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Impacts of trace metal pollution, urbanization and aquifer recharge on Spokane River macroinvertebrates

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IMPACTS OF TRACE METAL POLLUTION, URBANIZATION AND AQUIFER RECHARGE ON SPOKANE RIVER MACROINVERTEBRATES

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Abstract

This study analyzed macroinvertebrate communities of the Spokane River to determine: 1) accumulation of toxic trace metals in macroinvertebrate tissue, 2) effects of aquifer recharge, and 3) impact of urbanization. I predicted that aquifer inflows of cold, clean water would mitigate effects of pollution, and that reaches above the City of Spokane would be less negatively affected by urbanization; both were unsupported. I also predicted that effects of toxic trace metals would decrease within distance downstream; this prediction was supported. I sampled 12 riffles of the Spokane River between the Washington/Idaho border and Riverside State Park in late summer 2010. Six riffles were downstream of the City of Spokane (downstream), 3 were within suburban areas upstream of the City of Spokane with aquifer recharge (upstream gaining), and 3 were within suburban areas upstream of the City of Spokane without aquifer inputs (upstream losing). Four macroinvertebrate metrics indicated that the upstream gaining reach had the most anthropogenic degradation, with the highest proportion of dominant taxon ($p=0.01$), and proportion Chironomidae ($p=0.0453$), as well as the lowest proportion Clinger ($p=0.01$) and Shannon Weaver Diversity values ($p=0.001$). The downstream reach had the highest family taxa richness ($p=0.01$), EPT taxa richness ($p=0.001$) and proportion Trichoptera ($p=0.01$). These results supported the hypothesis that metal effects would decrease with distance downstream from the source, the Lake Coeur d'Alene basin, indicating that toxic metal effects are more significant than urbanization effects on Spokane River macroinvertebrates. Patterns of trace metal concentrations in macroinvertebrates tissues in this study are consistent with patterns from 1979-1981 and 1999. Concentrations in the tissues of *Hydropsyche cockerelli* declined with distance downstream of Lake Coeur d'Alene for Cd, Pb and a combined index of Cd, Pb and Zn ($p=0.016$; $p=0.054$; $p=0.0426$). The robust data in this study reflect an effective sampling protocol to address differences between reaches. With a large current investment in restoring the Spokane River, repeating this bioassessment could be an effective tool to address the ecosystem health of the river and effectiveness

of restoration practices, particularly if the number of sampling sites can be expanded in areas of significant degradation.

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Introduction

This study had two purposes: the primary goal was to perform a biological assessment of the Spokane River from the Idaho-Washington border downstream past the City of Spokane. This assessment specifically addressed effects of urban influences, toxic trace metals, and aquifer inputs on macroinvertebrate communities. In addition, I analyzed the concentrations of toxic trace metals in two macroinvertebrate species to determine if accumulations were different in reaches with different pollution profiles.

Bioassessments, rather than physical or chemical assessments, are a powerful device in evaluating the ecosystem health of rivers. Physical disturbance is a natural component of river habitats, and concentrations of chemical contaminants may fluctuate through time as they enter and are exported from the reach by stream flow (Rosenberg and Resh 1992). Biological communities that inhabit a reach must survive and respond to natural and anthropogenic stressors throughout their life cycle, integrating the effects of multiple impacts, including impacts that may not have been previously identified (Rosenberg and Resh 1992). The Clean Water Act (1973) requires monitoring of the ecological integrity of fresh water in the United States to achieve the final goal of the water body being fishable and swimmable; required monitoring includes a biological component, which is usually fulfilled by monitoring of fish or macroinvertebrate communities in the ecosystem in addition to habitat and water quality.

Macroinvertebrates are the most widespread group of organisms used in bioassessment of streams (Barbour et al. 1999). They are ubiquitous in freshwater environments and exist in a diverse range of habitats within a river, but each species has a narrower range of environmental conditions in which it can persist (Merrit et al. 2008). Some macroinvertebrates have multiple life cycles in a season while others live up to three years, so community compositions can be related to temporal changes in the environment (Merrit et al. 2008). Macroinvertebrates must survive conditions present throughout the length of their life cycle, macroinvertebrate bioassessment reflects a longer time scale than chemical analysis of the water column or faster-reproducing taxa such as algae and microbes (Rosenberg and Resh 1992). These communities essentially act as a continuous monitor of the water they inhabit. Macroinvertebrates include the lowest trophic level of animals in the system, so they may be the first to incorporate contaminants that can harm higher tropic levels of animals, including humans (Merrit et al. 2008).

Because organic pollution, mining wastes, and other specific impacts affect macroinvertebrate communities predictably, a number of indices of organic pollution, and metal tolerance, and other specific pollutions types have been developed based on presence or absence of macroinvertebrate families or species (Rosenberg and Resh 1992). The Saprobien System, an indicator organism system, was developed in Europe in the early 1900s and was used to correlate the relative abundance of species to water quality based on known tolerances of organic pollution (Rosenberg and Resh 1992). Pollution tolerance indices are most accurate if applied to regions with similar geomorphology and climate; in North America multiple pollution tolerance indices have been developed (Rosenberg and Resh 1992). The Hilsenhoff Family Biotic Index (HFBI) is the most widely used biotic index in North America (Hilsenhoff 1977 & 1987, Barbour et al. 1999). Because it uses family-level taxonomy, this index can be applied to a larger geographical context than the Midwestern U.S. where it was developed (Hilsenhoff 1977 and 1987; Barbour et al. 1999). General ecological concepts have also been applied to macroinvertebrate biomonitoring. High taxa richness (# species/area), diversity (e.g. Shannon-Weaver diversity index), and evenness (even distribution of individuals among taxa) are thought to reflect intact, highly-functioning ecological communities and are widely used indices (Rosenberg and Resh 1992). The proportion of organisms found in the ecosystem that are tolerant to a certain type of pollution is also an indicator of the ecosystem heath of the river (Iwasakiy et al. 2009).

Macroinvertebrate biological monitoring has been applied to a wide diversity of ecological problems. One example is that macroinvertebrates have been used to assess effects of climate change. In multiple studies, biological indicator metrics related to stream health were unaffected by climate shifts, but presence/absence of indicator species was altered. Daufrense et al. (2007) monitored macroinvertebrate abundances in the French Rhône River for 20 years (1985 -2004). Environmental factors such as an increase in water temperature from 1985 to 2004 due to climate change and an improvement in water quality between 1985 and 1991 had confounding effects on macroinvertebrate community structure. This made it difficult to determine effects more specific than a decrease in the resilience of community structure of macroinvertebrates due to climate change (Daufresne et al. 2007). It was hypothesized that macroinvertebrate communities would be visibly affected by climate change in Mediterranean-climate regions worldwide because these regions are strongly affected by climate change (Lawrence et al. 2010). Effects such as a significant increase in temperature and precipitation were compared to macroinvertebrate abundance data collected over 20 years. The data collected from 4 sites along 2 small streams in Northern California did not reflect changes in California Biotic Integrity Indices due to altered temperature and precipitation. However, the authors developed presence-absence indicator genera for these climactic shifts. Widely used biological metrics, such as percent EPT (Ephemeroptera Plecoptera Trichoptera) and taxa richness, and local indices such as the Californian Benthic Invertebrate Index of Biological Integrity, were still effective in identifying local anthropogenic stressors, despite climactic disruption (Lawrence et al. 2010). In addition to climate, groundwater, urbanization, and heavy metal pollution are other factors that can influence macroinvertebrate communities.

Groundwater Effects on Macroinvertebrate Communities

The chemical and physical attributes of aquifer-derived ground water could have substantial effects on macroinvertebrate communities. Colder water typically supports greater diversity of macroinvertebrates than warmer water; a major reason is the higher solubility of oxygen in colder water. Even though aquifer input initially has low concentrations of O_2, turbulence in riffle environments will quickly bring O_2

concentrations into equilibrium with the atmosphere, while water temperature increases much more slowly (Merritt et al. 2008).

Metabolism, growth, development, and reproduction, quantity and quality of food, are also greatly influenced by temperature (Resh and Rosenberg 1984). Macroinvertebrates are adapted to a wide range of temperatures (<-20 to almost 50 °C) but the majority of species are found at cooler temperatures (0 to 20°C). The widespread adaptation of cool-adapted species could be related to the role of cool streams as the ancestral habitat for many aquatic insect taxa (Merritt et al. 2008). Species that are tolerant of warmer water temperatures may have faster growth rates and shorter generation times in these environments (Merrit et al. 2008), leading to high abundance and dominance of the community by these tolerant taxa (Rosenberg and Resh 1992). Many older macroinvertebrate metrics, such as Hilsenhoff Family Biotic Index (HFBI) and diversity indices (e.g. Shannon-Weaver diversity) are sensitive to types of pollution such as organic enrichment and nutrient loading that reduce oxygen availability (Rosenberg and Resh 1992, Barbour et al. 1999). Acidity (pH) and alkalinity can have significant effects on macroinvertebrate communities, but the magnitude of variation within the Spokane River would not be expected to have substantial effects (Bell and Nebeker 1969, Feldman and Connor 1992, Feio et al. 2007, Bellucci et al. 2011). Calcium availability can be a limiting factor for invertebrates with high calcium demand for exoskeletons or shells, such as some snails and crustaceans (Allan and Castillo 2007). These factors support the expectation that aquifer influence would have a positive effect on macroinvertebrate communities.

Urbanization

Storm water drainage and wastewater disposal are the primary pathways through which urbanization degrades river ecosystems (Parr et al. 2016). Urbanization results in an increase of impervious surface cover and the effects on river ecosystems and the subsequent effects on the stream character and biota have been dubbed “the urban stream syndrome" (USS) (Meyer et al. 2005, Pennino et al. 2016). Typical changes that occur in urban catchments are altered biotic communities, hydrology, water chemistry and channel morphology (Wallace et al. 2013). Streams suffering from USS have a flashier hydrograph, elevated concentrations of nutrients, salt and contaminants, reduced biotic richness and increased dominance of tolerant species (Wallace et al. 2013, Gabor et al. 2017). All these factors contribute to the degradation of stream ecosystems. Excess storm water runoff and increased sediment transport is considered a principle cause of stream degradation through erosion which can cause a permanent geomorphic change (Vietz et al. 2015). In a study done on streams in the Toronto region a significant negative relation was found between road density (a proxy for urbanization) and macroinvertebrate family richness and diversity; at high levels of road density only the most common and tolerant species and families remained (Wallace et al. 2013). Zinc and copper were found to found to increase and have a strong relationship with road density ($R2 > .731$ and $R2 > .676$) (Wallace et al. 2013). Specifically, zinc is known to migrate into the Spokane River during storm events that carry particulate matter from galvanized buildings, stockpiles of galvanized metals and tire wear from streets and parking lots (Fernandez and Hamlin 2009). Storm water runoff, either from direct impervious surface runoff to streams or impervious inputs piped into the stream, can have significant negative effects on the stream ecosystem (Wallace et al. 2013).

Macroinvertebrate metrics have been effective in determining anthropogenic influence in the form of urbanization. Specifically, percent of urban cover, conductivity, and percent of fines in riffles are associated with urban influence, and are correlated with reduced macroinvertebrate richness and diversity, as well as an increased abundance of tolerant organisms (Walters et al. 2009; Purcell et al. 2009). As urban streams are affected by a variety of stressors, finding key indicators reflective of specific alterations that can then be quickly used for diagnosing stream health could be important for restoration. Macroinvertebrate assemblages have been found to be one of those key indicators (Walters et al. 2009; Santoro et al. 2009).

Metal Effects

Toxic trace metals may have profound effects on macroinvertebrate communities. For example, on the Basento River in Southern Italy there is extensive metal pollution from urban, industrial and agricultural runoff specifically As, Cd, Cr, Cu, Pb, and Zn (Santoro et al. 2009). Macroinvertebrates with different eating strategies, classified by functional feeding guilds, consumed and bioaccumulated these metals at different rates. As, Cd, and Cr were found in higher concentrations in the tissues of the collector/gatherer functional feeding group than in tissues in any other functional feeding group, suggesting that the higher rate of biological uptake is associated with macroinvertebrates that feed on or in the benthic substrate of a river. There was also a linear relationship between the concentrations of As, Cd, Cr, and Zn in sediments and macroinvertebrate tissue (Santoro et al. 2009).

In smaller tributary streams in the Hasama River catchment in Japan, the effects of the metals, Pb and Zn, on drift-prone insects were studied. Metal pollution reduced mayfly (Ephemeroptera) richness most, with mayflies in the family Baetidae dominant in metal-impacted streams (making up 72% of the mayfly assemblage in the summer and 86% in the spring). Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness and abundance (common metrics of stream health) were lower in metal-impacted streams. There was also a decrease in caddisfly+mayfly richness and abundance and Chrominidae richness, differing from Chironomidae (midge larva) abundance, which increased (Iwasaki et al. 2009).

In the southern Rocky Mountains, toxic metals, particularly aluminum, copper, iron, and zinc may be the most important physical or chemical factor determine spatial patterns in macroinvertebrate diversity (Clements et al. 2000). In this ecoregion, mayfly abundance and diversity was particularly reduced by metals. Clements et al. (2000) assigned streams to background, low, medium, and high levels of metal toxicity, based on comparing stream water concentrations with EPA toxicity criteria. Mayfly diversity and abundance, particularly the family Heptageniidae, were strongly affected by even moderate levels of metal contamination. In the "medium" metal toxicity category streams, multiple parameters describing the macroinvertebrate community were significantly impacted, including: lower abundance of Ephemeroptera, Trichoptera, and Plecoptera; lower abundance of the Functional Feeding Group "Scrapers", lower abundance of predators, and lower abundance and taxa richness of Ephemeroptera (Clements et al. 2000).

On the Clark Fork River, Montana, the concentration of mining-deposited metals (As, Cd, Cu, Pb, Zn, and total metals) in six macroinvertebrates taxa was evaluated. Mining tailings of Cu, Cd, and Pb are found in river sediments and the floodplain top soils, and were found in invertebrates at correlated concentrations (Poulton et al. 1995). The metals that are consumed by fish, through their primary food source of macroinvertebrates, result in reduced abundances of age 0 rainbow and brown trout. Therefore, macroinvertebrates are used to monitor heavy metal contamination in streams and determine relative risk of metal availability to higher trophic levels. Riffle habitats were determined to be the most comparable area for sampling macroinvertebrates between sites (Poulton et al. 1995). This study attempted to provide an overall ranking of the relative impacts for each site based on metal concentrations in invertebrate tissue and benthic community structure. This study design was robust enough to discriminate between impacts related to metals and organic pollution. Metrics with the best indicators of heavy metal impacts were taxa richness, EPT richness, Chironomidae richness, % of the most dominant taxon, and density (#/m^2) (Poulton et al. 1995).

This study specifically examined effects of the Spokane River-Rathdrum Prairie Aquifer, urbanization of the watershed, and toxic trace metal contamination on macroinvertebrate communities in the Spokane River. Each of these influences should affect macroinvertebrates in predictable ways.

Spokane Rathdrum-Prairie Aquifer

Compared to surface water flowing within the Spokane River, groundwater input from the Spokane-Rathdrum Prairie aquifer is colder, lower in oxygen, more alkaline, has higher pH, and has higher concentrations of calcium and magnesium (McNeely and Nezat 2018, *in press*). Aquifer discharge is also relatively less polluted than the Spokane River by metals or urban pollution sources (Aquifer Atlas Team 2015). Benthic macroinvertebrates are an ideal group to assess ecosystem health of the Spokane River, as they are strongly affected by the main potential influences of aquifer recharge, urbanization, and metal contamination. Chemical analyses of both the water column and sediments can provide information on what contaminants are present, while concentrations in the tissues of macroinvertebrates provide more specific information about what contaminants are biologically available and therefore could alter the food web of a river ecosystem (Rosenberg and Resh 1992).

I have developed my thesis with three components. The first component is a macroinvertebrate bioassessment of the Spokane River which compares upstream and downstream communities of macroinvertebrates to determine the effects of cumulative urban influence on those communities, as well as comparing macroinvertebrate communities affected and unaffected by aquifer inputs from the Spokane Valley Rathdrum Prairie Aquifer. The second component is an analysis of toxic trace metals contained in the tissue of two abundant macroinvertebrates in the Spokane River. Finally, I compared metal concentrations between two taxa with different feeding modes and characterize their diets to help determine routes for bioaccumulation of metals. The known distribution of metal contamination in sediment was used (Grosbois et al. 2001) to confirm the effectiveness of using *Hydropsychidae* caddisfly tissue to indicate where these contaminants get into the biotic portion of the system (Maret et al. 2003, Cain and Luoma 1998). This project will establish efficient and informative protocols for macroinvertebrate biological monitoring of the Spokane River ecosystem over time. A more specific discussion of the Spokane River, my hypotheses, methods and future application of the study design follows.

Spokane River

Hydrogeology and Land Use within the Spokane River Basin

The Spokane River is located in eastern Washington and begins at the northern outlet of Lake Coeur d'Alene (Lake CDA, Figure 1). The river is about 180 km long and is 110 km downstream from the Coeur d'Alene mining district. The river flows predominantly westward from Lake CDA (Idaho) to the Columbia River (Lake Roosevelt, Washington). The Spokane River watershed drains periglacial outburst flood deposits that are interbedded with glaciolacustrine material laid down by a series catastrophic outbursts of Montana's glacially dammed Lake Missoula known as the Missoula or Ice Age Floods. These floods occurred between 34,000 and 14,000 years ago (Bretz, 1969). Deposits overlie the Columbia River basalt formations generally west of Upriver Dam and Cretaceous and Eocene granite and granodiorite generally east of the dam (Johnson et al. 1998, Grosbois et al. 2001)

This study focuses on the middle portion of the river from the Idaho/Washington border to just downstream of the City of Spokane (Figure 1). This study area includes influences from both the Spokane Valley Rathdrum Prairie Aquifer and the Hangman Creek watershed and is made up of forest, agriculture, urban and range lands (Fernandez and Hamlin 2009, Grosbois et al. 2001).

The most urbanized stretch of the Spokane River is contained in the cities of Spokane Valley and Spokane (Fernandez and Hamlin 2009) and ends before the influence of Nine Mile Dam and Lake Spokane. The interaction between the Spokane River and the Spokane Valley Rathdrum Prairie Aquifer contributes relatively clean, cold water to the river intermittently throughout the study area (Aquifer Atlas Team 2015). Compared to surface water flowing within the Spokane River, groundwater input from the Spokane-Rathdrum Prairie aquifer is colder, lower in oxygen, more alkaline, has higher pH, and has higher concentrations of calcium and magnesium (McNeely and Nezat 2018, *in press*). Aquifer discharge is also relatively less polluted than the Spokane River by metals or urban pollution sources (Aquifer Atlas Team 2015).

To study the ecosystem health of a river, specifically the Spokane River, requires an understanding of the many different accumulating influences to that body of water. Three specific influences on the Spokane River are recognized in this study. 1) The landscape the river flows through contributes natural sediments and discharge and determines the structure of the channel, including tributaries, aquifers, and riparian areas are critical influences. 2) Human influences on rivers have created a new level of complication to our understanding of these systems, such as increased urbanization adjacent to a river that can disrupt linkages between the stream and adjacent riparian zones and aquifers; impermeable surfaces that decrease groundwater recharge and increase surface runoff; and degraded riparian corridors that alter the natural latitudinal interactions with the surrounding landscape or floodplain (Naiman et al. 2005). Further, longitudinal impasses, such as dams, prevent migration of plant propagules, isolate fish communities, hold back sediments, increase temperatures, reduce dissolved oxygen and increase nutrient concentrations (Naiman et al. 2005). Urbanization can also increase the amount of pollution being directly discharged into a river through industrial and municipal discharges as well as storm water discharges. 3) The Spokane River is directly impacted by historical silver, lead, and zinc mining and processing in watershed of its tributary, the South Fork Coeur d' Alene River (Beckwith 1998). This river, like many urban rivers, has been used as a dilution source for wastes such as storm water, industrial discharges and treated and untreated sewage.

All these influences have contributed to the degradation of the river ecosystem (USGS 2003, Aquifer Atlas Team 2015). Portions of Spokane River between the Idaho/Washington State line and Nine Mile Falls Dam are currently on the US Environmental Protection Agency's 303d list of designated polluted water bodies under the Clean Water Act for: dioxin, PCBs and excessive temperatures. In addition, Total Daily Maximum Load plans are in place for excessive toxic metal pollution and low dissolved oxygen concentrations in portions of the river (http://www.ecy.wa.gov/programs/wq/303d/index.html, accessed 4 December 2017).

Spokane Valley Rathdrum Prairie Aquifer

An important influence on the Spokane River is the dynamic interchange with the unconfined Spokane Valley Rathdrum Prairie Aquifer. The aquifer has one of the fastest flow rates in the United States, flowing up to 60 feet per day in some areas (SVRP Aquifer Atlas Team 2015). The volume of the entire Aquifer is about 10 trillion gallons making it one of the most productive aquifers in the country (Spokane County, https://www.spokanecounty.org/1219/Spokane-Valley-Rathdrum-Prairie-Aquifer, accessed December 22, 2017). It is made of an underground layer of gravels, cobbles, and boulders deposited by the Glacial Lake Missoula floods. The aquifer interacts with the Spokane River in gaining and losing reaches. A gaining reach occurs when the water table of the aquifer is higher than the Spokane River so water flows into the river from the aquifer through springs and seeps. Losing reaches are areas where the water table is lower than the river and consequently water percolates from the river down into the aquifer (Aquifer Atlas Team 2015). Compared to surface water flowing within the Spokane River, groundwater input from the Spokane-Rathdrum Prairie aquifer is colder, lower in oxygen, more alkaline, has higher pH, and has higher concentrations of calcium and magnesium (McNeely and Nezat 2018, *in press*). Aquifer discharge is also relatively less polluted than the Spokane River by metals or urban pollution sources (Aquifer Atlas Team 2015).

Urban Influence

The Spokane River flows through industrial, suburban, and urban areas throughout the study reach, mostly within the cities of Liberty Lake, Spokane Valley, and Spokane. At the time of this study, it also had two wastewater treatment plants, Liberty Lake and the City of Spokane's Riverside Park Water Reclamation Facility that discharged into the Spokane River and potentially impacted the macroinvertebrate communities in this study. A third wastewater treatment plant opened in Spokane Valley (Spokane County Regional Water Reclamation Facility) after the samples for this study were collected.

Urban influences from the City of Spokane are different and possibly more extensive than the City of Spokane Valley. The section of the Spokane River that passes through the City of Spokane has the highest concentration of influence from storm water as well as combined sewer overflow pipes (CSO) that empty water into the river (City of Spokane 2007). CSOs are antiquated sewer lines that do not separate storm water and sewage en route to the City of Spokane waste treatment plant. During storm events untreated sewage and storm water empty directly into the river (City of Spokane 2007). In 2010 there were 278 discharge events where CSOs discharged directly into the Spokane River. From 2003 -2012 there was an average of 296 discharge events per year (City of Spokane 2007). Currently, the City of Spokane is implementing an Integrated Water Quality Plan that should result in an average of ≤ 1 CSO discharge event per year (CMH2Hill 2014).

Storm water and sewage were not discharged directly into the Spokane River within the city of Spokane Valley at the time of this study. The City of Spokane Valley treated storm water on site through dry wells or directed it into smaller tributary streams eventually leading to the Spokane River (Fernandez and Hamlin 2009). After samples were collected for this study, on December 1st 2011, the Spokane County regional water reclamation facility (SCRWRF) began treating wastewater with the treated affluent outflows directed into the Spokane River. Spokane Valley is also the site of the most significant current industrial discharges, including the Liberty Lake Sewer and Water District, Kaiser Aluminum Trentwood Works, and Inland Empire Paper Company. These were all permitted point source dischargers at the time of this study (Washington Department of Ecology, https://ecology.wa.gov/Water-Shorelines/Water-quality/Water-improvement/Total-Maximum-Daily-Load-process/Directory-of-improvement-projects/Spokane-River, accessed December 22, 2017).

Historically, there was significant agriculture within Spokane Valley but less so at the time of this study. Agriculture is still a significant land use in portions of the watershed upstream of Lake Coeur d'Alene, and throughout the Hangman Creek watershed. Agriculture without sufficient riparian buffers increases sediment, pesticide, and nutrient inputs to streams (Santoro et al. 2009).

Toxic trace metal contamination

Toxic trace metal contamination is prevalent in the Spokane River and has a long history of monitoring. Lake Coeur D' Alene, the source of the Spokane River, has been profoundly polluted by heavy metals as a result of historic mining practices in its watershed. In 1940, Ellis characterized the lower Coeur d' Alene (CDA) river and southern end of Lake CDA as devoid of life from mining pollution. Recommended remediation was implemented in 1960s (Beckwith 1998). Within the CDA basin, invertebrate communities effectively delineate small tributary streams with and without the influence of mining waste (Maret et al. 2003). From the early 1880s–to the late 1960s mine tailings were dumped directly into streams within the CDA River watershed, and in 1973 a fire at the Bunker Hill Mines in Smelterville, ID resulted in substantial lead deposition in the CDA River valley (Farag et al. 1998). Consequently, the sediments of CDA Lake, the source of the Spokane River, are highly contaminated with Pb, Zn, As, Cd, Sb, and Hg. Elevated concentrations of these metals are associated with suspended sediment from the outflow of Lake CDA to the Spokane River, and have been detected as far downstream as Lake Spokane (Grosbois et al. 2001). Concentrations of metal enrichment in the Spokane River generally decrease with distance downstream from Lake Coeur d'Alene (Grosbois et al. 2001). This is because metals such as lead and cadmium are hydrophobic and associated with sediments; they settle out of flowing water and are also diluted by uncontaminated sediments from elsewhere in the basin (Grosbois et al. 2001).

Bioavailability of Trace metals in Spokane River Macroinvertebrates

In addition to the community-based bioassessment, I also investigated whether metal concentrations in invertebrate tissue are spatially variable within this portion of the Spokane River. The highest concentrations of metals have been found in the Spokane River Basin between the Idaho-Washington border and Long Lake. In this area Pb, Zn, and Cd have been found in fish tissue (Fernandez and Hamlin 2009) and surface sediments (Grosbois et al. 2000). Other metals (found in fish &/or sediments) at high concentrations are As, Sb, and Hg. As benthic macro-invertebrates live and feed in metal-contaminated sediments, some of these organisms accumulate metals in their tissues (Maret et al. 2008**,** Rosenberg and Resh 1992, and Santoro et al. 2009) that can lead to trophic transfer in higher order organisms. The large production rates of macroinvertebrates and specific life history of having both an aquatic and terrestrial phase make them a popular food source of both aquatic (such as fish) and terrestrial (such as birds and bats) consumers (Walters et al. 2010, Tsui et al. 2009). This indicates that macroinvertebrates are a major pathway for the movement of metals to higher trophic levels in the Spokane River, as they are in other river systems.

The toxic metal concentrations analyzed in macroinvertebrate tissues in this study are Cd, Cu, Pb and Zn, as they are found in elevated concentrations on the Coeur d' Alene River sediments; the metals Mg and Ca were also included because they could be associated with aquifer inputs (Grosbois et al. 2001, Brennan et al. 1995, McNeely and Nezat 2018, *in press*). Levels of Cd, Cu, and Zn are suspected to be particularly high in macroinvertebrate tissue because of the high concentrations found previously in the Coeur d'Alene River Basin in sediments, biofilm, and invertebrates (Beltman et al. 1999, Besser et al. 2001 & Farag et al. 1998). Maret at al. (2003) found that tissues of a common, metal tolerant group of net spinning caddisflies (Trichoptera), *Arctopsyche grandis* and *Hydropsychidae spp.,* collected from tributaries of the CDA River affected by mining waste had higher concentrations of the metals Cd, Pb, and Zn in their cytosol than those from reference tributaries without mining. The analysis of metals in macroinvertebrate tissue in this study was modeled after Maret et al. 2003.

