sample laboratory blanks and the detection level for each metal from the ICP-MS. Laboratory blanks were collected from an acid washed polypropylene sample bottle filled with NanoPure water. Collection techniques for laboratory blanks were the same as the surface water grab samples.

Sample	Type	Metal	Total Metal Concentrations (µg/L)	Dissolved Metal Concentrations (µg/L)	Detection level (µg/L)
Grab Samples	**Field Blanks**				
		Aluminum	<dl*	<dl*	1.36
		Antimony	<dl*	<dl*	0.023
		Arsenic	<dl*	<dl*	0.033
		Barium	<dl*	<dl*	0.24
		Beryllium	<dl*	<dl*	0.048
		Cadmium	<dl*	<dl*	0.041
		Calcium	<dl*	<dl*	5.21
		Chromium	<dl*	<dl*	0.019
		Cobalt	<dl*	<dl*	0.018
		Copper	<dl*	<dl*	0.13
		Iron	<dl*	<dl*	1.46
		Lead	<dl*	<dl*	0.050
		Manganese	<dl*	<dl*	0.030
		Magnesium	<dl*	<dl*	1.68
		Molybdenum	<dl*	<dl*	0.15
		Nickel	<dl*	<dl*	0.021
		Potassium	<dl*	<dl*	5.63
		Selenium	<dl*	<dl*	0.50
		Silver	<dl*	<dl*	0.039
		Sodium	<dl*	<dl*	7.77
		Thallium	<dl*	<dl*	0.20
		Thorium	<dl*	<dl*	0.005
		Uranium	<dl*	<dl*	0.003
		Vanadium	<dl*	<dl*	0.013
		Zinc	<dl*	<dl*	0.49

*<dl= below detection level

Table 19. The total number of SLMD field blanks (n=13) which were above the detection level for each metal from the ICP-MS. Field blanks were assembled and stored at WWU, transported to sample sites, placed into scintillation vials during site visits, and transported back to WWU. Field blanks underwent the same extraction and analysis process as all the deployed SLMDs. Due to instrument limitations for the number of samples that can be analyzed in one run, SLMDs were analyzed on two different days and both detection levels are presented

Sample	Type	Metal	Total number of samples >dl*	Detection level[1] (µg/L)	Detection level[2] (µg/L)
SLMDs	**Field Blanks**				
		Aluminum	11	1.08	0.91
		Antimony	2	0.023	0.026
		Arsenic	2	0.052	0.019
		Barium	4	0.42	0.28
		Beryllium	2	0.075	0.11
		Cadmium	2	0.036	0.030
		Calcium	2	2.29	7.94
		Chromium	5	0.021	0.033
		Cobalt	2	0.014	0.005
		Copper	1	0.16	0.15
		Iron	5	1.20	1.01
		Lead	1	0.13	0.069
		Manganese	10	0.060	0.020
		Magnesium	2	1.16	1.03
		Molybdenum	3	0.046	0.021
		Nickel	2	0.057	0.020
		Potassium	5	2.12	7.34
		Selenium	2	1.11	0.69
		Silver	3	0.013	0.035
		Sodium	1	5.34	7.04
		Thallium	2	0.27	0.007
		Thorium	2	0.005	0.007
		Uranium	2	0.005	0.005
		Vanadium	3	0.012	0.009
		Zinc	9	0.71	0.015

*>dl: below detection level

[1] Detection level for samples analyzed on November 12th, 2015

[2] Detection level for samples analyzed on November 13th, 2015

Table 20. The number of SLMD lab blanks (n=12) which were above the detection level for each metal from the ICP-MS. Lab blanks were assembled and stored at WWU and underwent the same extraction and analysis process as all the deployed SLMDs. All lab blanks were analyzed on November 13th, 2015, so the detection level for each metal during that run is presented.

Sample	Type	Metal	Number of samples above detection level	Detection level (µg/L)
SLMDs	**Field Blanks**			
		Aluminum	12	0.91
		Antimony	1	0.026
		Arsenic	0	0.019
		Barium	0	0.28
		Beryllium	0	0.11
		Cadmium	0	0.030
		Calcium	3	7.94
		Chromium	4	0.033
		Cobalt	0	0.005
		Copper	0	0.15
		Iron	4	1.01
		Lead	0	0.069
		Manganese	5	0.020
		Magnesium	1	1.03
		Molybdenum	0	0.021
		Nickel	0	0.020
		Potassium	0	7.34
		Selenium	1	0.69
		Silver	3	0.035
		Sodium	1	7.04
		Thallium	0	0.007
		Thorium	0	0.007
		Uranium	0	0.005
		Vanadium	3	0.009
		Zinc	12	0.015

SLMD extraction solution replicates and periphyton digestions solution replicates

Based on relative percent differences lower than 15%, replicate SLMD extraction solution samples were in agreement for metal quantities for most of the metals analyzed. Nineteen or more of 23 SLMD extraction solution replicate samples had relative percent differences below 15% for aluminum, arsenic, barium, calcium, cobalt, iron, lead, magnesium, manganese, nickel, uranium, vanadium and zinc (Table 23); this indicates that the aliquot of SLMD extraction solution used to detect the quantity of metals accumulated on SLMDs was precise for these metals (Table 23). Cadmium, chromium and potassium all had eight or more replicates with relative percent differences greater than 15% (Table 23). This could have resulted from metal quantities being close to the detection level of the ICP-MS thereby decreasing the accuracy of the results, or the aliquots of SLMD extraction solution were not representative of the metal content in the entire composite sample for cadmium, chromium and potassium.

Metal quantities detected in periphyton replicates show that the periphyton subsamples that were acid digested to determine metal content were representative of the metal content in the entire dried periphyton sample. Five or more periphyton replicates had low relative percent differences (<15%) for cadmium, calcium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, and zinc (Table 24). Two replicates, b10 and b11, had high relative percent differences (>15%) for 16 of the 21 metals analyzed indicating that these samples may not have been mixed well before subsamples were obtained for acid digestion and metals analysis (Table 24). Overall, periphyton subsamples appear to be representative of the metal quantities in the entire dried periphyton sample.

Replicate samples from the same periphyton digestion solution showed that the periphyton digestion solutions were well mixed prior to obtaining an aliquot for analysis and there was minimal contamination. None of the periphyton digestion solution replicates had relative percent differences greater than 15% for 12 of the 21 metals analyzed (Table 25). Five of six periphyton digestion solution replicates exceeded the 15% relative percent difference criteria for sodium (Table 25). For the remaining eight metals, less than three of the periphyton digestion solution replicates had a relative percent difference above 15% (Table 25). Although precautions to prevent contamination were taken, sodium is ubiquitous and contamination could have resulted from bare hands touching vials or lids.

Analysis of the Apple Leaves Standard reference material showed greater than 15% deviation for all detectable metals except zinc (Table 26). These results indicate that there may have been sources of other metals that could have contaminated the reference material during the drying, acid digesting, or instrumental analysis process. However, periphyton replicates and replicates of periphyton digestion solutions indicate little contamination so this is unlikely. Because concentrations from the ICP-MS become less reliable the closer a concentration is to the detection level of a particular metal, these results could be explained by low metal concentrations in the reference material that were near the detection level of the ICP-MS thus producing less accurate measurements of metals.

