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Analysis of the Biological Data Collected from the Animas and San Juan Rivers Following the Gold King Mine Release

Collection of fish tissue samples from the Animas River

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Final Report

Analysis of the Biological Data Collected from the Animas and San Juan Rivers Following the Gold King Mine Release

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Notice

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Executive Summary

In response to the Gold King Mine (GKM) release on August 5, 2015, EPA mobilized field crews to sample water, sediment, and biological data from river segments impacted by the plume. Rivers downstream of the GKM release included the Animas River near Silverton, CO to its confluence with the San Juan River in Farmington NM, and the San Juan River from the Animas confluence to Lake Powell in Utah. A detailed examination of the water chemistry and sediment data collected from the Animas and San Juan rivers is presented in the EPA ORD report *Analysis of the Transport and Fate of Metals Released from the Gold King Mine in the Animas and San Juan Rivers* (EPA/600/R-16/296).

In this report, EPA presents its analysis of available biological data collected from the Animas and San Juan rivers to assess how the aquatic life responded to the GKM release. Biological communities provide a measure of water quality and aquatic habitat quality by responding to extreme events, such as the GKM release, and integrating stressors over time. Data gathered for this analysis include the EPA near-term (post-GKM release fall 2015) and long-term (fall 2016) biological monitoring of 30 locations, as well as biological data collected by federal, state, and tribal partners. The sampling and analysis approach was designed to evaluate potential changes in the species compositions, population abundance, and the concentration of metals in the tissue by comparing the post-GKM release data to the pre-release conditions.

The upper Animas River immediately below the confluence with Cement Creek experienced the highest metal concentrations, the greatest number of water quality standards excursions, and the greatest deposition of GKM sediment, during and immediately following the GKM release. A significant increase in copper and decreases in manganese concentration were observed in benthic macroinvertebrate tissue in the near-term 2015 samples. Although these conditions existed, the pre- and post-GKM release analyses did not reveal any clear changes in the aquatic community. The lack of a biological response is largely because the aquatic life in this section of the river has been impacted for decades by legacy contamination from historic mine ore processing and ongoing acid mine drainage contamination. The sensitive macroinvertebrate and fish species that would be expected to respond to the GKM release were already extirpated from the upper reaches of the Animas River.

Study Questions

Did the GKM release add to biological degradation in the already contaminated upper Animas River?

Did the GKM release degrade biological communities in other segments of the Animas and San Juan rivers that had not been known to have historic metal impacts?

Biological Response to the GKM Release

- Some fish accumulated metals the weeks after the GKM event. Levels in fish declined to background conditions when samples were collected again the following spring and never triggered human health consumption advisories.
- There were no measurable changes to benthic macroinvertebrate assemblages after the GKM release.
- There were no clear impacts on fish populations after the GKM release.
- Differences in sampling methods used by the states and other partners across years limited what could be interpreted in the report.

In the middle Animas River, we also did not observe a clear loss of, or change in the more sensitive macroinvertebrate and fish taxa that start to appear as one moves away from the concentrated historic mining operations in the headwaters. Our review of the Animas River adult fish population data collected by Colorado Parks and Wildlife near Durango agrees with existing state analyses, reports, and press releases that concluded fish were not exposed to acutely toxic concentrations in 2015. Naturally reproducing fish species (suckers and sculpin) and trout fry continue to be found in the Animas River at pre-release abundance levels weeks after and a year following the GKM release, however small bluehead suckers less than <200 mm were not observed in the 2016 data. The lack of a substantial biological response in this section of the river can be attributed to dilution of the plume, the dominant form of the metals was particulate rather than dissolved, and exposure duration was short, which resulted in fewer excursions of water quality standards.

Our analysis of fish tissue data collected by New Mexico Department of Game and Fish showed that many metals were significantly elevated in bluehead sucker and flannelmouth sucker liver and speckled dace muscle tissue samples collected in weeks after the GKM release in the lower Animas River. The degree of metal accumulation in liver differed by species, sampling location, and among the metals, with aluminum, cadmium, lead and manganese exhibiting the greatest concentrations. Cadmium and mercury in liver tissue and selenium in muscle were greater in the San Juan than in the Animas. When fish were sampled the following spring and fall in 2016, the concentration of metals in muscle/filet samples were similar to pre-release concentrations and were low throughout both rivers. For the most part, the elevated liver concentrations in 2015 did not translate to elevated muscle concentrations. Metal concentrations in muscle tissue never triggered human health consumption advisories. There were no fish population data available from this section of the Animas River to help us understand if the metal concentrations in fish tissue were sufficiently high to adversely affect the fish populations.

By the time the GKM plume reached its confluence with the San Juan River, total metal concentrations had declined by three orders of magnitude from what they were when the plume entered the Animas because of the combined effects of the dilution, chemical reactions, and deposition. The excursions of aquatic life water quality criteria in the San Juan were limited to metals that are also naturally high in the sediment and water.

The U.S. Fish and Wildlife Service fish population data for the San Juan River show that fish abundance in 2015 and 2016 was generally within pre-release levels. The exception to this was the abundance of bluehead sucker, flannelmouth sucker, and speckled dace in the middle reaches of the San Juan River. These species had historically low abundance in this area in both 2015 and 2016. The razorback sucker, Colorado pikeminnow and channel catfish, however, had high abundance in 2015 and 2016, which are potential predator/competitor species. We cannot conclude that changes in the physical (i.e., release from the Navajo dam resulting in a short duration of increased flow) and chemical conditions in the San Juan River during and after the plume contributed to changes in species abundance as, the aquatic life water quality criteria excursions were limited and the flow increase was similar to a moderate-sized storm event. It is as plausible that a combination of ecological (increase of predator/competitor species) and physical interactions, and/or fisheries management actions (stocking of razorback and pikeminnow), contributed to the observed changes.

With respect to metals accumulated in biota one-year post-GKM release, metal concentrations measured in benthic macroinvertebrate tissue and fish tissue generally track the gradient of concentrations measured in sediment and water through the watershed. The highest metal concentrations in tissue were typically observed in the upper Animas and the lowest concentrations were observed in the San Juan. Localized high metal concentrations were observed in the post-release tissue data; however, the location at which the high concentrations were observed was not consistent among years highlighting the high intra- and inter- site variability in tissue concentrations. In fall 2016, many metals were elevated in benthic macroinvertebrate tissue when compared to the pre-release concentration; however, the high concentrations were also observed in the upstream and tributary samples suggesting that something other than the GKM release contributed to the concentration change. Likely explanations include differences in sample collection methodologies between years and taxonomic differences between sampling locations. A comparison of pre- and post-GKM fish muscle data among data provider showed similar concentrations that did not exceed human health consumption screening advisory levels.

The EPA 2016 sampling was the first effort to obtain biological data that covered the entire Animas and San Juan rivers in a single sampling event with consistent sampling methods. Our ability to conduct a watershed-scale analysis of data collected by all partners was limited by the different sampling and analytical methods and revealed the need for a consistent sampling approach. This was especially true for studies focusing on bioaccumulation of metals. Future watershed-scale monitoring efforts should include the development of consistent sampling methods when an objective is to compare results to data collected from other areas of the watershed.

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Acknowledgments

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Acronyms

AMD	acid mine drainage
CMP	conceptual monitoring plan
DOC	dissolved organic carbon
dw	dry weight
GKM	Gold King Mine
GLM	generalized linear model
RKM	river kilometer
MDL	minimum detection limit
MMI	multimetric index
ppb	parts per billion
ppm	parts per million
ww	wet weight

CHAPTER 1 OVERVIEW

The impact of historic mining on water resources in the Animas River watershed in the San Juan Mountains of southwest Colorado has been a concern for decades. Beginning in the 1870s, the headwaters of the Animas River near the town of Silverton became home to a dense network of hard rock mines from which gold, silver, lead, zinc, and copper ores were extracted from the highly mineralized geologic formations found in the Colorado mineral belt (Figure 1.1). Mining operations in this area ceased by the early 1990s, leaving hundreds of abandoned mines that historically have discharged an average of 5.4 million gallons of acidic mine drainage (AMD) per day into the headwaters of the Animas River (USGS 2007). AMD contains high concentrations of heavy metals, such as iron, aluminum, zinc, lead, cadmium, copper, and many others. Metals generated from AMD and historic ore processing have impacted the Animas River and its aquatic life for more than a century. The U.S. Geological Survey (USGS) focused considerable research activity in the upper Animas watershed from 1995 to 2007, to guide restoration plans to abate AMD and reduce metals contamination (USGS 2007) in this heavily impacted area. Research included the collection of physical, chemical and biological data as well as metals accumulation in biota and toxicity tests.

As a result of these studies, federal and state governments, as well as stakeholder groups have conducted remediation activities in the watershed. The Animas River Stakeholder Group, the Bureau of Land Management, the Colorado Division of Reclamation/Mining and Safety, and EPA Region 8 have completed remediation projects in the watershed (EPA Region 8, Upper Animas Mining District: Draft Baseline Ecological Risk Assessment, http://www2.epa.gov/region8/upper-animas-mining-district-draft-baseline-ecological-risk-assessment). The Colorado Department of Public Health and the Environment has developed more than twenty-five Total Maximum Daily Loads (restoration plans required for waterbody segments considered impaired under the Clean Water Act) to help guide restoration activities towards meeting water quality standards. However, for some waters, including Cement Creek, the State of Colorado has followed procedures under the Clean Water Act to remove aquatic life support as a designated use for the waterbody because it is not an attainable goal (Colorado Department of Public Health & Environment, https://www.colorado.gov/pacific/cdphe/tmdl-san-juan-and-dolores-river-basins).

On August 5, 2015, an EPA team investigating the Gold King Mine as a source of metals inadvertently triggered a release of 3 million gallons of acidic, mine-influenced waters. These waters had been trapped by the collapsed mine structure and rock blocking the opening (or adit) of the mine, damming the water behind the collapse and causing the waters to become pressurized. Over an eight-day period, the plume from the release flowed down the Animas River to the San Juan River. The EPA report *One Year After the Gold King Mine Incident* (EPA 2016f) provides an overview of the EPA's response to the GKM release and additional information on the environmental conditions of the watershed prior to and after the incident.

The EPA, the states, and tribes began monitoring metals in water and river bed sediments throughout the affected rivers to assess risk to public health as compared with water quality criteria and probable effect concentrations. In September 2015, the EPA released a follow-up draft conceptual monitoring plan (CMP) that specified how the agency would gather scientific data on physiochemical and biological parameters downstream of the GKM release. The CMP was finalized March 2016 and incorporated comments received from local, state and tribal stakeholders; knowledge gained from the first round of sampling in fall 2015 and increased familiarity with the historic data. (https://www.epa.gov/sites/production/files/2016-03/documents/post-gkm-final-conceptual-monitoring-plan_2016_03_24_16.pdf). The primary objective of the CMP was to provide biological data that span the watershed that can be used to compare current conditions to conditions that existed in the watershed prior to the GKM release. These data were also collected for use by EPA, states, tribes, and local entities to supplement a general assessment of water quality, sediment quality, and biological conditions in the watershed.

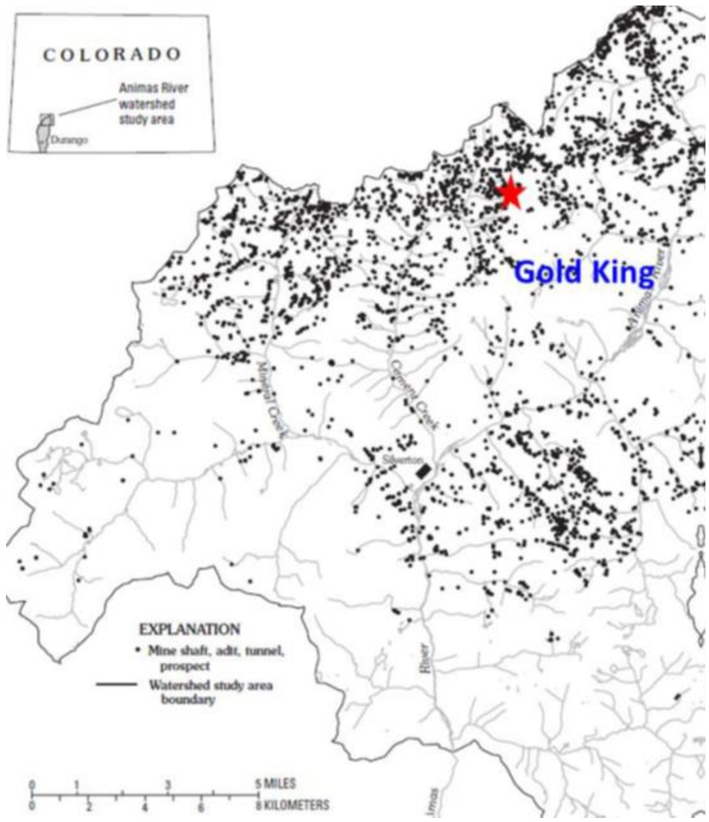

Figure 1.1. Map of mines within the Animas River headwaters, many of which are abandoned. Mining has not been economically viable in this area since the early 1990s. (Map modified from USGS 2007).

The EPA Office of Research and Development (ORD) report *Analysis of the Transport and Fate of Metals Released from the Gold King Mine in the Animas and San Juan Rivers* (EPA/600/R-16/296) provides a detailed examination of the water chemistry and sediment data collected from the Animas and San Juan rivers before, during and after the release (EPA 2016c).

This report presents EPA's analyses of the biological data collected from the Animas and San Juan rivers during the GKM release and in the months following, using data collected by states, tribes, federal partners, and EPA. Post-release data providers included Colorado Parks and Wildlife (CPW), Colorado Department of Public Health and Environment (CDPHE), New Mexico Environment Department (NMED), New Mexico Department of Game and Fish (NMDGF), Southern Ute Indian Tribe (SUIT), Navajo Nation Environmental Protection Agency (NNEPA), and U.S. Fish and Wildlife Service (USFWS). Biological data presented here include fish and benthic macroinvertebrate community data, biological tissue data, and physical habitat. States and tribes have already reported key findings of agency studies of the immediate impacts of the event to their stakeholders.

The objective of this report was to consolidate available data into an integrated analysis of the biological response to the GKM release by exploring the following questions:

1. *Did the GKM event add to biological degradation in the already contaminated upper Animas River?*
2. *Did the GKM release degrade biological communities in other segments of the Animas and San Juan rivers that had not been known to have historic metal impacts?*
3. *Were acute impacts to the biological communities observed during the initial GKM release when metals concentrations were highest?*
4. *Were long-term changes in biological communities observed a year after the GKM release?*

Report Outline

Chapter 2 provides an overview of the Animas and San Juan rivers watersheds, emphasizing the factors that influence aquatic habitat and metals characteristics that contribute to the distribution and vitality of macroinvertebrate and fish communities. The overview provides a brief review of two decades of extensive study of the impact of acid mine drainage and historical mining practices on the biological conditions in the mining district in the headwaters of the Animas River by USGS, EPA and academic researchers. This chapter also reviews the physical and chemical conditions that may have influenced biological communities during and after the GKM release.