Known concentrations of heavy metals in sediments and fish tissue in the Spokane River (Fernandez and Hamlin 2009) would be complimented by an understanding of the concentrations found in invertebrate tissue for two primary reasons. Unlike most fish, many macroinvertebrates are primary consumers in a river food web and therefore they are often the first animals within the food web to incorporate heavy metals into their tissues (Poulton et al. 1995). Second, they are less mobile than fish, so the concentrations found in their tissues are more likely to reflect the bioavailability of metals at the location where they were collected (Rosenberg and Resh 1992). Macroinvertebrate trace metal cytosolic concentrations also correlated with concentration gradients found in sediments contaminated with trace metals in the Clark Fork River (Cain and Luoma 1998). The Washington State Department of Ecology under the Urban Waters Initiative set out to find the sources of contaminants in the Spokane River and prioritize restoration sites. Heavy metals are considered a contaminant of concern and therefore studies addressing the source of these contaminates in the river have been conducted (Fernandez and Hamlin 2009). Health advisories for the consumption of fish tissues are also in effect in the Spokane River because of toxins found in fish tissue, including toxic metals (WSDOH 2009). Understanding how these metals are accumulated and transported in the ecosystem as well as the locations in which this occurs will be helpful for prioritizing restoration and to prevent further contamination of the ecosystem and food webs.

Objectives and Hypotheses

The goal of this study was to examine effects of the Spokane River-Rathdrum Prairie Aquifer, urbanization of the watershed, and toxic trace metal contamination on macroinvertebrate communities in the Spokane River. Each of these influences should affect macroinvertebrates in predictable. Specifically macroinvertebrate bioassessment was used to comparatively address the ecosystem health of three reaches of the Spokane River in Spokane County, Washington. Widely recognized invertebrate metrics for water quality monitoring were applied to quantify macroinvertebrate community health of macroinvertebrate samples collected using a protocol I designed specifically for the Spokane River.

First Hypothesis: Macroinvertebrate communities are less degraded in reaches gaining water from the aquifer than those that do not.

Second Hypothesis: These communities are significantly degraded by urban influences below the urban core of the city of Spokane as compared to all upstream reaches.

Third Hypothesis: Metal impacts decline with distance downstream of Lake Coeur d'Alene and these metal impacts are reduced in reaches with substantial aquifer inputs.

Fourth Hypothesis: Heavy metal concentrations found in the tissues of the two focal macroinvertebrates decline with distance downstream of Lake Coeur d'Alene.

When selecting my study design for testing these hypotheses, I attempted to balance the amount information that could be gathered with a practical approach that could be repeated for long term monitoring to support effective management. Decisions were therefore made in my study design to insure methods would be easily repeatable by an inexperienced field crew.

Consistent, repeatable protocols are well-established for addressing the ecological health of wadeable (first or second order) streams (Barbour et al. 1999). Methods for addressing the ecological health of large rivers such as the Spokane River are much less developed (Fisher 1997). There are many difficulties associated with field sampling in large streams compared to wadeable streams. Equipment that is used in small streams is not able to stand up to the higher flows, and the deep benthos of the thalweg is much less accessible. Sampling all types of habitat or a proportion of the entire community is much more time consuming and often impractical particularly for a procedure designed to be repeated annually. Generally there is a shift from sampling procedures that collect macroinvertebrates from the entire channel to those that sample the shoreline at shallow depths with dip nets or artificial substrates (Rosenburg and Resh 1992). Because the community assemblages collected are rarely representative of the entire macroinvertebrate community in a river, protocol that tests hypotheses based on comparisons is more feasible (Rosenberg and Resh 1992). Comparisons between impacted and unimpacted streams or reaches can be informative, quantitative and repeatable without representing the entire fauna of the river (Rosenberg and Resh 1992). Riffles (fast flowing shallow areas with significant turbulence and oxygen) are often the most productive and diverse river habitat for macroinvertebrates (Thorp et al. 2010). To determine relevant impacts of metals on the benthic community structure in metal impacted streams it is recommended that macroinvertebrate samples be collected from riffle habitats owning to riffles close relation to metal availability and transfer to higher trophic levels (Poulton et. al. 1995). These characteristics, along with their more consistent accessibility, make riffles an appropriate sampling location in large rivers. Substrate type can be another challenge in large rivers, and will usually influence sampling technique. The large cobble of the Spokane River lent itself well to randomized stratified sampling. In this study I sampled the entire surface of boulders in a specific size class. This is an informative method that can relate macroinvertebrate biomass to sampling area (McNeely et al. 2007).

The extent of urban development often results in the absence of reference streams for urban ecosystems though it is still essential to determine a target condition for management (Purcell et al. 2009). The large size of the Spokane River, the inputs of the Spokane River Rathdrum-Prairie Aquifer, and the diversity of potential impacts preclude finding a comparable reference stream. Without the aid of a suitable reference stream, I designed the bioassessment portion of this study specifically to understand differences among reaches of the Spokane River. The aim of this design is to determine the significance of aquifer recharge and urbanization as these are important yet understudied influences on the Spokane River. In particular, the portion of the river between downtown Spokane and Nine-Mile Dam were not included in previous macroinvertebrate sampling of the river, for both community characterization (Gibbons et al. 1984) and determination of tissue metal concentration (Kadlec 2000). If the protocol of my study is an informative tool, it can be repeated to determine how the ecosystem health of this river changes over time. Using principles found in Rosenberg & Resh (1992) we can also determine what species previously collected should be monitored as indicator species.

For assessment of macroinvertebrate tissue metal concentrations, I followed Maret et al. (2003) in focusing on net-spinning Hydropsychid caddisflies. I limited my sampling to a single species (*Hydropsyche cockerelli*) to reduce variability in diets among sites (Schefter and Wiggins 1986). I also determined if a larger-bodied easily collectable macroinvertebrate, *Pteronarcys californica* (a long-lived stonefly nymph), had similar metal concentrations and therefore could be used instead. In order to better understand variation in metal concentrations found in invertebrate tissues and potential bioaccumulation pathways, I also analyzed the diets of the two focal invertebrate species. Based on previous literature, *Hydropsyche cockerelli* would be expected to filter phytoplankton, bacteria, and other organic particles from the water, while *Pteronarcys californica* would be expected to feed on large organic particles, such as detrital terrestrial leaves in the benthic sediments of the river (Merritt et al. 2008). These dietary preferences might suggest that *H. cockerelli* and *P. californica* would experience different dietary exposure to metals, which could contribute to different concentrations of metals being incorporated into tissues. If *P. californica* accumulate toxins at similar levels to *H. cockerelli*, they may offer several advantages as a study organism for future studies. *P. californica* is easy to identify in the field, has larger biomass which may be particularly important for contaminants present in small concentrations such as PCBs (Fernandez and Hamlin 2009), and can be reared in the laboratory more easily for ecotoxicology studies.

Methods

Study Design

To address the effects of the urban core of the City of Spokane on the macroinvertebrate community, I sampled six sites above downtown Spokane and six sites below downtown Spokane. To determine effects of aquifer recharge on the macroinvertebrate community, three of the upstream sites were located in a losing reach and three were located in a gaining reach. All three of the losing reaches were upstream of the gaining reaches so would be unaffected by the water chemistry of the aquifer recharge in the gaining reach. The six upstream reaches were then compared to the six downstream reaches to determine if urban pollution from the city of Spokane is having a negative effect on macroinvertebrate communities in the Spokane River and aquifer recharge is having a positive effect on macroinvertebrates communities in the Spokane River. I also compared the degree of metal pollution impact on the macroinvertebrate community among all reaches.

Twelve riffles were sampled in the Spokane River, six upstream of the City of Spokane, and six downstream. Upstream sites were divided between gaining sites that receive aquifer input (three sites), and losing sites where water flows from the river into the aquifer (three sites). All downstream riffles (six sites) are in upwelling reaches. Locations of these sites are indicated in Figure 1.

Future Applications of Study Design

This assessment can provide a tool to evaluate the restoration practices encouraged by state, federal, and local laws by monitoring the changes to the ecosystem along the river in areas most affected by development and population growth (Beckwith 1998; Fernandez & Hamlin 2009). The serious environmental degradation of the South Fork of the CDA River, Lake CDA, and subsequently the Spokane River may be improving as mining waste is no longer being discharged into the river system without environmental safeguards, and federal and state restoration programs are in place (Beckwith 1998)). However, sediments contaminated with Ag, As, Cd, Hg, Pb, Sb, and Zn is already ubiquitous in the CDA drainage basin. During high flows they are washed downstream into the Spokane River (Grosbois et al. 2001). These contaminants generally decrease with distance downstream from Lake Coeur d'Alene but have been found at elevated levels in the Spokane River as far as Lake Spokane. These contaminants may continue to wash into the river despite restoration in the upper CDA watershed (Grosbois et al. 2001). Heavy metal pollution is not just associated with mining waste but also results, to a lesser degree, from treated wastewater, storm water and industrial dischargers (Baysal et. al. 2013, Brown and Peake 2006, Forstner 1983). Pollution sources on the Spokane River can be difficult to pinpoint and that may make prioritizing locations for restoration difficult, as well as making it difficult to monitor how successful these efforts might be.

The monitoring of these effects using ecosystem assessment techniques based on macroinvertebrates community composition, I have personally found to be inexpensive and easily explained to a general audience making the ecosystem health of the Spokane River accessible and direct. This strategy for monitoring may foster local interest and participation in the future restoration efforts. This study may produce information that can assist water-quality and natural resource management decisions. Its duplication may help to assess the effectiveness of those decisions.

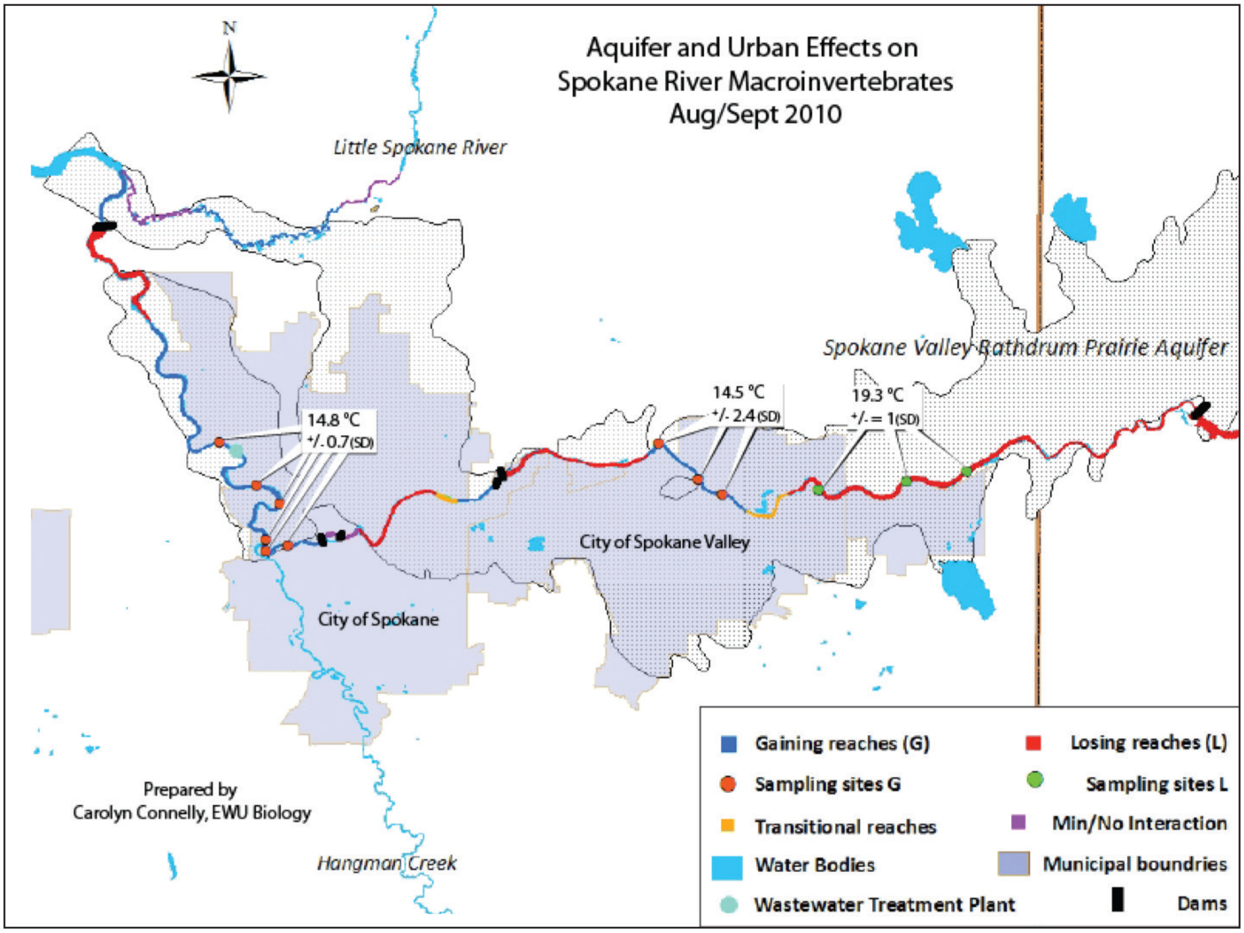

Figure 1. Sample sites upstream of the downtown urban core of the City of Spokane. Upstream gaining (G) reaches, Upstream losing (L) reaches and downstream of the urban core of the City of Spokane gaining (G) reaches are indicated. Spokane County, WA (2010).

Habitat Sampling

All habitat variables are displayed by sample site ID in Table 1.

Temperature

Water temperature was determined at the substrate of each site by burying a soil thermometer about two cm into the sediment a meter from shore at three locations along the transect: the farthest upstream end of each site and farthest downstream end of each site as well as one point in the middle of the transect.

Discharge

Discharge of the Spokane River in cubic feet per second (CFS) was assessed for the closest USGS sampling stations to the study area, during the invertebrate sampling period for this study August-September 2010 (https://waterdata.usgs.gov/nwis accessed 10/16/17). Post Falls, ID is just upstream of the first upstream losing reach, and the Hangman Creek confluence is within the downstream study area.

Dissolved Nutrients

At the upstream end of each site, I collected water for dissolved nutrient analysis. Samples were filtered in the field with a 0.7 μg glass fiber filter into acid-washed HDPE bottles. Samples were placed on dry ice in the field and stored frozen until analysis. Soluble reactive phosphorous concentrations were determined using the molybdate method (APHA 2008). Ammonium concentrations were determined using a modified Holmes method (Holmes et al. 1999, Taylor et al. 2007). Nitrate and nitrite concentrations were determined using an Alpkem 3 Flow Analyzer with and without cadmium reduction (OI Analytical 2009b).

Table 1. Sample Collection Sites – Collected in field: Description, Date, Temp, Mean median particle diameter and water velocity. USGS CFS data: https://waterdata.usgs.gov/nwis accessed 10/16/17; river miles: https://www.topozone.com/washington/spokane-wa/stream/spokane-river/ accessed 9/8/17.

Site ID	Description	River Miles	River km	Sample Date	Temp °C	CFS Post falls	CFS Upstream of Hangman Cr	Mean median particle diameter (cm)	Mean water velocity (m/sec)
UL1	West of State Line	96	154.5	9/2/10	20.0	628	892	20.5	0.25
UL2	Liberty Lake Exit	93	149.7	8/18/10	19.8	652	1000	22.7	0.48
UL3	Barker Road	90.5	145.7	8/21/10	18.0	617	945	21.3	0.15
UG1	Mirabeau Park	87.5	140.8	8/30/10	12.7	622	906	22.2	0.42
UG2	Kaiser	86	138.4	8/23/10	16.0	652	943	21.6	0.27
UG3	Coyote Rock	84.5	136.0	8/24/10	16.0	634	896	18.8	0.61
D1	Lower Crossing	73	117.5	8/15/10	16.0	744	1150	21.3	0.23
D2	Sandifur Bridge	72.5	116.7	8/14/10	14.7	738	1170	20.8	0.44
D3	Latah Creek	72	115.9	8/16/10	14.8	682	1150	16.4	0.57
D4	Nursing School	70	112.7	8/17/10	15.2	652	1000	20	1.26
D5	Northside CSO	69	111.0	9/1/10	14.3	634	920	19.8	0.61
D6	Below RPWRF	67	107.8	8/31/10	14.0	634	926	20.6	0.74

Invertebrate Sampling

I collected all quantitative macroinvertebrate samples between August 14th and September 2nd, 2010. Samples were taken in late summer because water levels were low enough for Spokane River riffles to be sampled safely. In addition, most univoltine insects would have already emerged reducing the potential for differences in phenology among sites to confound the results (Barbour et al. 1999). At each site I selected a shallow, turbulent riffle with boulder and cobble substrate. I then established a 100 m transect from downstream to upstream along the stream bank. At each site I collected 8 samples from natural river boulders with a medium diameter of 20.5 cm (ranging from 13 to 38 cm), starting at the downstream end of the riffle. Most riffles were longer than 100 m, so only the downstream 100 m portion of the riffle was included. The exception was one site whose length totaled 60 m; therefore its total length was included. This site is directly below the waste treatment plant for the City of Spokane, so its location was important to include despite its shorter length.

Stones were selected through a stratified random protocol (Rosenberg and Resh 1992). At eight randomly determined points along the 100 m transect, I located the nearest boulder at a depth of 64 cm and measured flow velocity 2.5 cm above the boulder using a Marsh-McBirney 2000 flow meter. To sample the boulder, a large benthic sampling net (45.5 cm wide by 24 cm high; 500 µm) was held firmly against the benthic substrate downstream from the boulder, the bolder was then lifted and placed in the net, and the substrate beneath it was disturbed by hand. This entire sample was carried to shore and all macroinvertebrates were then removed from the stone and net and stored in 70% ethanol for later identification. The surface area of the rock was then determined by the aluminum foil method (Hauer & Lamberti 2007). Dry rocks were wrapped completely with one layer of foil avoiding overlap and trimming excess foil. The foil was then removed and the weight of that one layer of foil representing surface area is compared to a square of the same foil cut and measured to know dimensions. The unknown surface area of the rock (Ar) was determined using equation 1.

Equation 1.

A r = (Ak/Wk) x Wrf Ar/2 = estimate for the colonized surface area

Ak= known area (cm2), Wk= known weight (mg for mass), and Wrf = weight of rock foil; this was repeated for all 8 sites moving upstream.

Community Sample Processing

Macroinvertebrate samples were sorted using a dissecting microscope at 10x magnification. Invertebrates were identified to lowest practical taxonomic level (usually genus), and abundances recorded. Three undergraduate volunteers assisted in processing the samples. Primary identification references included Merritt et al. (2008), Thorpe and Covich (2001), and Schefter and Wiggins (1986). Invertebrates of a subset of 8 samples were identified by EcoAnalyst (Moscow, ID) taxonomists according to their standard protocols. This allowed for an independent check on my identifications, and to determine higher taxonomic resolution for some groups (e.g. Chironomidae).

Biomass collection for Metal Analysis

Biomass samples for metal analysis were taken on the same day as quantitative community sampling described above. At each of the 12 riffles, collectors started at 3 random points along a 100m stream bank transect, and searched for target species within the wadeable portion of the river using kick nets and turning over rocks by hand, moving upstream, with the goal of collecting at least 0.5 g of tissue per sample for each species. The target species were separated from sediments and other organisms at the site. I took care to avoid any contact with metal or other possible contaminants, and periodically rinsed specimens with river water during processing. Samples were stored in clean Whirl-Packs on dry ice, and then transferred to an -80˚C freezer for storage. If possible, 2 samples composited of 3-15 individuals of *Hydropsyche cockerelli* and 1-5 individuals of *Pteronarcys californica* species were collected at each site. The wet weights of the samples were measured as well as the average % moisture of *H.*

cockerelli and *P. californica.* Average dry weight to wet weight was determined for individual samples to relate to heavy metal concentration to dry weight for each sample. In August 2011, samples of *P. californica* were collected from the St. Joe River (ID) as a reference reach (*Hydropsyche* samples could not be found at that time). In 2012 from 8/3/2012 to 9/5/2012 all study reaches were resampled to obtain larger sample sizes. *H. cockerelli* and *P. californica* were collected at all 12 study reaches on the Spokane River as well as samples from the North and South fork of the CDA River (ID) and the St. Joe River. *Hydropsyche,* but not *H. cockerelli* were found in the South Fork CDA River and *P. californica* was found at the North and South Fork of the CDA River as well as the St. Joe River.

Analysis of Invertebrate Tissue Metal Content

Macroinvertebrate tissues collected in the summer of 2010, 2012 & 2013 were analyzed for heavy metal content to complement macroinvertebrate bioassessment. I analyzed the metal concentrations found in macroinvertebrate tissues following protocol from Maret et al. (2003), with some modifications. Following the protocol in Cain and Luoma 1998 specimens (both *Hydropsyche cockerelli* caddisflies *Pteronarcys californica* stoneflies) from each sampling site were partially thawed, and rinsed with cold deionized water (each about 400 mg wet weight). Each sample was then digested in 5 or 10 ml of 70% trace metal grade nitric acid on a hot plate under a sterile fumigation hood, and after invertebrate tissue was completely dissolved the nitric acid was evaporated. The remnant of invertebrate tissue was again dissolved in 10ml of 10% trace metal grade nitric acid, filtered (Acrodisk 0.45 µm), and then analyzed by a Thermo Fisher iCAP 6200 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP – OES) by Dr. Carmen Nezat at Eastern Washington University or the Analytical Sciences Laboratory at the University of Idaho. Data values were converted from mg element per L solution to mg element per g of bug with the following formula (Equation 2).

Equation 2.

mg element/ g bug tissue =

((mg/L) x .01L)/(wet weight x average proportion dry mass))

Diet Samples

Diet samples consisting of two *Hydropsyche cockerelli* caddisflies and one *Pteronarcys californica* stonefly of each size class (if available) were also collected from 10 random transect points, turning over boulders in the wadeable portion of the river. These samples were placed in acid washed HDPE containers, frozen on dry ice and then taken back to the lab.

Diet Analysis

I used simple microscopy to characterize dietary habits of the same two macroinvertebrates for which I determined trace metal content. This was done to determine the primary constituents of each invertebrate's diets in the reaches in which I sampled indicating sources of heavy metal content in the tissue. Individuals for diet analysis were dissected within a few hours and foreguts were stored in 10% formaldehyde and refrigerated (4 °C). The contents of the foregut were examined using a compound microscope at 100x or 400x. To do this the intact gut was dissected and mounted with Taft's media (Hauer and Lamberti 2007). Gut samples were processed in two ways. For some *Hydropsyche cockerelli*, a Whipple grid was used to determine the relative abundance of material types including sediment, terrestrial detritus, fine particulate organic matter, and major groups of algae. For the remaining *H. cockerelli*, and all *Pteronarcys californica*, the relative abundance of algae constituents was determined by standard protocols by EcoAnalyst (Moscow, ID) taxonomists.

Metrics for Bioassessment

Relevant Metrics were applied to the macroinvertebrate taxa values (Table 2) focusing specifically on metrics that are sensitive to metal, urbanization and anthropogenic effects. Aquifer influence may be seen from an absence of these three effects as it should have a diluting influence on other pollution sources and cold water generally has a positive effect on macroinvertebrate communities (Merrit et al. 2008).

Urban influence throughout the basins effects on macroinvertebrate communities was analyzed by applying urban influence-sensitive matrices developed by Purcell et al. 2009 to macroinvertebrate community taxa values. The three metrics they determined to be most effective were those that represent major ecological attributes of macroinvertebrate assemblages when compared to an urban gradient (population density, road density, and urban land use). Specifically, EPT richness (number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera) has a strong negative response to anthropogenic influence (Iwasaki et al. 2009, Poulton et al. 1995, Purcell et al. 2009). In addition, filterer richness and proportion of the individuals that can be classified as clingers both decrease with an increase in urbanization (Purcell et al. 2009). “Filterers” are a functional group of macroinvertebrates that filter organic particles (seston) carried downstream by flow as their main source of food (Cummins 1973, Merrit et al. 2008). “Clinger” indicates a functional group of macroinvertebrates that cling tightly the surface of the substrate (Merrit et al. 2008).

Toxic metal influence on macroinvertebrate communities was analyzed by applying metal-sensitive metrics: decrease in family richness, decrease in Chironomidae richness, decreases in proportion Ephemeroptera, proportion EPT, and proportion Trichoptera, and increases to proportion Diptera, proportion Chironomidae, and a Metal Tolerance Index (Poulton et al. 1995, Clements et al. 2000, Iwasaki et al. 2009, Clements et al. 2013).

I compared macroinvertebrate communities between the gaining and losing reaches of the Spokane River above the urban core of the city of Spokane as well as the macroinvertebrate communities above and below the urban core of the City of Spokane. Macroinvertebrate communities were also characterized by other indices reflecting ecosystem health and metal tolerance (Hilsenhoff 1987, Maret et al. 2003), including Hilsonhoff Family Biotic Index, Shannon-Weaver Diversity, and Chironomidae richness.

Metals Tolerance Index (MTI) is an index of community tolerance to metal contamination, primarily copper and similar acting metals. As with the HBI, values range from 0-10, with higher values indicating a possible effect due to the presence of metal contaminants. Values used for this calculation are those currently in use by Montana Department of Environmental Quality (McGuire 2009). Streams generating index values above 6 are regarded as having some metal pollution affecting the macroinvertebrate community (McGuire 2009). Index values below 4 indicate no metal pollution affects (McGuire 2009). The Metals Tolerance Index is a diagnostic metric used to identify samples with a high degree of organisms tolerant of metals. This index is on a scale from 0 to 10 with higher values indicating more tolerant organism, and more polluted conditions (McGuire 2009). This index (equation 3) was only applied to a small group of samples (8) that were sent to EcoAnalysts for more precise identification.

Equation 3.

$$\text{Metal tolerance Index (MTI)} = \sum_{i=1}^{t} (RA_i * t_i)$$

Where t is the number of taxa, $\mathbf{RA_i}$ is the fraction or relative abundance of individuals of the "i"th taxon in the sample, $\mathbf{t_i}$ is the tolerance index value of that taxon

Shannon-Weaver Diversity index is a classic community diversity index which is an information statistic. It assumes all species are represented in a sample and that they are randomly sampled. As with other diversity indices, higher values correspond to greater diversity. Values decrease with increasing anthropogenic perturbation. (Lydy et. al. 2000). Diversity Indices originally were designed for terrestrial ecosystems and have been found to be less accurate in aquatic environments, and as such, should not be used without other water quality community composition indicators (Washington 1984). The Shannon- Weaver Diversity index (equation 4) was selected in particular to compare to a comprehensive macroinvertebrate study done on the Spokane River in 1984, this was the diversity index selected to best represent the community structure (Gibbons et. al.

1984).

Equation 4.

Shannon Index = $-\sum_{i=1}^{s} p_i (\ln p_i)$

P is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), and s is the number of species.

Hilsenholf Family Biotic Index (HFBI) is the most widely used Biotic Index in the United States, and is used to describe responses to general anthropogenic perturbation. It is most sensitive to organic pollution, sedimentation, and other factors that reduce dissolved oxygen availability. It is an average, weighted by abundance, of the tolerance values assigned to each taxon (equation 5). HBI scores range from 0 to 10, with values closest to 0 reflecting communities highly intolerant of any organic enrichment (Hilsenhoff 1977; Hilsenhoff 1987) A HFBI score <4.0 is considered to represent very good water quality.

Equation 5.

Hilsenholf Family Biotic Index (HFBI) = $(\sum x_i t_i)/n$

Where x_i is the number of individuals within a taxon, t_i is the tolerance value of a taxon and **n** is the total number of organisms in the sample.