Table 21. Mean mass of metals and relative percent differences (rpd) from SLMD extraction solution replicates (n=2). Two replicates were generated from 23 composited SLMD extraction solutions. To generate replicates, two separate aliquots of SLMD extraction solutions were diluted with NanoPure water and analyzed by the ICP-MS for metals. Relative percent differences greater than 15% are bolded and underlined.

	Aluminum		**Arsenic**		**Barium**		**Calcium**		**Cadmium**		**Chromium**		**Cobalt**		**Iron**	
Sample ID	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)
33	186.91	6.07	0.29	11.60	5.99	10.87	106.88	8.85	0.24	3.36	0.38	13.03	0.32	**16.63**	207.72	11.14
112	209.55	7.50	0.45	12.23	1.38	5.33	50.67	12.46	0.36	6.86	0.32	**27.82**	0.26	0.52	275.14	2.32
203	371.30	0.29	1.40	2.20	1.53	4.06	590.45	4.27	0.11	**19.77**	0.60	1.57	0.38	4.23	570.81	3.24
213	143.69	3.02	0.42	9.35	0.24	2.72	776.59	0.59	0.02	**23.71**	0.23	8.44	0.14	1.71	188.71	0.30
242	430.74	5.73	1.64	0.38	0.94	4.63	515.88	1.60	0.07	9.15	1.13	4.37	0.50	1.22	608.38	1.99
291	780.51	4.77	3.84	4.32	5.69	3.50	1298.43	3.42	0.16	14.41	0.87	3.14	0.40	5.16	1026.45	3.76
293	870.27	6.35	4.65	6.84	6.74	6.59	2337.10	7.16	0.42	1.44	1.03	5.77	0.34	2.56	1188.29	5.07
321	1404.04	5.42	4.99	6.65	3.50	6.11	761.88	3.92	0.39	10.00	5.09	2.72	1.64	5.77	2021.95	3.36
403	153.34	6.91	0.27	5.49	0.42	**34.80**	334.84	5.09	0.09	**29.99**	0.38	**17.42**	0.38	4.00	275.55	8.31
432	339.90	1.91	1.35	6.76	1.33	1.28	4524.01	1.66	0.92	11.11	0.18	6.74	0.29	5.09	143.88	5.49
433	348.59	8.21	1.12	**15.17**	0.00	0.00	786.55	10.29	1.01	5.05	0.14	**21.83**	0.35	9.08	131.46	2.36
441	113.00	4.86	0.28	9.73	0.00	0.00	116.44	9.48	0.11	**25.38**	0.16	**18.88**	0.31	11.07	157.57	4.28
491	337.48	2.31	0.65	6.65	0.67	0.87	575.96	3.50	0.00	0.00	0.91	**35.00**	0.41	6.76	531.68	4.05
582	107.79	3.42	0.35	1.73	0.37	**22.75**	255.17	0.82	0.02	**29.71**	0.33	**33.98**	0.13	5.36	125.30	5.75
613	129.11	1.11	0.16	2.55	0.16	**23.35**	861.15	2.08	0.00	0.00	0.10	8.07	0.05	7.63	102.82	0.98
671	1026.03	0.39	1.78	1.00	2.36	11.53	1306.38	4.88	0.14	0.43	3.51	1.66	0.97	0.73	1449.98	1.21
743	893.97	3.86	6.36	9.10	3.70	13.49	1469.15	11.95	0.53	5.49	0.70	4.96	2.31	9.26	719.32	0.94
841	1130.26	8.56	1.76	4.11	8.14	7.23	1283.47	6.22	0.13	15.36	2.80	11.71	2.05	0.28	2172.94	9.78
842	926.77	14.96	1.47	0.12	6.45	4.35	1218.80	0.48	0.11	**29.00**	2.50	**18.43**	1.73	4.34	1763.89	**15.83**
901	161.14	11.19	0.76	5.68	0.16	**40.27**	168.58	1.27	0.01	**20.02**	0.21	**26.45**	0.18	7.26	223.65	**19.41**
921	48.25	2.56	0.34	8.82	0.00	0.00	66.27	2.84	0.31	11.20	0.08	**28.14**	0.13	7.39	45.82	10.03
983	486.11	1.66	2.94	7.54	3.75	13.60	324.60	5.47	0.32	17.65	0.58	4.28	0.27	8.87	660.80	0.13
993	145.35	5.52	0.39	3.75	0.62	10.89	290.17	0.18	0.02	**149.63**	0.12	3.41	0.06	0.11	129.79	13.61

Table 21. Continued.

	Lead		Magnesium		Manganese		Nickel		Potassium		Uranium		Vanadium		Zinc	
Sample ID	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)	Mean (µg/d)	rpd (%)
33	0.00	0.00	71.04	14.02	13.09	11.65	1.52	14.02	25.10	**33.99**	1.27	11.21	0.62	18.65	83.62	9.69
112	0.29	3.91	66.69	0.33	26.59	3.11	0.98	2.72	27.76	2.73	1.53	2.56	0.58	5.90	1461.42	2.52
203	0.13	1.27	169.40	3.81	18.21	2.96	2.01	6.05	46.17	0.76	0.15	3.20	1.26	3.03	25.15	10.60
213	0.00	0.00	62.65	1.22	7.01	0.78	0.94	3.50	15.78	12.42	0.09	6.36	0.52	3.59	8.40	8.50
242	0.55	1.01	216.12	0.06	37.89	1.18	2.12	1.55	43.38	4.61	0.16	0.49	1.48	3.08	14.05	1.11
291	1.78	3.20	269.08	4.47	32.84	4.68	0.75	4.05	107.95	5.07	0.56	6.00	2.24	6.14	25.32	6.55
293	1.92	7.68	323.37	6.66	35.13	5.12	1.00	8.05	162.68	11.81	0.51	3.52	2.48	5.46	41.50	3.85
321	1.05	1.32	825.04	2.21	56.62	3.34	6.58	2.74	255.48	5.19	0.27	4.86	6.10	4.18	42.45	1.77
403	0.00	0.00	75.10	8.96	40.93	4.36	2.05	8.44	30.50	7.77	0.24	6.44	0.65	10.16	7.00	2.58
432	4.22	2.02	30.47	7.95	41.45	0.98	3.29	0.74	7.47	0.52	0.03	4.44	1.04	1.14	52.05	3.43
433	3.84	8.15	28.08	9.53	35.93	7.99	3.68	5.24	7.61	**40.93**	0.03	8.22	0.78	8.21	59.86	6.55
441	0.00	0.00	36.90	7.00	17.96	4.38	0.86	10.25	30.52	1.51	0.88	5.64	0.32	9.79	340.59	4.78
491	0.00	0.00	161.50	0.55	18.03	3.52	1.89	**16.64**	28.45	5.13	0.10	11.30	1.31	1.61	3.26	8.10
582	0.00	0.00	46.61	0.51	9.78	5.08	1.22	0.23	23.97	**62.86**	0.05	4.33	0.37	1.22	4.61	6.39
613	0.00	0.00	25.53	6.16	6.86	1.81	0.08	4.12	17.52	**69.33**	0.15	5.19	0.38	0.71	1.51	4.34
671	0.14	**196.40**	602.46	4.57	30.75	1.22	4.71	2.76	123.76	**18.33**	0.10	5.69	4.31	1.44	11.91	3.92
743	11.83	4.35	148.49	2.95	135.85	10.24	6.80	**17.02**	71.01	**27.62**	0.06	1.14	1.32	0.30	49.17	12.13
841	0.90	**27.97**	629.52	7.83	157.90	4.34	5.57	4.48	159.02	13.48	0.35	7.50	4.15	6.96	12.43	5.94
842	0.66	**39.76**	507.08	14.70	134.40	1.31	4.95	6.22	134.93	8.55	0.39	0.37	3.56	12.14	10.37	7.54
901	0.00	0.00	65.37	11.31	8.95	9.66	1.19	2.59	36.04	**32.82**	0.12	3.14	0.51	10.63	4.06	2.67
921	0.00	0.00	27.83	1.09	3.82	3.46	2.68	2.79	51.91	**18.64**	0.05	9.91	0.27	7.30	58.47	0.32
983	0.76	**21.58**	185.23	5.78	22.89	5.31	0.62	4.42	88.26	**25.51**	0.19	13.77	1.34	3.71	28.10	8.29
993	0.00	0.00	39.14	4.49	16.49	3.56	0.12	**17.77**	43.60	**17.41**	0.14	0.09	0.32	6.43	3.27	0.77