Chapter 3 discusses the available biological data and methods of analysis used to synthesize a river-wide assessment of the GKM release. Biologic data was collected by states, tribes, watershed groups, the USFWS and EPA before, during, and after the GKM release.

Chapter 4 provides an overview of the post-GKM physical habitat conditions data collected by EPA and contractors.

Chapters 5 and 6 present the benthic macroinvertebrate assemblage and fish community analyses, respectively. Pre- and post-GKM release analyses are provided for sections of the Animas and San Juan River with historic data.

Chapters 7 and 8 present analyses of the metals accumulated in benthic macroinvertebrate and fish tissue, respectively.

Chapters 9 and 10 synthesize the findings and present watershed wide longitudinal trends in metal concentration a year following the release, after the GKM deposit moved through the system. Chapter 10 also provides recommendations for future biological monitoring in the San Juan watershed.

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CHAPTER 2 BACKGROUND WATER QUALITY, SEDIMENT QUALITY AND ECOLOGY IN THE ANIMAS AND SAN JUAN RIVERS

2.1 Watershed features

The Animas River is a major tributary to the San Juan River that originates within the San Juan Mountains in southwestern Colorado. After flowing southward for 200 km, the Animas joins the San Juan River at Farmington, NM. (Figure 2.1). The San Juan River then flows westward for nearly 400 km through increasingly arid s terrain until it flows into Lake Powell in Utah. The Animas and San Juan rivers downstream of their confluence are hereafter referred to as the study area.

The baseline physical and chemical characteristics within the rivers establish the foundation for the expected composition and abundance of the aquatic biota. Physical habitat characteristics such as water temperatures, channel slope and river bed morphology, and composition influence the spatial distribution of aquatic communities at a watershed and local scale. River ecosystems change significantly along the 600-km length as the Animas and San Juan rivers transition through more than 5,100 ft (1,500 m) of elevation change and flow through diverse climatic, geologic and geomorphic conditions. Anthropogenic alterations to these conditions (e.g., riparian disturbance, channelization, flow modification and water quality degradation) affect the abundance and distribution of species that would normally be expected to occupy those habitats.

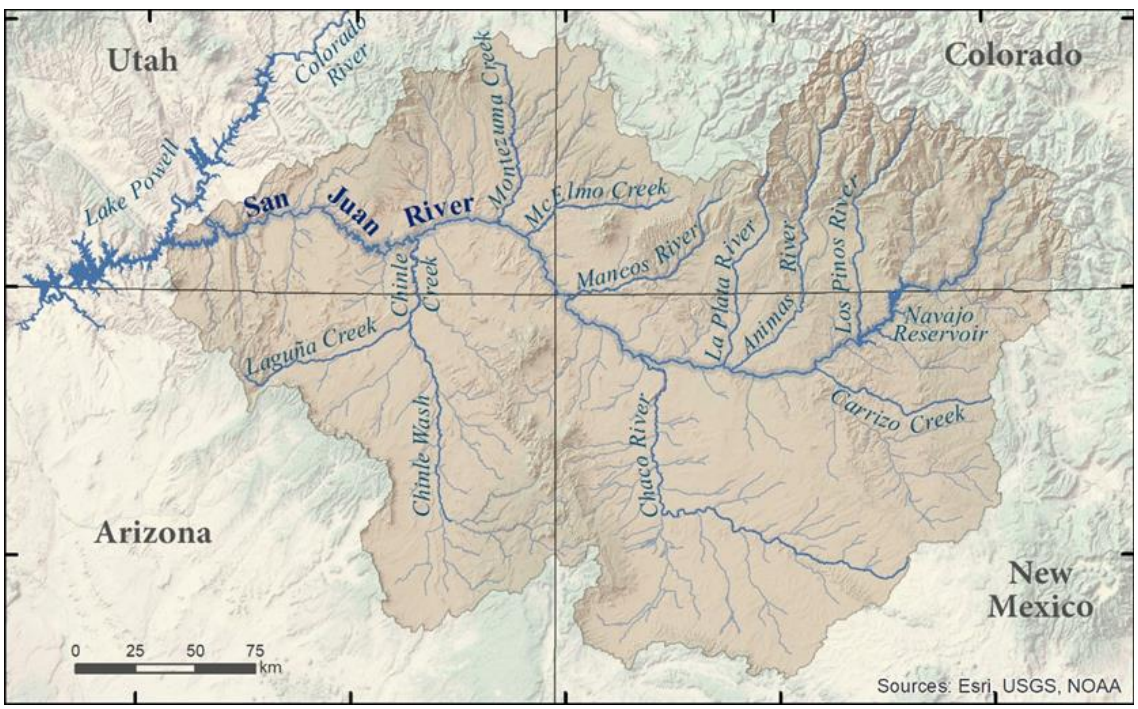


Figure 2.1. General map of the San Juan River watershed.

Cement Creek (RKM 12.5)

Animas: at Bakes Bridge (RKM 64)

Animas: south of Durango (RKM 110)

Animas: near Cedar Hill (RKM 147)

Animas: at Farmington (RKM 190)

Upper and middle Animas River

The Animas River originates high in the San Juan Mountains of southern Colorado near the town of Silverton, Colorado. Aquatic habitats lie within the alpine and subalpine forests with most of the watershed managed as the San Juan National Forest. From 8,500 ft (2,600 m) elevation in Silverton, the Animas River flows southward for approximately 50 km, descending through a steep and narrow canyon carved into the Precambrian basement rocks. The Animas abruptly exits the canyon at Baker's Bridge (RKM 64) and flows onto a wide alluvial valley near Hermosa Springs about 30 km north of Durango, CO. The channel is heavily braided for about 10 kilometers before establishing a meandering form that persists to Durango. The high gradient segment above Bakers Bridge is generally referred to as the upper Animas, while the lower gradient segment that extends downstream through Durango and the Southern Ute Indian Reservation to the Colorado/New Mexico border is referred to as the middle Animas.

Within the upper Animas and tributaries (e.g., Cement Creek and Mineral Creek), summer water temperatures are relatively cool (< 17ºC; Figure 2.2) and river morphology is steep (0.7 to 1.6% gradient) and characterized by riffles, cascades, and falls with coarser substrates composed of gravel, cobble and boulders. Upon exiting the canyon, the middle Animas segment transitions to warmer water, fine substrate size, and transitional biotic communities.

Lower Animas River

The Animas River becomes more constrained with a straighter course within the incised valley from Durango, CO to Farmington, NM where it joins the San Juan River 190 km from the headwaters origin. The segment between Cedar Hill and Farmington is generally referred to as the lower Animas River. The lower reaches of the Animas are warm (maximum temperatures 26ºC; Figure 2.2), channel slopes are moderate (0.4%) and habitat conditions continue to transition to low gradient, fine substrate channels.

San Juan River

The San Juan River has been regulated by the Navajo Dam located approximately 60 km upstream of its confluence with the Animas at Farmington since the 1960's. The Animas routinely supplies approximately 50% of the flow of the combined rivers and is the primary unregulated source of perennial flow to the San Juan. The Navajo Dam has altered the flow regime of the San Juan River downstream of the dam, changing its ecology from a warm, muddy and highly seasonal river to one with relatively constant flows. The San Juan River retains more of its unregulated nature below the confluence of the Animas.

San Juan: in Farmington (RKM 194)

San Juan: at Shiprock (RKM 246)

San Juan: at Montezuma Creek (RKM 346; August 2015)

Photo: Utah Department of Environmental Quality

San Juan: at Bluff, Utah (RKM 377; USGS Gage)

Spring snowmelt from the Animas and monsoonal storms in the more arid tributaries are the primary source of flow variability. Maximum water temperatures can exceed 30ºC. The San Juan River flows westerly towards its junction with the Colorado River near Mexican Hat, UT within a valley that for most of its length is shallowly incised into a series of sedimentary rock formations at various depths. The river flows through highly erodible marine and continental sedimentary rocks and carries a high sediment load during seasonal storms. Valley width ranges from tens to hundreds of meters and channel widths range from 50 to 100 m with low gradient ranging from 0.07 to 0.16% and dominated by fine-grained particles. Braided channels are common in most of the intermittent tributaries and probably was in much of the San Juan River mainstem before flow control. Now the mainstem channel alternates between stable multi-threaded channels with vegetated island bars and straight intervening segments.

Land Use

Most of the combined Animas and San Juan River watershed are remote and uninhabited. Vegetation is characterized by subalpine forests in the headwaters of the Animas that is managed primarily by the US Forest Service and shrubland, rangeland and grassland in the rest of the area (EPA 1979). The watershed is lightly populated, with most settlements concentrated along the San Juan and Animas rivers. Farmington, New Mexico is the largest city and other major population centers include Durango, Colorado, and Aztec, and Shiprock, New Mexico. Irrigated agriculture is a major land use in the middle and lower reaches of the Animas River, withdrawing water through a system of ditches and canals. There are also numerous wells drilled into the river floodplains that supply public, domestic and irrigation users.

The San Juan River flows through the states of New Mexico and Utah and the tribal lands of the Navajo Nation and Ute Mountain Ute Tribe. Within this generally arid area, the river supports irrigated farming. A large canal diverts water from the San Juan River near Waterflow, NM to supply regional irrigation water needs. Near Mexican Hat, UT, the San Juan River ultimately flows into Lake Powell created by the Glen Canyon Dam at Page, AZ at elevation of 3,400 ft. Population density is sparse downstream of Shiprock, NM. Most of the lower San Juan River in Utah flows through inaccessible canyons that largely preclude habitation.

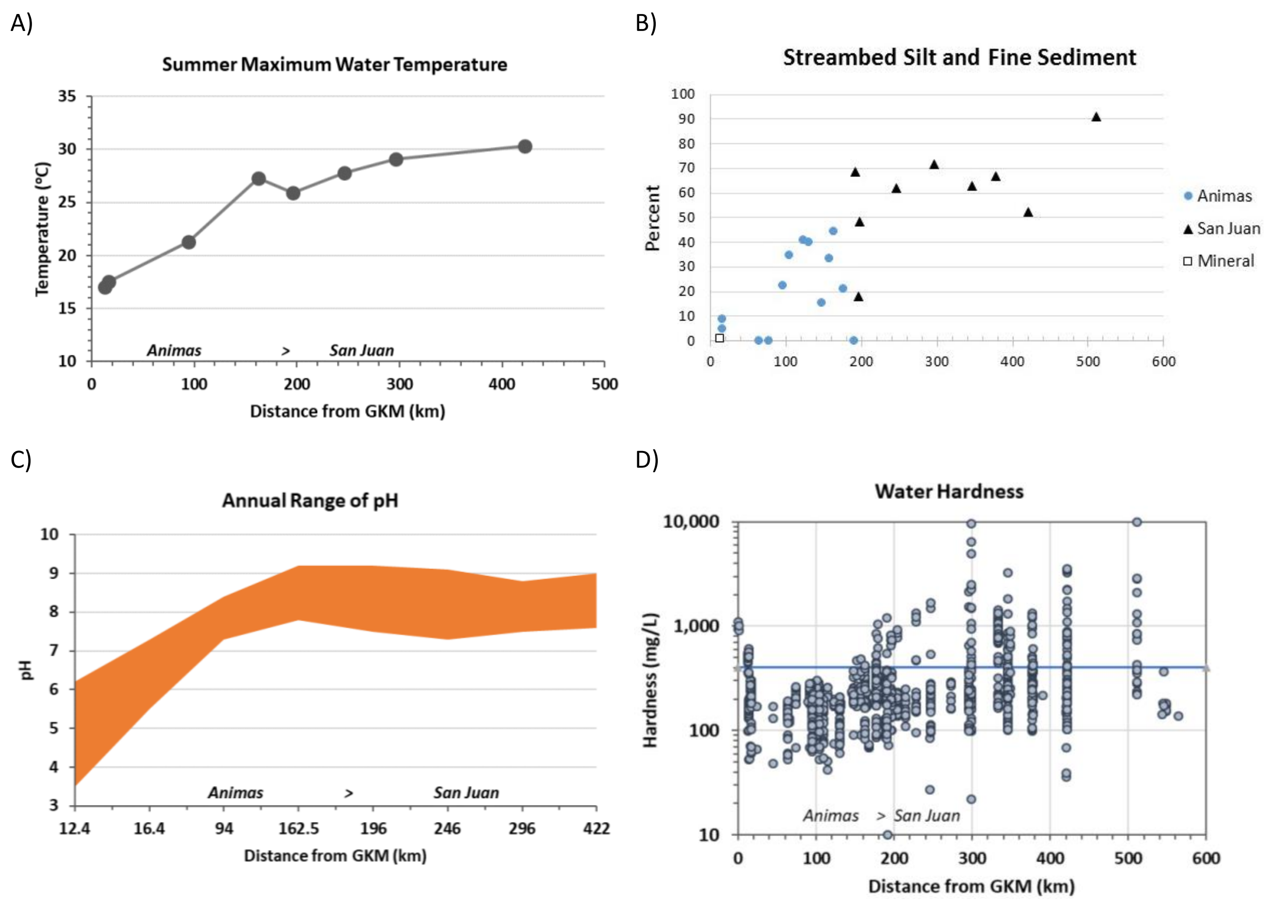


Figure 2.2. Water quality conditions along the length of the Animas River (RKM 0 to 195) and the San Juan River below its confluence with the Animas at Farmington (RKM 195-650): A) average maximum observed water temperatures each year at USGS gages; B) streambed sediment distribution was obtained from EPA post-release habitat surveys; C) range of pH observed annually measured by sondes deployed at USGS gages 2016-2018; D) water hardness in 2015-2016 samples.

2.2 General distribution of aquatic life

Fish need plants, insects and benthic macroinvertebrates to eat; in-stream and streambank cover for shelter; appropriate streambed substrate conditions for spawning; and overhanging vegetation to shade the water in which they live. The changes in temperature, dissolved oxygen, pH and myriad other physical and chemical constituents in water along the river continuum influence the changes in species composition, species abundance, and physical habitat that are observed as one moves from the headwaters down to Lake Powell. The general longitudinal change in water quality and sediment conditions along the Animas and San Juan rivers is provided in Figure 2.2. Water temperature increases while particle size of the benthic sediment decreases with distance from headwaters. Watershed geology strongly influences water quality. The volcanic geology within the upper Animas generate river flow with low pH and high metals content. The sedimentary rocks characteristic of most of the watershed buffer pH. The importance of headwaters geology in determining water quality will be discussed later in this chapter.