Chironomidae richness is the total number of identifiably distinct taxa in the insect family Chironomidae (Order = Diptera). The family Chironomidae is the most common group of aquatic insects in freshwater systems around the globe, often accounting for over 50% of the total taxa richness in a benthic sample. In North America there are over 2,000 species occupying a wide variety of habitats, feeding functions, and ecological niches (Merritt et al. 2008). Increased environmental stress will reduce Chironomidae richness (Iwasaki et al. 2009; Poulton et al. 1995). A subset of samples were sent to EcoAnalysts for identification of Chironomidae to genus. This was done to see if this might be a useful metric to determine differences between study reaches.

Data Analysis for Bioassessment

One-way Analysis of Variance was used to compare mean macroinvertebrate metrics among the three study reaches (Upstream losing, upstream gaining, and downstream). For this analysis, the means of each metric were calculated for each riffles, and riffles were used as single replicates. Reach was the independent variable, and each of the 15 invertebrate metrics was analyzed as a dependent variable. An arcsin-square root transformation was applied to all proportional metrics (e.g. proportion dominant taxon) prior to ANOVA. A $\log_{10}$ transformation was applied to all densities (#/area) before ANOVA. When 1-way ANOVA was significant ($p < 0.05$), I used a Tukey post-hoc test to identify, which reaches differ significantly. All ANOVA and Tukey analysis were performed with JMP 6.0 (SAS Institute).

Table 2. Community composition metrics used to characterize anthropogenic effects on macroinvertebrate communities of the Spokane River.

Metric	Urbanization	Toxic trace metal contamination	General anthropogenic disruption	Reference
Density (total # invertebrates/m^2)		Decreases		Poulton et al. 1995
Family Taxa Richness		Decreases	Decreases	Poulton et al. 1995, Barbour et al. 1999
EPT Family Richness	Decreases	Decreases		Iwasaki et al. 2009, Poulton et al. 1995, Barbour et al. 1999
Chironomidae Richness		Decreases		Iwasaki et al. 2009, Poulton et al 1995
Proportion Dominant Taxa		Increases		Poulton et al. 1995, Barbour et al. 1999
Proportion Ephemeroptera		Decrease	Decreases	Barbour et al. 1999
Proportion EPT	Decreases	Decreases	Decreases	Barbour et al. 1999, Lydy et al. 2000
Proportion Trichoptera		Decreases		Iwasaki et al. 2009
Proportion Diptera		Increases		Barbour et al. 1999
Proportion Chironomidae		Increases		Iwasaki et al. 2009, Barbour et al. 1999
Proportion Clinger	Decreases		Decreases	Purcell et al. 2009, Barbour et al. 1999
Filterer Richness	Decreases			Purcell et al. 2009
Shannon-Weaver Diversity			Decreases	Barbour et al. 1999, Rosenburg & Resh 1993
Hilsenhoff Family Biotic Index (HFBI)			Increases	Hilsenhoff 1987, Hilsenhoff 1988
Metal Tolerance Index		Increases		McGuire 2009

Data Analysis Metal Concentrations

To determine if whole body metal concentrations decline with distance downstream from the contamination source, I performed linear regressions with distance downstream from Lake Coeur D' Alene as the independent variable and whole-body metal concentrations as the dependent variables, specifically focusing on metals that were detectable in macroinvertebrate tissue in the majority of study sites and have known sources in Coeur D'Alene mining sediments or urban discharge (cadmium, copper, lead, and zinc). I also combined tissue concentrations for the metals most associated with mining sediments in the Spokane River – cadmium, lead, and zinc (Kadlec 2000), through a simple index that developed to give equal weight to each element (Equation 6). First, I calculated the mean tissue concentration for each of these elements. Then for each site, I divided the tissue concentrations by the mean concentration for each element, added the three values (one for each Cd, Pb and Zn), and divided by three. This produced a value that would be equal to 1 if all the tissue concentrations at the site equaled the mean concentrations for all 3 elements. A value > 1 indicated higher concentrations, and < 1 indicated lower concentrations, with all 3 elements weighted equally. I also performed a linear regression of this value ("CLZ Index") against distance downstream from Lake Coeur d'Alene. All regressions were performed with JMP 6.0 (SAS Institute).

Equation 6.

CLZ index value **= ((site cadmium concentration/mean cadmium concentration) + (site lead concentration/mean lead concentration) + (site zinc concentration/mean zinc concentration))/3**

To determine the feasibility of using *Pteronarcys californica* stoneflies for future studies in this river system, I tested whether the concentrations of heavy metals found in *Pteronarcys californica* tissues and those of *Hydropsyche cockerelli* caddisflies are correlated with each other. I performed linear regressions with concentrations of each metal in *H. cockerelli* tissue as the independent variable and concentration of the same metal in *P. californica* tissue as the dependent variable. All regressions were performed with JMP 6.0 (SAS Institute).

To determine if aquifer influx reduced metal bioavailability, I analyzed the differences between the concentrations found in *H. cockerelli* tissue in the upstream gaining and losing reaches, excluding the downstream reach with a simple t-test. These tests were performed with JMP 6.0 (SAS Institute).

Diet Data Analysis

Relative abundances of the algae found in each invertebrate's gut content and the functional food groups generally eaten by *Hydropsyche cockerelli* were determined. Those algal values were compared between *Pteronarcys californica* and *Hydropsyche cockerelli* to determine if they were eating the same foods in similar proportions. Functional food group were determined to see where *Hydropsyche cockerelli* was feeding in the river ecosystem and determine if the where primary consumers. Sample size was not large enough for statistical comparisons.

Results

Habitat

Discharge ranged from 600 to 1200 CFS during the sampling time period with lower CFS near the upstream limit of the study sites (Post Falls, ID, USGS #12419000) and higher CFS near the downstream end of the sites just upstream of the confluence with Hangman Creek (USGS #12422500). Discharge data show substantial inflows into the study reach from aquifer inputs, and a slight decline in discharge during the sampling interval (Figure 2).

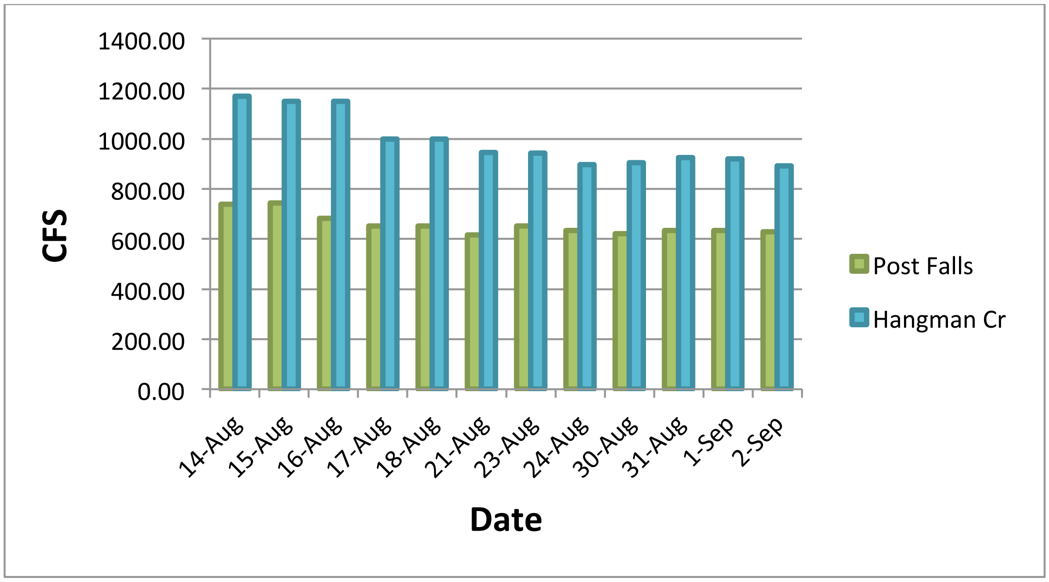

Figure 2. Discharge of the Spokane River in cubic feet per second (CFS) at closest USGS stations to the study area, during invertebrate sampling September 2010. Post Falls, ID is just upstream of the first upstream losing reach, and the Hangman Creek confluence is within the downstream study area.

There was substantial variation among sites in mean water velocities measured at the substrate, but no consistent variation among reaches. Sites with both the highest and lowest mean water velocities were found in the downstream reach with riffle D4's average flow rate at 1.26 m/s and D6's average flow rate at 0.15 m/s (Figure 3).

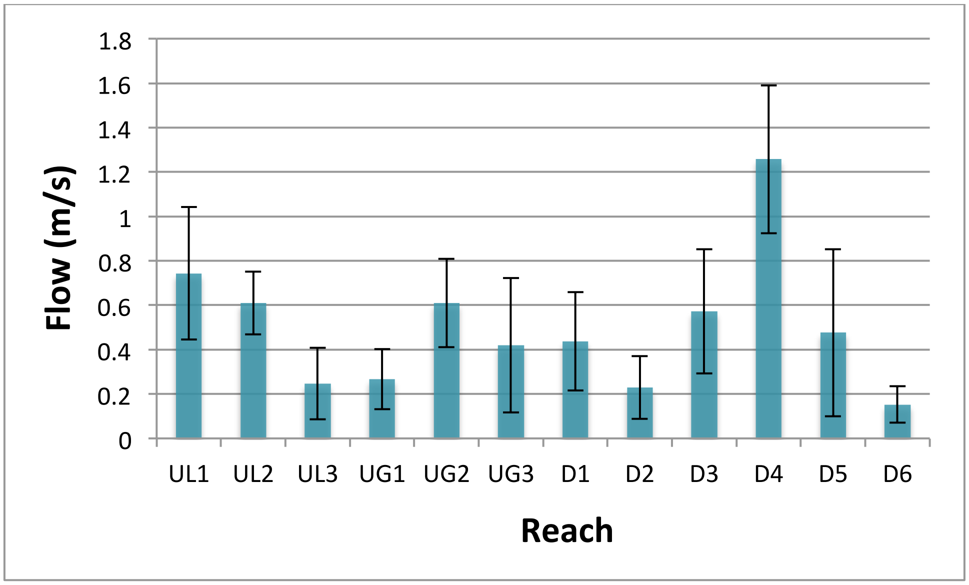

Figure 3. Mean water velocity (m/s ± SD) over cobbles sampled for invertebrates at each study site, Spokane River, WA, August - September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

Mean sampled particle sizes were similar among the sites, indicating that stratified sampling was effective in controlling particle size (Figure 4).

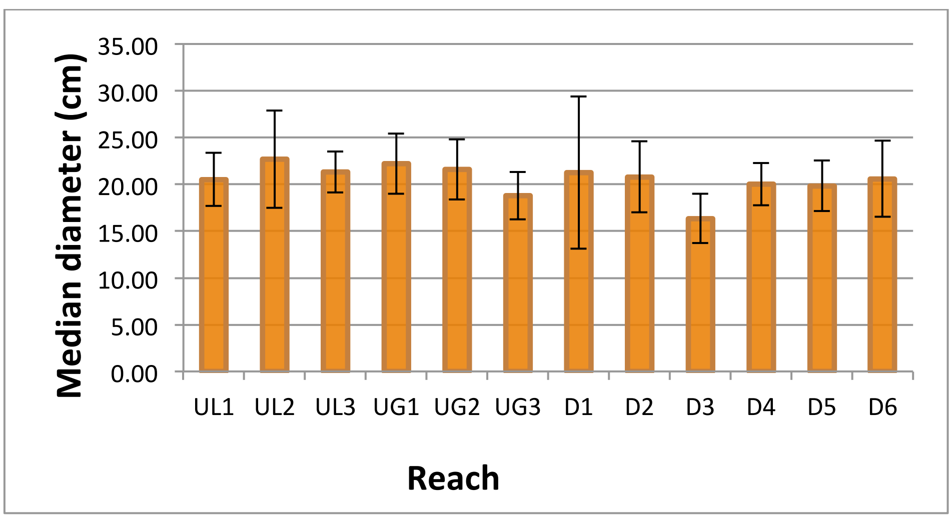

Figure 4. Mean median diameter (cm) of cobbles sampled for invertebrates in each study site, Spokane River, WA, August - September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

Water Quality

Dissolved oxygen, pH and conductivity values for the Spokane River were within a normal range during the sampling period (Table 3).

Table 3. Spokane River (WA) Dissolved Oxygen (mg/L), pH, and Conductivity (umhos/cm) data collected by Washington Department of Ecology at Riverside State Park on closest available dates before and after macroinvertebrate samples were collected in 2010. Riverside State Park is < 0.5 km downstream of most downstream site sampled.

Date	8/16/10	9/20/10
Oxygen (mg/L)	7.8	9.5
PH	7.7	7.1
Cond. (umhos/cm)	60	N/A

Water temperatures clearly demarcate aquifer influence; mean temperatures for upstream and downstream gaining reaches were 14.5 +/- 2.4 (SD) ˚C and 14.8 +/- 0.7 (SD)˚C, respectively. Mean water temperatures for upstream losing reaches were 19.3 +/- 1 (SD) ˚C (Figure 5).

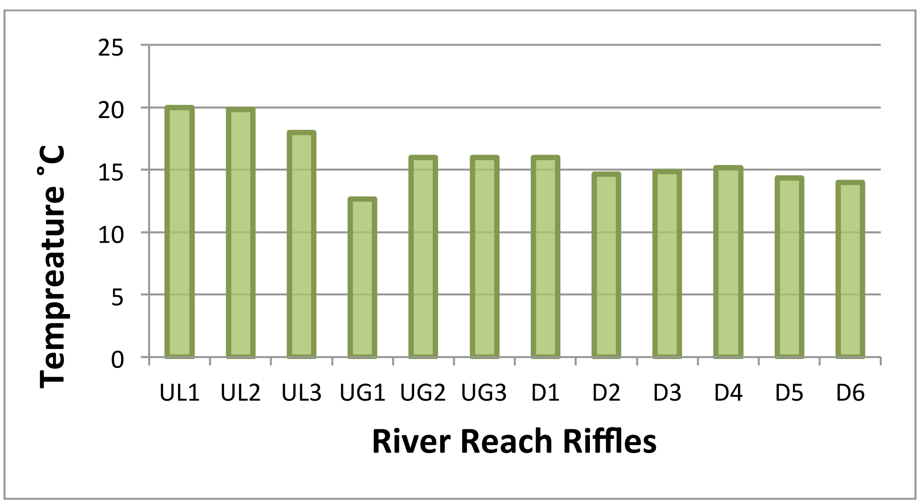

Figure 5. Water temperature at each of the 12 sample sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of

Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

Dissolved nutrient concentrations, including orthophosphate, ammonium, nitrite, and nitrate, were generally low at all study sites, with the exception of nitrate levels at site D6 below the outflow of treated wastewater from the Riverside Park Water Reclamation Facility (Table 4, Figure 6, Figure 7, Figure 8, Figure 9). More samples would have to be taken to determine if these concentrations are consistently high. There were no consistent differences in orthophosphate, ammonium, or nitrite concentrations among upstream losing, upstream gaining, or downstream reaches (Figure 6, Figure 7, Figure 9). However, nitrate levels consistently increased downstream, with the lowest levels in the upstream losing reach, and the highest levels in the downstream reach, even excluding site D6 (Figure 8).

Table 4. Nutrient Data: Phosphate (ppb), Nitrite (ppb), Nitrate (ppb) and Ammonium (ppb) at each of the 12 study sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

Reach Name	Phosphate (ppb)	Nitrite (ppb)	Nitrate (ppb)	Ammonium (ppb)
UL1	5.6	14	140	3.8
UL2	damaged	12	118	3.8
UL3	9.8	11	109	damaged
UG1	3.7	10	560	1.8
UG2	4.7	11	666	1.6
UG3	0.9	15	531	1.1
D1	< detection limit	16	812	0.6
D2	1.8	14	821	1.5
D3	1.4	12	801	2.1
D4	< detection limit	16	727	damaged

D5	damaged	14	868	2
D6	8.4	16	6528	3.4

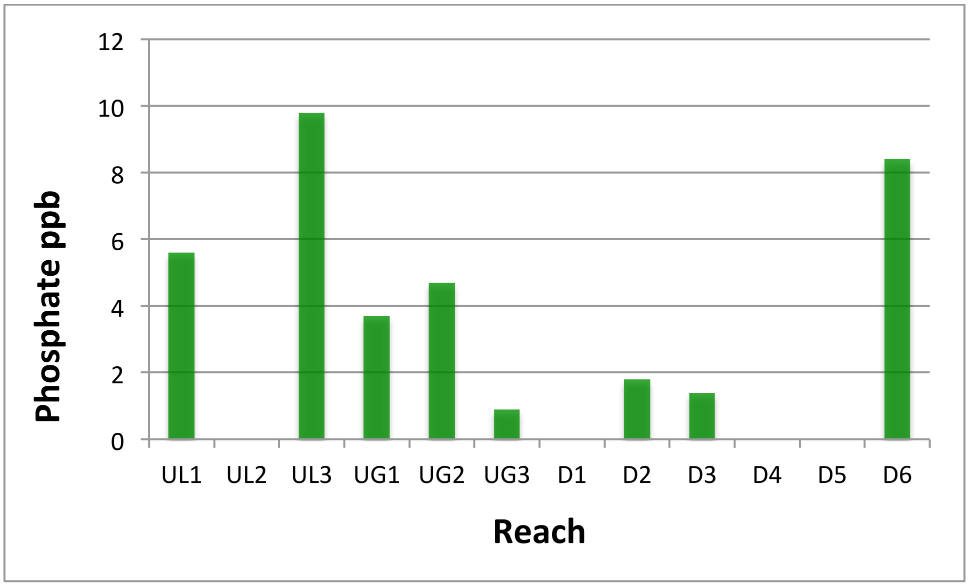

Figure 6. Phosphate (ppb) at each of the 12 sample sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

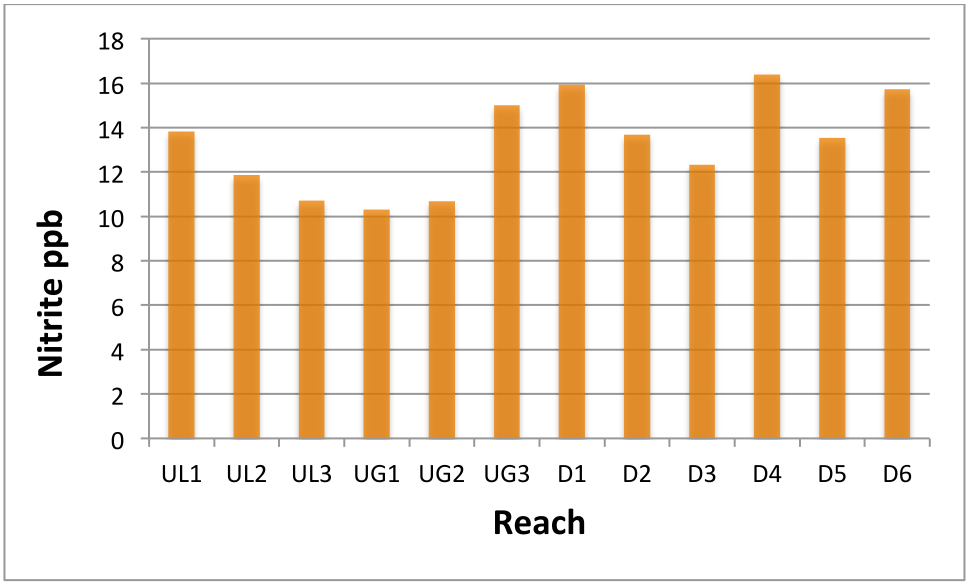

Figure 7. Nitrite (ppb) at each of the 12 sample sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

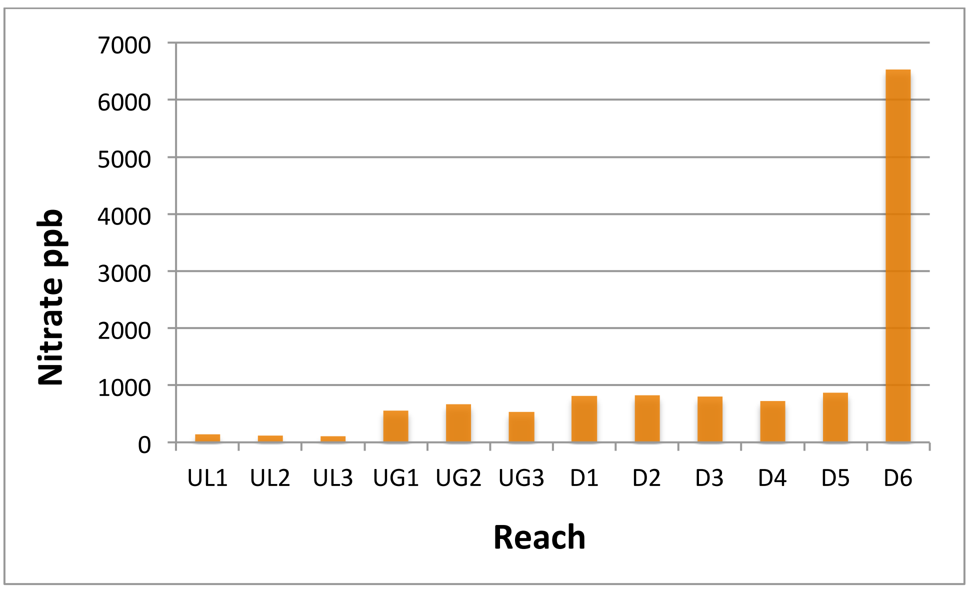

Figure 8. Nitrate (ppb) at each of the 12 sample sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

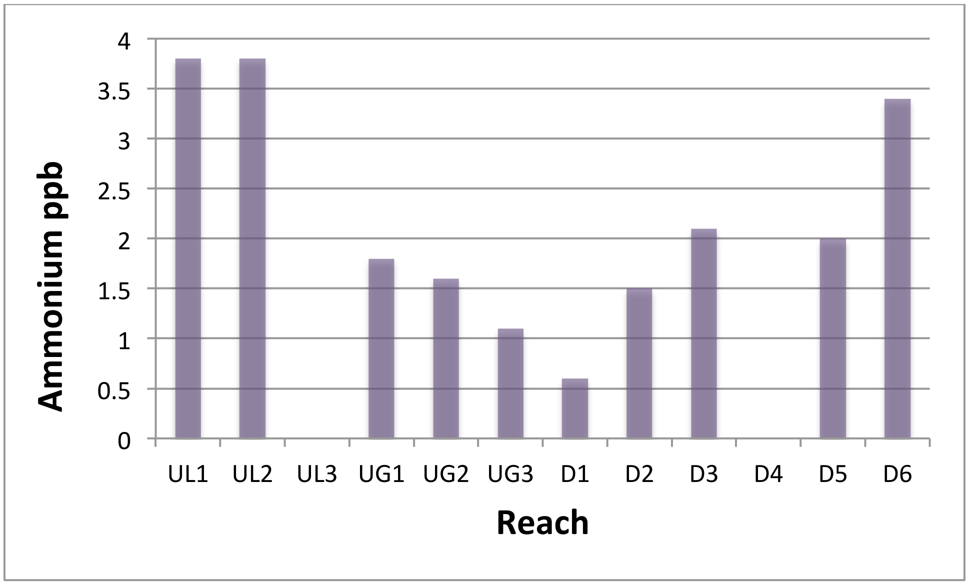

Figure 9. Ammonium (ppb) at each of the 12 sample sites on the date they were sampled (August-September 2010). D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

Macroinvertebrates

Density

Densities of macroinvertebrates generally decrease with increased anthropogenic perturbation, but can increase with excess nutrients or organic enrichment (see Table 3). The **density (total # invertebrates/m^2) of macroinvertebrates** was similar at all study sites with the exception of UG2 (Figure 10). Substrates at this site were densely covered with aquatic moss, which may have substantially increased available colonization area for invertebrates. There were no significant differences in macroinvertebrate densities among the sample reaches (UL, UG, D, Figure 11, Table 5).

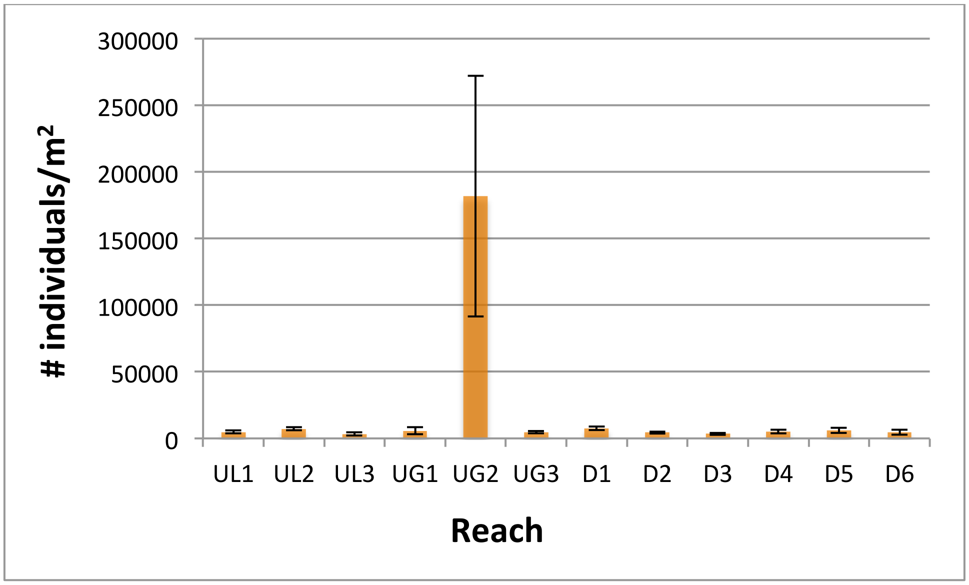

Figure 10. Mean (± SE) macroinvertebrate density on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

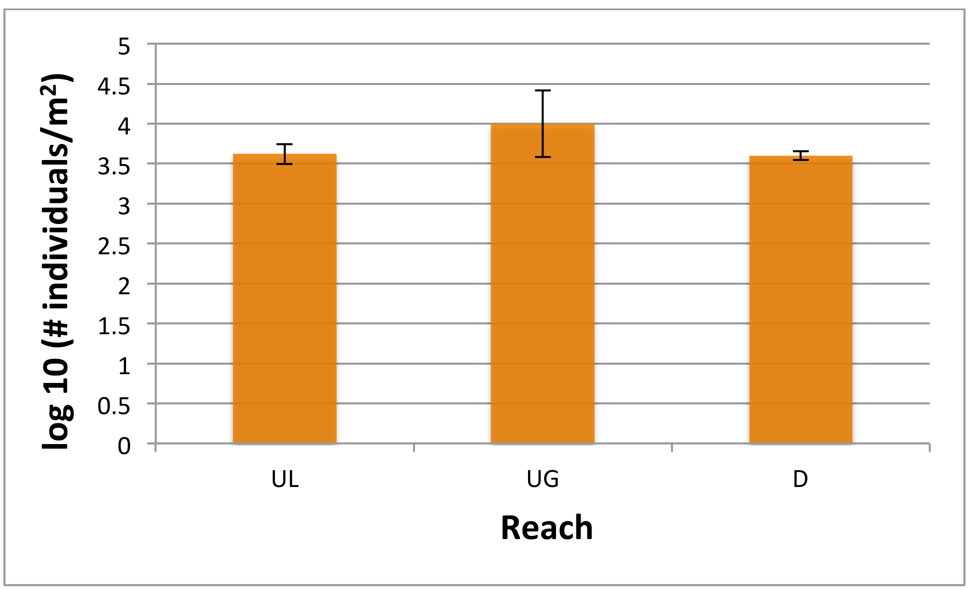

Figure 11. Mean (± SE) log-transformed macroinvertebrate density for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates upstream reaches receiving inputs of aquifer flow (n=3), and UL indicates upstream site not receiving aquifer flow (n=3).

Table 5. ANOVA of reach means for log-transformed macroinvertebrate density comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was $\log_{10}$ (invertebrates/m^2), and the independent variable was reach type.