Table 22. Mean mass and relative percent differences (rpd) of accumulated metals per gram of periphyton per days of deployment in periphyton replicate samples. Replicate periphyton samples (n=2, except b11 n=3) were generated from dried periphyon samples that had enough dry mass to be divided into two or three subsamples (0.25-0.5 g each). Each subsample was acid digested separately to form periphyton digestion solutions, which were diluted with NanoPure and analyzed by the ICP-MS for metals. Relative percent differences greater than 15% are bolded and underlined.

	Aluminum		**Arsenic**		**Barium**		**Cadmium**		**Calcium**		**Chromium**		**Cobalt**	
Sample ID	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)
b2	124.11	9.75	0.51	1.42	0.80	9.86	0.01	**34.69**	87.04	1.09	0.49	4.17	0.10	2.87
b10	71.79	**134.32**	0.08	**145.42**	0.30	**128.65**	0.00	0.00	54.71	**108.42**	0.32	**159.45**	0.07	**156.02**
b11	114.69	**21.95**	0.17	**16.61**	0.79	**63.54**	0.00	**300.00**	56.70	14.09	0.25	**32.30**	0.09	**34.87**
b14	80.95	1.72	0.20	1.72	0.80	14.60	0.00	0.00	124.27	**24.46**	0.43	**18.53**	0.04	5.17
b15	129.88	1.62	0.23	5.28	0.60	10.29	0.00	0.00	63.01	7.04	0.44	5.95	0.10	4.27
b16	169.91	6.07	0.45	**33.12**	0.54	12.92	0.00	0.00	95.22	12.20	0.74	5.22	0.14	11.94
b25	161.89	**16.92**	0.78	12.56	0.88	**16.87**	0.01	14.62	93.33	5.38	0.45	14.92	0.14	11.05

Table 22. Continued

	Copper		**Iron**		**Lead**		**Magnesium**		**Manganese**		**Nickel**		**Potassium**	
Sample ID	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)
b2	1.18	3.32	372.23	2.39	0.15	3.52	52.75	4.95	6.57	3.10	0.25	0.00	48.04	0.01
b10	0.68	**155.75**	150.34	**148.49**	0.04	**152.50**	35.28	**150.59**	3.68	**131.60**	0.18	0.14	9.00	3.18
b11	0.61	**29.72**	207.49	**23.05**	0.15	**63.75**	44.69	**25.70**	7.39	**17.56**	0.26	0.03	35.01	0.18
b14	0.59	7.35	296.24	13.19	0.04	1.05	22.20	12.44	4.69	4.63	0.11	0.00	16.95	0.00
b15	0.91	9.10	278.18	2.06	0.12	1.97	58.42	0.60	6.28	2.96	0.32	0.00	19.58	0.00
b16	2.01	2.87	370.55	3.55	0.09	**18.93**	99.68	4.00	5.99	7.11	0.63	0.01	22.06	0.12
b25	1.37	11.58	319.31	11.79	0.33	10.55	71.25	11.50	11.08	9.49	0.31	0.02	40.88	0.09

Table 22. Continued

Sample ID	Selenium		Silver		Sodium		Thorium		Uranium		Vanadium		Zinc	
	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)
b2	0.07	4.40	0.02	10.95	2.73	4.99	0.01	**29.67**	0.01	**23.03**	0.40	8.82	26.01	0.20
b10	0.05	**152.58**	0.00	**125.18**	0.00	0.00	0.00	**190.54**	0.01	**112.38**	0.27	**147.52**	17.91	**142.04**
b11	0.05	**18.84**	0.00	**15.76**	2.15	**41.72**	0.04	**114.57**	0.06	**18.36**	0.37	**24.03**	4.84	**23.26**
b14	0.06	12.17	0.00	4.99	0.32	**200.00**	0.00	**200.00**	0.02	7.91	0.18	7.97	42.30	10.22
b15	0.05	11.52	0.00	3.85	4.79	4.80	0.01	**17.44**	0.02	**18.31**	0.43	1.09	13.20	9.79
b16	0.06	**42.91**	0.00	**21.46**	6.09	14.20	0.00	4.97	0.00	10.27	0.71	5.42	22.69	4.72
b25	0.10	10.20	0.01	**21.53**	3.84	**15.02**	0.00	**76.08**	0.01	12.91	0.50	**17.51**	19.83	6.32

Table 23. Mean mass and relative percent differences (rpd) of accumulated metals per gram of periphyton per days of deployment in periphyton digestion solution replicates (n=3). Periphyton digestion solution replicates were generated using three separate aliquots of periphyton digestion solutions which were each diluted with NanoPure and analyzed separately on the ICP-MS for metals. Relative percent differences greater than 15% are bolded and underlined.