2.2.1 Fish communities

Fish communities transition from coldwater species in the headwaters of the Animas River to warmwater communities in the San Juan (Table 2.1). The upper Animas River lies entirely within alpine and subalpine habitats and would be expected to support coldwater species typical of the Colorado Rockies. Barriers to upstream movement in the Animas River canyon limited the composition of the native fish community to cutthroat trout that are currently found in high altitude tributaries with good water quality (vonGuerard *et al.* 2007). Brook trout were introduced to the upper Animas as early as 1885. Brook trout are well adapted to coldwater, small stream habitats. Populations are sustaining and brook trout are currently the predominant fish species, but are locally impacted by poor water quality (Besser and Brumbaugh 2007). Rainbow trout were also stocked in the upper Animas at various times but were not as successful.

Below the Animas canyon, native fish species include bluehead sucker, flannelmouth sucker, white sucker, speckled dace, and mottled sculpin. The Colorado Division of Wildlife (CDW) manages two segments of the middle Animas River between Durango and the Colorado/New Mexico border to provide a high quality recreational fishery of brown and rainbow trout. Natural reproduction of trout in the middle Animas River is low; therefore, the fishery is supported by annual stocking with fry/fingerling/sub-catchable salmonids" and catch limits are used to control angling pressure (CDW 2010, 2015). Cutthroat trout fingerlings have also been stocked since 2005. Regular inventories of the Animas River fish for the last several decades have shown that trout biomass and density vary from year to year due to multiple factors including water temperatures, stocking rates, and potentially metals from the upper Animas basin (CDW 2010). Summer water temperatures are near optimal for rainbow and brook trout in this segment (generally within 18^{o}C) but maximum temperatures can become stressful for rainbow trout during low flow years. The river is also heavily used for recreational boating and swimming.

The lower Animas River is wider and warmer than the middle Animas. New Mexico Environment Department (NMED) classifies this reach as marginal coldwater aquatic life (20.6.4 NMAC). Maximum summer temperatures reach 28^{o}C but seasonal temperatures during other times of the year are much more moderate. New Mexico Department of Game and Fish (NMDGF) stocks a two-mile reach of the Animas through Aztec, NM with catchable rainbow trout. The lower Animas has abundant populations of bluehead suckers, flannelmouth suckers, and speckled dace with white suckers also present. The U.S. Fish and Wildlife Service (USFWS) has stocked razorback sucker and Colorado pikeminnow in the lower Animas River annually since 2011.

Like the lower Animas, the upper San Juan River near Farmington is designated as marginal coldwater aquatic life and warmwater aquatic life by NMED. Summer maximum temperatures exceed 28^{o}C. The San Juan River provides habitat to at least eight native species including cutthroat trout, roundtail chub, speckled dace, flannelmouth sucker, bluehead sucker, mottled sculpin, Colorado pikeminnow and razorback sucker, with a possible ninth species being the bonytail chub. The non-native common carp and channel catfish have become widespread in the lower reach of the San Juan (USFWS 2006). Rainbow and brown trout occur near Farmington but abundance varies seasonally.

The San Juan River downstream of the Animas River is designated as critical habitat for federally endangered Colorado pikeminnow and razorback sucker. The San Juan River Recovery and Implementation Program (SJRIP) was established to support the recovery of the endangered Colorado pikeminnow and razorback sucker, in conjunction with water development projects in the basin. The USFWS with state and tribal partners manage the plan including conducting long-term fish community surveys from the Navajo Reservoir to Lake Powell. The braiding channel type characteristic of this low gradient river is important to successful reproduction of the native fish that use low velocity and backwater habitats created by the braiding channel morphology. Flow management at the Navajo dam contributes to the loss of braided-channels and associated habitat. Endangered fish are also subject to predation from non-native fish; channel catfish are actively removed to facilitate recovery of the endangered fish.

Table 2.1 Fish occurrence within the Animas and San Juan rivers, as available from various sampling data listed in Chapter 3.

Common Name	Scientific Name	General Area Within Watershed Collected	Status and Notes
Brook trout	*Salvelinus fontinalis*	Upper Animas	Introduced, population limited due to metals
Brown trout	*Salmo trutta*	Upper, middle, lower Animas, upper San Juan durring sometimes of year	Introduced, annual stocking program in middle Animas
Rainbow trout	*Oncorhynchus mykiss*	Upper, middle, and lower Animas	Introduced, annual stocking program in middle Animas
Cutthroat trout	*Oncorhynchus clarkii*	Upper and middle Animas	Native, confined to uncontaminated high elevation streams outside the mining district; occasionally stocked
Mottled sculpin	*Cottus bairdii*	Upper and middle Animas	Native; limited in upper Animas due to metals
Speckled dace	*Rhinichthys osculus*	Middle and lower Animas, upper San Juan	Native
Bluehead sucker	*Catostomus discobolus*	Middle and lower Animas, upper San Juan	Native, larger river habitats
Flannelmouth sucker	*Catostomus latipinnis*	Middle and lower Animas, upper San Juan	Native, larger river habitats
White sucker	*Catostomus commersonii*	Lower Animas	Native, larger river habitats
Razorback sucker	*Xyrauchen texanus*	Upper and lower San Juan	Native, endangered status since 1991 (flow modification)
Colorado pikeminnow	*Ptychocheilus lucius*	Upper and lower San Juan	Endangered status since 1967 (flow modification)
Channel catfish	*Ictalurus punctatus*	Upper and lower San Juan	Introduced, eradication program to reduce predation on native fish
Common carp	*Cyprinus carpio*	Upper and lower San Juan	Introduced, eradication program to reduce predation on native fish

2.2.2 Benthic macroinvertebrates

Streams can have several hundred different kinds of benthic macroinvertebrates with total numbers ranging in the thousands. Three orders of aquatic insects are common in the benthic macroinvertebrate communities. These orders are Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). EPT taxa (combined Ephemeroptera, Plecoptera, and Trichoptera) tend to prefer higher gradient, coarse substrate habitats and would be expected to be observed in high abundance in the coarser river substrates characteristic of most of the Animas River. These taxa are also generally considered to be sensitive to or intolerant of pollution and are widely used as indicators of water quality.

Macroinvertebrate communities have been sampled through the length of Animas River at various times over the past 20 years. Anderson *et al.* (2007) sampled the Animas River from the headwaters downstream to the boarder with NM and a single sample in New Mexico in 1996, finding some degradation of benthic macroinvertebrate communities relative to reference tributaries throughout. Various metrics of macroinvertebrate communities presented by Anderson *et al.* (2007) are shown n Figure 2.3. Species richness, number of taxa, and sensitive taxa are very low in the upper Animas River within the first 50 km of river length and tend to increase once the river enters onto the alluvial river valley near Bakers Bridge (e.g. caddisflies and mayflies in Figure 2.3). While the San Juan River is not represented in the graphs below, the expected composition of San Juan River macroinvertebrate communities would not be the same as that expected from the Animas River. The macroinvertebrate composition in the San Juan River would reflect the differing physical and chemico-physical factors such as the presence of increased fine-grained particles in the stream bed as well as warmer water temperatures.

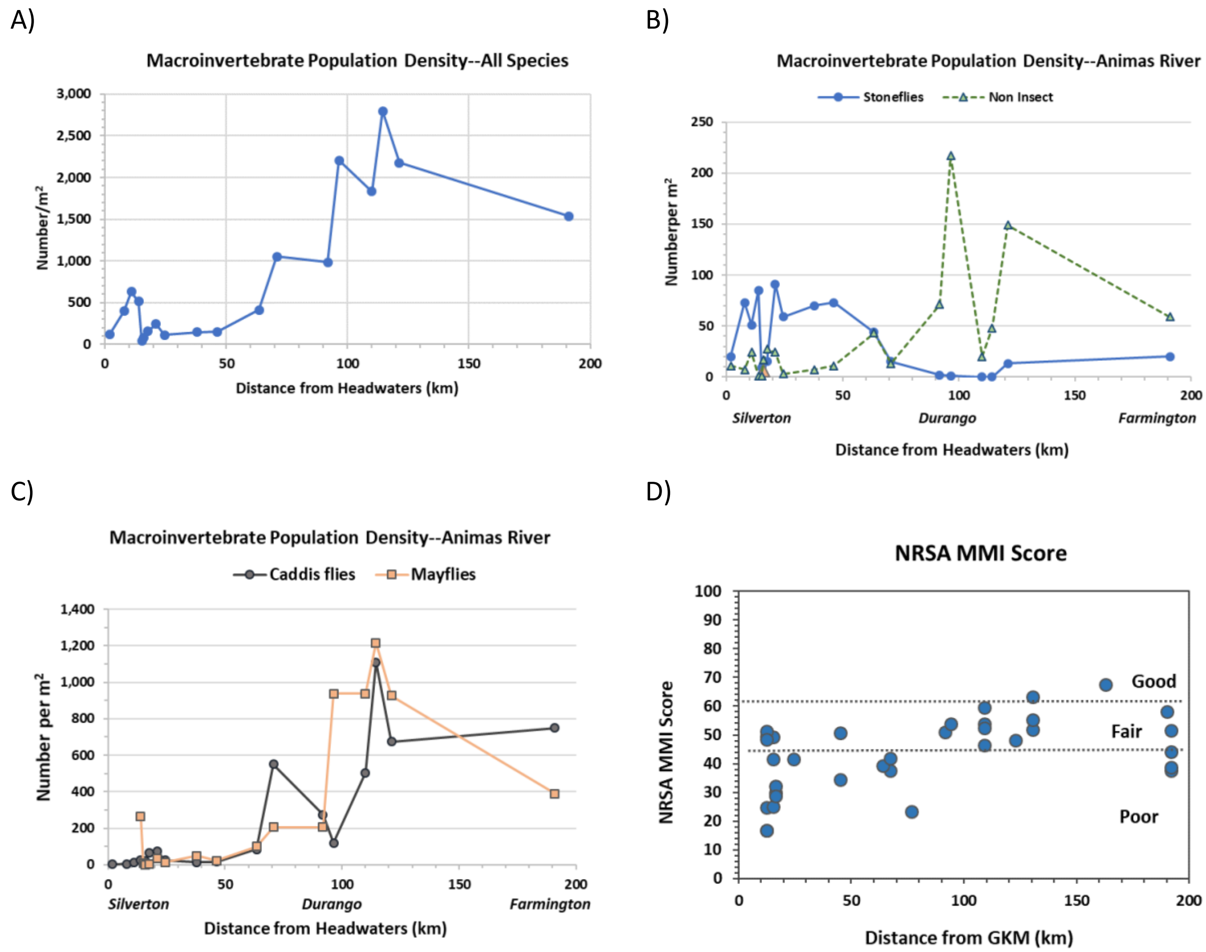


Figure 2.3. Characteristics of macroinvertebrate populations in the Animas River. Data in figures A-C present data from Anderson (2007), D) presents pre-release data collected by states and tribes.

The biological condition of the nation's flowing waters is assessed every five years during the EPA's National Rivers and Streams Assessment (NRSA). In this national survey, regionally specific benthic macroinvertebrate multi-metric indices (MMI) are the primary tool for assessing biological condition. Five to six individual assemblage metrics, such as taxonomic richness, composition and diversity, functional feeding groups, habits/habitats, and pollution tolerance, are combined to create each of the regionally specific MMIs (EPA 2016b). By combining metrics that represent different aspects of the benthic macroinvertebrate assemblage, each MMI integrates the influence of multiple chemical and physical stressors. Additionally, due to the unique life history characteristics of benthic macroinvertebrates (life cycles of weeks to a few years and relatively immobile), this assemblage integrates the spatial and temporal impacts of stressors more comprehensively than other biological assemblages, such as algae and fish. Regionally specific reference conditions were used to develop biological condition benchmarks for each regional MMI. NRSA MMI scores for the Animas River are shown in Figure 2.3, and have been categorized into good, fair, and poor condition based upon regionally relevant benchmarks, either the

Western Mountains or Xeric aggregate ecoregion (EPA 2016b). When applied to pre-release samples, NRSA MMI scores show a trend of increasing benthic macroinvertebrate condition on the Animas River as you get further from the headwaters (Figure 2.3 D).

2.3 Persistent stressors to aquatic life

There are numerous stressors to fish and benthic macroinvertebrates in the Animas and San Juan rivers. Like most watersheds, land use often impacts water quality through introduction of pollutants and loss of riparian function. Those factors as well as flow management and recreational river use affect water quality and aquatic communities, especially in the middle and lower reaches of the Animas and San Juan where populations centers are located. Nutrient loading from agricultural runoff is high in lower reaches of the Animas River. Sediment loads are high in the lower Animas and San Juan River during monsoonal storms that occur in the region during the summer/fall months. However, metals contamination of water and sediment due to the headwaters geology and past mining activity have had an impact on aquatic life in the upper Animas River, extending downstream for some distance.

2.3.1 *Metals in the watershed*

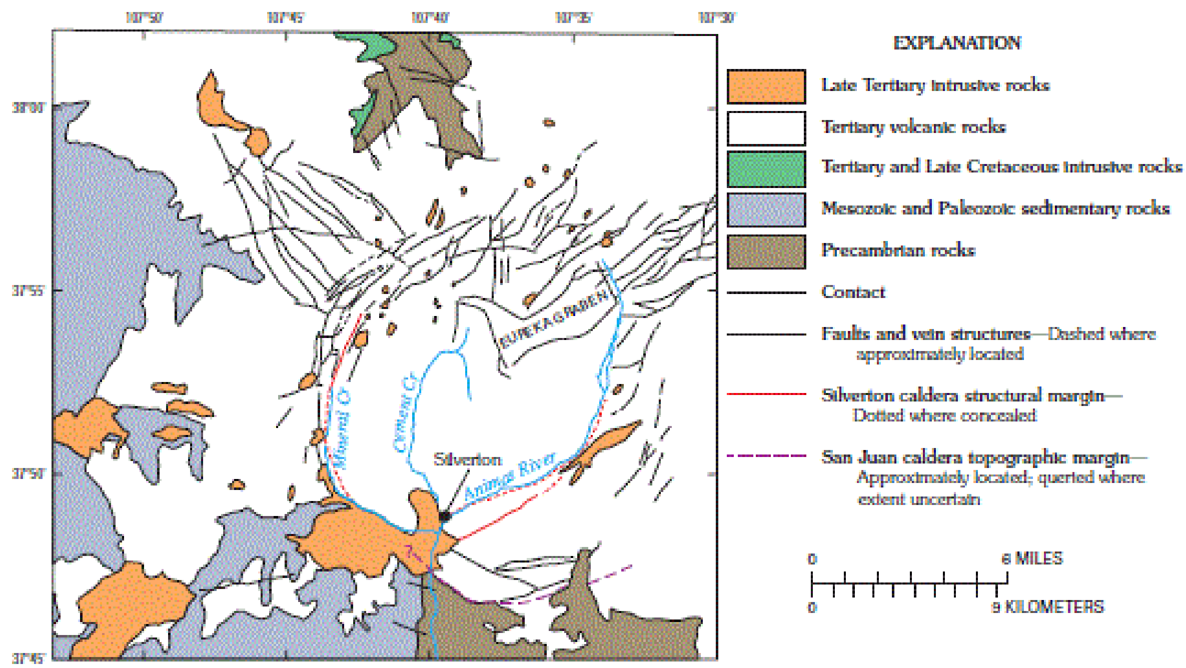
The Animas River originates in a regionally important geologic zone known as the Colorado Mineral Belt that was formed in a series of regional volcanic eruptions that took place during the late Paleogene (28-23M years ago). Regional volcanism left relict features including the remnants of a large caldera almost 19 km in diameter that is also the source of the Animas River. Within the caldera are mineralized sulfide ores that contain extensive areas of naturally acidic rocks and soils with vein-type deposits of gold, silver, zinc, and copper. The ore deposits were extensively mined for 120 years before the last mine was shuttered in 1991 (Luedke and Burbank 1999; von Guerard *et al.* 2007).