Source	df	SS	MS	F	p
Reach type	2	0.339	0.169	1.23	0.33
Residual	9	1.230	0.137		
Total	11	1.569			

Richness

Family taxa richness generally increases with increasing water quality, habitat diversity, and habitat suitability and is an effective metric in detecting effects of toxic trace metals (see Table 3). The mean number of invertebrate families per sample (family richness) was significantly higher in the downstream reach compared to the upstream gaining and losing reaches. There was no significant difference between upstream losing and gaining reaches, suggesting aquifer inputs did not affect family richness (Figures 12, Table 6).

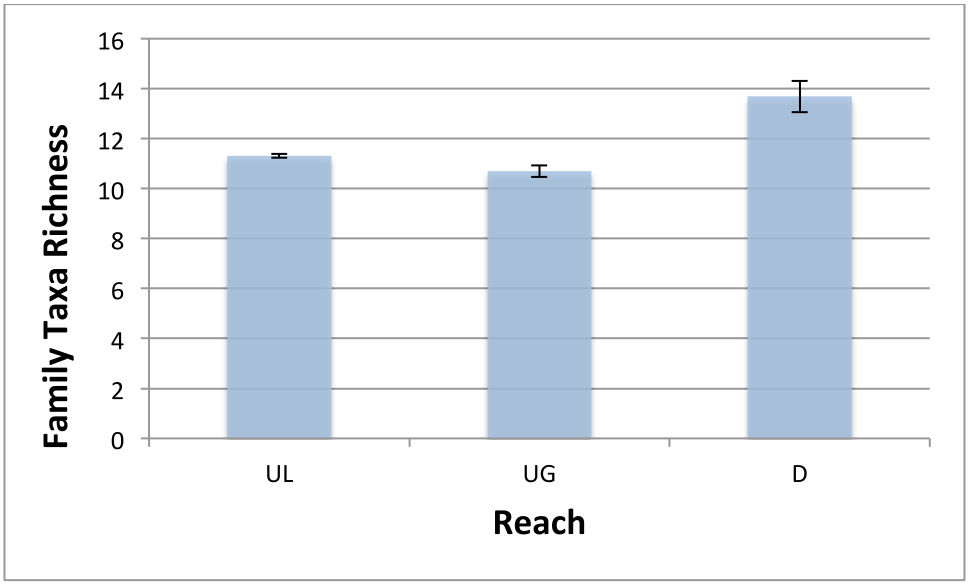

Figure 12. Mean (± SE) family taxa richness for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 6. ANOVA of reach means for family taxa richness comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean number of families per sample and the independent variable was reach type. Tukey test ($p<0.05$, $q^*=2.79$, least significant difference=1.86) comparing the three reach types found that the downstream reaches had significantly higher family richness than either upstream reach type. There was no significant difference between upstream gaining and upstream losing reaches.

Source	df	SS	MS	F	P
Reach type	2	22.1	11.07	8.28	<0.01
Residual	9	12.0	1.34		
Total	11	34.2			

Ephemeroptera/Plecoptera/Trichoptera (EPT) family richness is the number of taxa of mayflies, stoneflies, and caddisflies. These taxa are largely intolerant to poor water quality and are particularly sensitive to toxic trace metals and urbanization (Table 3, Clements et al. 2013). Higher EPT values are thought to exemplify a healthy benthic community with an EPT value of 30 being found on the St. Joe River at Calder (ID), a least impacted site in the watershed (USGS 2003). All Spokane river sites had low numbers of EPT taxa with the highest mean # of taxa per sample being 8.0 at downstream site D1 (Figure 13). There also was a significant ($p<0.001$) difference between upstream sampling sites' mean EPT scores (4.6 and 4.8) and the mean for the downstream sampling sites (6.9). There was no significant difference between the upstream gaining and losing reaches (Figure 14, Table 7)

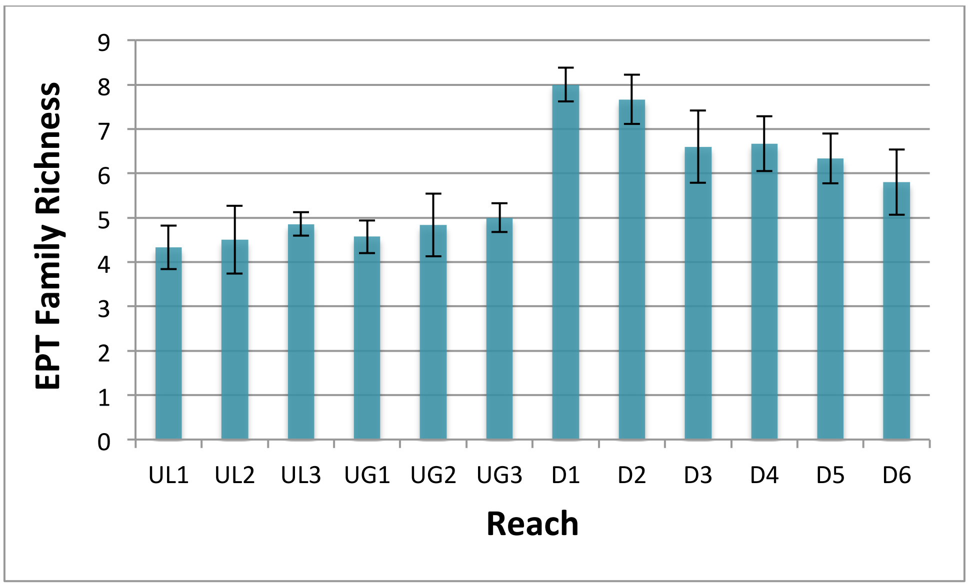

Figure 13. Mean (± SE) of EPT (Ephemeroptera, Plecoptera, and Trichoptera) family richness on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

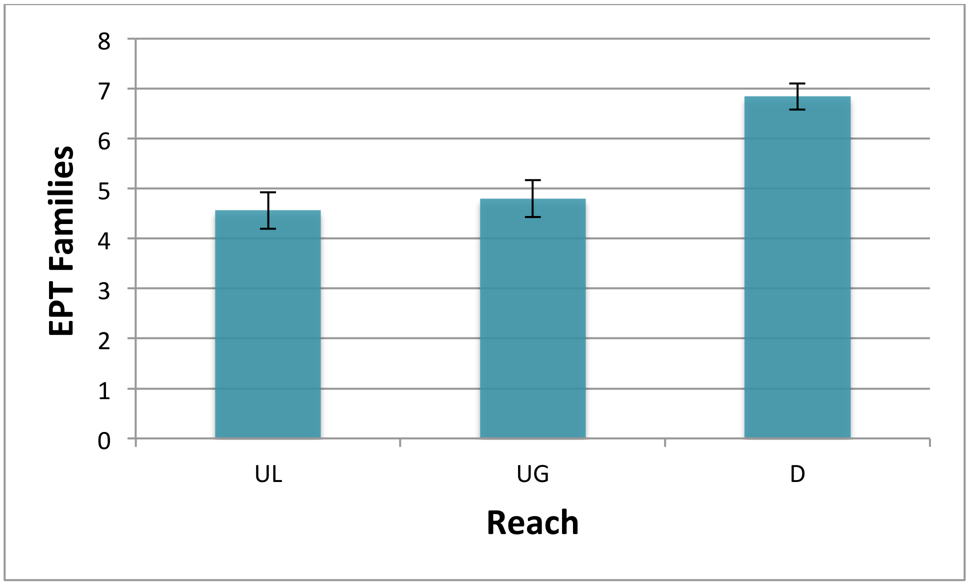

Figure 14. Mean (± SE) EPT (Ephemeroptera, Plecoptera, and Trichoptera) family richness for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 7. ANOVA of reach means for EPT (Ephemeroptera, Plecoptera, and Trichoptera) family richness comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing) The dependent variable for this analysis was the mean number of EPT families per sample and the independent variable was reach type Tukey test ($p<0.05$, $q^*=2.79$, least significant difference=1.03) comparing the three reach types found that the downstream reaches had significantly higher EBT richness than either upstream reach type. There was no significant difference between upstream gaining and upstream losing reaches.

Source	df	SS	MS	F	P
Reach type	2	14.11	7.055	17.2	<0.001
Residual	9	3.69	0.410		
Total	11	17.80			

Richness of the important Diptera family Chironomidae is also sensitive to trace metal contaminants (Table 3). Chironomidae were identified to genus for a subset of eight samples (out of a total of 80) by EcoAnalysts (Moscow, ID). Among these samples, Chironomidae richness varied from 5 to 14 genera. However, there was no significant difference between study reaches (1-Way ANOVA, total DF = 7, F = 0.652, P = 0.56, Figure 15).

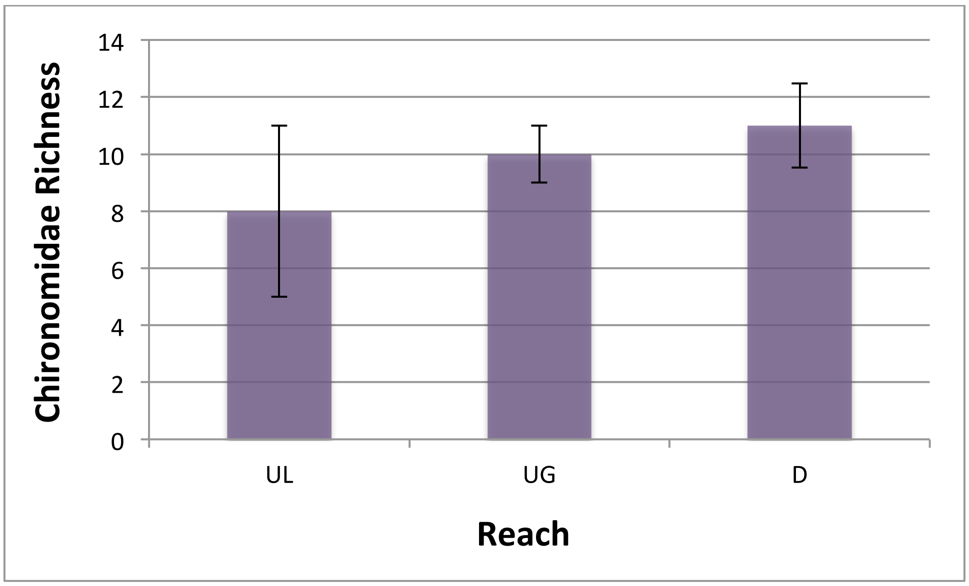

Figure 15. Mean (± SE) Chironomidae richness for samples collected upstream and downstream of the City of Spokane, August-September 2010. D indicates sampling downstream of the City of Spokane (n=4), UG indicates samples from upstream reaches receiving inputs of aquifer flow (n=2), and UL indicates samples from upstream reaches not receiving aquifer flow (n=2).

Dominance

Taxa that can tolerate poor conditions often thrive under those conditions, and may make up a high proportion of the number of individuals present. The proportion of individuals made up of the single most abundant taxon (proportion dominant taxon) typically increases with anthropogenic impacts and is sensitive to trace metal contamination (Table 3). The dominant taxa in this study consisted of the Dipteran families Chironomidae and Simuliidae, the Trichopteran family Hydropsychidae, and the Ephemeropteran family Baetidae, all taxa tolerant of metal contamination and tolerant or moderately tolerant of other anthropogenic impacts. For most sites, the dominant taxon made up a high proportion of the individuals, ranging from 0.37 (UL1) to 0.78 (UG2, Figure 16). Upstream gaining sites, located within Spokane Valley, had significantly higher mean proportions dominant taxon than either upstream losing sites or downstream sites (Figure 17, Table 8).

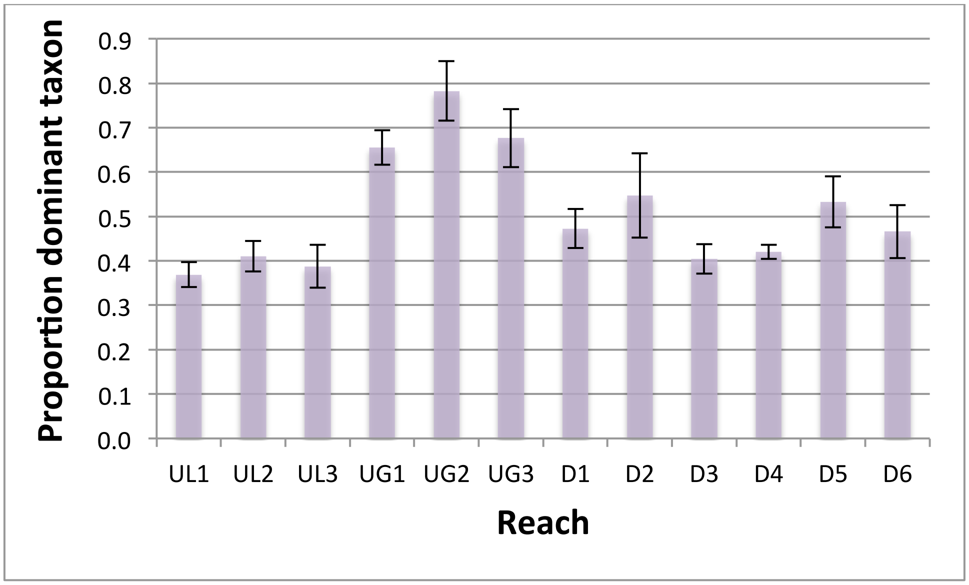

Figure 16. Mean (± SE) proportion dominant taxon on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a reach downstream of the City of Spokane, UG indicates an upstream reach receiving inputs of aquifer flow, and UL indicates an upstream reach not receiving aquifer flow.

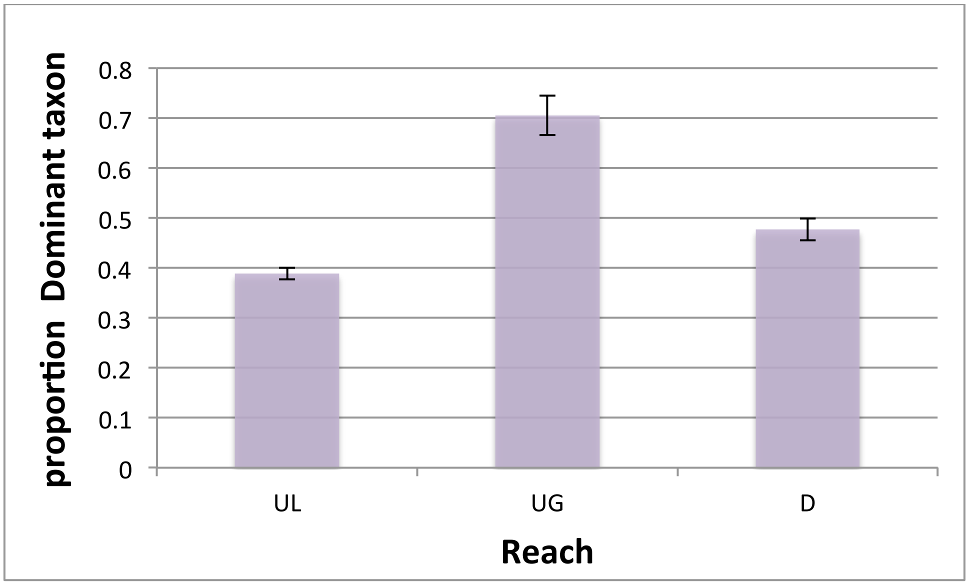

Figure 17. Mean (± SE) proportion dominant taxon for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 8. ANOVA of reach means for transformed proportion dominant taxon, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). Tukey test (p<0.05, q*2.79, least significant difference=0.136) comparing the three reach types found that the upstream gaining reaches had significantly higher proportion dominant taxon compared to upstream losing reaches and downstream reaches. There was no significant difference between upstream losing reaches and downstream reaches.

Source	df	SS	MS	F	P
Reach type	2	0.2473	0.1237	11.3	<0.01
Residual	9	0.0985	0.0109		
Total	11	0.3458			

Community Composition

Proportion EPT referrers to the proportion of the total number of individuals made up of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). These macroinvertebrates are mostly intolerant to anthropogenic perturbation and > 0.5 proportion EPT is considered representative of a healthy community (Barbour et al. 1999; Lydy et al. 2000). Mean proportion EPT varied from about 0.2 at site UG2 to almost 0.8 at site D3, with most sites above the 0.5 criterion (Figure 18). Unlike the EPT richness, proportion EPT did not vary significantly with reach type (Table 9, Figure 19). Most EPT individuals were mayflies in the family Baetidae and caddisflies in the family Hydropsychidae, which are relatively tolerant of anthropogenic impacts and metal pollution. I also analyzed the relative abundance of mayflies and caddisflies separately. Stoneflies were relatively scarce and did not occur in all samples.

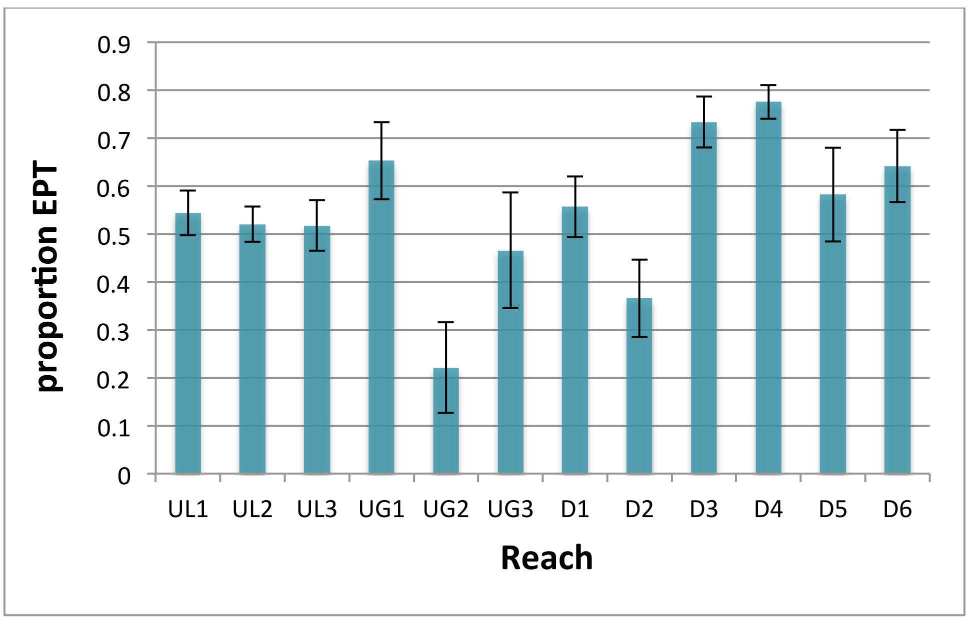

Figure 18. Mean (± SE) proportion Ephemeroptera/Plecoptera/Trichoptera (EPT) on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a reach downstream of the City of Spokane, UG indicates an upstream reach receiving inputs of aquifer flow, and UL indicates an upstream reach not receiving aquifer flow.

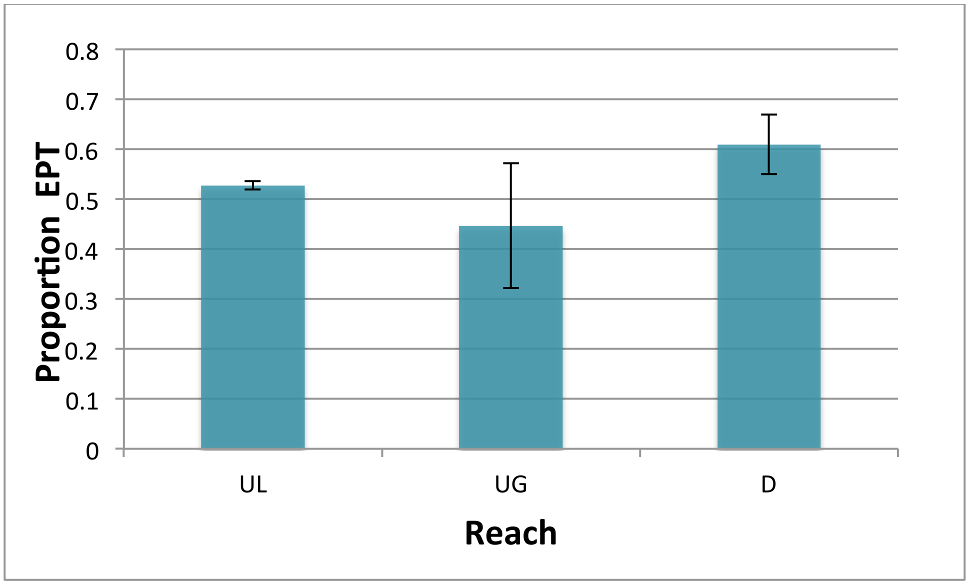

Figure 19. Mean (± SE) proportion Ephemeroptera/Plecoptera/Trichoptera (EPT) for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 9. ANOVA of reach means for transformed proportion EPT (Ephemeroptera, Plecoptera, and Trichoptera) comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean arcsin (square root) transformed proportion of the total number of individuals made up of EPT taxa for each reach. The independent variable was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.0307	0.0154	0.417	0.67
Residual	9	0.3310	0.0368		
Total	11	0.3618			

Only 2 species of Ephemeroptera were found in all 80 samples analyzed: *Baetis tricaudatus and Acentrella insignificans,* both in the family Baetidae. The very low mayfly diversity is a strong indicator of metal toxicity. The proportion of individuals made up of these mayflies was highly variable, ranging from < 0.1 at site D2 to >0.5 at site UG1 (Figure 20). There was no significant variation among reach types in the proportion Ephemeroptera (Table 10, Figure 21).

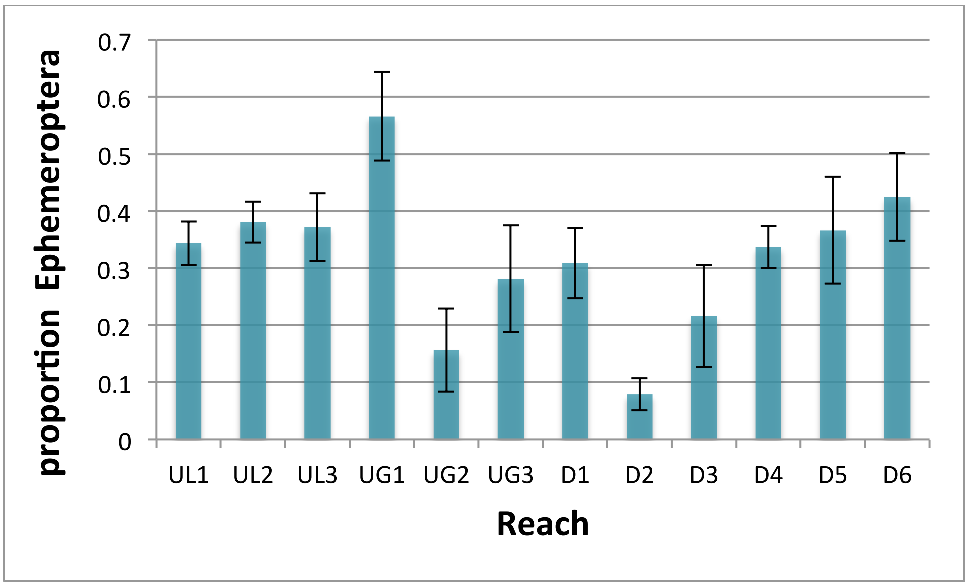

Figure 20. Mean (± SE) proportion Ephemeroptera on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

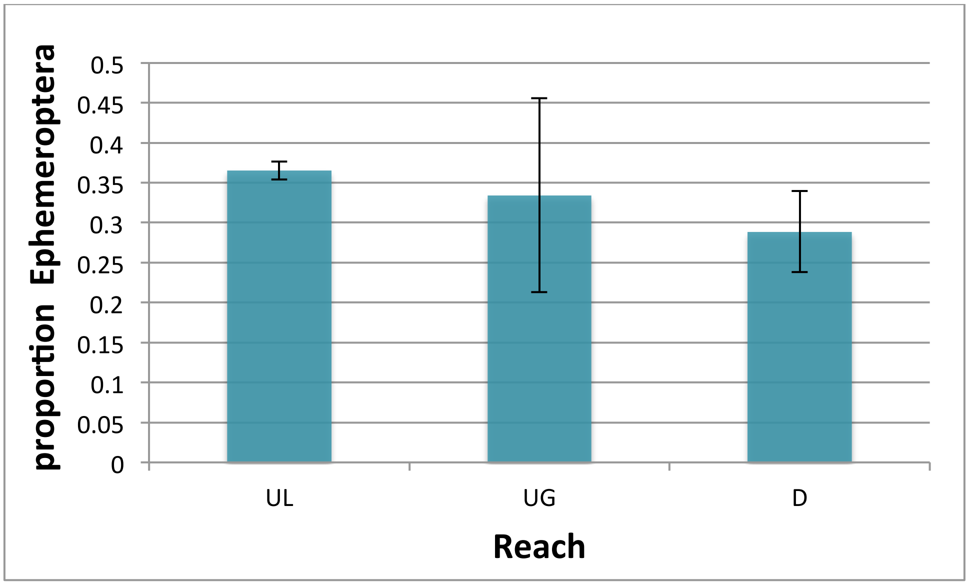

Figure 21. Mean (± SE) proportion Ephemeroptera for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 10. ANOVA of reach means for transformed proportion Ephemeroptera, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean of arcsin (square root) transformed proportion of the total number of individuals made up of Ephemeroptera taxa for each site. The independent variable was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.0238	0.0119	0.421	0.67
Residual	9	0.2543	0.0283		
Total	11	0.2781			

More diversity was present among Trichoptera than Ephemeroptera. Nine genera of Trichoptera were collected among all sites, representing 7 families. By far the most abundant Trichoptera were the relatively tolerant (particularly to metals) family Hydropsychidae. The proportion Trichoptera ranged from .063 at site UG2 to .496 at site D3 (Figure 22). In the downstream reach the proportion Trichoptera was significantly higher than in the upstream gaining or losing reach (Table 11, Figure 23). Proportion Trichoptera has been observed to decrease with increased metal pollution indicating the downstream gaining reach was least affected by metal pollution (Table 3). There was no significant difference between upstream gaining reaches and upstream losing reaches.