	Aluminum		**Arsenic**		**Barium**		**Cadmium**		**Calcium**		**Chromium**		**Cobalt**	
Sample ID	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)
b1	289.51	4.84	0.63	10.10	0.50	14.05	0.02	236.56	85.18	8.26	1.30	5.41	0.25	**20.79**
b5	235.64	1.13	1.78	3.15	0.94	11.07	0.10	2.36	111.45	13.91	0.30	2.42	0.80	2.08
b15	131.07	3.28	0.23	3.78	0.59	10.02	0.00	0.00	63.40	5.02	0.43	3.53	0.10	2.09
b17	165.69	7.94	0.74	1.63	1.31	4.96	0.00	0.00	75.83	8.80	0.29	2.22	0.07	1.50
b18	171.67	6.29	0.91	4.22	0.82	3.42	0.05	5.98	51.12	4.84	0.61	4.23	0.16	3.21
b28	343.01	9.60	0.55	6.93	2.01	10.12	0.00	0.00	126.11	7.96	1.18	8.38	0.51	9.00

Table 23. Continued

	Copper		**Iron**		**Lead**		**Magnesium**		**Manganese**		**Nickel**		**Potassium**	
Sample ID	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (μg/g/d)	rpd (%)
b1	2.42	4.33	578.65	1.36	0.12	38.60	203.39	1.98	9.28	0.97	0.86	8.66	21.99	**20.02**
b5	1.00	2.95	289.47	1.24	4.13	2.45	49.42	0.98	42.89	1.50	0.46	1.20	13.75	2.10
b15	0.96	1.61	285.25	2.99	0.13	4.66	59.71	3.36	6.49	3.44	0.32	1.47	20.71	4.47
b17	3.18	1.24	315.92	1.36	0.27	2.53	65.72	1.12	5.33	1.82	0.16	0.78	33.70	2.19
b18	3.18	3.05	742.34	4.00	0.66	4.81	71.78	3.33	16.24	3.93	0.60	3.12	18.97	7.05
b28	1.49	7.17	720.51	8.83	0.28	9.81	238.22	7.95	31.45	8.33	1.18	6.69	43.53	11.11

Table 23. Continued

	Selenium		**Silver**		**Sodium**		**Thorium**		**Uranium**		**Vanadium**		**Zinc**	
Sample ID	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)	Metal mass/ periphyton mass/day (µg/g/d)	rpd (%)
b1	0.04	**121.85**	0.02	**235.41**	6.72	**49.10**	0.02	**225.84**	0.02	**204.94**	1.20	5.39	22.63	1.65
b5	0.11	11.06	0.04	6.62	1.96	**38.51**	0.00	0.00	0.02	3.65	0.49	2.36	26.49	2.37
b15	0.06	13.48	0.00	0.00	5.00	5.79	0.02	6.74	0.02	4.42	0.44	2.90	14.01	2.18
b17	0.04	**23.01**	0.00	0.00	3.97	**82.26**	0.01	**20.04**	0.01	3.79	0.49	1.75	16.36	1.12
b18	0.07	5.60	0.03	4.04	1.12	**143.25**	0.01	2.07	0.04	3.34	0.69	4.60	27.49	2.81
b28	0.08	3.69	0.01	4.34	2.71	**60.70**	0.01	**19.36**	0.01	**17.05**	1.25	8.24	12.33	6.87

Table 24. Mass fraction of metals obtained after drying, acid digesting and analyzing Apple Leaves Standard Reference Material (n=2). Observed values were compared to expected values by calculating percent deviation.

Metal	Sample Mass (g)	[µg/L]$_{ICP-MS}$	Detection Limit (µg/L)	Observed Mass Fraction (µg/g)	Expected Mass Fraction (µg/g)	% deviation
Aluminum	0.3259	55.92	0.005	169.72	93.21	82.08
	0.3018	19.51		64.65	86.31	25.01
Arsenic	0.3259	<dl*	0.15	na**	0.012	na**
	0.3018	<dl*		na**	0.011	na**
Barium	0.3259	14.24	0.19	43.23	15.97	170.70
	0.3018	11.27		37.33	14.79	152.40
Copper	0.3259	0.78	0.097	2.38	1.84	29.35
	0.3018	0.36		1.18	1.70	30.59
Lead	0.3259	<dl*	0.092	na**	0.15	na**
	0.3018	<dl*		na**	0.14	na**
Molybdenum	0.3259	<dl*	0.51	na**	0.031	na**
	0.3018	<dl*		na**	0.028	na**
Manganese	0.3259	19.70	0.042	59.78	17.60	239.66
	0.3018	14.91		49.40	16.30	203.07
Nickel	0.3259	0.15	0.070	0.46	0.30	53.33
	0.3018	<dl*		na**	0.27	na**
Selenium	0.3259	1.20	0.96	3.65	0.016	22,712.50
	0.3018	<dl*		na**	0.015	na**
Vanadium	0.3259	0.056	0.015	0.17	0.085	100.00
	0.3018	<dl*		na**	0.078	na**
Zinc	0.3259	1.14	0.26	3.48	4.07	14.50
	0.3018	<dl*		na**	3.77	na**

*<dl: Below the detection limit

**na= not applicable

Discussion

Spatial and temporal changes in water chemistry were associated with mining activities. The kinds and concentrations of metals in grab samples distinguished grab samples associated with mining activities. Grab samples collected during the placer mining season (July to September) downstream of mining formed a distinct and almost exclusive cluster with three different clustering techniques. This indicates that the patterns within the data, which distinguished the grab sample group linked to mining activities from other groups, were robust because cluster membership was similar despite different approaches (hierarchical, Kmeans, and RIFFLE) to cluster development (Dr. Robin Matthews, Dept. of Environmental Sciences, Western Washington University, personal communication, October 2016).

Although Kmeans and RIFFLE clustering both clustered grab samples associated with mining activities together (“during_mining”), the consistency of the cluster solutions varied between the methods. For grab samples, Kmeans clustering produced more consistent cluster solutions than RIFFLE. Since RIFFLE defaults to using four variables (metals) with the highest PRE scores to generate clusters, more variables may be necessary to generate stable cluster solutions (Robin Matthews, Dept. of Environmental Sciences, personal communication, November 2016). However, the variability of the RIFFLE solutions could be attributed to different cluster development strategies for RIFFLE compared to Kmeans. Since RIFFLE is not a variance-based clustering technique and instead uses ranks and medians to develop clusters, it can discover patterns in the data using variables with small variances, like trace metals. However, if several patterns exist in the data, many significant cluster solutions may result (Matthews and Hearne 1991).

Metals in grab samples that distinguished areas of active mining through clustering have been associated with mining contamination in other areas. Metals that consistently contributed to the greatest cluster separation with Kmeans clustering and did not exhibit a watershed-wide seasonal trend were cobalt, chromium, and manganese. Recent studies have also associated elevated concentrations of cobalt, chromium and manganese in stream water to contamination from hard rock mines and waste piles. Gray and Eppinger (2011) detected elevated concentrations of cobalt in water downstream of cobalt mines. Similarly, Caruso et al. (2011) found that manganese concentrations in water samples collected downstream of manganese mining were higher than samples collected upstream. Chromium has been detected at elevated concentrations in stream samples collected downstream of a nickel hard rock mine (Gunkel-Grillon et al. 2016).