The ore bodies generate high concentrations of trace heavy metals in soils and water and naturally low pH in the streams that drain them. Three main headwater tributaries define the area containing the sulfide ores and the mining district. Mineral Creek and the Upper Animas River border the caldera on its western and eastern sides, respectively (Figure 2.4.A). Portions of their watersheds also drain the surrounding calcareous sedimentary rocks that buffer acidity and create locally variable conditions of metals concentrations and pH within these streams. Cement Creek dissects the caldera and has persistently high metals concentrations and very low pH. Cement Creek, Mineral Creek, and the upper Animas River converge in the valley where the town of Silverton is located (Figure 2.4.B).

Water quality in the upper Animas River and its tributaries is influenced by natural ores and historic mining. Mining activities have added substantially to metals concentrations in water and sediments in the aquatic environment. Mining operations left hundreds of abandoned mines with many miles of underground workings that have altered subsurface hydrology at a hillside scale. The mining voids collect and provide preferential flow paths for groundwater while the voids provide an ideal environment for oxygen enrichment that triggers the acid-producing reactions in the ore deposits. Abandoned mines have historically discharged an average of 5.4 million gallons of AMD per day into the headwaters of the Animas River (USGS 2007). AMD contains high concentrations of heavy metals, such as iron, aluminum, zinc, lead, cadmium, copper, and many others.

It was also common practice through much of the mining era to dump mine tailings and mine-waste rock that had been pulverized to remove sulfide ores directly into the rivers (von Guerard *et al.* 2007). By the time mining ended, more than 8.6 million short tons of mill tailings and waste had been discharged directly into the Animas River and its tributaries from the headwaters to Durango (Jones 2007). Substantial amounts of the discarded wastes have been subsequently transported downstream and dispersed in stream deposits (Church *et al.* 1997; UGSS 2007), while considerable amounts remain in place where they were dumped.

A)



B)

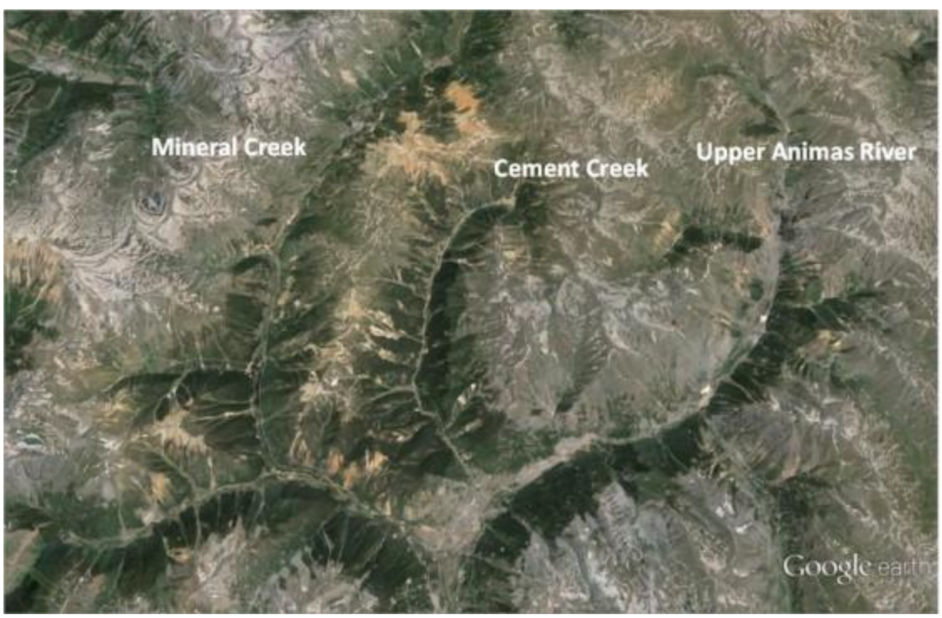


Figure 2.4. A) Generalized regional geology map of Animas River headwaters and surrounding regions near Silverton, CO (from: USGS 2007). B) Aerial view of the Silverton caldera area and the three main tributaries to the Animas River; Silverton is located in the center bottom of the image (Source: GoogleEarth).

Metals concentrations in the bed sediments of the Animas and San Juan rivers reflect the metals concentrations in soils and the underlying geology from which they are formed. A watershed view of metals concentrations in soils, river sediments and water as well as aquatic life are shown for 4 metals known to be important to aquatic life including Cu, Zn, Pb, Cd in Figures 2.5-2.8. Soil metal maps shown in panel A of each figure were obtained from the USGS Mineralogy website that spatially maps soil concentrations from compiled nationwide soil pit data.

Metals concentrations in the soils along the trace of the Animas and San Juan rivers range from among the highest measured in the United States within the sulfide ores in the headwaters to the lowest generally observed along the length of the San Juan River, especially where local lithology is dominated by continentally deposited sedimentary rocks. The concentrations of most trace metals are at extreme values in a circle centered within the Silverton caldera and the headwaters mining district. High metals concentrations are not constrained to the caldera but radiate outward for some distance. Concentrations decline sharply or gradually along the river path, depending on the metal. Soil concentrations reach moderate levels at some point along the Animas River within the middle to lower Animas. Most, but not all, trace metals show a similar pattern.

Available sediment and dissolved water data collected by various agencies in study of the river are shown in panels B and C, respectively. River data are plotted with the horizontal axis reversed to match the general east to west flow of the river. Sediment and water metal concentrations within the Animas decline from high values observed in the impacted mining district by two to three orders of magnitude by the time the Animas joins the San Juan River. This decline is due to dilution with water and sediments from surrounding low concentration geologic formations, as well as transformation of dissolved metals to solid forms from biogeochemical reactions in higher pH waters (Figure 2.2.C).

Metals in river sediments generally follow the same trajectory as those in soils in the river proximity (Figure B in each panel). The trace of the midpoint of the soils concentration categories in panel A are shown on the sediment graph (panel B). Metals in river bed sediments tend to be similar to those in the soils in the San Juan River while river bed sediment concentrations exceed soil concentrations in the Animas River. This could reflect the contamination of river sediments from mine waste disposal during the first 70 years of mining activity (Church *et al.* 1997, 2007, Jones 2007).

Dissolved water concentrations are also high in the headwaters within the ore deposits and decline with distance downstream. Dissolved concentrations vary over a wider range at a location reflecting the importance of seasonal runoff and storm events that may mobilize metals sequestered in the stream bed. Metals concentrations are generally similar through the length of the San Juan. Metals concentrations in the San Juan are most strongly influenced by episodic stormflow and suspended sediment loads (EPA 2016c).

The wide range and systematic declining pattern of background metals in water and sediment in the Animas River at the watershed scale identifies the general influence of environmental concentrations on biological communities. Fish and aquatic invertebrates readily assimilate metals from their environment (Elder 1989). While most research has focused on conditions within and immediately downstream of the mining district, a few have studied metals in the environmental and biota downstream to determine the extent of mining impacts throughout the Animas River (Church *et al.* 1997; Anderson 2007; MSI 2016). The metals concentrations in aquatic organisms has been measured for lengthy portions of the Animas River as part of environmental impact assessment for public projects (US Bureau of Reclamation 1996) and risk assessments in support of mining-related remediation activities in the mining district (USGS 2007, EPA 2015).

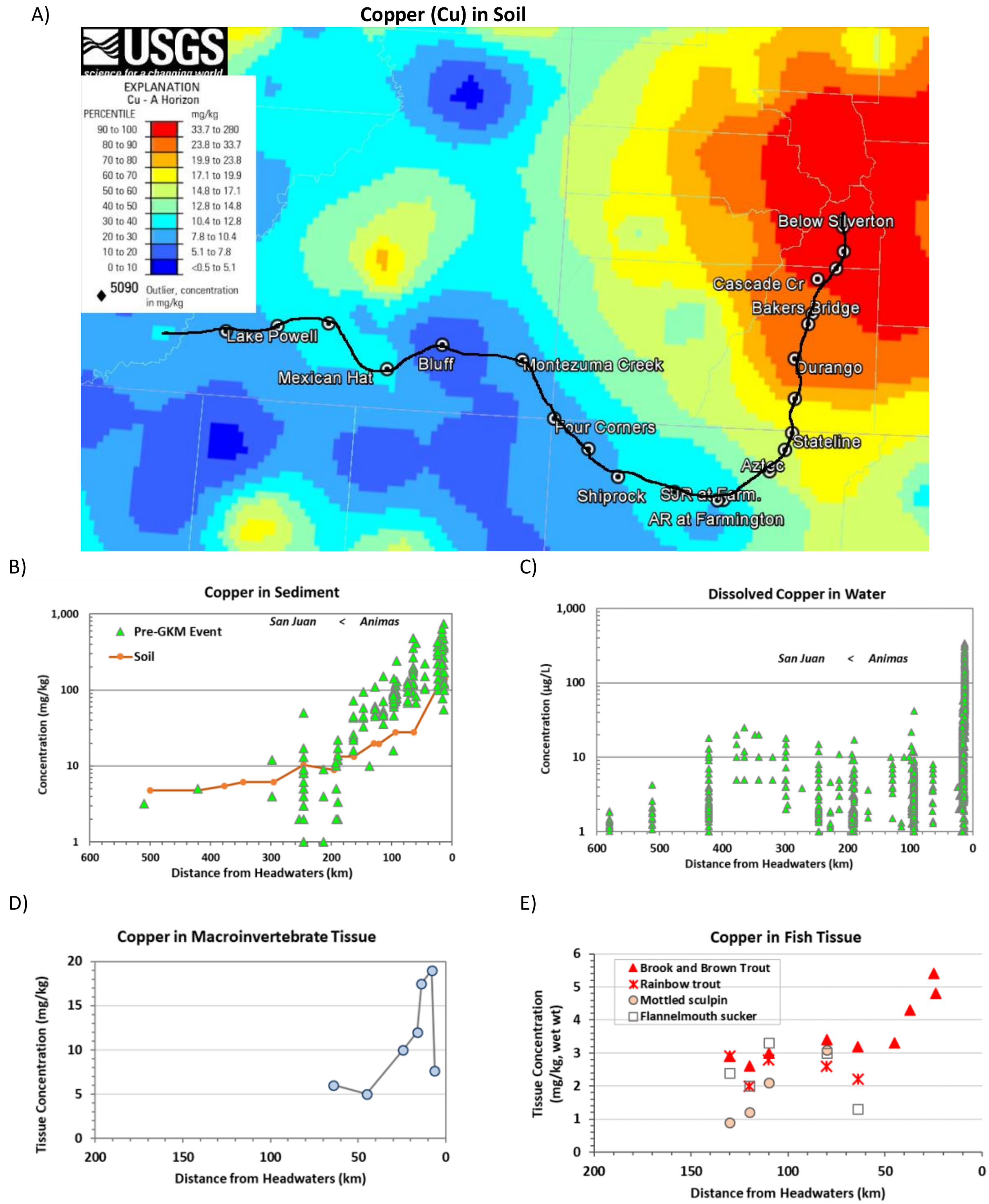


Figure 2.5. Longitudinal distribution of copper (Cu) in A) soils, B) river bed sediment, C) river water, D) macroinvertebrate tissue (MSI 2016) and E) fish tissue (US Bureau of Reclamation 1996). Horizontal (distance) axes are reversed to follow east to west path of river. Soils map was obtained from the US Geological Survey Mineral Resources On-Line Spatial Data website (https://mrdata.usgs.gov/soilgeochemistry).

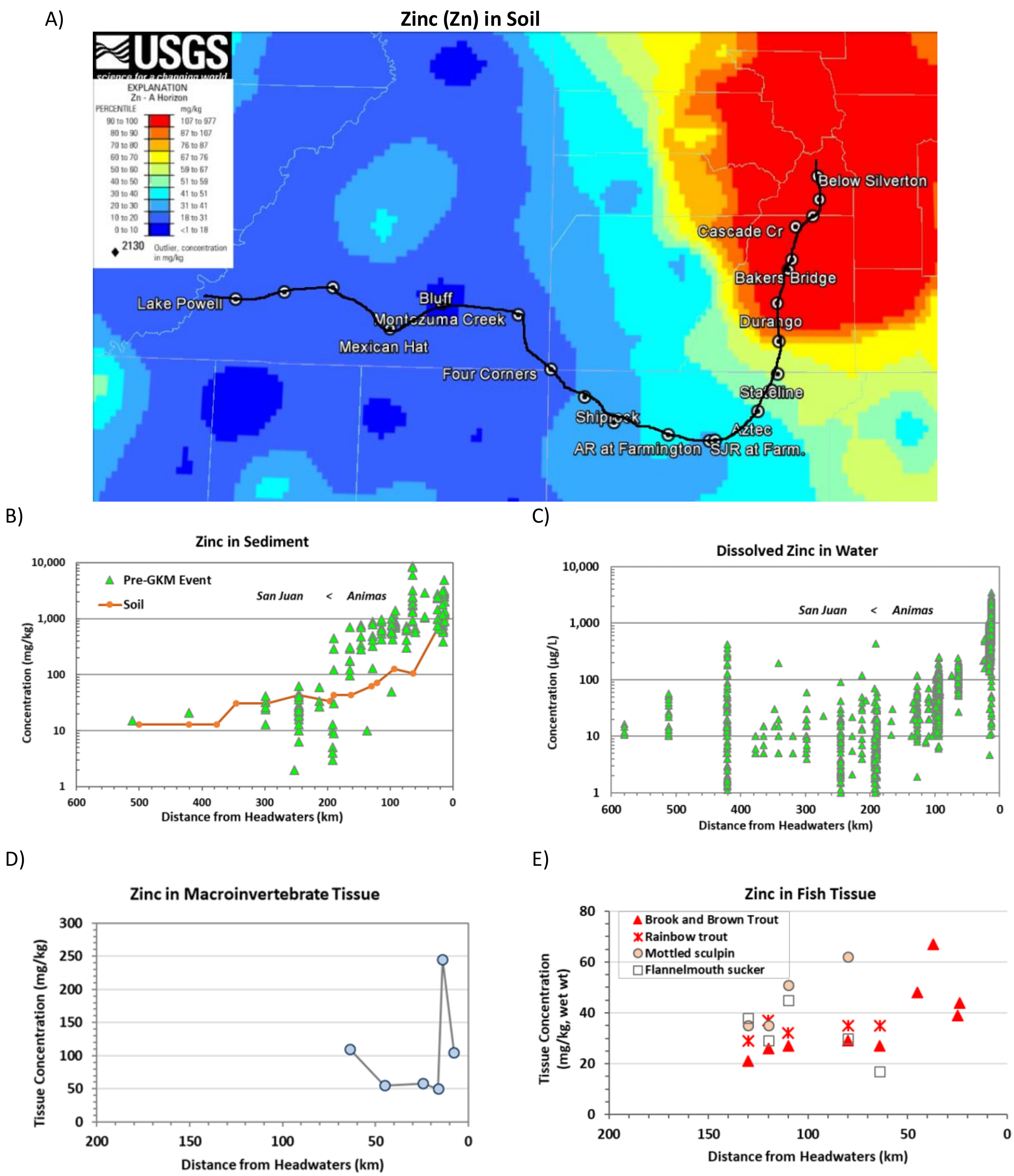


Figure 2.6. Longitudinal distribution of zinc (Zn) in A) soils, B) river bed sediment, C) river water, D) benthic macroinvertebrate tissue (MSI 2016) and E) fish tissue (US Bureau of Reclamation 1996). Horizontal (distance) axes are reversed to follow east to west path of river. Soils map was obtained from the US Geological Survey Mineral Resources On-Line Spatial Data website (https://mrdata.usgs.gov/soilgeochemistry).