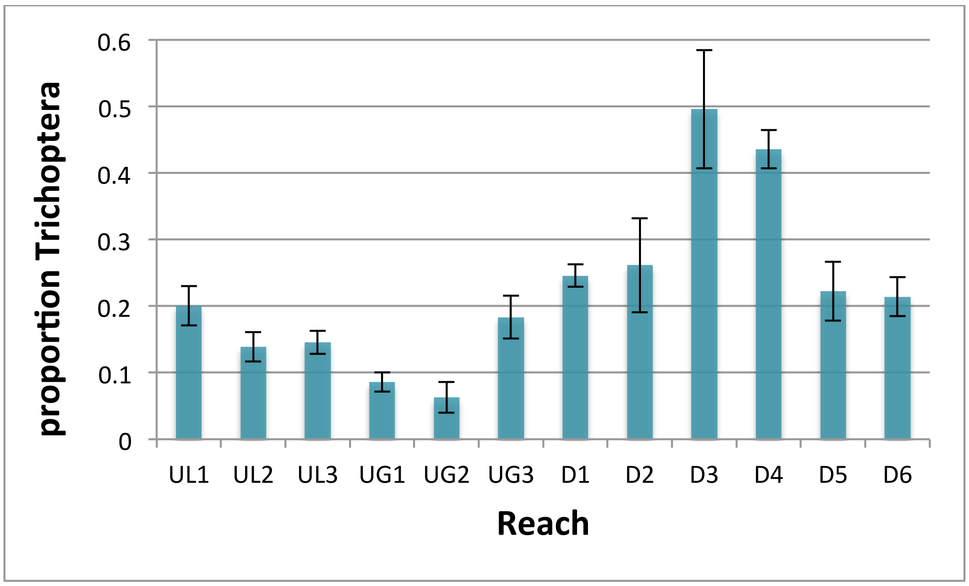

Figure 22. Mean (± SE) proportion Trichoptera on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

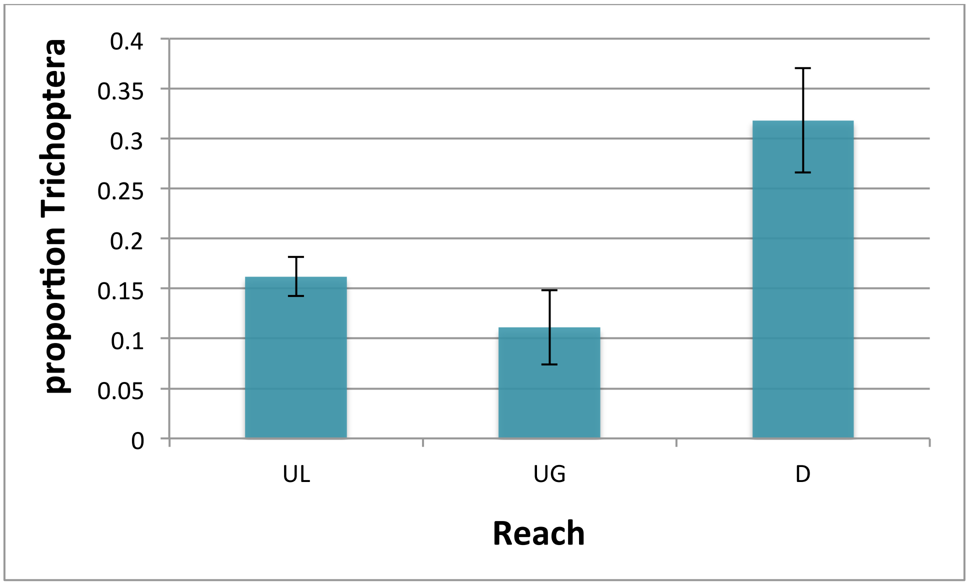

Figure 23. Mean (± SE) proportion Trichoptera for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 11. ANOVA of reach means for transformed proportion Trichoptera, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean of arcsin (square root) transformed proportion of the total number of individuals made up of Trichoptera for each reach. The independent variable was reach type. Tukey test ($p<0.05$, $q^*=2.79$, least significant difference=0.0214) comparing the three reach types found that the upstream gaining reaches had significantly lower proportion Trichoptera compared to downstream reaches. There was no significant difference between upstream gaining and upstream losing reaches, or between downstream reaches and upstream losing reaches.

Source	df	SS	MS	F	P
Reach type	2	0.166	0.832	6.08	0.0214
Residual	9	0.123	0.137		
Total	11	0.290			

In contrast with EPT taxa, the proportion Diptera, particularly families Chironomidae and Simuliidae, generally increases with anthropogenic perturbation (Table 3). Proportion Diptera ranged from < 0.2 at sites D4 to almost 0.8 at site UG2 (Figure 24). However, there were no significant differences in the proportion Diptera among the three study reaches (Table 12, Figure 25).

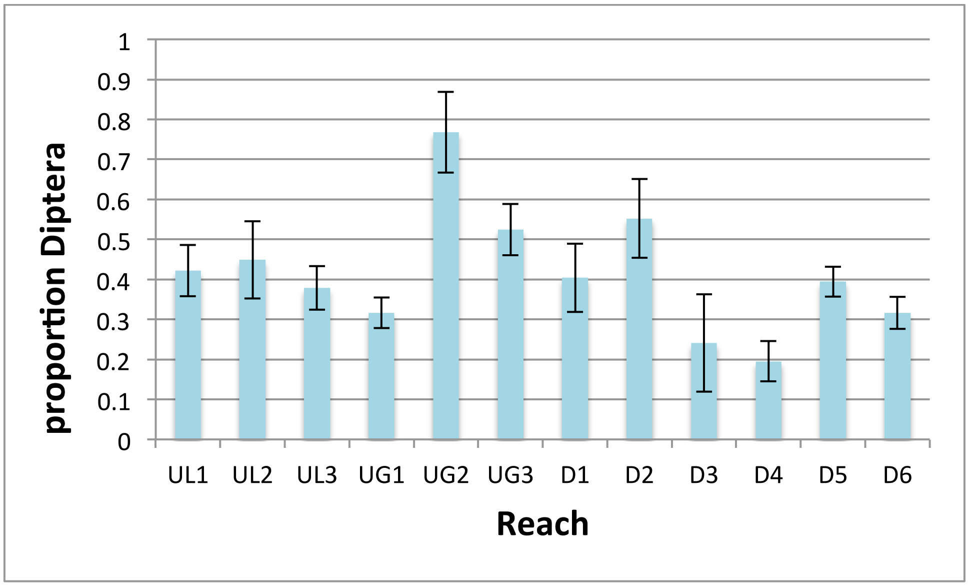

Figure 24. Mean (± SE) proportion Diptera on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a reach downstream of the City of Spokane, UG indicates an upstream reach receiving inputs of aquifer flow, and UL indicates an upstream reach not receiving aquifer flow.

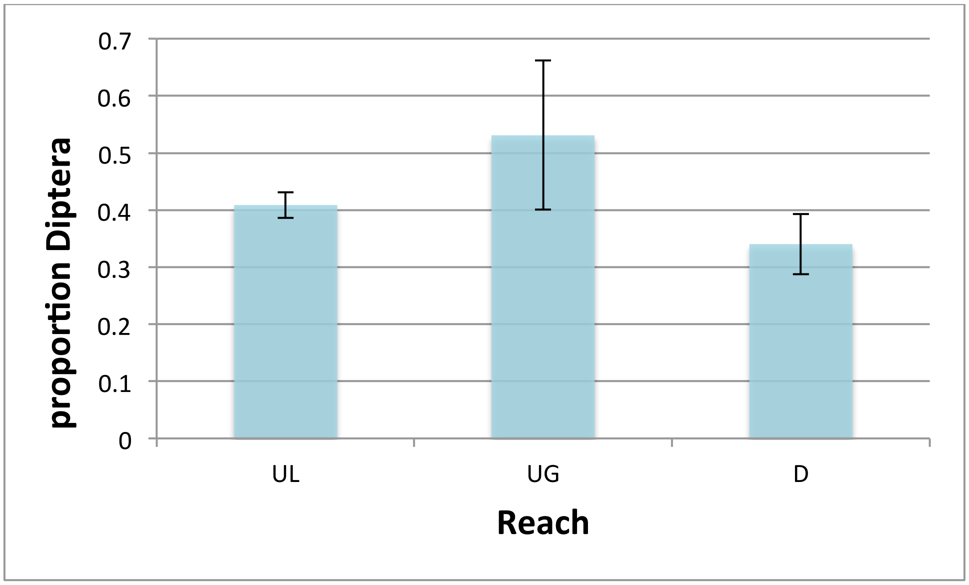

Figure 25. Mean (± SE) proportion Diptera for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 12. ANOVA of reach means for transformed proportion Diptera comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean of arcsin (square root) transformed proportion of the total number of individuals made up of Diptera taxa for each reach. The independent variable was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.0896	0.0448	1.71	0.23
Residual	9	0.2356	0.0262		
Total	11	0.3252			

Proportion Chironomidae ranged from .083 at site UL2 to .719 at site UG2 (Figure 26). The upstream gaining reach had a significantly (p=0.04) higher proportion Chironomidae compared to the upstream losing reach (Figure 27, Table 13). There was no significant difference between the upstream gaining reach and the downstream reaches or the downstream reaches and upstream losing reaches. This is the opposite of the pattern expected due to water temperature alone, which generally increases the abundance of multivoltine species such as many Chironomidae. Effects of water temperature, in absence of other factors, would predict a lower proportion Chironomidae in the colder upstream gaining reach compared to the warmer upstream losing reach. The high relative abundance of Chironomids in the upstream gaining reach may indicate stronger anthropogenic impacts in this reach.

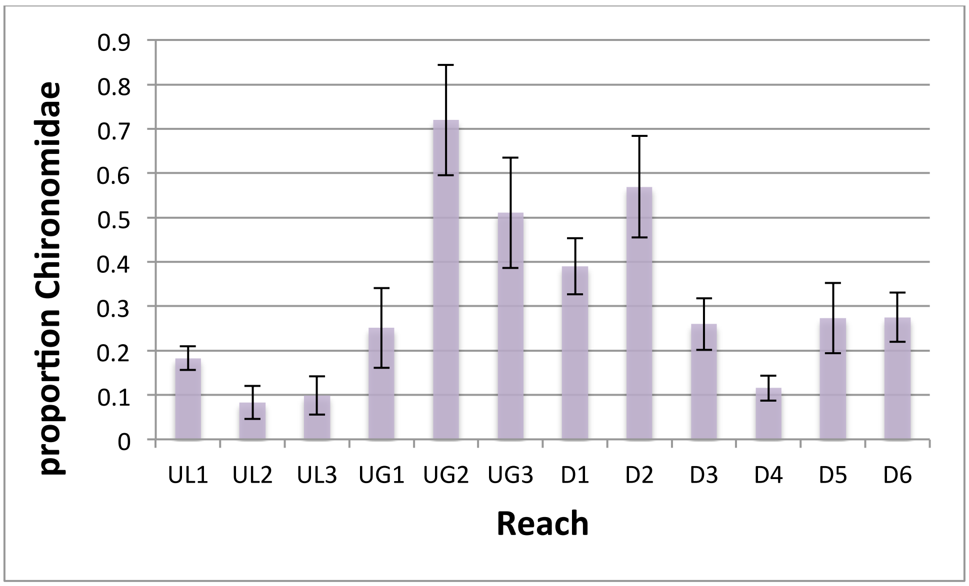

Figure 26. Mean (± SE) proportion Chironomidae on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

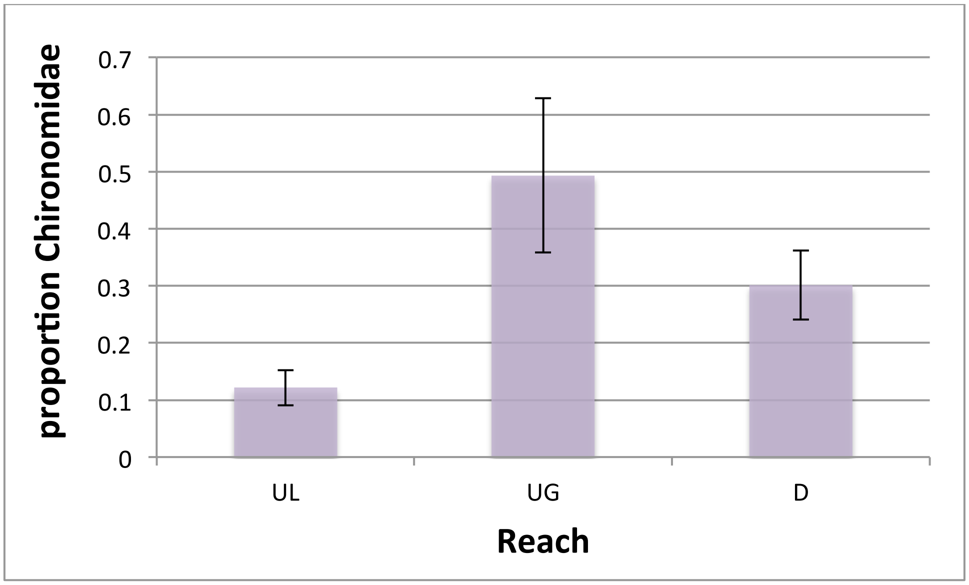

Figure 27. Mean (± SE) proportion Chironomidae for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 13. ANOVA of reach means for transformed proportion Chironomidae, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean arcsin (square root) transformed proportion of the total number of individuals made up of Chironomidae for each reach. The dependent variable was reach type Tukey test ($p<0.05$, $q^*=2.79$, least significant difference=0.423) comparing the three reach types found that the upstream gaining reaches had significantly higher proportion Chironomidae compared to upstream losing reaches. There was no significant difference between gaining reaches and downstream reaches, or between downstream reaches and upstream losing reaches.

Source	df	SS	MS	F	P
Reach type	2	0.306	0.1529	4.45	0.0453
Residual	9	0.309	0.0344		
Total	11	0.615			

Urbanization effects – Proportion clingers and filterer richness

The proportion of the invertebrate community that could be classified in the functional group "clingers" (**proportion clingers**) and the number of taxa in the functional group "filterers" (**filterer richness**) were used as specific indicators of urbanization impacts, following Purcell, et al. (2009). Proportion clingers ranged from .117 at site UG2 to .537 at site D4 (Figure 28). The upstream gaining reach had the lowest proportion clingers compared to upstream losing reaches and downstream reaches (Figure 29, Table 14), suggesting this reach may have been most impacted by urbanization. There was no significant difference between the upstream losing reaches and downstream reaches. Filterer richness ranged from 1 to 4 taxa per sample, with most samples containing 3 taxa. Hydropsychid caddisflies were the most abundant filterers, followed by Simuliid dipterans. There was little variation among sites in mean filterer richness and no significant differences in mean filterer richness among the study reaches (Figure 30, Figure 31, Table 15).

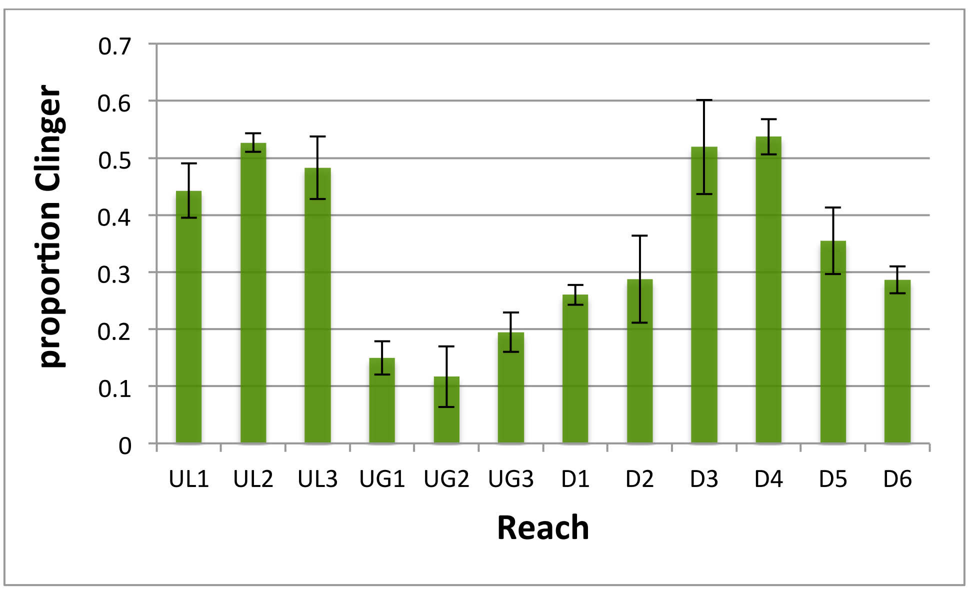

Figure 28. Mean (± SE) proportion Clinger on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

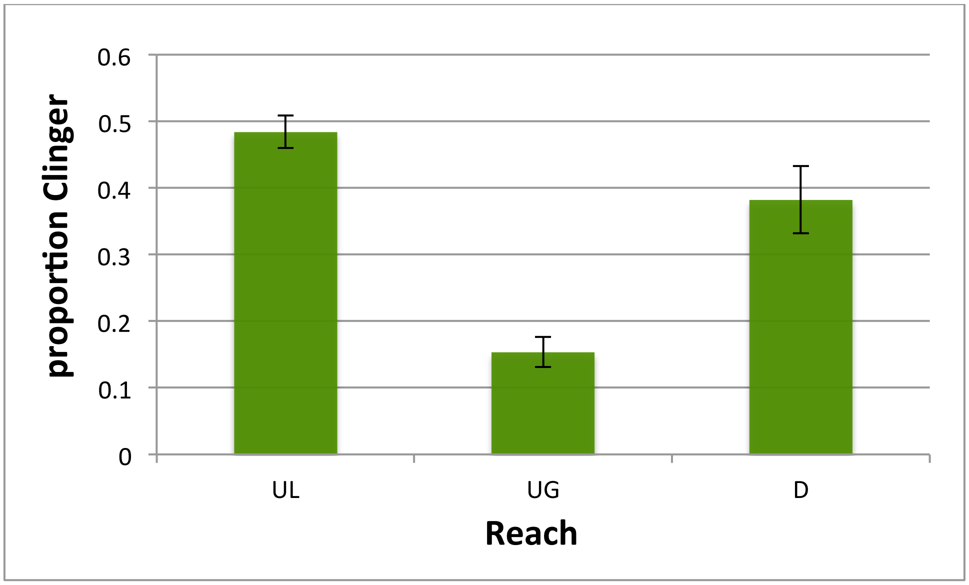

Figure 29. Mean (± SE) proportion Clinger for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 14. ANOVA of reach means for transformed proportion Clingers, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean of arcsin (square root) transformed proportion of the total number of individuals made up of the Clinger functional group for each reach. The independent variable was reach type. Tukey test (P<.05, q*=2.79, least significant difference=0.238) comparing the three reach types found that the upstream gaining reaches had significantly lower proportion Clingers compared to upstream losing reaches and downstream reaches. There was no significant difference between upstream losing reaches and downstream reaches.

Source	df	SS	MS	F	P
Reach type	2	0.2473	0.1237	11.3	<0.01
Residual	9	0.0985	0.0109		
Total	11	0.3458			

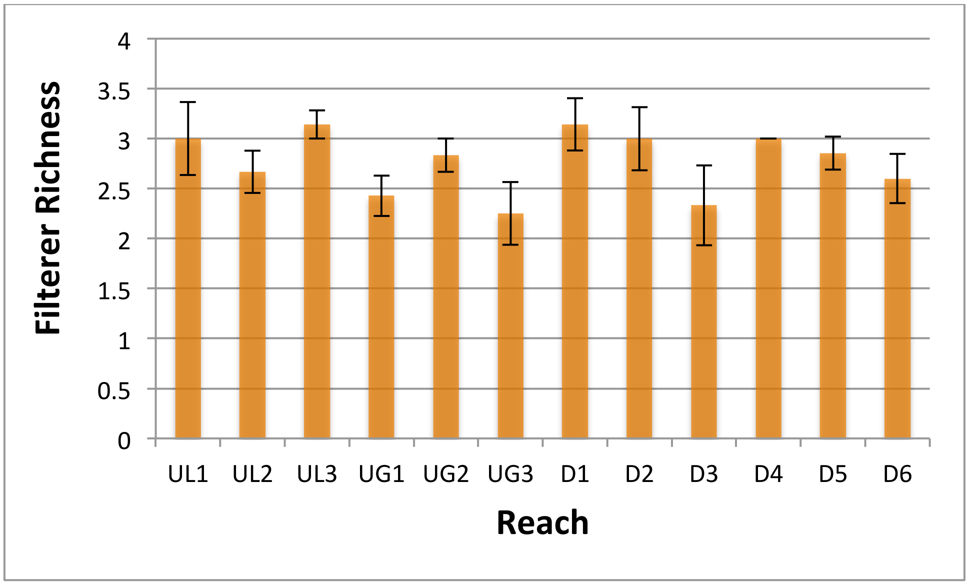

Figure 30. Mean (± SE) Filterer Richness on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

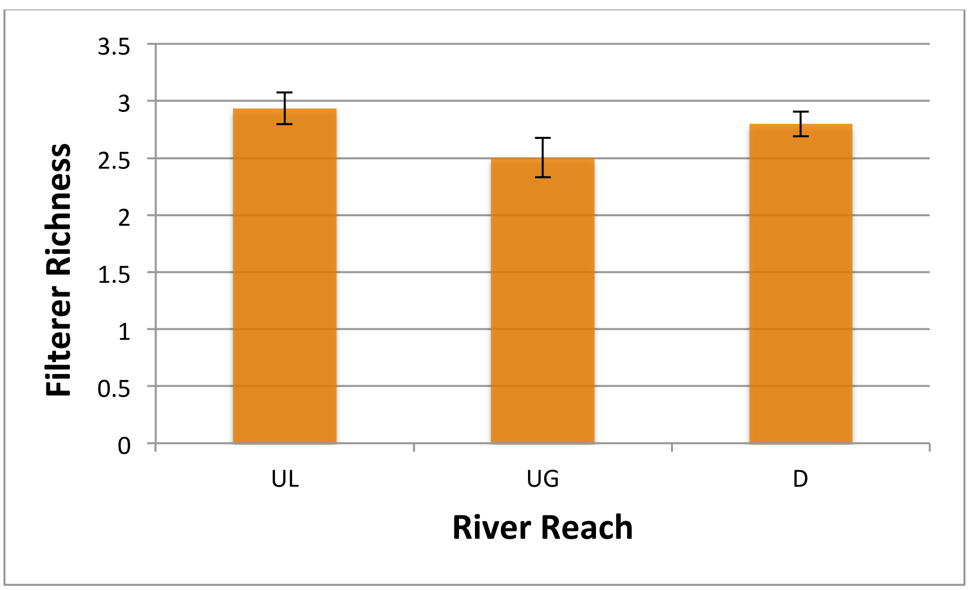

Figure 31. Mean (± SE) Filterer Richness for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 15. ANOVA of reach means for Filterer richness comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing) the dependent variable for this analysis was the mean number of families classified in the Functional Feeding Group per sample and the independent variable was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.301	0.150	2.08	0.18
Residual	9	0.650	0.72		
Total	11	0.951			

Diversity and Biotic Indices

Shannon-Weaver Diversity is a community diversity index; higher values correspond to greater diversity. Values decrease with increasing anthropogenic perturbation (Table 3). The upstream gaining reaches had significantly ($p<0.001$) lower Shannon-Weaver Diversity than either upstream losing reaches or downstream reaches (Figure 32, Figure 33, Table 16). There was no significant difference between upstream losing reaches and downstream reaches.

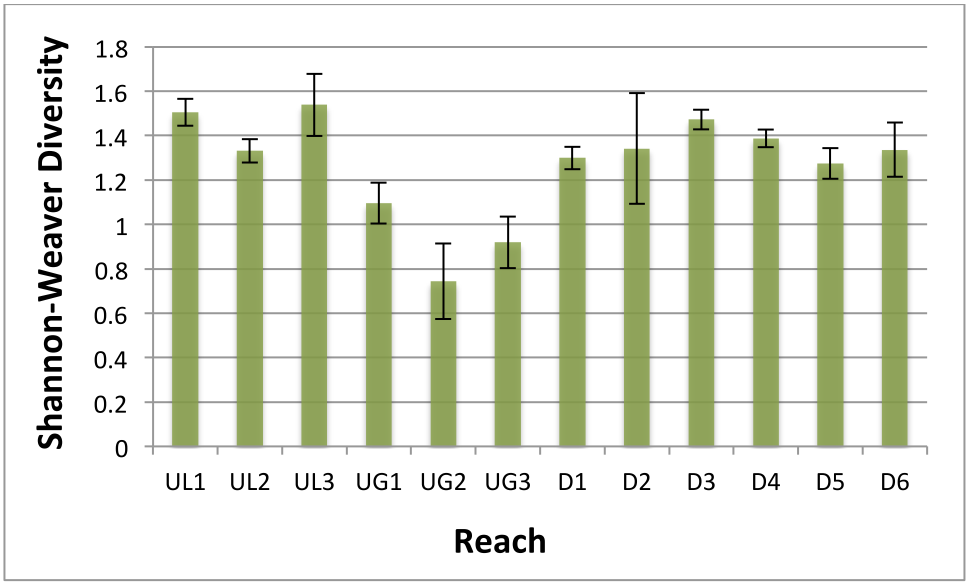

Figure 32. Mean (± SE) Shannon-Weaver Diversity on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

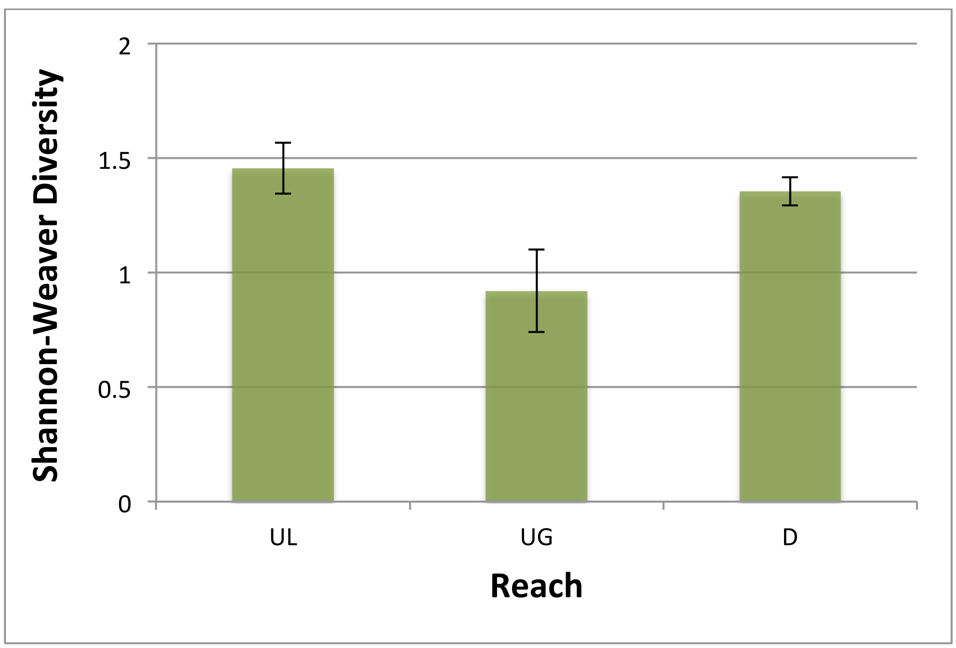

Figure 33. Mean (± SE) Shannon-Weaver H' (log 2) diversity index values for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 16. ANOVA of reach means for Shannon-Weaver Diversity comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean Shannon-Weaver Diversity score for each reach and the independent variable was reach type. Tukey test ($p<0.05$, $q^*=2.79$, least significant difference=0.249) comparing the three reach types found that the upstream gaining reaches had significantly lower Shannon-Weaver Diversity than either upstream losing reaches or downstream reaches. There was no significant difference between upstream losing reaches and downstream reaches.

Source	df	SS	MS	F	P
Reach type	2	0.513	0.257	21.5	<0.001
Residual	9	0.107	0.012		
Total	11	0.620			

Hilsenholf Family Biotic Index (HFBI) is the most widely used Biotic Index in the United States, and is used to describe responses to general anthropogenic perturbation, especially organic enrichment. According to the HFBI, all study sites were categorized as "Good" with some organic pollution (Table 17, Figure 34, Figure 35). There were no significant differences among study sites (Table 18).

Table 17. Hilsenhoff Family Biotic Index Values.