Clustering of grab samples also suggested the presence of unidentified mining activities. The remoteness of the Ruby Creek watershed made identification of mining locations challenging. The sites that I identified as being influenced by mining were strongly evident in the clustering results; these cluster results also suggest placer mining may be occurring in other areas, specifically above the Upper Canyon site. Samples collected during the placer mining season (July-September) from the Upper Canyon site clustered with the samples collected during the placer mining season downstream of mining. Furthermore, indications of unidentified placer mining activity are also supported by conductivity measurements. Conductivity was higher at all sites downstream of placer mining and the Upper Canyon site, so conductivity could serve as an indicator of placer mining activity. Conductivity, which is a measure of ions such as the dissolved forms of many metals, could increase below placer mining because of sediment disturbance. Placer mining disturbs the sediment which increases subsurface flow which contains higher concentrations of total dissolved solids (Harter 2003; Gilman 2005). In addition, before the placer mining season began (June), I accessed the Upper Canyon sampling site by an unmaintained trail overgrown by vegetation and partially covered by landslide runout. In July I noted active placer mining downstream and that the trail had been cleared. This could be linked to mining activities because the trail has been decommissioned in areas above Canyon and North Fork of Canyon Creeks, and no trail improvements were made by the

Forest Service during that time.

Due to the abundance of hard rock and placer mines throughout the Ruby Creek watershed, I designed my study to capture watershed-scale effects. Spatial and temporal patterns in metal concentrations in grab samples were evident throughout the watershed. Some temporal trends of metal concentrations were consistent at all sampling sites regardless of mining activity; barium, calcium, magnesium, molybdenum, potassium, and sodium increased at all sites, and aluminum decreased at all sites throughout the summer. These patterns appear to be unrelated to mining and could be explained by streamflow changing from surface runoff, as snowmelt ended, to baseflow conditions that are driven by groundwater. Baseflow conditions appeared to begin unusually early in the summer of 2015 because of a record low snow pack (NOAA 2016). The Canyon Creek hydrograph indicates that baseflow conditions started before equipment deployment in early July and persisted throughout the summer, as shown by little daily or three-month variation in water stage and water temperature. Baseflow conditions are maintained by subsurface flow. The typical major constituents within this water source are calcium, magnesium, sodium, and potassium, and trace constituents can include barium and molybdenum (Harter 2003). Thus, increasing concentrations of barium, calcium, magnesium, molybdenum, potassium and sodium could be explained by an increase in the proportion of streamflow contributed by subsurface flow. The highest concentrations of aluminum were found during the months of May and June, when streamflow is mostly driven by surface runoff. The source of this aluminum may be geological or atmospheric (ATSDR 2008).

The most evident utility of SLMDs for my research was that they identified metals that were not detected in grab samples. In my study, 10 metals that have established Aquatic Life Criteria—aluminum, arsenic, cadmium, chromium, copper, iron, lead, nickel, silver, and zinc—had a higher frequency of detection by SLMDs compared to grab samples (EPA 2016). This underscores that grab samples capture water chemistry at one point in time and may miss episodic pulses of metals. However, SLMDs selectively accumulated metals as shown by higher detection of antimony, molybdenum, selenium, and sodium in grab samples compared to SLMDs.

The utility of SLMDs for this research was limited by a lack of understanding of metal uptake kinetics. Prior to this study, there have been three published documents regarding the construction, utility, and analysis of SLMDs (Brumbaugh et al. 2000; Brumbaugh et al. 2008; Marshall and Miller 2012). Currently, sampling rates are not quantifiable because of uncertainty regarding how variability of metal concentrations, temperature, and water velocity affect metal accumulation on SLMDs (Brumbaugh et al. 2000; Dr. William Brumbaugh, personal communication, January 2016). SLMDs deployed downstream of both placer and hard rock mining accumulated less chromium, cobalt, iron, magnesium, manganese, nickel, and vanadium than the other SLMD sample groups. Sites with both placer and hard rock mining activity upstream were located in headwater streams with lower mean stream temperatures (<10°C) compared to the other sites (>10°C). Cooler temperatures may have limited diffusion of the chelating agent to the outer surface of the membranes thus resulting in less metal accumulation on the SLMDs (Brumbaugh et al. 2000; Dr. William Brumbaugh, personal communication, January 2016).

SLMDs deployed below areas of placer mining were distinguishable by the kinds and quantities of metals accumulated. Clustering of SLMDs correctly identified the placer mining SLMD sample group based on metals that have Aquatic Life Criteria (EPA 2016). Despite the limited sample size (n=4), SLMDs downstream of placer mining were assigned to a single cluster, and clusters were distinguished based on metals that have established toxicity criteria for aquatic life: aluminum, cadmium, chromium, nickel, lead, and zinc (EPA 2016).

Other factors may affect metal accumulation on SLMDs besides metal concentrations in surface waters. If metal concentrations in surface waters were the only factor affecting metal uptake, and metal concentrations in the grab samples I collected characterize average metal concentrations in the streams over the month-long deployment of the SLMDs, the SLMDs should have clustered similarly to grab samples. While temperature and water velocity have been hypothesized to affect metal accumulation on SLMDs, my study also showed that SLMDs preferentially sample individual metals as shown by the percentage of samples with detectable metal concentrations for SLMDs compared to grab samples. Preferential sampling may have limited accumulation of metals that distinguished sites and time periods of mining for grab sample clusters, thereby explaining the dissimilarity between grab samples and SLMD clustering results.

My study supports the premise that periphyton can accumulate metals associated with mining activities and be a concentrated source of metals to higher trophic levels including the Rainbow Trout (*O. mykiss*) and Bull Trout (*S. confluentus*) that live in and spawn in the Ruby Creek watershed (Ashley Rawhouser, Aquatic Resources Division, North Cascades National Park, personal communication, October 2014). Periphyton communities collected downstream of hard rock and placer mining were distinguishable from other sites through clustering based on the kinds and quantities of metals accumulated. Since metals associated with mining activities may enter the food web through water, sediment and dietary exposures, these metals can transfer to higher trophic levels (Farag et al. 1994, 1999; Woodward et al. 1994, 1995). In other studies of biotic and abiotic components of mining-contaminated streams, periphyton contained the highest concentrations of arsenic, cadmium, copper, mercury, lead, zinc and antimony compared to other trophic levels (Farag et al. 1998; Dovick et al. 2015). The highest concentrations of arsenic specifically were found in periphyton, and then in tadpoles which feed on periphyton, followed by sediment, benthic macroinvertebrates, fish, and water (Dovick et al. 2015). The highest concentrations of cadmium and zinc were also in periphyton, followed by sediment, benthic macroinvertebrates, and fish (Farag et al. 1998). Additionally, metal concentrations in functional feeding groups of benthic macroinvertebrates, specifically the shredders and the scrapers which feed on periphyton, were greater than in other functional feeding groups, again supporting the premise that periphyton can be a source of metals to higher trophic levels (Farag et al. 1998).

Concentrations of two metals in the Ruby Creek watershed exceeded Aquatic Life Criteria (EPA 2016). Because the freshwater Criterion Continuous Concentration (CCC) for cadmium and lead were exceeded in two different streams, Granite and Slate Creeks, at different times of the summer, the potential for chronic exposures to these and other metals has to be considered. The CCC was established on the basis that aquatic organisms would be adversely impacted if exposed to concentrations higher than the criterion indefinitely (EPA 2016). My sample collection was limited to one grab sample per month, so the likelihood that more frequent collections across a broader range of sites with more focus on specific locations of mining activity would detect additional exceedances seems high. These criteria may have been exceeded at Granite Creek because of two potential sources: runoff from Washington State Route 20 and historical mining activities. Historical mines are present in the Granite Creek watershed, although the current status of mining is unknown (Moen 1969). Also, SR 20 is adjacent to Granite Creek along approximately 24 km of the highway upstream of my sample site, so road runoff containing cadmium from automobile brakes or tires is a potential source (McKenzie et al. 2009). Unlike the Granite Creek site, the Middle Slate site where the other exceedance occurred is remote and downstream of several hard rock mines and placer claims. Although no active placer mining was observed during June, the exceedance could have resulted from mine drainage from hard rock mines upstream of the sampling site.