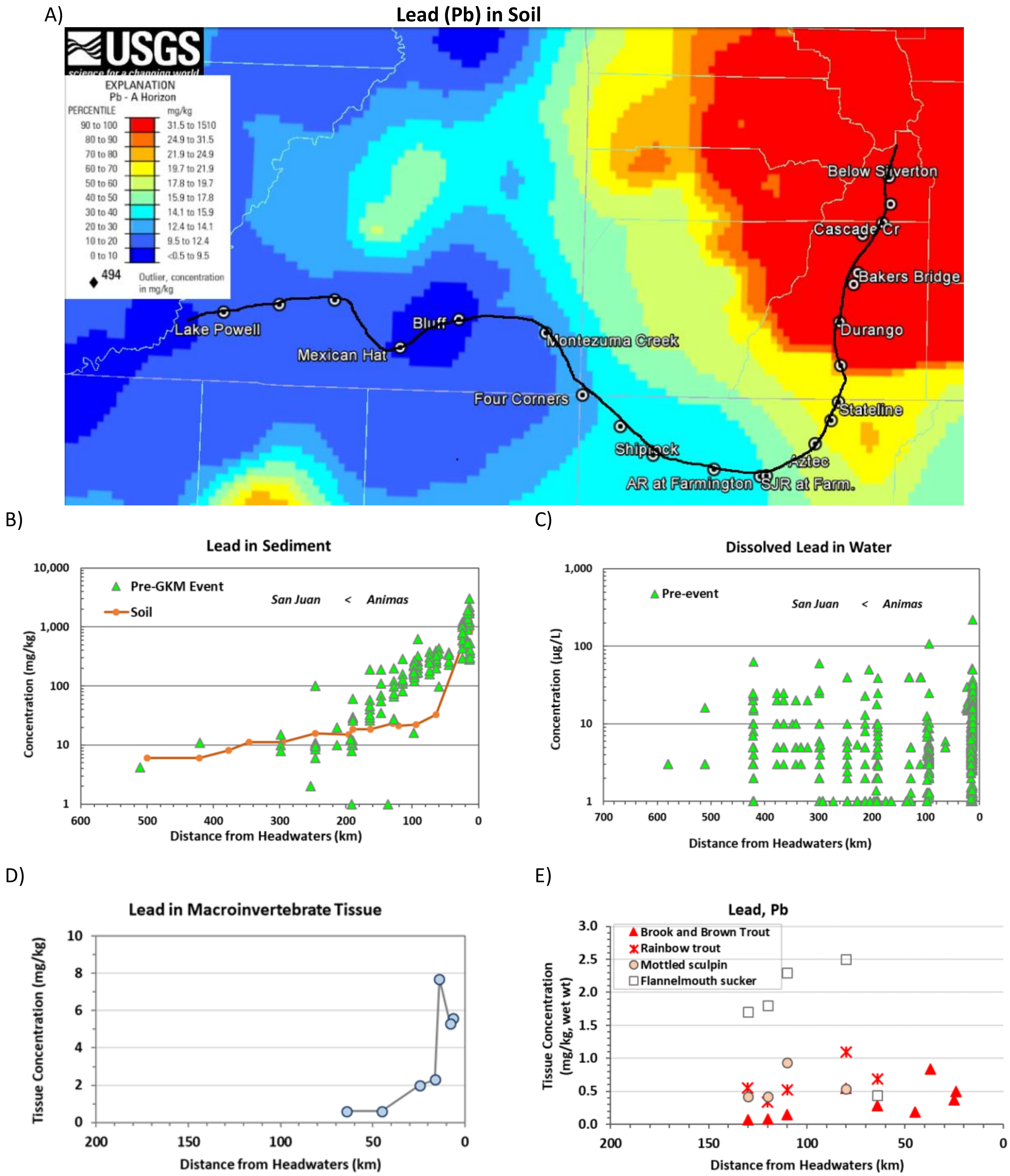


Figure 2.7. Longitudinal distribution of lead (Pb) in A) soils, B) river bed sediment, C) river water, D) benthic macroinvertebrate tissue (MSI 2016) and E) fish tissue (US Bureau of Reclamation 1996). Horizontal (distance) axes are reversed to follow east to west path of river. Soils map was obtained from the US Geological Survey Mineral Resources On-Line Spatial Data website (https://mrdata.usgs.gov/soilgeochemistry).

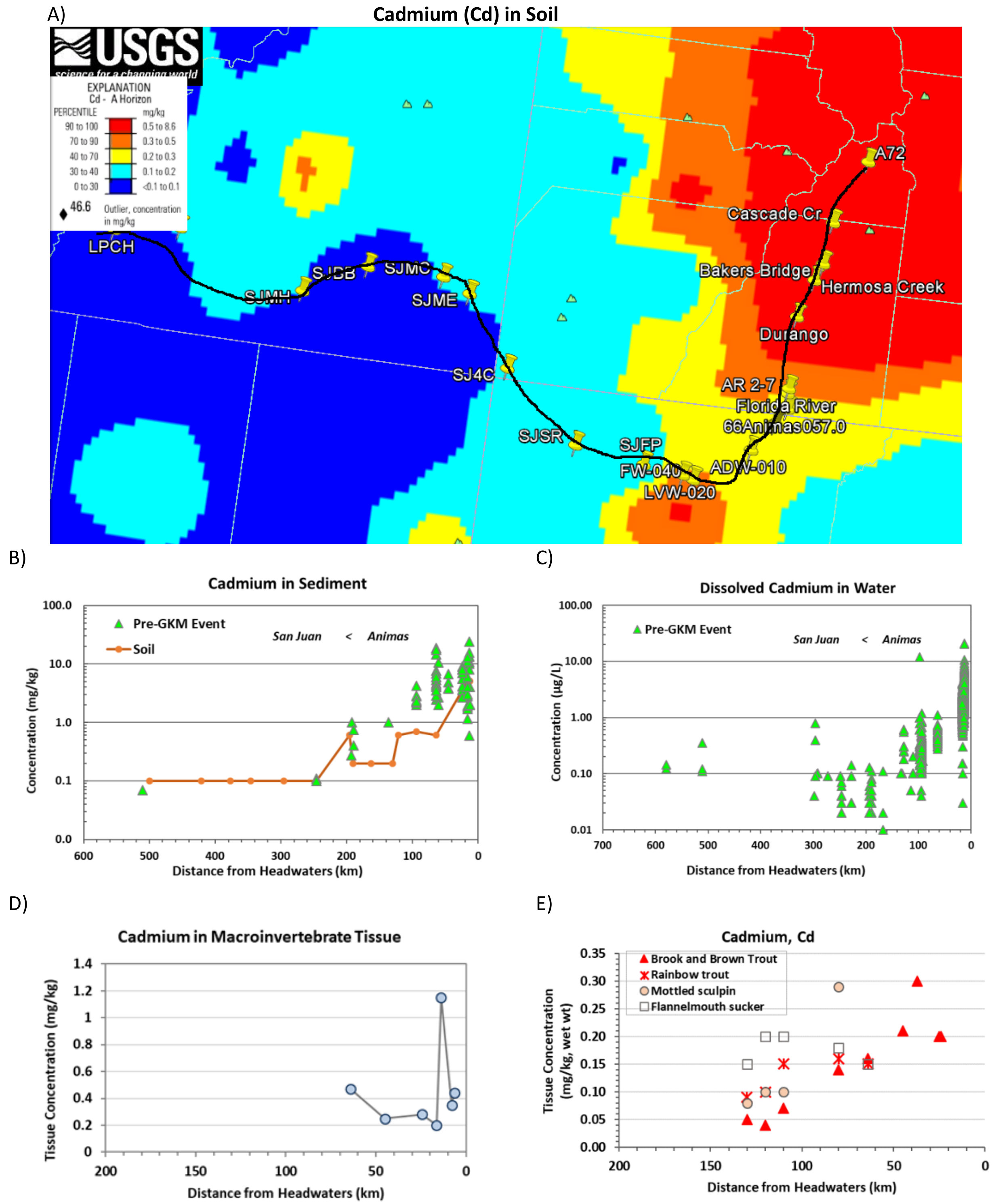


Figure 2.8. Longitudinal distribution of cadmium (Cd) in A) soils, B) river bed sediment, C) river water, D) benthic macroinvertebrate tissue (MSI 2016) and E) fish tissue (US Bureau of Reclamation 1996). Horizontal (distance) axes are reversed to follow east to west path of river. Soils map was obtained from the US Geological Survey Mineral Resources On-Line Spatial Data website (https://mrdata.usgs.gov/soilgeochemistry).

Metals in fish tissue were sampled in the 1990's at multiple locations from the headwaters to Farmington, NM for the U.S. Bureau of Reclamation La Plata Project Environmental Impact Assessment (US Bureau of Reclamation 1996). Mountain Studies Institute (MSI) collected macroinvertebrate populations in the upper and middle Animas in 2014, including metals in macroinvertebrate tissue (MSI 2016). Data from these sources are shown with soil, sediment and water concentrations in panels D (macroinvertebrates) and E (fish) in Figures 2.5-2.8. Note that tissue data were only available from the Animas River.

These surveys show that fish and macroinvertebrates in the Animas River assimilate metals and generally had higher body burdens of copper, cadmium, and zinc in the headwaters of the Animas where concentrations in water and sediment are greatest. Lead and arsenic (not shown) were present in macroinvertebrates but not in fish. Highest concentrations of metals in benthic macroinvertebrates (peaks in D panels) were sampled from the monitoring location upstream of Cement Creek (A68). Field studies have also established that the number of taxa and abundance increase with distance downstream from the mining district (Besser and Leib 2007, Anderson 2007). Increasingly healthy aquatic communities generally follow the longitudinal trends towards lower water and sediment concentrations and body burdens moving downstream from the mining district (Figure 2.3).

2.3.2 Metal toxicity to aquatic life in the Animas River

Metal toxicity and bioaccumulation in aquatic environments is complex, and is influenced by multiple routes of exposure (diet and solution) and physiochemical characteristics that control bioavailability (e.g., temperature, pH, dissolved organic carbon, inorganic cations and anions) (Luoma 1983; Paquin *et al.* 2002, Luoma and Rainbow 2005). Although some metals are essential for life, all metals are toxic at sufficiently high concentrations (Luoma 1983**).** Metals are partitioned between solid and dissolved phases in aquatic environments. Free metal ions in the water are highly bioavailable and may be the most important control on bioaccumulation and toxicity, especially for some metals including cadmium, copper, iron, manganese and zinc (Luoma 1983). Dissolved metals cause acute toxicity to fish by exposure to the gill, which damages gill tissue and alters gill function. Metal exposure also occurs through ingestion of particulates in sediment and suspended particulates. Intake through digestion and biomagnification through the food chain is an important exposure route for some metals including selenium and mercury (Luoma 1983). Generally, dissolved metals are considered more toxic, more reactive, and more mobile than particulate metals**.**

Laboratory and field toxicity studies of aquatic communities in the mining impacted reaches of the upper Animas (Besser and Leib 2007; Courtney and Clements 2022; EPA 2015) and elsewhere (Mebane *et al.* 2012, 2017, Cadmus *et al.* 2016) have shown that persistently high concentrations of metals in water, sediment and food resources degrade benthic organisms and fish populations. The USGS Professional Report 1651 (USGS 2007) summarizes the historic field sampling and toxicity testing in the upper Animas in support of AMD remediation activities in the Bonita Peak district near Silverton, CO. The EPA has also performed additional toxicity tests, extensive monitoring, and risk analysis for aquatic life in the upper Animas River (EPA 2015).

The USGS studies in the Animas River concluded through field observations and supporting toxicity tests that Cu had the main impact on trout, while zinc was most important for macroinvertebrates and amphipods (Besser *et al.* 2007; Besser and Leib 2007). USGS studies also identified potential impacts from dissolved aluminum and deposited Al and Fe oxides. Toxicity tests of upper Animas River water and sediment performed by EPA (2015) identified potential effects from Al, As, Cd, Cu, Pb, Mn, Ni, Se, Ag, and Zn.

Both efforts documented that persistently high metal concentrations in the upper Animas River are toxic to many taxonomic groups. The direct toxic effects of metals cause mortality, reduced growth, decreased reproductive output, and eliminate sensitive species from the aquatic community. This results in reduced diversity and abundance of benthic macroinvertebrates and fish (Besser and Leib 2007; Courtney and Clements 2002). There is significant variation in sensitivity to metals among taxa and complex responses within the biological community that result in high spatial and temporal variability within the aquatic community (Besser and Leib 2007). For example, results of an *in situ* toxicity test in the Animas River presented by Courtney and Clements (2002) identified that the greatest toxic effects are observed on mayflies (Heptageniidae, Ephemerellidae) and stoneflies (Taeniopterygidae). Furthermore, food abundance and quality in the Animas River was found to be reduced compared to reference tributaries, which also likely contributed to the absence of sensitive species (Courtney and Clements 2002). EPA (2015) found that rainbow and brown trout were more sensitive to metal concentration measured in the Animas River than brook trout.