Biotic Index	Water Quality	Degree of Organic Pollution
0.00 – 3.50	Excellent	None Apparent
3.51 – 4.50	Very Good	Possible Slight
4.51 – 5.50	Good	Some
5.51 – 6.50	Fair	Fairly Significant
6.51 – 7.50	Fairly Poor	Significant
7.51 – 8.50	Poor	Very Significant

8.51 – 10.00	Very Poor	Severe

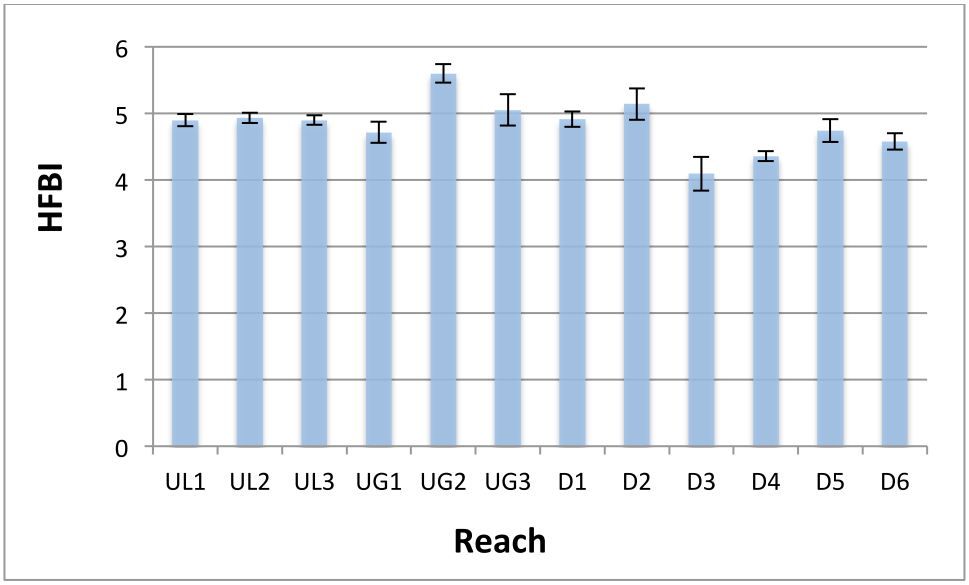

Figure 34. Mean (± SE) Hilsenholf Family Biotic Index (HFBI) on individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010. D indicates a site downstream of the City of Spokane, UG indicates an upstream site receiving inputs of aquifer flow, and UL indicates an upstream site not receiving aquifer flow.

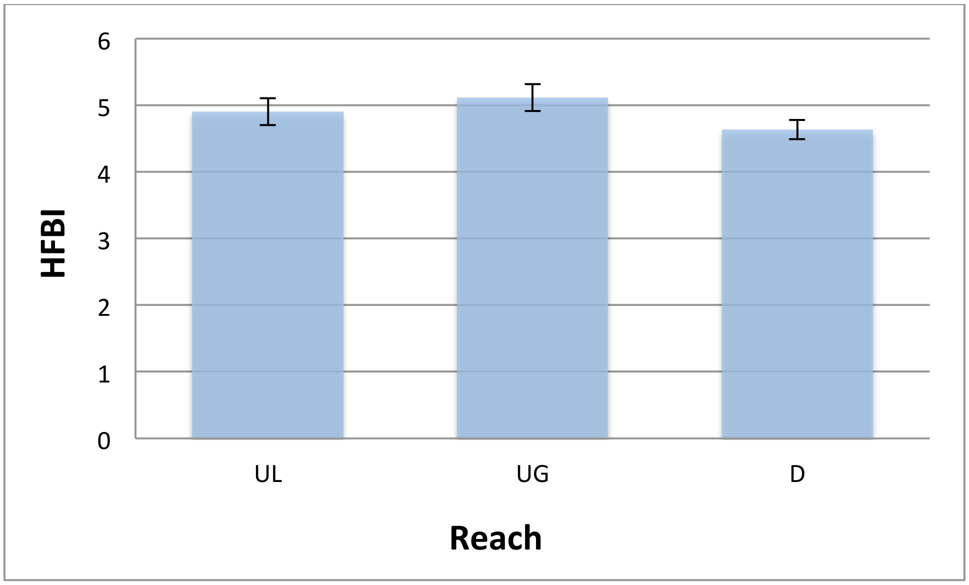

Figure 35. Mean (± SE) Hilsenhoff Family Biotic Index values for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 18. ANOVA of reach means for Hilsenhoff Family Biotic Index comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing). The dependent variable for this analysis was the mean Hilsenhoff Family Biotic Index for each reach the independent was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.493	0.246	1.984	0.19
Residual	9	1.117	0.124		
Total	11	1.160			

A **Metal Tolerance Index** was applied to a small group of samples sent to EcoAnalysts for more precise identification (n = 8). This index was developed by the Montana Department of Environmental Quality and tolerance values for individual taxa are not widely available, so I was unable to apply the index to all samples. With this limited sample size, there were no clear patterns among the reaches (Figure 36).

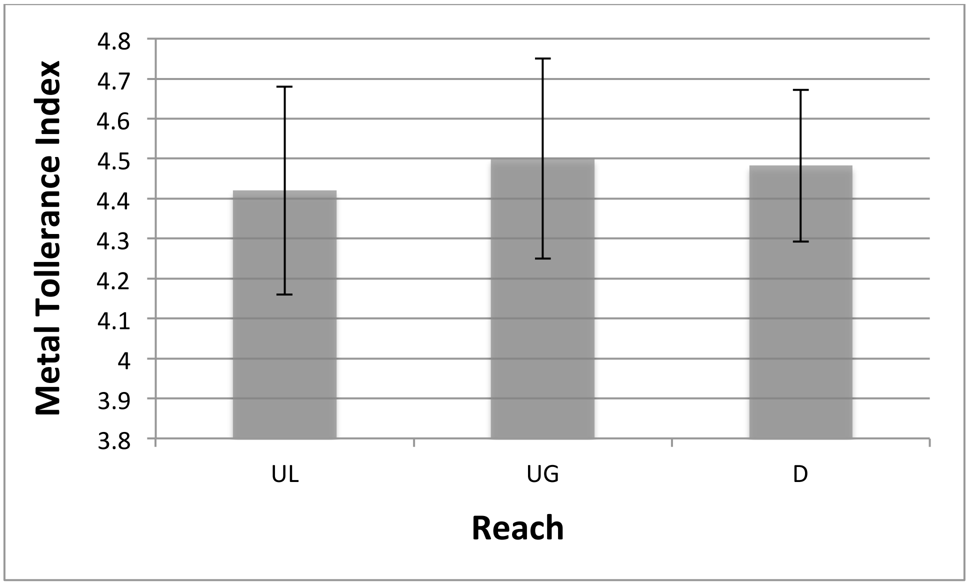

Figure 36. Mean (± SE) Metal Tolerance Index values for samples collected upstream and downstream of the City of Spokane, August-September 2010. D indicates sampling downstream of the City of Spokane (n=4), UG indicates samples from upstream reaches receiving inputs of aquifer flow (n=2), and UL indicates samples from upstream reaches not receiving aquifer flow (n=2).

Metal Tissue Concentrations

Hydropsyche cockerelli tissue metal concentrations

Hydropsyche cockerelli larvae were present at all study sites, with densities ranging from 30 individuals/m^2 at site UL3 to 2650 individuals/m^2 at site UG2. There was no significant variation among the three study reaches in abundance of *H. cockerelli* (Figure 37; Table 19). At all Spokane River sites tissue concentrations of metals were lower than the South Fork of the Coeur d'Alene River but because of small sample sizes these values can only demonstrate trends (Table 20). Mean concentrations of cadmium in *H. cockerelli* tissue were highest in the upstream losing reaches and lowest in the downstream reaches (Figure 40). There was no consistent pattern of variation in *H. cockerelli* tissue lead and zinc concentrations among reaches (Figure 38, Figure 39). *H. cockerelli* tissue concentrations of copper, calcium, and magnesium were highest in the downstream reach and lowest in the upstream losing reach (Figure 41, Figure 42, Figure 43).

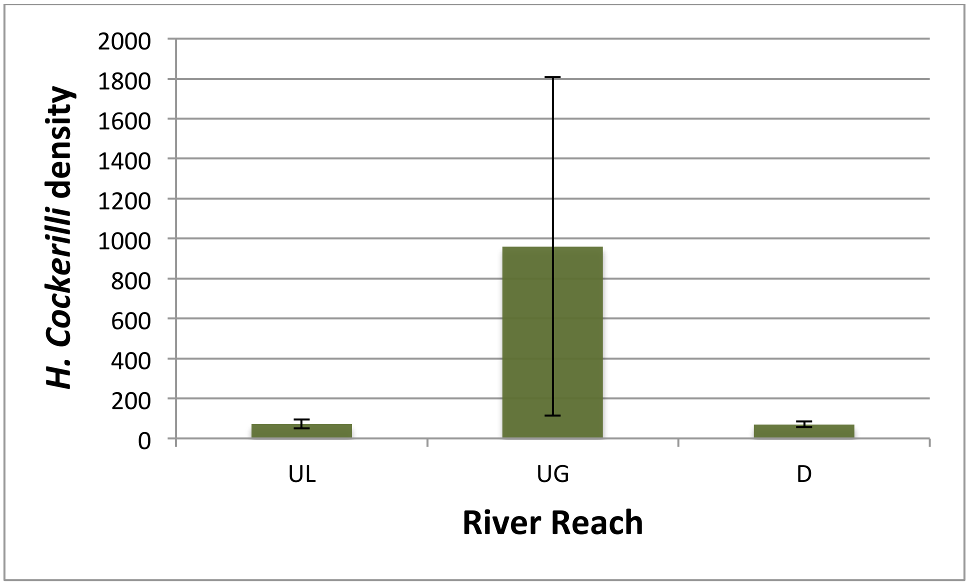

Figure 37. Mean (± SE) *H. cockerilli* density for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=6), UG indicates an upstream reach receiving inputs of aquifer flow (n=3), and UL indicates an upstream reach not receiving aquifer flow (n=3).

Table 19. ANOVA of reach means for log-transformed proportion *Hydropsyche cockerelli* density, comparing reaches downstream of the City of Spokane, and upstream of the City of Spokane with flow from the aquifer (upstream gaining) and without aquifer flow (upstream losing) the dependent variable for this analysis was mean $\log_{10}$ (# H. cockerelli/m^2) for each reach. The independent variable was reach type.

Source	df	SS	MS	F	P
Reach type	2	0.136	0.0680	0.386	0.69
Residual	9	1.585	0.1761		
Total	11	1.721			

Table 20. Mean of Pb, Zn, Cd, Cu and Mg concentrations found in *Hydropsyche cockerelli* tissue for samples collected in reaches on the South Fork of the Coeur d' Alene River (August 2012) as compared an average of all the Spokane River data collected August-September 2010, 2012.

	mg Pb/g bug tissue	mg Zn/g bug tissue	mg Cd/g bug tissue	mg Cu/g bug tissue	mg Ca/g bug tissue	mg Mg/g bug tissue
Spokane	0.008	0.093	0.000	0.003	0.268	0.360
SF CDA	0.057	0.182	0.002	0.005	0.149	0.185

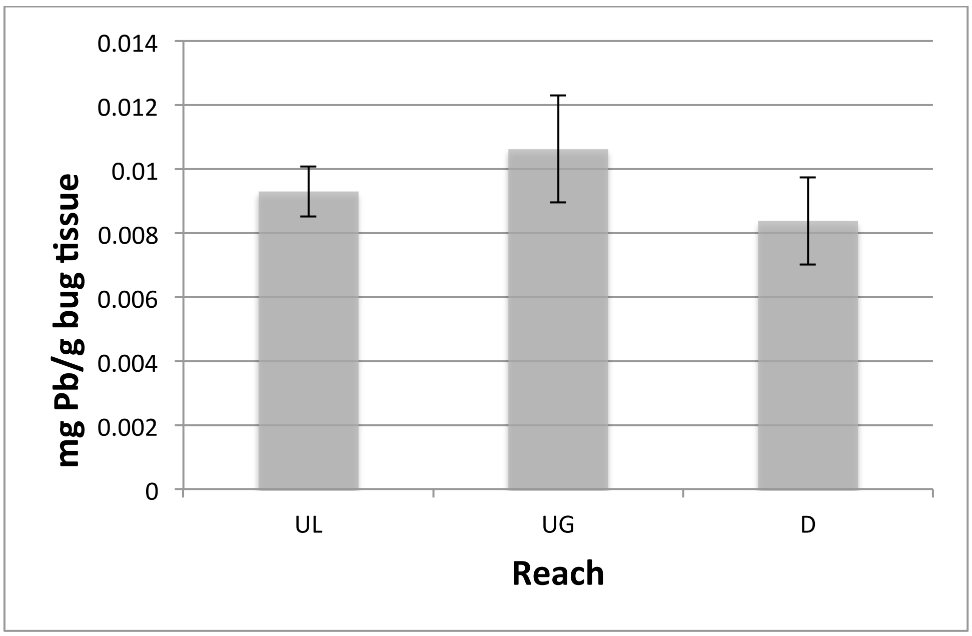

Figure 38. Mean (± SE) of Pb concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=18), UG indicates an upstream reach receiving inputs of aquifer flow (n=10), and UL indicates an upstream reach not receiving aquifer flow (n=9).

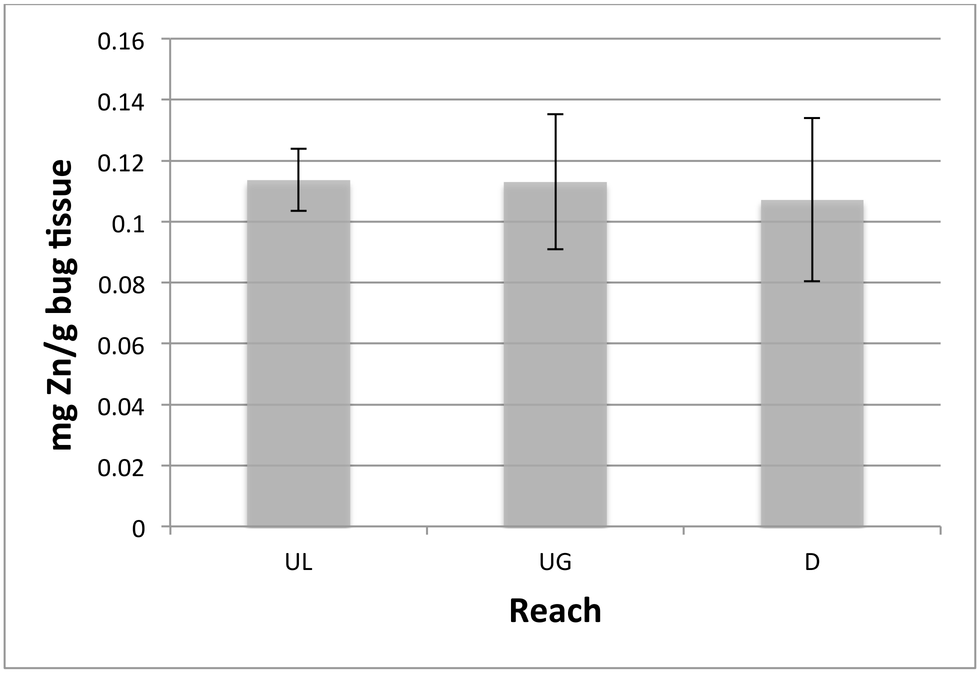

Figure 39. Mean (± SE) of Zn concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=18), UG indicates an upstream reach receiving inputs of aquifer flow (n=10), and UL indicates an upstream reach not receiving aquifer flow (n=9).

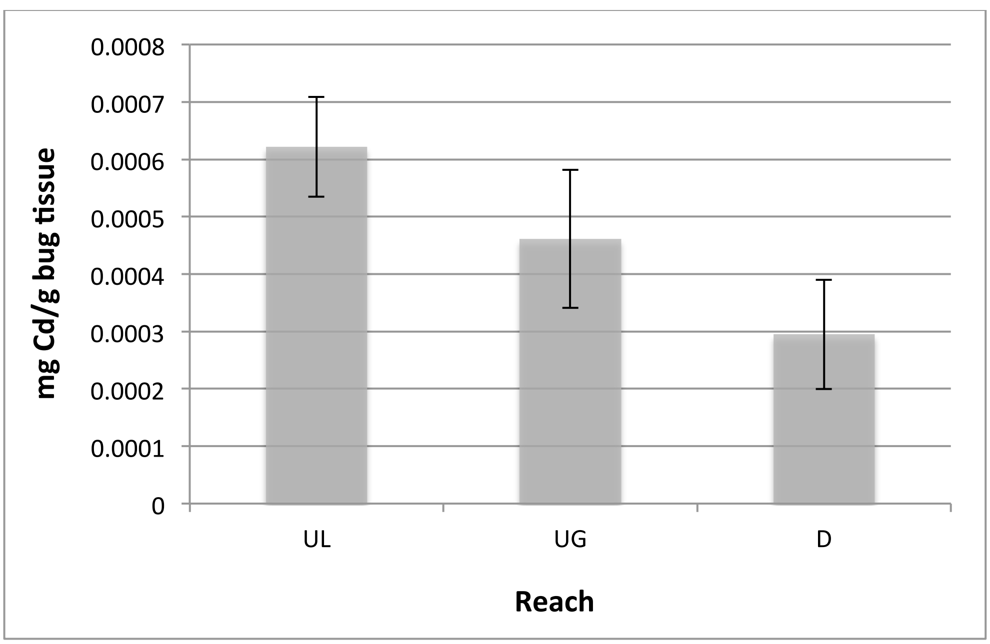

Figure 40. Mean (± SE) of Cd concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September

2010. D indicates reaches downstream of the City of Spokane (n=18), UG indicates an upstream reach receiving inputs of aquifer flow (n=10), and UL indicates an upstream reach not receiving aquifer flow (n=9).

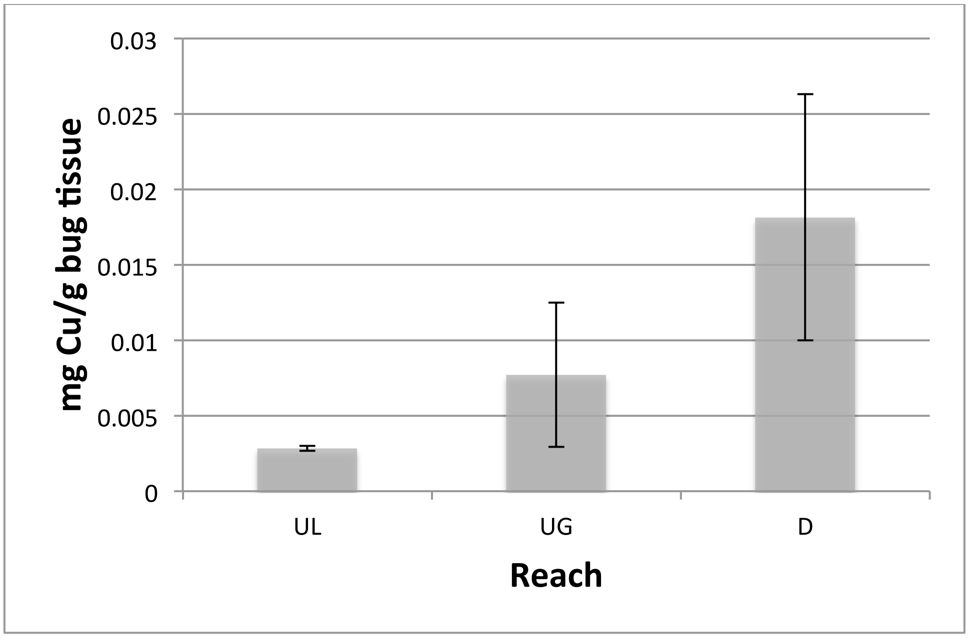

Figure 41. Mean (± SE) of Cu concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=18), UG indicates an upstream reach receiving inputs of aquifer flow (n=10), and UL indicates an upstream reach not receiving aquifer flow (n=9).

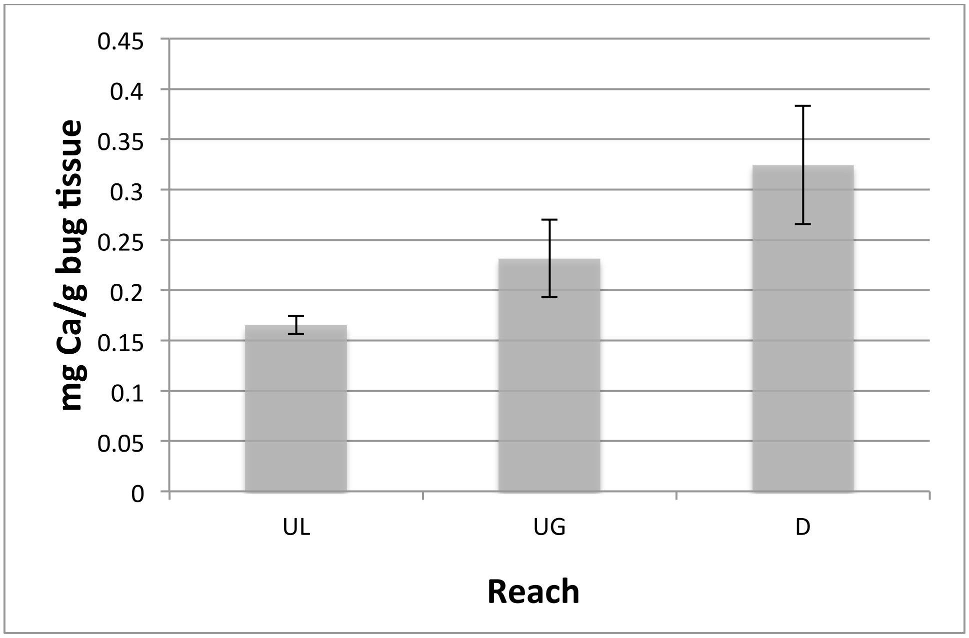

Figure 42. Mean (± SE) of Ca concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=12), UG indicates an upstream reach receiving inputs of aquifer flow (n=8), and UL indicates an upstream reach not receiving aquifer flow (n=9).

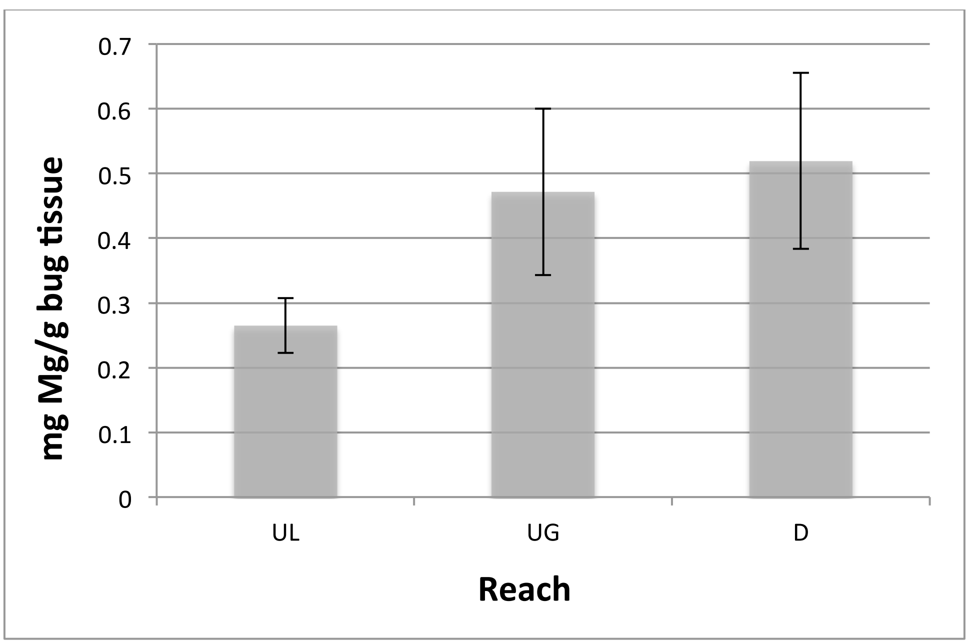

Figure 43. Mean (± SE) of Mg concentrations found in *H. cockerelli* caddisfly tissue for 3 sample reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates reaches downstream of the City of Spokane (n=18), UG indicates an

upstream reach receiving inputs of aquifer flow (n=10), and UL indicates an upstream reach not receiving aquifer flow (n=9).

***Pternonarcys californica* tissue metal concentrations**

Concentrations of average metals in tissues of *Pteronarcys californica* as compared to St. Joe (least impacted sight) and the North Fork and South Fork (most impacted sites) of the Coeur d'Alene river show higher or equal concentrations of Pb, Zn & Cd on the Spokane River as compared to the St. Joe River and lower concentrations on the Spokane River as compared to the North & South Forks of the Coeur d'Alene Rivers. Unfortunately because of the small sample sizes this data can only demonstrate trends (Table 21). *Pteronarcys californica* were only found in the upstream gaining reach and downstream reach. *P. californica* tissue concentrations of lead and magnesium were similar between the two reaches (Figures 44, Figure 48). Tissue calcium concentrations were not available for several of the specimens and were not included in the analysis. *P. californica* tissue zinc, cadmium, and copper concentrations were all higher in the upstream gaining sites compared to the downstream reach (Figure 45, Figure 46, Figure 47). I performed linear regression analysis to assess correlation between metal tissue content of *Pteronarcys* and *Hydropsyche*, but no significant correlation was found.

Table 21. Mean of Pb, Zn, Cd, Cu and Mg concentrations found in *Pteronarcys californica* tissue for samples collected from reaches on the North Fork of the Coeur d' Alene River, the South Fork of the Coeur d'Alene River (August 2012) and the St. Joe River collected (August 2011) compared an average of all the Spokane River data collected August-September 2010. August-September 2010, 2012.

Sample Location	mg Pb/g bug	mg Zn/g bug	mg Cd/g bug	mg Cu/g bug	mg Mg/g bug
Spokane	0.004	0.057	0.000	0.008	0.301
NF CDA	0.058	0.408	0.004	0.004	0.530
SF CDA	0.026	0.301	0.004	0.007	0.332
St. Joe	0.000	0.042	0.000	0.008	0.495

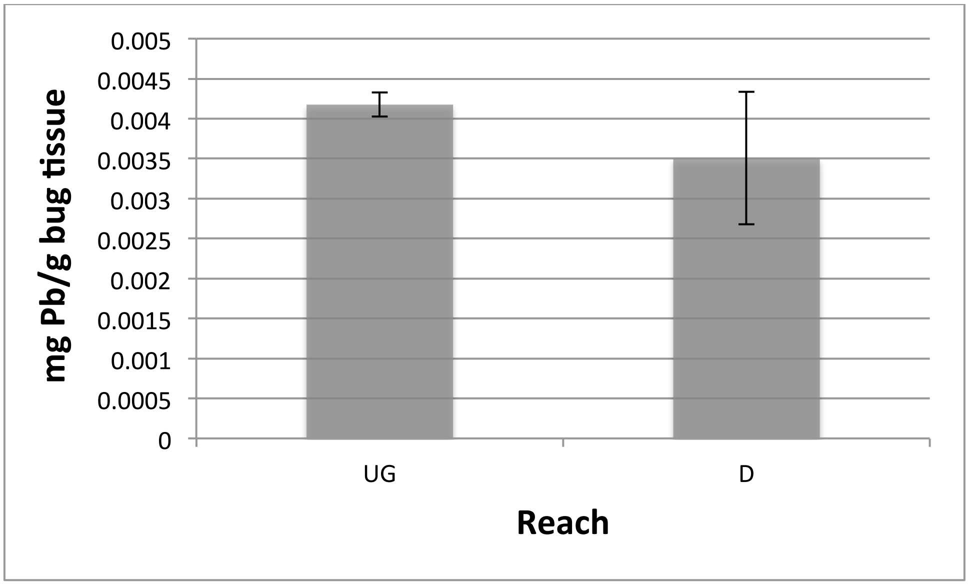

Figure 44. Mean (± SE) lead concentrations found in *Pteronarcys* tissue for 2 reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates stoneflies collected downstream of the City of Spokane (n=18) and UG indicates stoneflies collected from upstream reach receiving inputs of aquifer flow (n=4).