Without exception, the clear, cold, well-oxygenated streams in the Ruby Creek watershed are highly suited to supporting native trout; however, fish in the Ruby Creek watershed could be affected by waterborne and dietary exposures to metals. Water concentrations that exceed Aquatic Life Criteria indicate that aquatic organisms, including all life stages from eggs to adults of both Bull

Trout and Rainbow Trout, could be adversely impacted if exposed to these concentrations for extended periods. Additionally, chronic exposures through dietary sources such as the metals concentrated in periphyton, and likely in the organisms at higher trophic levels that consume periphyton like benthic macroinvertebrates, could affect developing fry. Reduced growth and survival of juvenile Rainbow Trout was observed after waterborne and dietary exposures to benthic macroinvertebrates contaminated with aluminum, arsenic, cadmium, copper, lead and zinc (Woodward et al. 1994). Additionally, early life stage fish may receive a larger dose of metals than adults because they feed exclusively on small invertebrates, and smaller invertebrates accumulate higher concentrations of metals than larger invertebrates (Farag et al. 1998).

Silverdaisy Creek also contains metals that potentially could be toxic to organisms. Arsenic, cadmium, lead and zinc, accumulated in higher quantities on SLMDs and in periphyton at Silverdaisy Creek compared to the upper Skagit River. Thus, because of its suitable habitat for spawning and rearing in the reach that I sampled, Silverdaisy Creek water and biota may be a source of arsenic, cadmium, lead, silver, and zinc to higher trophic levels such as Bull Trout and Rainbow Trout.

Summary

My study confirmed that metals were present in the water and benthos, and that site-specific and temporal differences can be linked locations of hard rock and placer mining activities. Metal concentrations in surface waters differed between sites upstream and downstream of mining at times depending on when mining was or was not occurring. In the Ruby Creek watershed, metal concentrations in surface waters were high enough to be capable of adversely affecting aquatic organisms. Metals that were present in streams were not always detected in grab samples, but their presence was confirmed by SLMDs and periphyton. Cluster analyses of SLMDs and periphyton from the Ruby Creek watershed each distinguished two different groups of samples, samples collected downstream of placer mining (SLMDs) and samples collected downstream of hard rock mining (periphyton). Lastly, periphyton can be a concentrated source of toxic metals to other aquatic organisms at higher trophic levels through dietary exposures.

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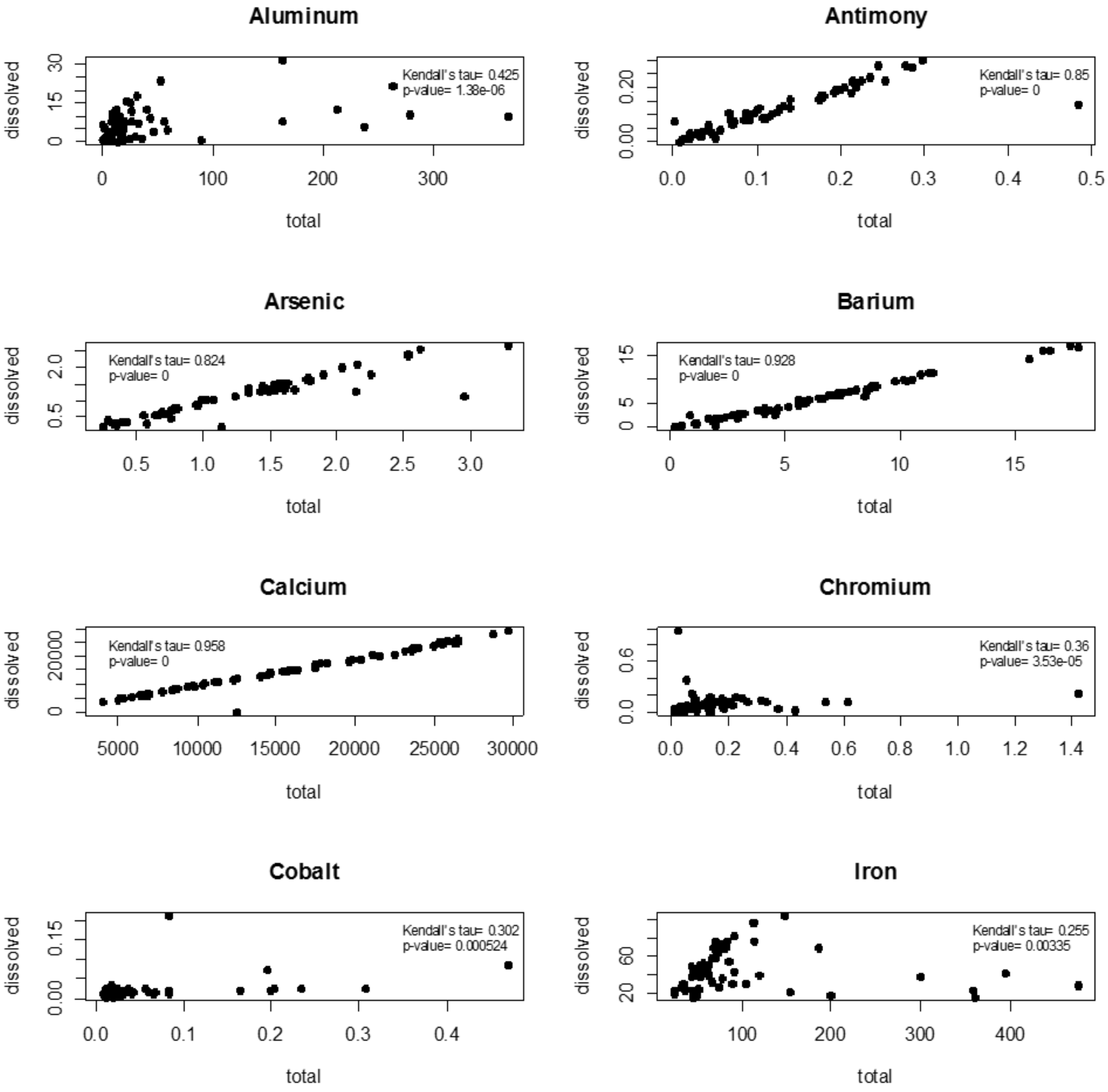
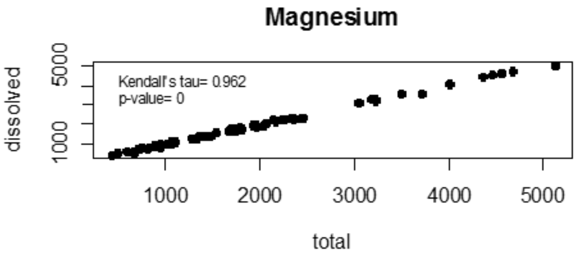
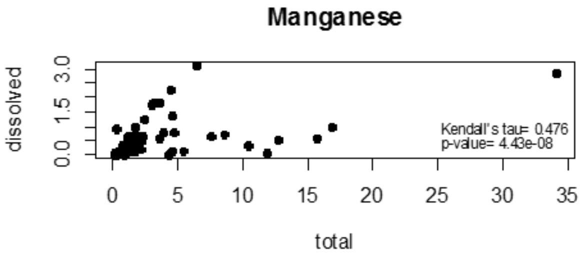
Appendices