Some macroinvertebrate studies have concluded that water exposure is the primary route of exposure in the Animas River (Courtney and Clements 2002) while other studies have emphasized the importance of dietary exposure from sediment and food resources (Besser and Leib 2007). Data from the Animas River presented in Besser *et al.* (2001) show that there is a strong relationship between metal concentrations in the pore water, metals in sediment, and metals in the periphyton and in some of the macroinvertebrate species. They observed that there were high concentrations of metals in the periphyton and that these concentrations tended to match those of sediment. The interrelationship between metal concentrations in sediment and water blurs inference from general surveys of causative exposure factors.

2.4 Metal water quality criteria and sediment thresholds for aquatic life

Water quality criteria are limits on chemicals or conditions in a waterbody that are derived to protect the designated uses for the waterbody, such as aquatic life use. Numeric criteria are defined by a magnitude, duration, and frequency of exposure. Pursuant to section 304(a) of the Clean Water Act, the EPA publishes national pollutant criteria recommendations to protect aquatic life. EPA aquatic life criteria include acute (short-term, or 1 hour) and chronic exposure (long-term; 96 hour) recommendations for the protection of aquatic life (https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table). States and tribes with jurisdiction of the Animas and San Juan rivers have generally adopted EPA's 304(a) criteria recommendations, or updated versions of the EPA's recommendations that take into consideration the most recent toxicity data.

Water quality criteria for aquatic life target exposure to dissolved metals. Research has shown that toxicity of most metals varies with the presence of dissolved calcium and magnesium carbonates (hardness) which compete for binding sites on the gill surface. This interaction results in hardness modified water quality criteria recommendations. Because of changes in species and hardness along the length of the Animas and San Juan rivers, water criteria vary spatially and temporally but are more likely to be exceeded in the upper and middle Animas as geology produces inherently higher metals concentrations, water relatively lower hardness, and lower pH (Figure 2.3) that is conducive to maintaining metals in the dissolved and more bioavailable solid phases. Metals criteria can vary widely over the applicable hardness range. For example, at hardness concentrations 25 to 400 mg/L, acute water quality criteria for dissolved cadmium and zinc ranges from 0.5 to 6.5 µg/L and 36 to 378 µg/L, respectively. There are no comparable EPA, state or tribal criteria for sediment; however, sediment probable effects concentrations (PECs) have been used to evaluate risk of sediment metals to aquatic life (Table 2.2), including use in the EPA BERA (EPA 2015). PECs are concentrations in sediment above which adverse effects are expected to occur more often than not (MacDonald *et al.* 2000).

Table 2.2. Sediment probable effects concentration (PEC) benchmarks for aquatic life from MacDonald *et al.* (2000).

Metal	Probable Effect Concentration (mg/kg)
Aluminum	60,000
Arsenic	33
Cadmium	4.98
Copper	149
Iron	250,000
Lead	128
Manganese	1,200
Mercury	1.06
Nickel	48.6
Zinc	459

Biota in some portions of the Animas River headwaters and its tributaries, and river segments immediately downstream from the mining district, are impacted by metals and pH to some degree, but sustainably support aquatic life (USGS 2007, EPA 2015). Since 1998, the State of Colorado has designated some segments of the upper Animas River, including Cement Creek, as persistently impaired for certain metals, including lead, iron and aluminum, and has followed procedures under the Clean Water Act to remove aquatic life support as a designated use for the waterbody because it is not an attainable goal (Colorado Department of Public Health & Environment, https://www.colorado.gov/pacific/cdphe/tmdl-san-juan-and-dolores-river-basins).

2.5 Gold King Mine release

The EPA Office of Research and Development (ORD) report *Analysis of the Transport and Fate of Metals Released from the Gold King Mine in the Animas and San Juan Rivers* (EPA/600/R-16/296) provides a detailed examination of the water chemistry and sediment data collected from the Animas and San Juan rivers before, during and after the release (EPA 2016c). In that report, ORD used a combination of empirical and modeled observations to describe the GKM plume as it traveled from Cement Creek to the San Juan River. The GKM release had the potential to impact the biological communities in the Animas and San Juan rivers through direct acute and chronic toxic effects typically associated with the dissolved fraction of the total metal. Generally, dissolved metals are considered more toxic, more reactive, and more mobile than particulate metals. Physiological effects can also be observed with the deposition of metal-bearing colloids that have a smothering effect on organisms and degrade aquatic habitat. Below we summarize the key findings in the EPA ORD report that provide insight to the biological results (see the full ORD report for all key findings).

2.5.1 GKM plume water chemistry

The GKM plume[1] was generally characterized by metals concentrations that rose abruptly, peaked quickly and fell rapidly within a period of about 12 hours as the central core of the plume moved past locations within the watershed. Concentrations in the downstream rivers then tapered back towards pre-event levels over days to weeks after the passing of the plume.

Once the GKM plume entered the Animas River, both dissolved and colloidal/particulate peak metals concentrations began to decline rapidly (e.g., within ~ 12 hours) as chemical reactions and hydraulic processes diluted, transformed, and deposited material. As the plume travelled, chemical transformations of dissolved metals began as soon as the acidic GKM plume mixed with the more alkaline waters of the Animas River at Silverton. As the plume traveled, acidity was neutralized and pH increased through hydrolysis chemical reactions that consumed hydrogen ions and stimulated the formation of iron, aluminum, and manganese hydr(oxides) and other incipient minerals. The incipient amorphous minerals that formed as the plume flowed included colloids, precipitates, and adsorbed phases that sequestered the trace metals including lead, copper, arsenic, zinc and others. The iron and aluminum reacted with the river water to cause the characteristic bright yellow color that was visible for days as the plume traveled down the river system.

Dilution of the GKM plume with river water also began as soon as it flowed into the larger Animas River and then shortly joined Mineral Creek in the Silverton area. Within 4 km distance of travel the plume was diluted to 38% of its original strength. Dilution reduced initial plume concentrations to 18% by Durango, 95 kilometers from the Gold King mine, and 15% by Farmington, 190 km from the source. Although the plume was visually similar as it traveled through the Animas River, no two places along the river experienced exactly the same plume when measured by the concentration or form of metals in the water.

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[1] In this document, we use GKM plume when discussing the eight-day period when the released metals traveled through the Animas and San Juan rivers. GKM release is also used when discussing the entire event, including the months after the plume when GKM deposits were present in the watershed.

As the aluminum and iron hydro(oxide) minerals formed between Silverton and Durango, some remained suspended in the water; some precipitated and settled to the river bed in slower waters along the edge and bottom of the channel, in side channels, and behind flow obstructions; and some adhered or cohered to rocks. By the time the GKM plume reached its confluence with the San Juan River, total metal concentrations in the water had declined by 3 orders of magnitude from what they were when the plume entered the Animas (Figure 2.9).

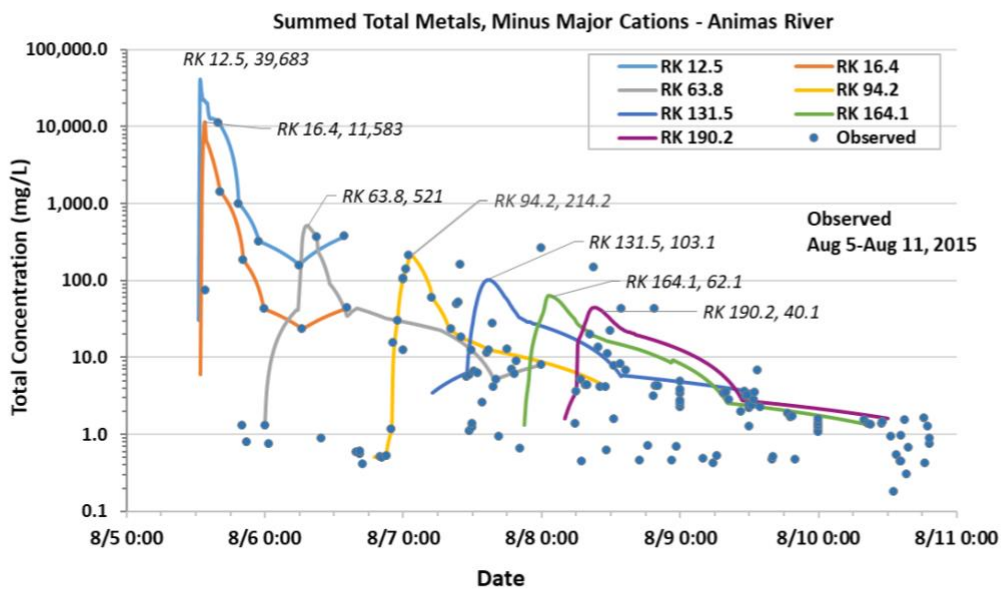

Figure 2.9. Observed and empirically-modeled summed total metals minus major cations, in the Animas River as the GKM plume passed from August 5–10, 2015 (from EPA 2016c).

Photo from CDPHE (2016a)

2.5.2 GKM sediment deposits

Only 10% of the GKM release reached Lake Powell with the plume. The other 90% was deposited onto the streambed along the length of the Animas and San Juan rivers (Figure 2.10), where it remained in place for various lengths of time depending on location (i.e., 3 weeks to 10 months). Subsequent effects on the biological communities could occur from chronic exposures to metals sequestered in the streambed. Freshly-deposited sediments dominated by iron and aluminum hydrous oxides are likely to be highly enriched with other more toxic metals, and may form a reservoir contributing to longer-term effects on stream biota. Any toxicity associated with the deposits should decline over time as they age.

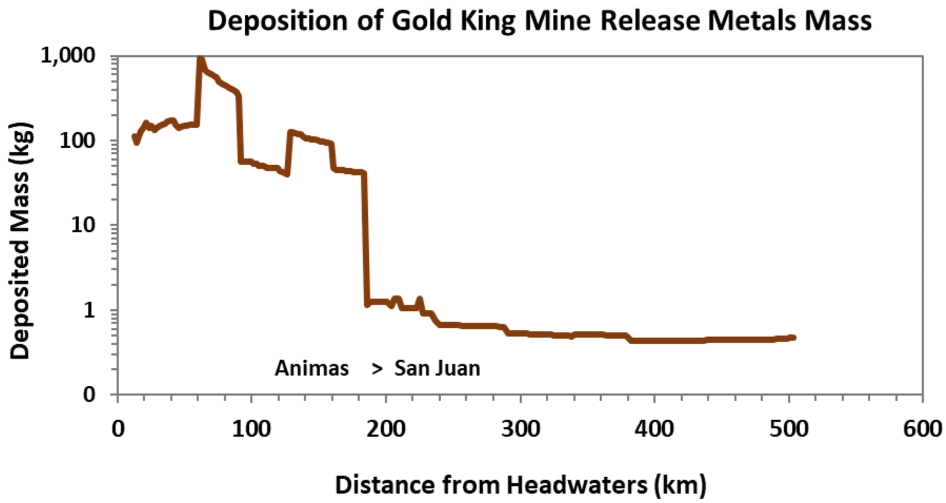

Figure 2.10. Estimated deposited mass of metals from the GKM release as it passed through the Animas and San Juan rivers. Deposited mass was estimated in 2-km segments of river by the Water Analysis Simulation Program (WASP) model as reported in the EPA GKM release fate and transport study (EPA 2016c).

Eighty percent of the GKM release (~390,000 kg) was deposited in the Animas River between Silverton and Durango. This portion of the river also stores a large amount of legacy contamination from historic mine ore processing and ongoing acid mine drainage contamination (Church *et al.* 1997; 2007), to which the GKM release added new material. The high concentrations of metals evident in Figures 2.5-2.8 reflect natural and mining related deposits in this river segment. GKM deposits in the Animas from the Colorado/New Mexico border northward to Silverton largely remained in place until 2016 snowmelt runoff began, as there were no storm events large enough to move them through Fall 2015.

The mass of the GKM metal deposits amounted to 10% of the metal mass that already contaminated the upper and middle Animas river. Targeted sampling of GKM deposits had high concentrations of metals. Samples representative of the general deposition observed on the riverbed collected in the months after the GKM release were not statistically different from pre-event concentrations where there were sufficient data for comparison (EPA 2016c; Rodriguez-Freire *et al*. 2016). Dissolved metals in river water were statistically lower in the upper Animas in the months following the GKM-event, possibly due to their adsorption onto the new deposits.

An additional 5% of the released metals (~25,000 kg) was deposited in the lower Animas River within New Mexico. Sediment metal concentrations measured in the reach from RKM 152 south of Cedar Hill, NM, to RKM 162 near Aztec, NM, were elevated after the GKM plume passed, as shown in Figure 2.11. Lead and zinc in the sediments exceeded recommended PECs for aquatic life (Table 2.2) within this reach for a time after the plume passed. Another 5% of the released mass may have deposited along the length of the San Juan River, but sediment samples only showed some evidence of this from Farmington (RKM 196) to Fruitland, NM (RKM 214). Deposits in the lower Animas and San Juan rivers remained in place for 3 weeks until they were mobilized during a monsoonal event and delivered to Lake Powell. Sediment samples collected after the event unambiguously showed that the concentrations of all metals in the sediments of both rivers were at background levels following the storm.