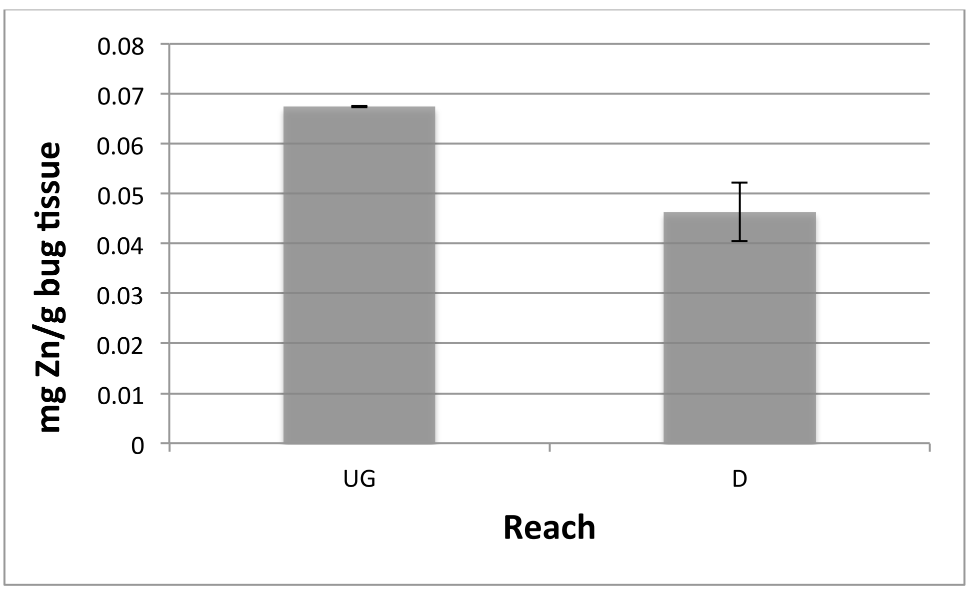

Figure 45. Mean (± SE) of zinc concentrations found in *Pteronarcys* tissue for 2 reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates stoneflies collected downstream of the City of Spokane (n=18) and UG indicates stoneflies collected from upstream reach receiving inputs of aquifer flow (n=4).

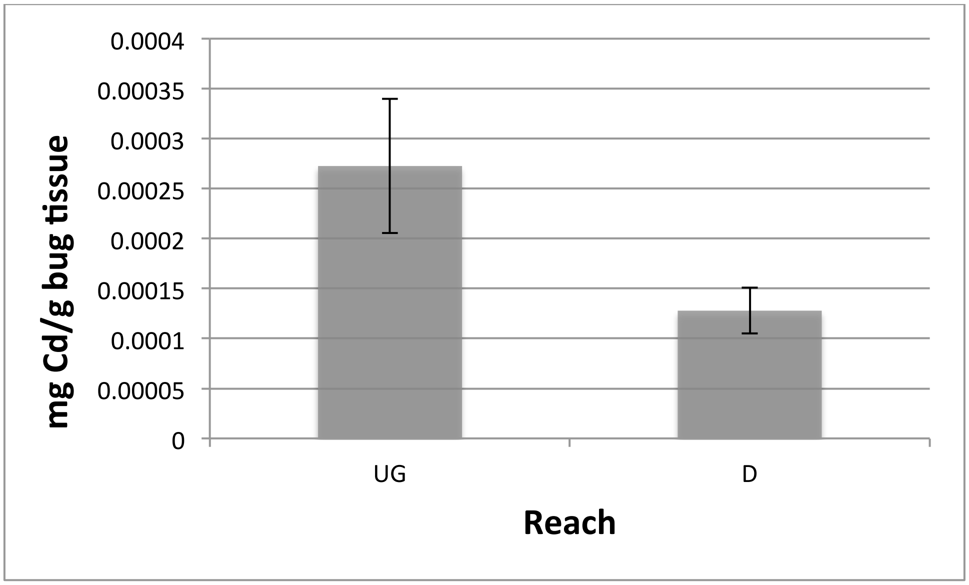

Figure 46. Mean (± SE) cadmium concentrations found in *Pteronarcys* tissue for 2 reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates stoneflies collected downstream of the City of Spokane (n=18) and UG indicates stoneflies collected from upstream reach receiving inputs of aquifer flow (n=4).

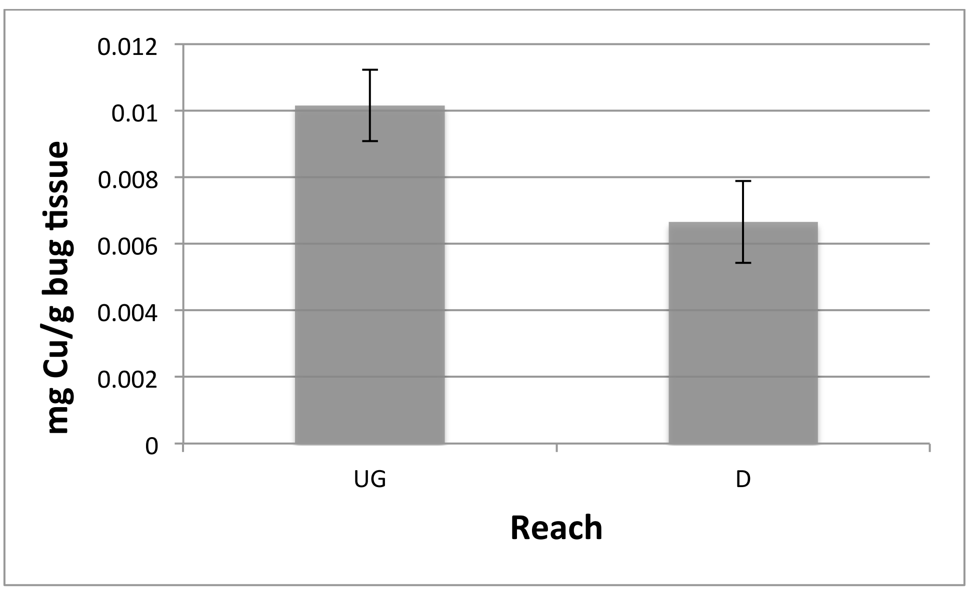

Figure 47. Mean (± SE) copper concentrations found in *Pteronarcys* tissue for 2 reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates stoneflies collected downstream of the City of Spokane (n=18) and UG indicates stoneflies collected from upstream reach receiving inputs of aquifer flow (n=4).

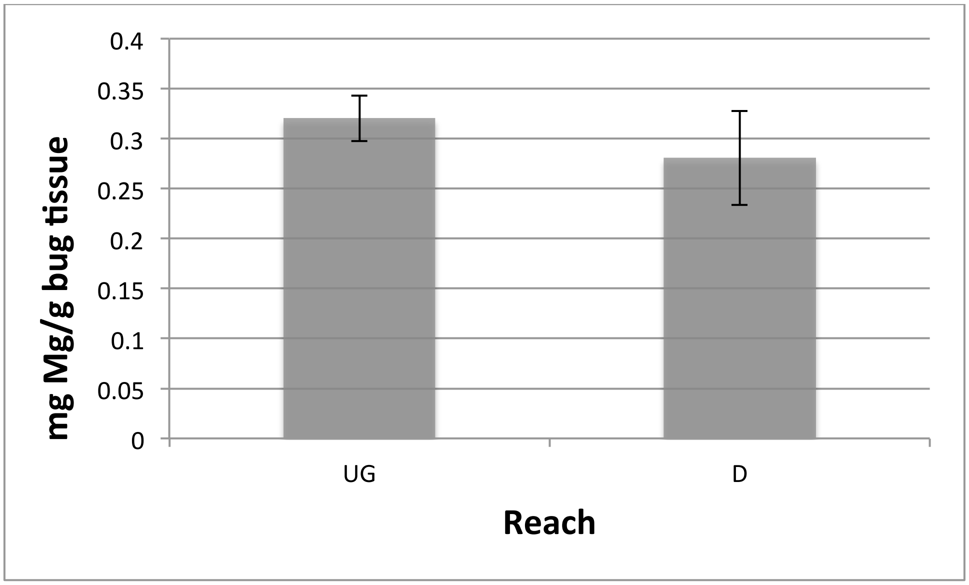

Figure 48. Mean (± SE) magnesium concentrations found in *Pteronarcys* tissue for 2 reaches upstream and downstream of the City of Spokane, August-September 2010. D indicates stoneflies collected downstream of the City of Spokane (n=18) and UG indicates stoneflies collected from upstream reach receiving inputs of aquifer flow (n=4).

Aquifer Effects on Metal uptake in invertebrate tissue

To determine if aquifer influx reduced metal bioavailability, I analyzed the differences between the concentrations found in *H. cockerelli* tissue in the upstream gaining and losing reaches, excluding the downstream reach with a simple t-test. There was no significant difference between the two reaches indicating that aquifer effects did not affect bioavailability.

Relationship between Hydropsyche cockerelli tissue concentrations of metals and distance downstream from mining pollution source

Linear regression was used to compare distance downstream from the outlet of Lake Coeur d'Alene to metal concentrations found in *Hydropsyche* tissue.

Hydropsyche cockerelli tissue concentrations of cadmium and lead showed significant linear decline with distance downstream (Figures 49, Figure 50), but copper and zinc did not show significant linear decline (Figures 51, Figure 52).

CLZ index value is a combination of tissue concentrations for the metals most associated with mining sediments in the Spokane River – cadmium, lead, and zinc (Kadlec 2000). There was a significant linear decrease in this value with distance downstream (Figure 53).

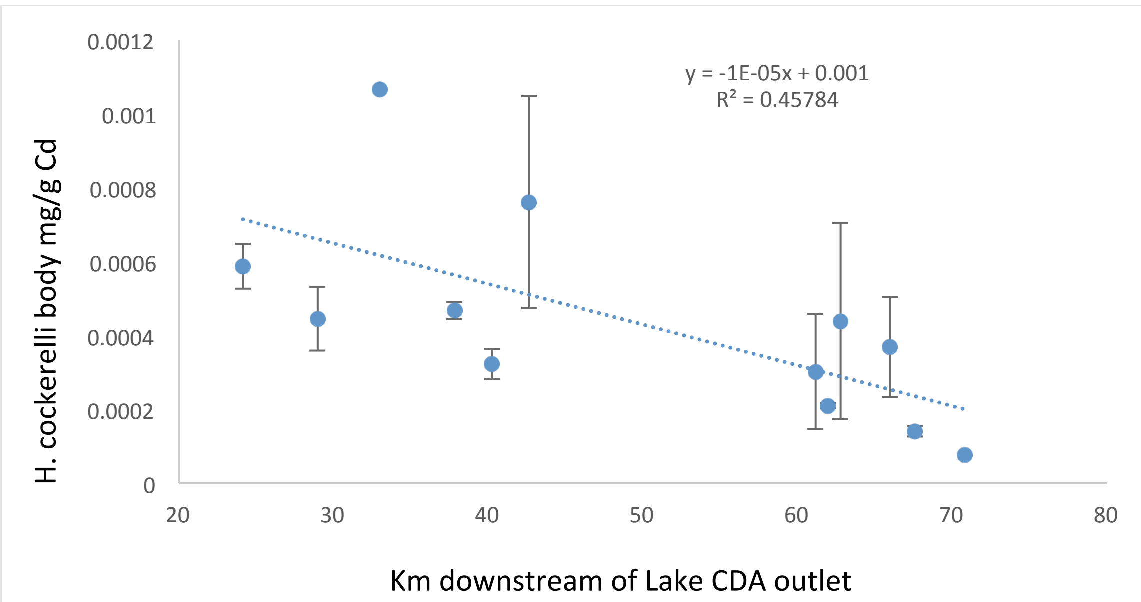

Figure 49. Mean Cd (± SE) concentrations found in *Hydropsyche cockerelli* tissue collected from the Spokane River as a function of distance downstream from Lake Coeur d'Alene. There was a significant linear relationship between distance and tissue Cd concentration (df = 11, F = 8.45, p= 0.016).

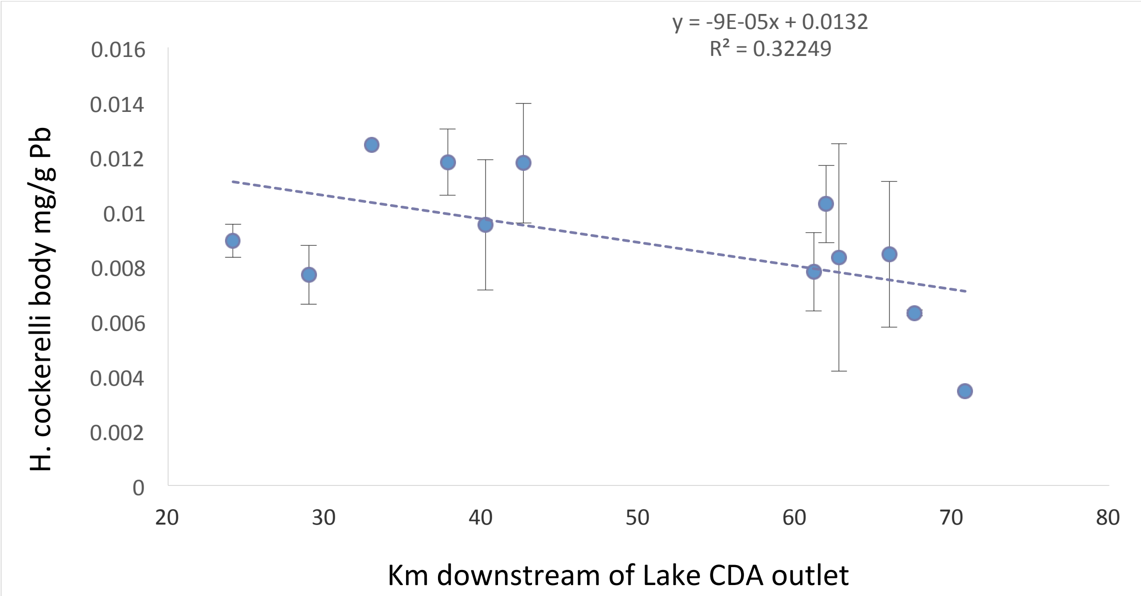

Figure 50. Mean Pb (± SE) concentrations found in *Hydropsyche cockerelli* tissue collected from the Spokane River as a function of distance downstream from Lake Coeur d'Alene. There was not a significant linear relationship between distance and tissue Cd concentration (df = 11, F = 4.76, p = 0.054).

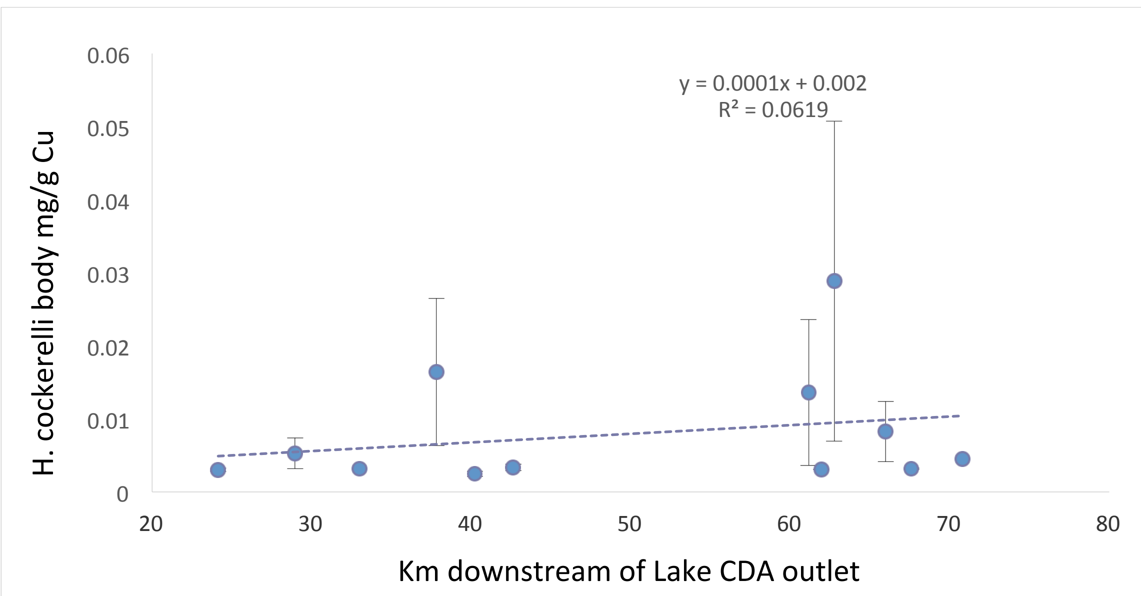

Figure 51. Mean Cu (± SE) concentrations found in *Hydropsyche cockerelli* tissue collected from the Spokane River as a function of distance downstream from Lake Coeur d'Alene. There was not a significant linear relationship between distance and tissue Cu concentration (df = 11, F = 0.6598, p = 0.436).

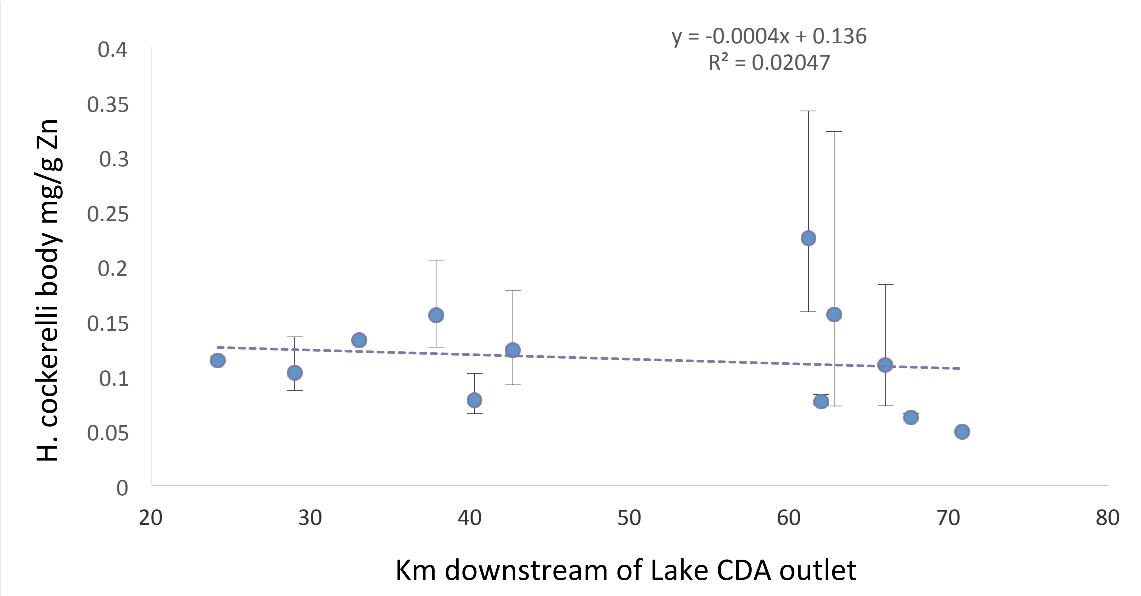

Figure 52. Mean Zn (± SE) concentrations found in *Hydropsyche cockerelli* tissue collected from the Spokane River as a function of distance downstream from Lake Coeur d'Alene. There was not a significant linear relationship between distance and tissue Zn concentration (df = 11, F = 0.209, p = 0.657).

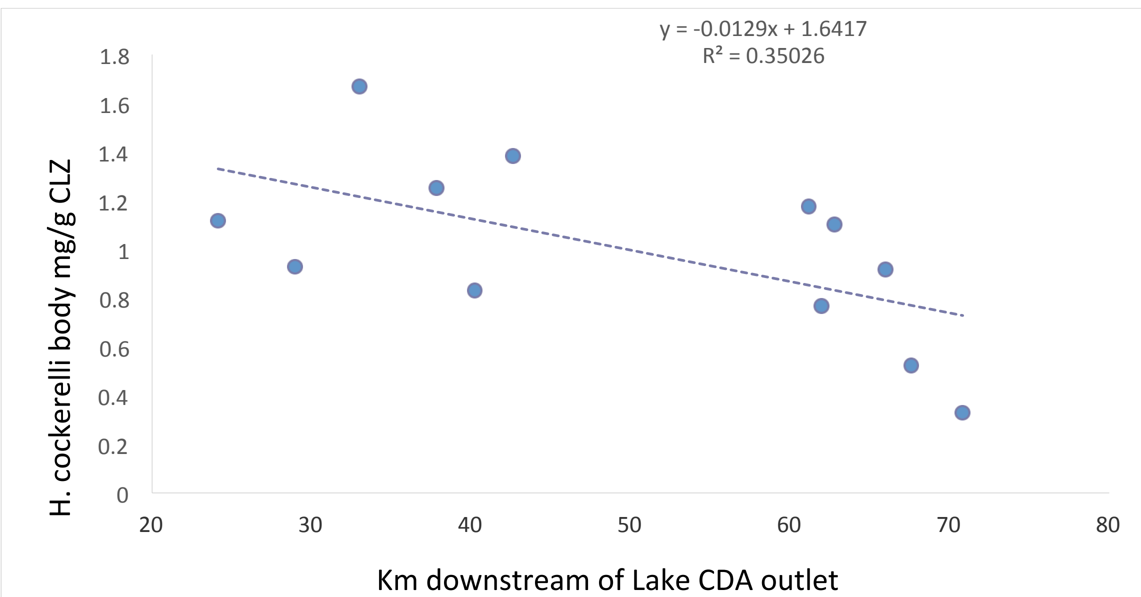

Figure 53. Mean Cd, Pb and Zn (CLZ score) (± SE) concentrations found in *Hydropsyche cockerelli* tissue collected from the Spokane River as a function of distance downstream from Lake Coeur d'Alene. There was a significant linear relationship between distance and tissue CLZ concentration (df = 11, F = 5.391, p = 0.0426).

Diet Analysis

Hydropsyche cockerelli gut contents were dominated by amorphous detritus (Figure 54), indicating the animals fed largely on fine particulate organic matter, likely primarily seston. There was little animal material in their guts, indicating they were exhibiting little facultative carnivory. Algae made up approximately 20% of the gut material. Finer examination of algae within the guts found them to be primarily pinnate diatoms and other benthic taxa, not centric diatoms likely to have been derived from phytoplankton (Figure 55). These caddisflies were likely feeding primarily on benthic, rather than planktonic, production, based on the composition of algae in their diets.

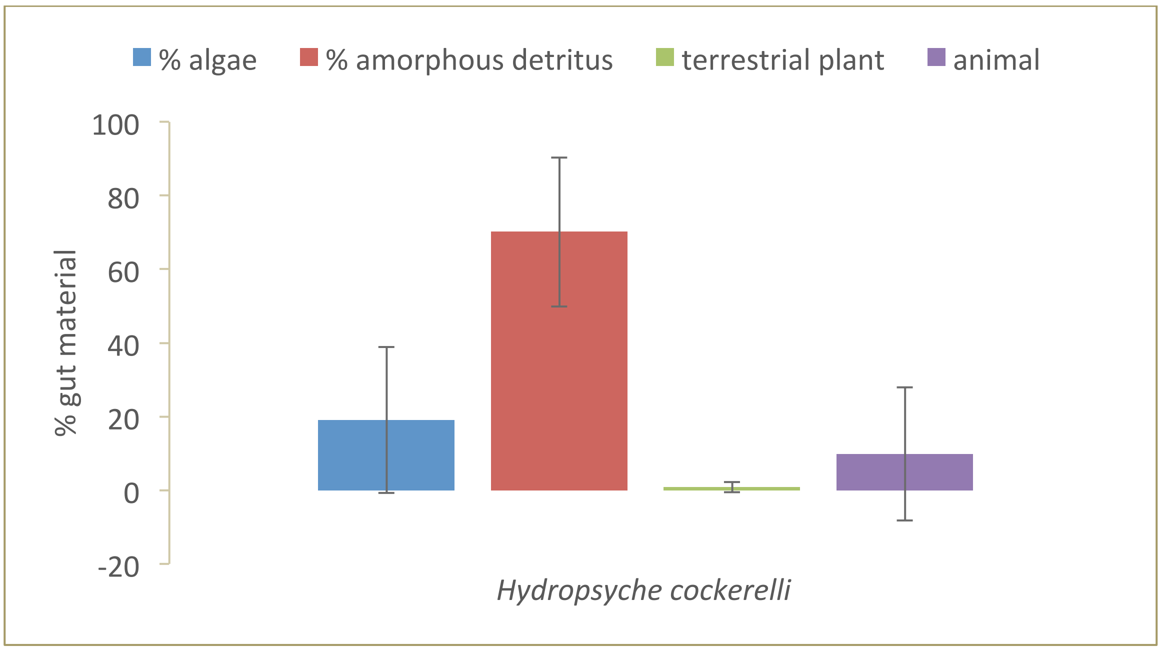

Figure 54. EWU analyzed data Mean (± SD) of % of gut material for *Hydropsyche cockerelli* collected from individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010

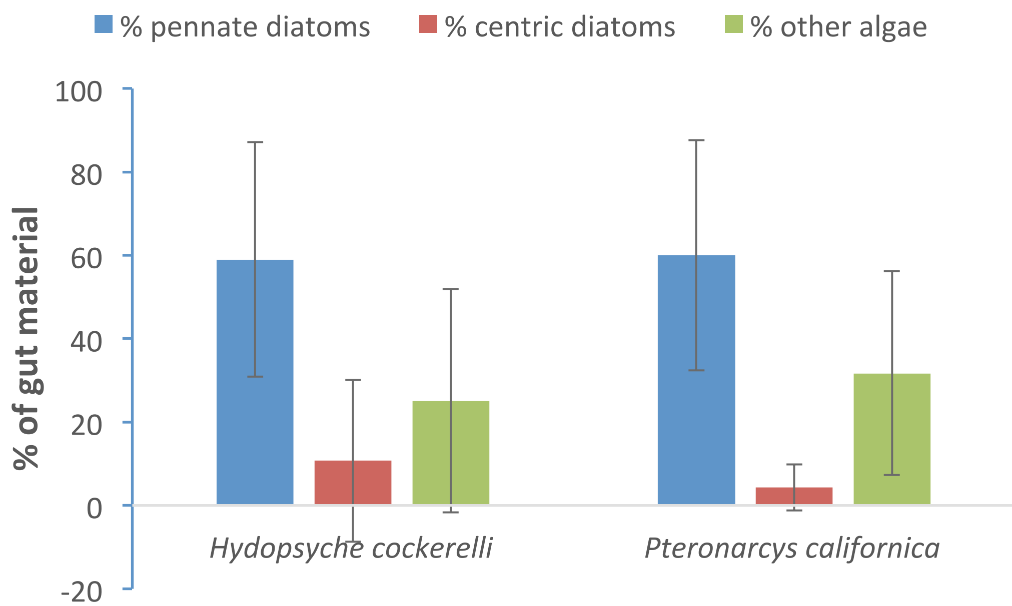

Figure 55. Ecoanalyst analyzed algae data Mean (± SD) of % of gut material for *Hydropsyche cockerelli* and *Pteronarcys californica* collected from individual stones collected in riffles upstream and downstream of the City of Spokane, August-September 2010.

Discussion

Macroinvertebrate communities clearly indicated impacts of toxic trace metals throughout the study reach, with impacts decreasing with distance downstream. In addition, water quality and ecosystem integrity appeared lowest in the upstream gaining sampling reach, which flows through Spokane Valley, WA and receives substantial inputs from the Spokane River Rathdrum Prairie Aquifer. Macroinvertebrate communities in this area indicated greatest degradation, despite aquifer inputs of cold water, which would be expected to benefit macroinvertebrates (Rosenberg and Resh 1984, Merrit et al. 2008). These results contradict two of my initial hypotheses. I predicted that point and non-point discharges throughout the City of Spokane (below the upstream gaining reach) would have the most significant effect on water quality, making the downstream reach the most degraded reach. I also predicted that aquifer inflows of cool, clean, water would mitigate the effects of pollution. Interestingly, neither of these predictions was supported by macroinvertebrate community data. Macroinvertebrate community metrics indicated the highest water quality and ecosystem integrity overall was found in the downstream reach. Spokane River macroinvertebrate communities did not indicate strong impacts of urbanization from the City of Spokane, or evidence of water quality problems such as low oxygen, high water temperatures, eutrophication, or organic enrichment. However, several community metrics indicated substantial degradation. Toxic trace metals are likely the most important, but not the only, significant anthropogenic impact on Spokane River macroinvertebrate communities.