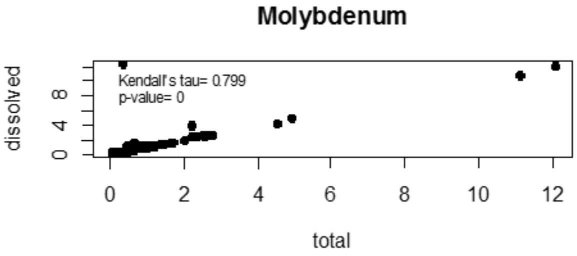
Appendix A. Relation of dissolved metal concentrations to total metal concentrations in surface water grab samples collected in the Ruby Creek watershed from May to September, 2015 (n=54) for the 16 metals that were used for statistical analyses. Correlation coefficients and probability values using Kendall's tau are shown in each plot.

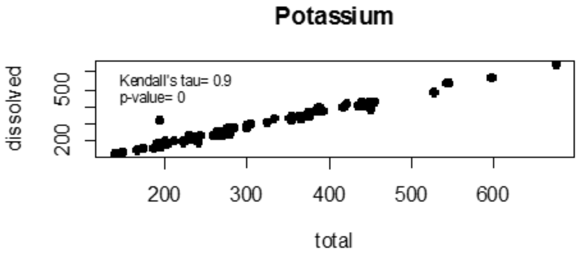


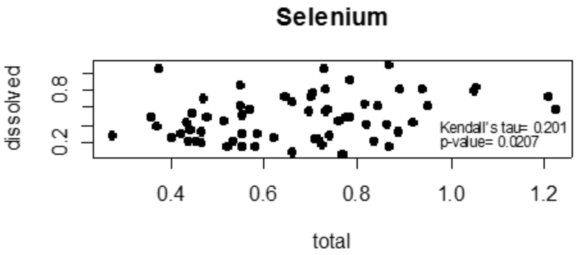
Appendix A. continued

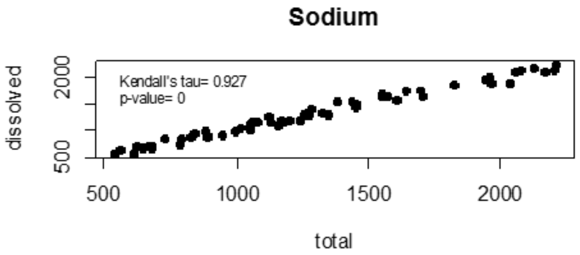


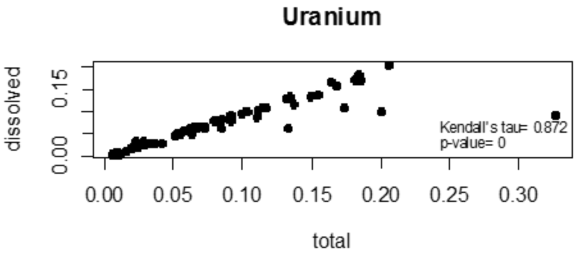


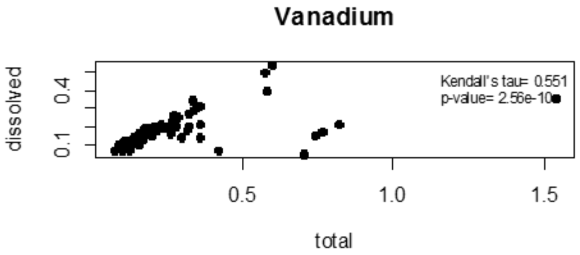




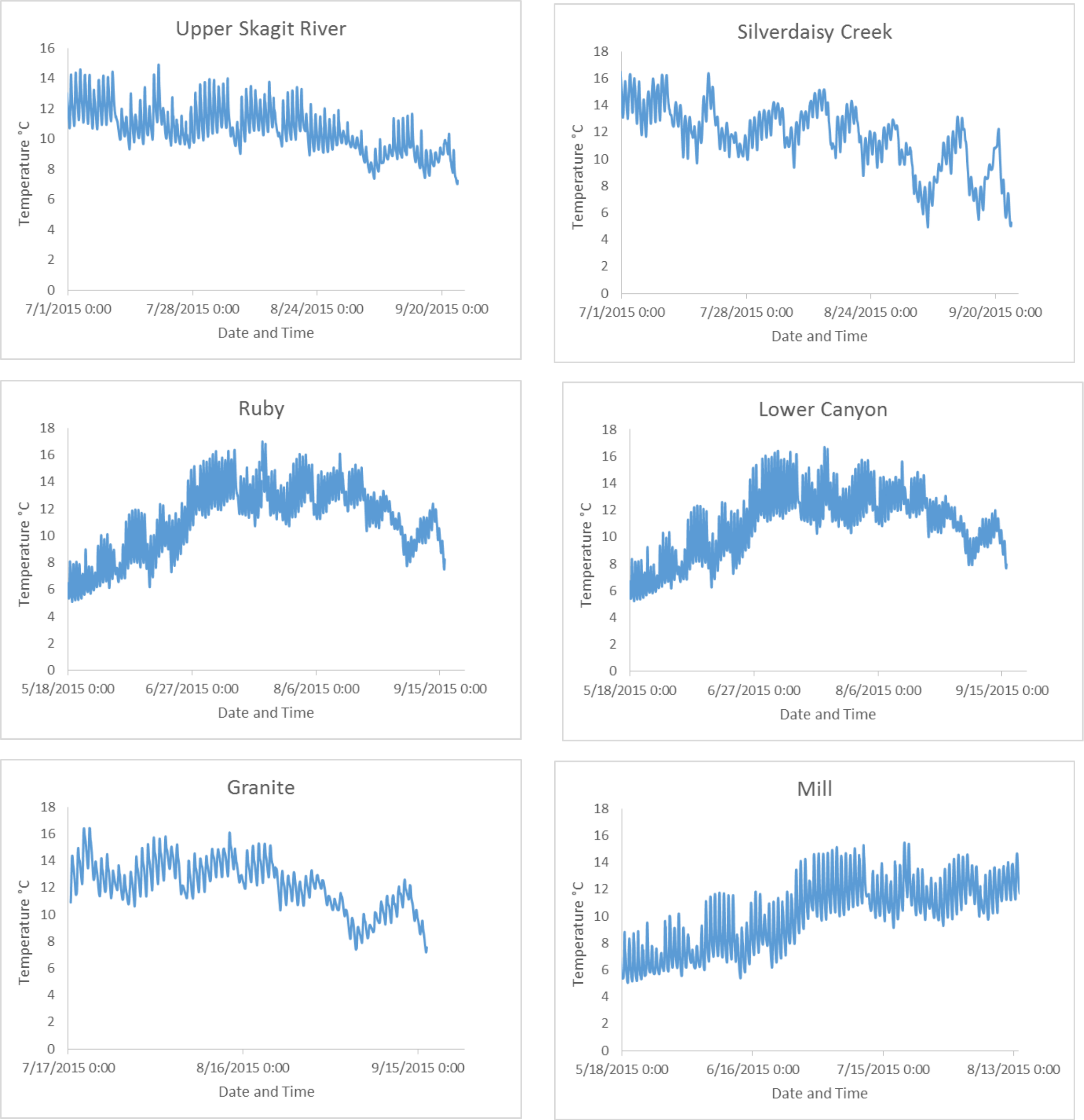




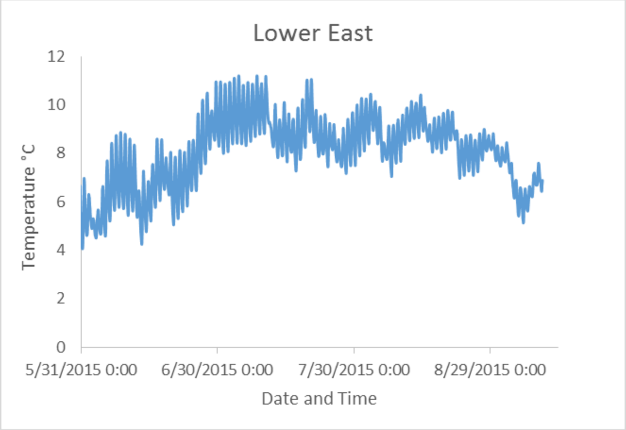