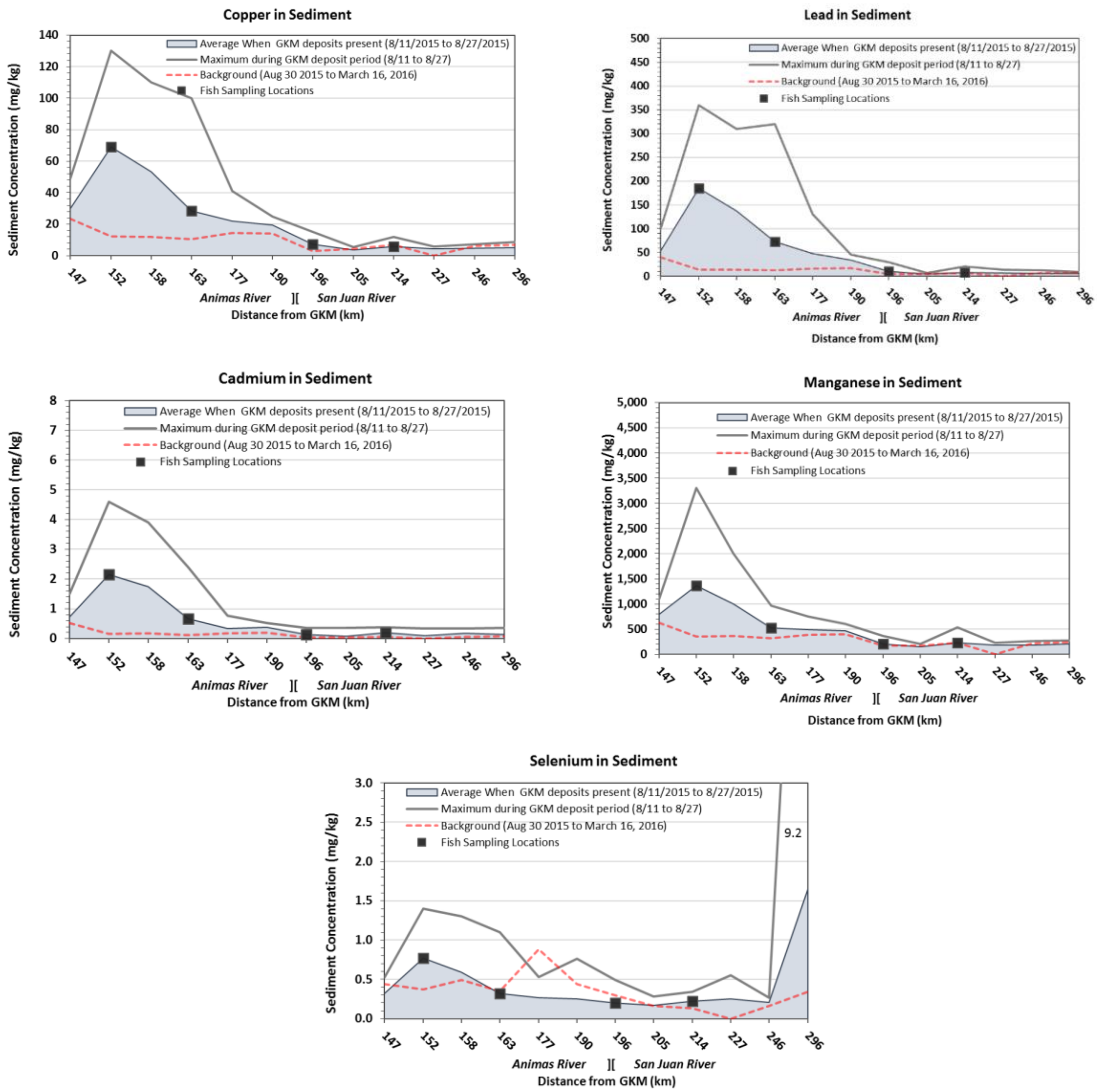

Figure 2.11. Concentrations of copper, lead, cadmium, manganese and selenium in sediment at the time of benthic macroinvertebrate (Chapter 7) and fish tissue sampling (Chapter 8) in the New Mexico segments of the Animas and San Juan rivers. The post-GKM period includes data collected from August 8 to August 27, 2015. March 2016 represents background concentrations. Black squares on the sediment figures indicate the location of fish sampling and the sites shown for water concentration.

2.5.3 GKM release water quality effects to aquatic life

For most metals, the peak concentrations that were observed as the GKM plume moved through the system were not greater than aquatic life water quality criteria at most locations in the Animas or San Juan rivers. Given the predominance of colloidal/particulate metals in the plume, criteria based on total concentration were exceeded more often than those based on the dissolved fraction. Because of the short duration of the plume, aquatic life was more vulnerable to acute, shorter-term concentrations during movement of the plume itself. Most of the observed excursions were associated with the state acute aquatic life criteria for aluminum. Excursions of acute aluminum criteria occurred throughout the Animas River and in the San Juan River down to Shiprock, NM (296 RKM). The frequency of the excursions varied due to the change in concentration and differences in the state and tribal water quality criteria for aluminum. The mainstem of the Animas immediately below Silverton experienced the most excursions of metals criteria including acute aluminum, cadmium, copper, lead, manganese, and zinc. Within the spatial trend of declining concentrations with distance, variation in excursions reflected the differences in criteria among states and tribes along the route. The duration of water quality exceedances was generally associated with the core of the plume where concentrations were highest and lasted several hours. [2] For example, total and dissolved metals concentrations in the Animas River are shown as the plume passed through Durango in Figure 2.12. At this location, concentrations of several metals were briefly close to, but did not exceed, the acute aquatic life criteria that are based on the dissolved concentration. Aluminum was the exception given the high total recoverable concentrations and the criterion is based on total recoverable, rather than dissolved concentration.

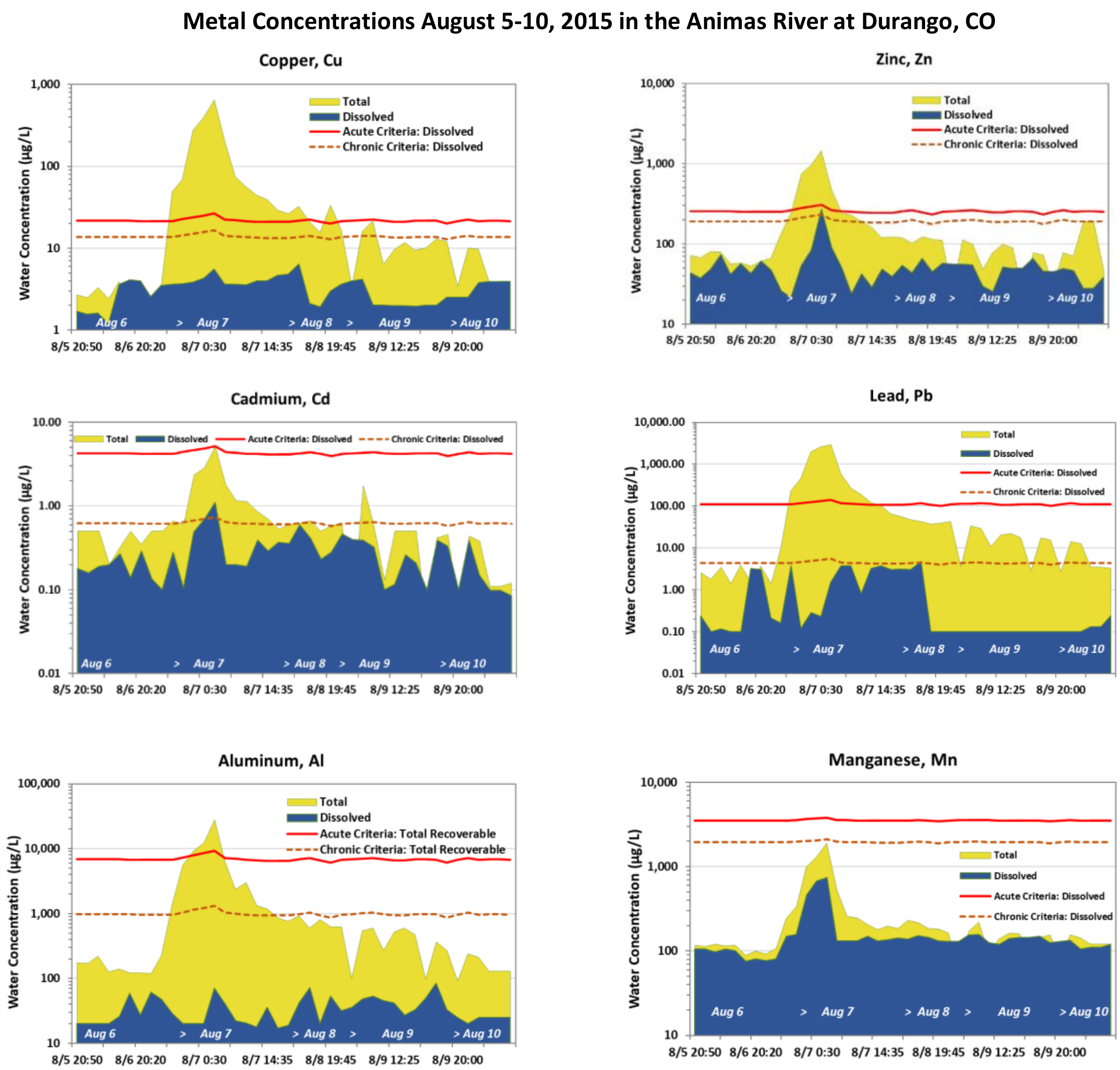

Figure 2.12. Total and dissolved water concentrations of four metals in the Animas River at Durango, CO from August 5-10, 2015 as the GKM plume passed through. Conditions represent the metal exposure during the CPW sentential caged trout study presented in Chapter 6. Acute and chronic water quality criteria are shown as solid and dashed red lines.

[2] Aquatic life criteria duration varies among the states and tribes water quality standards. The EPA's 304(a) aquatic life criteria recommendation for most metals is 1 hour for acute criteria and 96 hours for chronic criteria. The longest duration of exceedance for chronic criteria was 44 hours for iron concentrations measured at RKM 132. See Tables 7-5 through 7-8 in EPA (2016c) for additional details.

2.5.4 GKM release exposure to aquatic life relative to background conditions

EPA, states, and tribes began monitoring metals in water and river bed sediments throughout the affected rivers to assess risk to public and aquatic health as benchmarked by the water quality criteria. Monitoring agencies collected samples over varying intervals, beginning at six-hours prior to the front end of the plume, and continuing daily or weekly during later phases over the next year. We evaluated the metal exposure to aquatic life before, during, and in the year following the event by comparing measured concentrations to protective water and sediment benchmarks. Exposure identified in a single sample was measured with a Hazard Quotient (HQ).

$$HQ = Observed\ Concentration/Benchmark\ Concentration$$

Where the benchmark was EPA's acute or chronic aquatic life criteria recommendation calculated at the ambient water hardness or at hardness = 400 mg/L when hardness was greater than 400 mg/L for water or the PEC for sediment (Table 2.2). This calculation for a single sample defines the magnitude but not the duration of exposure. HQs equal to or greater than 1.0 for an individual sample identify a potential for ecological risk in that established thresholds have been exceeded at that location. Samples with an HQ less than 1 do not indicate a potential risk.

Acute and chronic HQ's calculated for pre-event, during and immediately after the event (the plume up to 1-month post release), and post event (2 months to 1 year following the GKM release) are shown in Figures 2.13 and 2.14, respectively. HQs for sediment are shown in Figure 2.15. The pre-event HQs were calculated using the same concentration data shown in earlier Figures 2.5-2.8. Figures 2.13 through 2.15 represent nearly 4,000 samples collected before and after the event.

Acute and chronic HQs greater than 1 occurred frequently in the Animas headwaters within about 40 km from GKM for most metals both before and after the release (Figures 2.13 and 2.14). Generally, HQs for all metals followed similar longitudinal patterns and remained within the same range of variability at a location before and after the GKM release.

Outside the Animas headwaters, acute HQs greater than 1 for all metals were rare. Chronic HQs greater than 1 were frequently observed for zinc and lead throughout the system. Chronic HQs greater than 1 in the lower Animas occur with high flow events typically associated with monsoonal storms. Lead HQs greater than 1 in the San Juan are associated with mobilization of dissolved lead from the bed sediments during monsoonal storms.

Sediment HQs after the GKM release were the same magnitude as prior to the release except for lead (Figure 2.15). Lead was elevated in sediment from 50 to 200 km distance from headwaters (Bakers Bridge to Farmington) during the immediate 1-month period after the event (also shown in Figure 2.11), and greater than the sediment PEC.

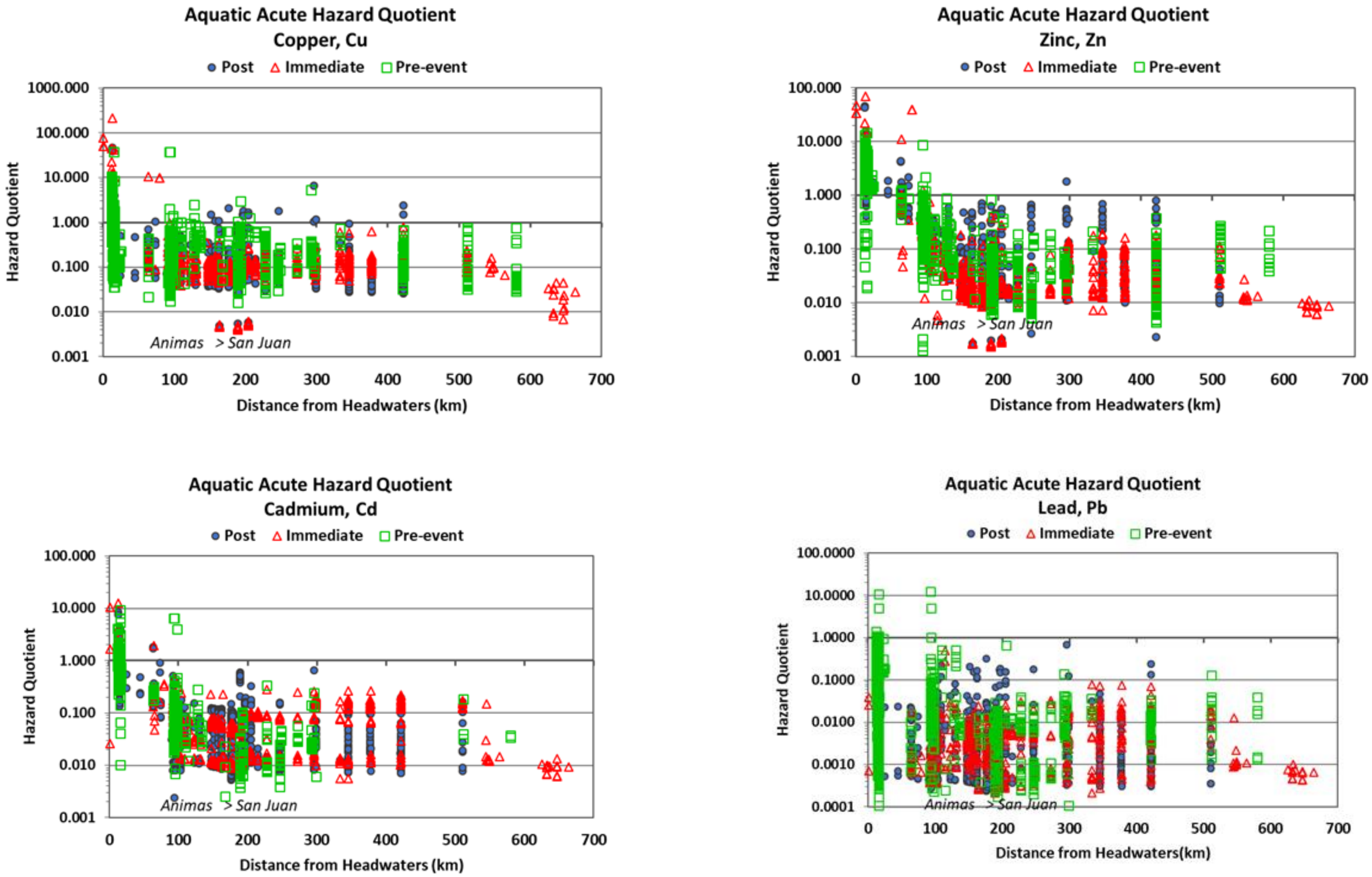

Figure-2.13. Acute aquatic life hazard quotients (HQ) for water samples collected from the Animas and San Juan rivers. The HQ was computed as observed concentration divided by the acute water quality criterion. An HQ greater than 1 indicates the water quality benchmark was exceeded. Pre-event samples were collected for several decades prior to the GKM release and include the same data shown in Figures 2.5 -2.8. Immediate samples were collected from August 5 to August 28, 2015. Post samples were collected from September 2015 to September 2016.