I predicted that point and non-point discharges throughout the City of Spokane (below the upstream gaining reach) would have the most significant effect on water quality, making the downstream reach the most degraded reach because of the current consistent pollution sources concentrated there at the time of this study's sample collection and also because of the abundance of literature describing the significant negative effects of concentrated stormwater and CSO's on river ecosystems (Wallace et al. 2013; Vietz et al. 2015, Pennino et al. 2016, Gabor et al. 2017). Additionally, the reach down stream of the City of Spokane has not previously been included in a macroinvertebrate bioassessment study, so it was unknown if the urban pollution concentrated in storm water and CSO's would have a significant effect.

My **first hypothesis** was that macroinvertebrate communities would be less degraded in reaches gaining water from the aquifer. In contrast, four macroinvertebrate community metrics indicated the greatest impacts in the upstream gaining reach. Proportion dominant taxon (increases with metal pollution and general anthropogenic impacts) and proportion Chironomidae (increases with metal pollution) had the highest values in the upstream gaining reach. Proportion Clinger (decreases with urban and anthropogenic impacts) and Shannon-Weaver Diversity (decreases with anthropogenic impacts) also indicated that the upstream gaining reach was most negatively affected by anthropogenic impacts. This macroinvertebrate community data did not support my hypothesis and it does not indicate what is the particular cause of deration within this reach. It is interesting that this reach is farther from the source of trace metal pollution the upstream losing reach yet still has the most metrics indicating degradation in this reach. It would be interesting to further investigate sources of pollution in this reach.

My **second hypothesis** was that macroinvertebrate communities would be significantly degraded by urban influences below the core of the City of Spokane as compared to upstream reaches. Three other macroinvertebrate community metrics differed significantly among study reaches: family taxa richness (decreases with urbanization & anthropogenic impacts), EPT taxa richness (decreases with urbanization and metal pollution), and proportion Trichoptera (decreases with metal pollution). All were significantly higher in the downstream gaining reach, indicating the **least** anthropogenic degradation in this reach. The macroinvertebrate community data does not support my hypothesis and can only confirm that urban pollution is not having the significantly negative effect I had predicted within this reach. The macroinvertebrate community data is consistent with the assumption that the decline in sediment and water concentrations of toxic metals as distance increases downstream from Lake Coeur d'Alene would have a significant and predictable effect and is also consistent with previous studies indicating that toxic trace metals are the most important contaminant affecting Spokane River invertebrate communities. Overall, my data strongly support my **third hypothesis**- that effects of toxic trace metals on macroinvertebrate communities will decline with distance downstream from Lake Coeur d'Alene.

Macroinvertebrate communities in the Spokane River have been assessed at least 2 times in past decades, providing the potential for observing changes through time, although there were some methodological differences among the studies. Previous sampling took place in 1979-1981 (Gibbons et al. 1984) and 1998-1999 (Kadlec 2000, USGS 2003), approximately 30 and 10 years prior to this study (sampled 2010). Macroinvertebrate community composition in the Spokane River was very consistent among these studies and over this 30-year period. It is also interesting to note that despite being exposed to climate change, commonly used biological metrics, such as percent EPT (Ephemeroptera Plecoptera Trichoptera) and taxa richness are still able to detect local anthropogenic stressors (Lawrence et al. 2010).

In 1979-1981, the dominant orders of macroinvertebrates were Ephemeroptera, Trichoptera and Diptera (Gibbons et al. 1984). Plecoptera were found in low numbers and a limited number of species; lower than would be expected in a river of this type and with the apparent water quality measured at the time (Gibbons et al. 1984). Also uncommon for rivers of this size and quality was that *Baetis* was the only mayfly observed. *Hydropsyche* sp. and *Cheumatopsyche* sp. were the most common Trichoptera, and Chironomidae were the most common Diptera (Gibbons, et al 1984). These aspects of community composition were the same in my 2010 study. In fact, the complete list of taxa collected by Gibbons, et al. (1984) was remarkably similar to those I collected. The only group found in their samples but not in this study, was the freshwater Cnidarian *Hydra* which was collected only from pools.

In this 2010 bioassessment, Shannon-Weaver Diversity (H') in the Spokane River ranged from 0.74 to 1.54, with both highest and lowest values in the downstream gaining reach. During the 1979-1981 bioassessment, all stations also had low values of the H', ranging from 0.00 to 2.84 with a mean of 1.54. Wilhm (1970) reviewed Shannon-Weaver Diversity values from polluted and unpolluted streams found H' of 0.00 to 1.60 in polluted streams and 2.60 to 4.61 in unpolluted streams. H' values in the Spokane River from both ~ 1980 and 2010 indicated a polluted or stressed environment. Gibbons et al. (1984) attributed low H' to high dissolved zinc concentrations.

Kadlec (2000) and USGS (2003) report data for macroinvertebrates communities in the Spokane River during 1998-1999. Both reports concluded that toxic trace metals severely impacted Spokane River macroinvertebrate communities, likely reducing secondary production and food availability for game fish. In addition to macroinvertebrate community data, Kadlec (2000) also reported the results of lab toxicity assays using sediments from the Spokane River. Very similar to 2010 patterns, Kadlec reported low Ephemeroptera, Plecoptera, and Trichoptera (EPT) relative abundance, high Chironomidae relative abundance, low taxa richness, and high proportion dominant taxon in the Spokane River from Post Falls, ID through several sites in Spokane Valley, WA, corresponding to both upstream losing and upstream gaining reaches in this study. No sites corresponding to the downstream reach of this study were included.   Interestingly, no Plecoptera individuals of any species were collected from the Spokane River in the 1998-1999 study (Kadlec 2000).

The USGS report on this data (2003) compared macroinvertebrate communities from the Spokane River to “least-impacted” sites on the North Fork Coeur d’ Alene River at Enaville, ID and the St. Joe River near Calder, ID. The Spokane River had lower macroinvertebrate taxa richness even though it had greater abundances (USGS 2003). The EPT richness was 2 to 3 times lower at Spokane River sites compared to the least impacted sites (USGS 2003). Comparison to regional collections of Maret et al. 2001 indicated the Spokane River should be able to support at least five taxa of stoneflies, but none were found in this study. Observed habitat substrate size and lack of imbededness did not indicate habitat degradation, so the USGS concluded that low EPT taxa richness indicated substantial water quality impairment, likely due to metal contamination (USGS 2003). EPT richness in 2010 was between 3 and 10 and highest in the Spokane River in the downstream reach, below the City of Spokane and furthest from the metal pollution source. Metrics with the best indicators of heavy metal impacts were taxa richness, EPT richness, Chironomidae richness, % of the most dominant taxon, and density (#/m^2) (Poulton et al. 1995). Among the most conspicuous impacts of metals on the Spokane River macroinvertebrate community is the remarkably low diversity of mayflies, with only representatives of the metal-tolerant family Baetidae present. Clements et al. (2000) describe similar effects of metals on mayfly richness in the southern Rocky Mountains.

It is clear that macroinvertebrates are at risk from toxic trace metals and other contaminants in the water column, through dietary exposure and direct exposure for invertebrates that spend some or all of their life cycle in the sediments (Kadlec 2000). Lab toxicity assays found Spokane River sediment from Spokane Valley sites (same area as upstream gaining and losing sites) and 7-mile Bridge (downstream of all sites in this study) to be toxic to invertebrates (Kadlec 2000). Upstream sites (Spokane Valley) had higher sediment metal concentrations and bioavailability (Kadlec 2000).

Dissolved nutrients

Dissolved nutrient concentrations were generally low at all the study sites with the exception of nitrate levels at site D6 below the outflow of the waste treatment plant. As I did not try to determine the influence of the waste treatment plant specifically, there were not more sites selected below the outfall of the plant. Sampling over a longer period of time would also have been needed to determine if these rates were consistently high.

Although nutrient levels were low during late summer sampling in this study, they are likely much higher at least at some sites during high winter and spring flow (McNeely et al. 2017). A confounding factor in determining effects of nutrient loading, particularly on macroinvertebrate communities, is that there is substantial evidence to support the conclusion that high metal concentrations, particularly zinc, are suppressing primary productivity in the river (Kadlec 2000). According to Kadlec (2000), the City of Coeur d'Alene Waste Water Treatment Plant might, in the absence of high dissolved metal concentrations, contribute sufficient nutrients to enhance primary productivity but no such enhancement is apparent. This is probably because the high concentrations of zinc, and perhaps cadmium and lead, inhibit the growth of algae (Kadlec 2000). Metal pollution could mask the effects of nutrient loading.

Metal concentrations in macroinvertebrate tissue

My **fourth hypothesis** that trace metal concentrations in the tissues of the two focal macroinvertebrates would decline with distance downstream of Lake Coeur d'Alene was confirmed for *Hydropsyche cockerelli*. However, the same pattern was not observed for *Pteronarcys californica*, likely because it was not found in a large enough range of sites. In *H. cockerelli*, tissue concentrations of cadium, lead, and an index combining cadmium, lead, and zinc decreased with distance downstream. However, tissue concentrations of zinc and copper did not follow the same pattern. This could be because zinc and copper are also associated with urban storm water runoff and industrial discharges (Brown and Peak 2006; Forester and Whitman 1983; Baysal et. al. 2013).

Pteronarcys californica were only found in the upstream gaining reach and downstream reach. Lead concentrations were similar between these two reaches, but concentrations of zinc, cadmium, and copper were higher in the upstream gaining reach compared to the losing reach. There was no significant correlation between metal tissue content for *H. cockerelli* and *P. californica*.

Patterns of trace metal concentrations in this study largely agree with patterns from 1979-1981 and 1999 sampling (Gibbons et al. 1984, Kadlec 2000, USGS 2003). In 1979-1981, trace metals were sampled in the water column and zinc was found to be high relative to other metal concentrations and water quality standards (5 to 225 ug/l), correlated to flow (increasing with higher flow), and mostly found in dissolved form (Gibbons et al. 1984). The USGS found that concentrations of lead and zinc in the tissue of Hydropsychid caddisflies from the Spokane River were five times the average concentrations in the tissues of Hydropsychids from in the least impacted sites in the Spokane River watershed (USGS 2003). The concentrations in caddisflies collected in 1999 were 3 micrograms per gram for lead and 180 micrograms per gram for zinc (USGS 2003). Contrary to my predictions, and the conclusions of Kadlec (2000), metal bioavailability did not appear to be reduced by aquifer inputs to the Spokane River within Spokane Valley. There was no difference between the concentrations found in *H. cockerelli* tissue in the upstream gaining and losing reaches.

Methodological recommendations

Pteronarcys californica and *Hydropsyche cockerelli* had similar diets despite their differing feeding strategies. However, *P. californica* was not found in significant numbers at all study sites and does not appear to be suitable as a focal organism for bioaccumulation of metals in future studies. The more widely used *Hydropsyche* was found in abundance in all three-study reaches. This study organism has been used in other regional studies analyzing metal content in macroinvertebrate tissue making it a good choice for comparing results from the Spokane River to other metal impacted rivers (Gibbons 1984, Cain & Luoma 1998, Kadlec 2000, USGS 2003).

Macroinvertebrate community sampling techniques differed among the three studies reviewed here (Gibbons et al. 1984, Kadlec 2000, USGS 2003, and this study), but all contain similar elements. Gibbons et al. (1984) used multiple sampling techniques, and found the Thompson's suction sampler most effective. This sampler used suction and scraping to remove invertebrates from natural river stones in-situ, without removing them from the river bottom. This technique was done at a more significant depth than my 2010 sampling and therefore required scuba divers to collect samples, a labor-intensive and expensive process. However, of the techniques used by Gibbons et al. (1984), the suction sampler was the most similar to my sampling protocol as it sampled macroinvertebrate communities in situ from natural substrate. The similar results and taxa lists produced by the two studies suggest that my sampling protocol, confined to the wadeable portion of the river, is adequate for bioassessment and the scuba technique is not necessary. My sampling protocol was carried out with undergraduates trained in the field, making it affordable as well as easily repeatable. Detailed methodology is not available for the 1998-1999 sampling, but USGS (2003) states that they followed the general recommendations of Cuffney et al. (1993). This reference does not provide detailed protocols, but outlines a general sampling technique that would be very similar to the one I used, and would be confined to the wadeable portion of the stream.

The robust data collected in this study reflect an effective sampling protocol. Macroinvertebrate community metrics indicated that there are significant detectable differences between sampling reaches therefore it is important to resample these reaches. The later summer sampling period avoided high flows. Water quality parameters oxygen, pH and conductivity were within a normal range during the sampling period. The substrates sampled were similar among the sites indicating the stratified sampling technique was effective in controlling particle size. Finally, water temperature confirmed aquifer influence at the sites where inflows from the aquifer to the river were expected (Aquifer Atlas Update Team 2015). Significant management improvements after 2010, such as the Spokane County Water reclamation facility; the

City of Spokane's "Cleaner River Faster" plan, including reduction in CSOs and construction of holding tanks for storing urban runoff; and improvements to the City of Spokane's Water Reclamation Facility, could be monitored by repeating the bioassessment protocol I developed.

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Appendix A. Taxa List by sample

* Sample ID done by EcoAnalysts for more precise identification

** Samples sub-sampled because of large macroinvertebrate numbers

Date	Gaining/ Losing	Downstream/ Upstream	Sample ID	Batidae	Hydropsychidae (other)	*Hydropsyche cockerelli*
8/15/10	Gaining	Downstream	D1 5	1625	660	30
8/15/10	Gaining	Downstream	D1 29	507	296	29
8/15/10	Gaining	Downstream	D1 40	262	333	15
8/15/10	Gaining	Downstream	D1 46	336	169	17
8/15/10	Gaining	Downstream	D1 50	359	285	21
8/15/10	Gaining	Downstream	D1 78	552	258	16
8/15/10	Gaining	Downstream	D1 92	11	17	16
8/14/10	Gaining	Downstream	D2 20 *	100	123	0
8/14/10	Gaining	Downstream	D2 21	31	93	9
8/14/10	Gaining	Downstream	D2 51	68	100	14
8/14/10	Gaining	Downstream	D2 52	59	134	13
8/14/10	Gaining	Downstream	D2 82	7	8	0
8/16/10	Gaining	Downstream	D3 2	6	23	7
8/16/10	Gaining	Downstream	D3 4	0	3	14
8/16/10	Gaining	Downstream	D3 14 *	338	161	0
8/16/10	Gaining	Downstream	D3 47	158	190	14
8/16/10	Gaining	Downstream	D3 57 *	28	202	0
8/16/10	Gaining	Downstream	D3 93	116	154	9
8/17/10	Gaining	Downstream	D4 6	58	110	1
8/17/10	Gaining	Downstream	D4 8	243	193	2
8/17/10	Gaining	Downstream	D4 13	366	393	0
8/17/10	Gaining	Downstream	D4 51	181	226	2
8/17/10	Gaining	Downstream	D4 84	179	315	27
8/17/10	Gaining	Downstream	D4 89	589	479	28
9/1/10	Gaining	Downstream	D5 5	8	45	1
9/1/10	Gaining	Downstream	D5 8	548	182	8
9/1/10	Gaining	Downstream	D5 12	926	137	14
9/1/10	Gaining	Downstream	D5 41	186	99	20
9/1/10	Gaining	Downstream	D5 52	62	44	6
9/1/10	Gaining	Downstream	D5 68	162	34	0
9/1/10	Gaining	Downstream	D5 72	208	215	0
8/31/10	Gaining	Downstream	D6 11	158	133	12
8/31/10	Gaining	Downstream	D6 35	486	389	5
8/31/10	Gaining	Downstream	D6 37	234	69	3
8/31/10	Gaining	Downstream	D6 40	328	94	3
8/31/10	Gaining	Downstream	D6 41	35	9	0
8/30/10	Gaining	Upstream	UG1 5 *	264	29	0
8/30/10	Gaining	Upstream	UG1 8	796	43	18
8/30/10	Gaining	Upstream	UG1 33	779	156	53

8/30/10	Gaining	Upstream	UG1 63	331	13	6
8/30/10	Gaining	Upstream	UG1 65	358	17	9
8/30/10	Gaining	Upstream	UG1 76	79	15	6
8/30/10	Gaining	Upstream	UG1 85	128	22	8
8/23/10	Gaining	Upstream	UG2 4 **	15600	3960	2520
8/23/10	Gaining	Upstream	UG2 6	386	204	94
8/23/10	Gaining	Upstream	UG2 24 **	1176	294	42
8/23/10	Gaining	Upstream	UG2 39 **	2024	368	276
8/23/10	Gaining	Upstream	UG2 62	1452	97	0
8/23/10	Gaining	Upstream	UG2 94	315	82	10
8/24/10	Gaining	Upstream	UG3 3	46	30	10
8/24/10	Gaining	Upstream	UG3 20	306	90	41
8/24/10	Gaining	Upstream	UG3 46	377	80	24
8/24/10	Gaining	Upstream	UG3 53	69	79	16
8/24/10	Gaining	Upstream	UG3 60 *	433	210	0
8/24/10	Gaining	Upstream	UG3 79	22	18	11
8/24/10	Gaining	Upstream	UG3 86	21	20	7
8/24/10	Gaining	Upstream	UG3 95	9	26	18
9/2/10	Losing	Upstream	UL1 8 *	124	51	0
9/2/10	Losing	Upstream	UL1 14	235	119	21
9/2/10	Losing	Upstream	UL1 50	382	278	23
9/2/10	Losing	Upstream	UL1 55	205	91	16
9/2/10	Losing	Upstream	UL1 60	97	70	8
9/2/10	Losing	Upstream	UL1 72	275	30	0
8/18/10	Losing	Upstream	UL2 3	404	41	0
8/18/10	Losing	Upstream	UL2 6	476	120	8
8/18/10	Losing	Upstream	UL2 33	192	73	0
8/18/10	Losing	Upstream	UL2 46	545	308	35
8/18/10	Losing	Upstream	UL2 64	383	126	21
8/18/10	Losing	Upstream	UL2 66	557	117	20
8/21/10	Losing	Upstream	UL3 16	248	33	10
8/21/10	Losing	Upstream	UL3 61	95	11	2
8/21/10	Losing	Upstream	UL3 66	9	20	0
8/21/10	Losing	Upstream	UL3 77*	233	41	0
8/21/10	Losing	Upstream	UL3 83	515	95	5
8/21/10	Losing	Upstream	UL3 89	**193**	61	8
8/21/10	Losing	Upstream	UL3 91	61	11	2

Cheumatopsyche	*Ceraclea*	Glossosomatidae	Rhyacophilidae	Hydroptilidae (*Leucotrichia*)	Psychomyiidae (*Psychomyia*)
12	2	0	5	3	0
13	2	2	2	3	0
6	4	1	0	6	0
2	10	3	1	8	1
3	7	3	0	11	2
3	16	19	1	2	2
4	2	4	0	5	4
16	11	30	2	5	4
7	1	33	0	8	1
7	16	19	0	2	3
14	16	56	1	11	6
0	8	8	0	2	0
5	4	36	0	0	0
0	1	13	1	1	0
8	2	45	1	2	1
10	5	37	1	0	0
13	9	101	3	1	3
5	0	2	0	3	0
2	0	5	1	0	0
5	2	4	0	0	0
6	6	6	0	1	0
7	1	14	0	1	0
10	2	13	3	1	0
19	2	34	1	1	0
1	0	6	0	0	0
1	0	1	4	0	0
8	6	16	1	0	2
12	5	15	1	0	0
4	1	1	0	0	0
4	1	13	1	0	1
0	1	5	0	0	0
21	3	1	0	0	2
13	3	0	0	1	1
3	0	0	0	0	2
7	1	1	0	0	9
0	0	3	0	0	0
0	6	5	1	0	0
6	9	0	0	0	0
4	1	0	0	0	0

5	1	3	0	0	0
0	4	0	1	0	0
0	1	0	0	0	0
0	1	0	0	0	0
120	120	0	120	0	0
7	22	0	1	0	0
168	0	0	0	0	0
0	92	0	0	0	0
44	16	0	4	0	0
5	0	0	0	0	0
1	0	1	0	0	0
4	0	1	0	0	0
9	6	2	0	1	0
0	2	3	0	0	0
17	8	3	0	0	2
0	1	1	0	0	8
7	2	0	1	0	3
0	0	3	0	0	24
4	11	1	0	0	0
1	2	0	0	0	0
0	6	0	0	0	0
6	11	0	1	0	0
15	8	0	1	1	0
0	17	0	0	0	0
0	5	2	1	0	0
8	1	0	0	0	0
0	0	2	0	0	0
79	8	0	0	0	0
34	16	1	1	1	0
18	0	0	0	0	0
4	1	2	0	0	0
5	7	0	0	0	0
5	3	0	0	0	0
16	14	0	0	1	0
28	14	0	0	27	0
12	0	0	1	4	0
4	3	0	1	2	0

Limnephilidae (*Onocosmoecus* sp.)	*Pteronarcys californica*	Perlidae (*Calineuria*)	Perlodidae (*Isogenoides*)	Perlodidae (*Arcynopteryx*)	Pyralidae (*Petrophila*)
0	5	0	0	0	0
0	1	0	0	0	0
0	4	0	0	0	2
0	1	2	0	0	3
0	0	1	0	0	4
0	0	0	0	0	13
0	0	1	0	0	1
0	1	0	0	0	16
0	3	0	0	0	0
0	1	0	0	0	11
0	4	0	0	0	15
0	0	1	0	0	8
0	0	1	0	0	4
0	0	0	0	0	1
0	0	0	0	0	2
0	0	0	0	0	1
1	0	0	0	0	1
0	0	0	0	0	1
0	0	0	0	0	0
0	0	0	0	0	0
0	2	0	1	0	0
0	0	0	0	0	0
0	4	0	0	3	0
0	5	0	0	1	0
0	1	0	0	0	3
0	1	0	0	0	0
0	4	0	0	1	0
0	0	0	0	0	0
0	0	1	0	0	0
0	0	0	1	0	1
0	0	1	0	0	0
0	2	0	0	0	29
0	2	0	0	0	1
0	0	0	0	0	2
0	1	0	0	0	4
0	1	0	0	0	0
0	0	0	1	0	0
0	0	1	0	0	0
0	1	0	0	0	0

0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	1	0	0	0
120	480	0	0	0	360
0	7	0	0	0	4
0	0	0	0	0	0
0	46	0	0	0	0
0	4	0	0	2	0
0	0	0	0	1	0
0	0	0	0	0	0
0	2	0	0	1	0
0	0	0	0	1	0
0	0	0	0	0	0
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0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	4
0	0	0	0	0	1
0	0	0	0	0	5
0	0	0	0	0	3
0	0	0	0	0	1
0	0	0	0	0	0
0	0	0	0	0	1
0	0	0	0	0	0
0	0	0	0	0	0
0	0	1	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	3
0	0	0	0	0	0
0	0	0	0	0	1
0	0	0	0	0	0

Chironomidae	Simulidae	Tipulidae (Antocha)	Elmidae	Hydrocarina	Isopoda (*Lirceus*)	Oligochaeta
403	50	4	1	11	1	0
409	6	3	0	50	0	1
505	12	3	1	96	0	0
719	9	3	0	30	2	0
432	6	8	0	42	0	1
453	10	7	0	87	0	0
133	0	5	0	0	0	0
114	0	3	0	17	2	7
158	0	4	0	2	0	4
578	0	6	0	13	1	3
546	3	7	1	31	0	0
684	5	0	0	6	0	1
80	0	1	5	9	0	7
10	0	0	0	0	0	0
76	13	5	8	12	0	0
65	1	8	1	5	0	1
115	0	5	2	1	0	0
237	2	1	3	8	0	1
69	32	1	3	1	0	0
35	50	1	7	1	0	0
107	27	4	22	2	0	0
66	78	1	5	2	0	1
29	30	3	23	7	0	1
170	74	0	38	25	0	4
28	147	0	0	1	0	0
1549	65	52	1	16	0	0
213	11	13	10	5	0	0
172	8	4	3	4	0	0
76	1	4	0	3	0	0
53	0	1	1	7	0	2
71	72	3	11	0	0	0
271	13	10	2	3	0	0
536	1	4	1	7	0	2
43	1	5	0	1	0	0
103	0	9	3	9	0	0
31	3	11	7	3	0	0
96	4	6	1	0	2	15
59	164	4	0	9	2	0
4086	160	32	1	12	0	0

61	8	2	4	2	10	0
101	10	3	0	8	1	0
35	20	1	0	2	0	0
36	15	0	2	3	9	5
72960	2160	1560	0	120	0	0
2403	120	12	6	25	1	0
19866	168	84	42	84	0	0
24656	598	0	0	230	0	0
42089	59	50	8	251	0	0
80	104	4	16	1	0	0
56	2	0	0	2	1	0
82	7	1	0	0	0	0
91	1	6	3	4	1	0
421	1	3	3	3	0	0
86	16	6	4	4	0	0
880	0	1	5	2	0	0
338	0	2	2	0	0	0
469	0	3	1	3	0	0
76	10	3	4	6	0	4
68	57	5	0	1	0	0
422	469	8	3	2	0	0
63	235	4	0	1	0	0
64	118	0	16	0	9	1
136	213	1	6	3	0	0
36	540	2	31	9	0	3
15	309	3	25	2	0	0
42	224	7	3	3	0	0
609	666	3	51	6	0	1
50	321	3	39	1	0	1
74	408	3	29	3	0	2
1	164	3	1	0	0	0
32	97	0	25	2	0	3
8	21	4	19	4	0	8
20	67	1	0	13	0	0
464	177	3	0	0	0	3
26	180	2	11	2	0	3
12	**62**	6	0	0	0	0

Turbellaria	Corixidae	Physidae	Planorbidae	Sphaeriidae	Crangonyctidae (*Crangonyx*)	Coenagrionidae (*Aegia* or *Argia*)
1	0	0	0	0	0	0
0	0	4	0	0	0	0
0	0	2	0	0	1	0
0	0	3	1	2	0	0
0	0	0	0	1	0	0
0	1	2	0	0	0	0
0	0	0	0	0	0	0
14	0	1	0	14	0	0
0	0	4	0	4	0	0
1	0	18	1	8	1	0
2	0	1	0	5	0	0
0	1	1	0	0	4	0
0	0	4	0	0	0	0
0	0	0	0	0	0	0
6	0	2	0	0	0	0
1	1	0	0	0	0	0
0	0	4	0	0	0	0
0	0	0	0	0	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
3	0	2	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
1	0	0	0	0	0	0
0	0	0	0	0	0	0
10	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
3	0	0	0	1	0	0
5	0	0	0	0	0	0
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0	1	1	0	0	0	0
2	1	0	0	0	0	0
0	0	0	0	0	0	0
1	0	0	0	0	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

2	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	1	0
0	0	0	0	0	0	0
1	0	0	0	0	0	0
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0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
1	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
5	0	1	0	1	0	0
2	0	0	0	1	0	0
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7	0	0	0	0	0	0
0	0	0	0	0	0	0
3	0	0	0	0	0	0
2	1	0	0	0	0	0
0	0	0	0	0	0	0
1	0	0	0	0	0	0
6	0	0	0	0	0	0
2	0	0	0	0	0	0
17	0	0	0	0	0	0
16	0	1	0	1	0	5
13	0	3	0	0	0	0
1	0	3	0	0	0	0
9	0	0	0	0	0	0
0	0	0	0	0	0	0

Empedidae (*Chelifera*)	Empedidae (*Hemerodromia*)	Empedidae (*Oreogeton*)	Ephydridae	Ceratopogonidae *(Bezzia/Palpomyia)*
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
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0	0	0	0	0
2	0	0	0	0
0	0	0	0	0
0	0	0	0	0

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0	0	0	0	0
0	0	0	0	0
240	0	0	0	0
2	0	1	0	0
0	0	0	0	0
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0	0	0	0	0
0	0	0	0	0
0	0	0	0	0

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