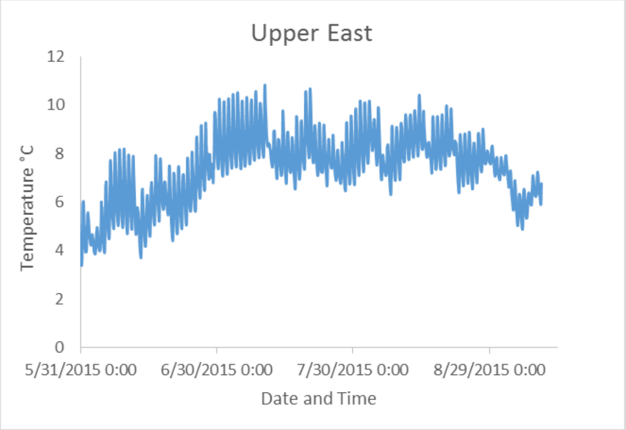


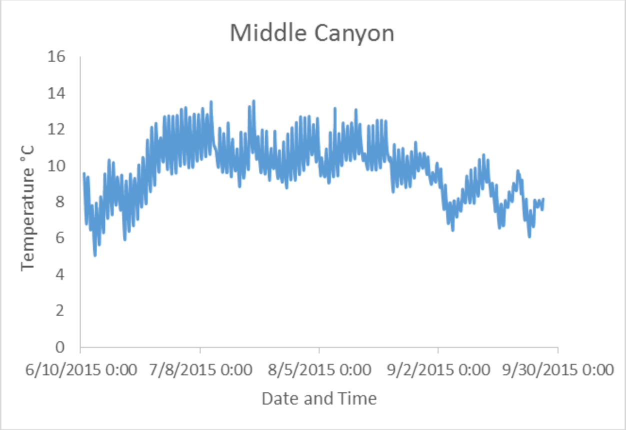
Appendix B. Water temperatures collected at hourly intervals (HOBO Water Temperature Pro v2 datalogger, Onset Computer Corporation) from early summer to early fall, 2015 on the bottom of the stream channel at 14 of 15 study sites in the Ruby Creek and Upper Skagit River watersheds. The Middle Slate temperature logger was removed from the sampling station during the month of July and was not re-deployed.

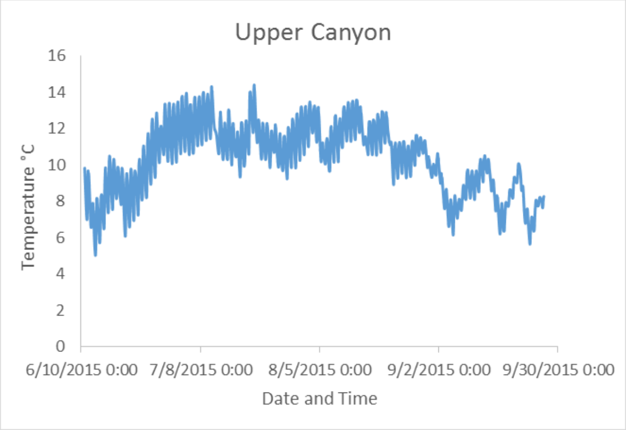


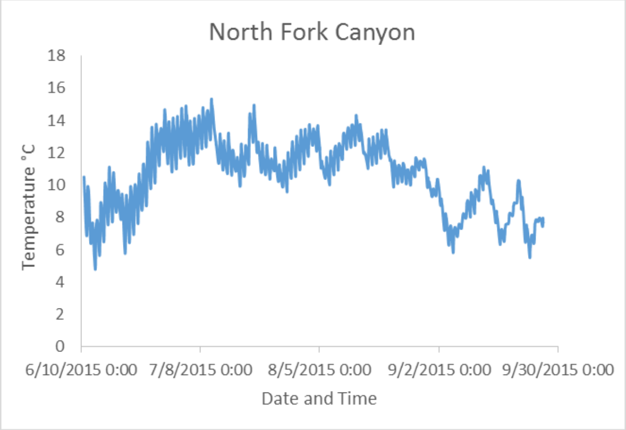
Appendix B. Continued

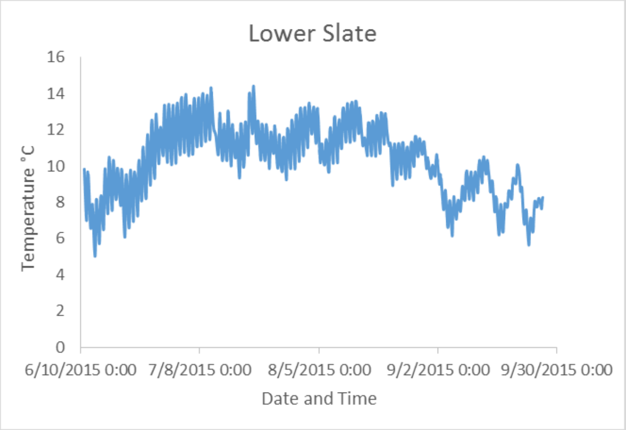












Appendix B. Continued