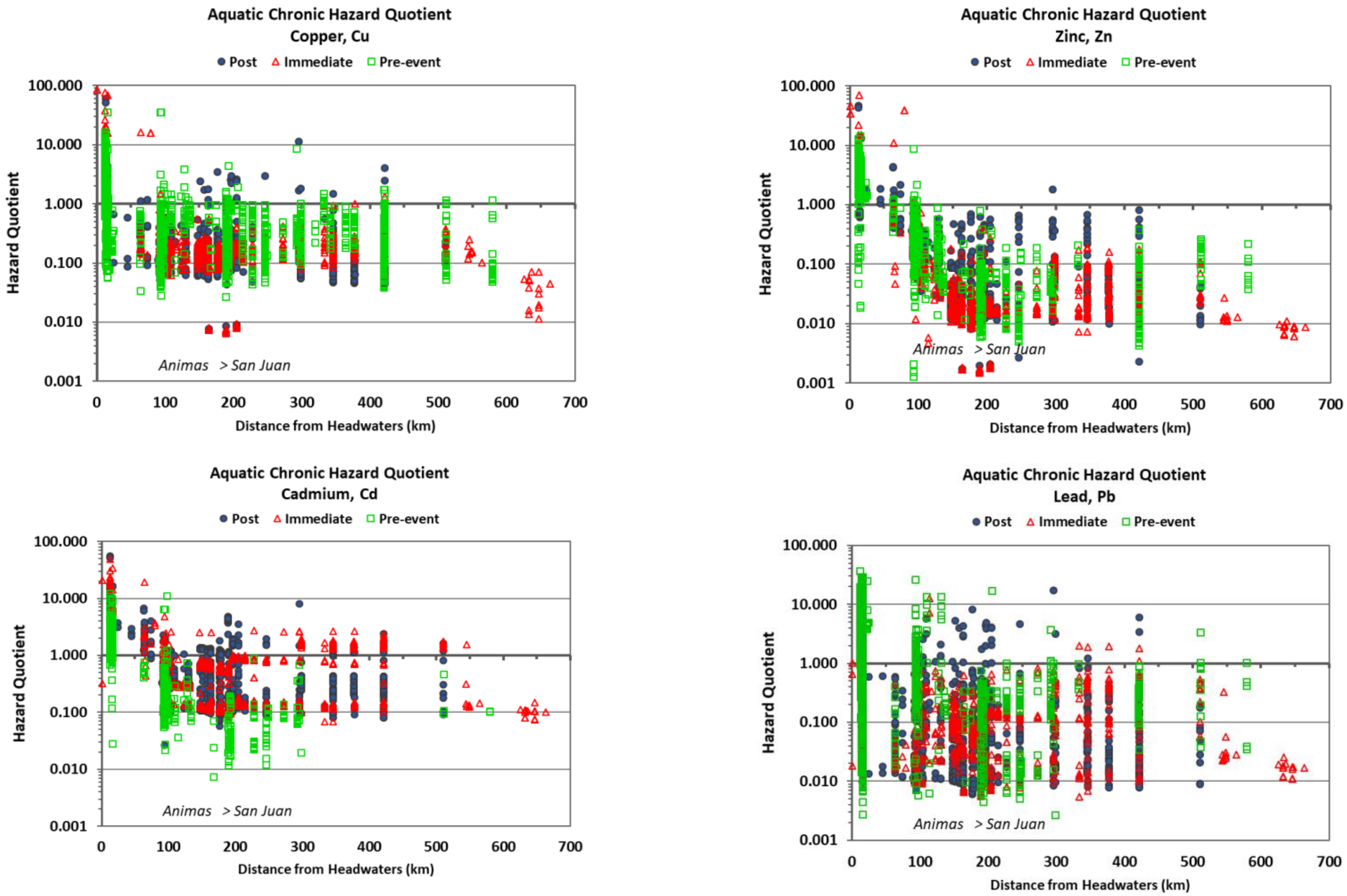

Figure-2.14. Chronic aquatic life hazard quotients (HQ) for water samples collected from the Animas and San Juan rivers. The HQ is computed as observed concentration divided by the chronic water quality criterion. A value greater than 1 indicates the criterion was exceeded. Pre-event samples were collected for several decades prior to the GKM release and include the same data shown in Figures 2.5-2.8. Immediate samples were collected from August 5 to August 28, 2015. Post samples were collected from September 2015 to September 2016.

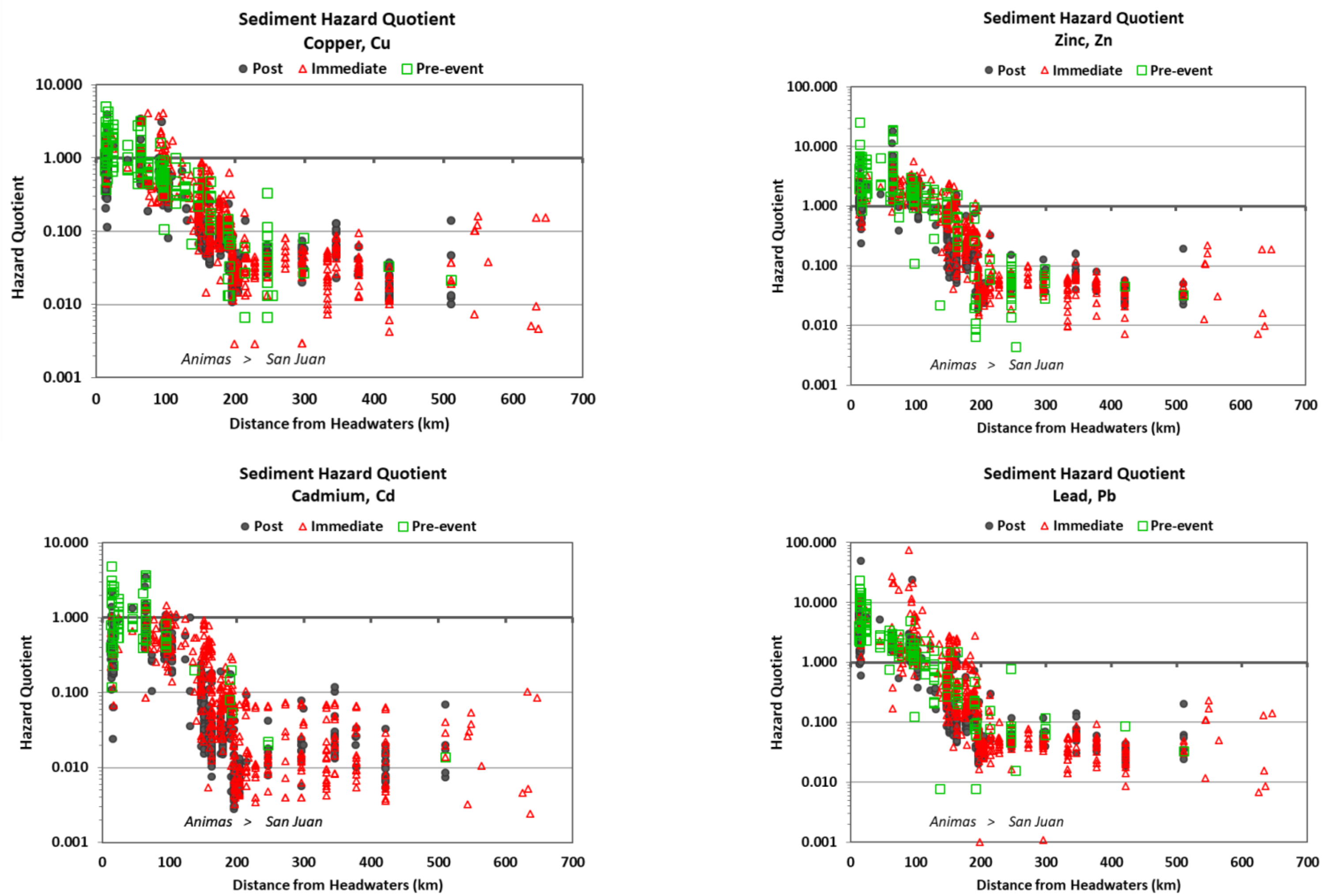

Figure 2.15. Hazard quotient (HQ) for sediment probable effects concentrations (PECs) for water samples in the Animas and San Juan Rivers. The HQ is computed as observed concentration divided by the water quality criteria. A value greater than 1 indicates the water quality criteria was exceeded. Pre-event samples were collected for several decades prior to the GKM release and include the same data shown in Figures 2.5-2.8. Immediate samples were collected from August 5 to August 28, 2015. Post samples were collected from September 2015 to September 2016.

2.5.5 Metals in water and sediment return to background

The mass of metals in the GKM release was removed from the Animas and San Juan rivers and delivered to Lake Powell in three primary events distributed over a 10-month period or the end of snow melt in 2016 (EPA 2016c). The first mass arrived with the plume approximately 8 to 9 days after the release. The second event was triggered by a series of monsoonal storms that began in late August 2015 described in Section 2.5.2. The storm flow resulting from three inches of rain in a few hours during this event resuspended GKM deposits in the Animas River below Cedar Hill, CO (RKM 140) to the confluence with the San Juan River in Farmington, NM (RKM 193) as well as the entire length of the San Juan River. The third event was associated with snowmelt runoff in 2016 that mobilized the remaining deposits in the Animas River over the winter months.

EPA was able to isolate the GKM release metals from background metals in water and sediment in these events and all data collected a year following the release with a metal “fingerprinting” technique (EPA 2016c). This technique associated the concentration of trace metals to that of aluminum or iron as representative of the dominant metals in the geologic substrate and the soils and sediments that weather from them. Water and sediment have typical relationships for each metal that have a strong central tendency over a range of background sediment levels explained by the elemental composition in the regional geology, as shown in Figures 2.5-2.8. If the trace metal ratio deviates from the central tendency, another source of contamination is suggested. The “fingerprinting” technique was particularly effective in detecting GKM release metals within the background concentrations of the San Juan River and was used to account for the GKM mass and track its movement through the river during the GKM plume and in the year following.

While water and sediment was extensively monitored for up to 1 year following the GKM event, various organizations conducted biological studies to evaluate potential impacts of the GKM event on the river biota. Sentinel studies of immediate survival of macroinvertebrates and fish as the plume passed were conducted near Durango, CO. Longer term studies included sampling 7-months post event period when GKM deposits were in place in Colorado and the lower Animas in New Mexico. EPA followed up with a river-wide survey of macroinvertebrates and fish tissue a year following the event in 2016 to assess for potential long-term impacts to aquatic life. EPA’s response sampling was the first time the biological communities were sampled through the entire length of the Animas/San Juan river system.

CHAPTER 3 OBJECTIVES, DATA, METHODS, AND ANALYSIS APPROACH FOR ASSESSMENT OF BIOLOGICAL DATA IN ASSOCIATION WITH THE GKM RELEASE

3.1 Study objectives

Aquatic biological communities provide a measure of river condition by responding to sudden changes in water quality, such as the plume of metals that moved through the rivers following the GKM release, and integrating persistent stressors over time, such as the legacy mining and ongoing acid mine drainage in the upper Animas watershed. Aquatic community condition is also a core measure of aquatic life use support in a given waterbody.

> **Study Questions**
>
> *HOW DID THE AQUATIC COMMUNITY, POPULATIONS, AND METAL SEQUESTRATION IN TISSUE RESPOND TO THE GKM RELEASE?*
>
> - *Did the GKM release add to biological degradation in the already contaminated upper Animas River?*
> - *Did the GKM release degrade biological communities in other segments of the Animas and San Juan rivers that had not been known to have metal contamination?*
> - *Were acute impacts to the biological communities observed during the initial GKM release when metals concentrations were highest?*
> - *Were long-term changes in biological communities observed a year after the GKM release?*

The EPA's primary objective was to gather and review all readily available biological data collected from the San Juan and Animas rivers to assess how the aquatic biota responded to the GKM release. Data gathered for this analysis included the EPA response sampling that targeted the near-term biological conditions immediately following the release (fall 2015) when deposits were still present in the Animas River and the long-term biological conditions occurring after the deposits have moved through the river system (fall 2016). Data collected by state and tribal partners were also included in our analyses. The sampling and analysis approach was designed to evaluate potential changes in the species compositions, population abundance, and the concentration of metals in the tissue by comparing the post-GKM release data to the pre-release conditions, when available. In many instances and particularly on the lower Animas and San Juan rivers, pre-release data were less available for pre- and post-GKM comparisons.

Monitoring and assessment efforts occurring prior to the GKM release identify pre-existing adverse impacts to water quality, sediment quality, and biological communities in this watershed (Besser *et al.* 2001; USGS 2007). Numerous sources of metals contamination are present within the watershed that have impacted environmental quality before the GKM release and continue to impact environmental quality post-GKM release (Chapter 2). Therefore, our ability to determine if current environmental impacts relate to the GKM release is confounded by the presence of on-going AMD sources in the upper watershed. Typical biological conditions in many areas of this watershed are neither pristine nor free of impairments. New data gathered post-GKM release are best understood by a comparison to previous conditions. It is well established that the historic and ongoing AMD in the upper Animas River has resulted in degraded benthic macroinvertebrate assemblages and portions of mainstem and tributaries that have not supported permanent fish populations (Anderson 2007, Besser and Leib 2007). Moving away from the historic mining operations, fish populations and benthic macroinvertebrate assemblages improve in the middle Animas. Metal concentrations decrease yet continue to be one of many stressors typically associated with more developed areas of watershed (CPW 2010). The aquatic communities in the lower Animas River and the

San Juan, on the other hand, are not known to be persistently disturbed by the AMD in the headwaters. Therefore, the primary assessment objective was to compare the pre-release/historic and post-GKM release biological data of the Animas River and San Juan River.

Our secondary objective was to present a watershed-wide analysis of all biological data collected from the GKM release impacted areas of the San Juan and Animas rivers, regardless of existing historic data for comparison. In this effort, we identified similarities and differences in existing state and tribal field collection methods and assessment approaches. This information can be used to inform future monitoring efforts in the San Juan watershed.

3.2 Sampling design

EPA mobilized field crews to sample water and sediment immediately after the GKM release occurred. Rivers impacted by the GKM release include the Animas River near Silverton, CO to its confluence with the San Juan River in Farmington, NM (190 RKM) and the San Juan River from the Animas confluence to Lake Powell in Utah (~650 RKM). The EPA identified 30 monitoring locations along Cement Creek, Mineral Creek, the Animas River, and the San Juan River based upon state, tribal or local interest; locations used in the emergency response; and long-term or pre-release data availability (Figures 3.1 and 3.2; Table 3.1). Sites that were not impacted by the GKM release were also sampled for a measure of background conditions in the watershed.

EPA targeted the response biological data collection (near-term sampling) at 22 sites in the fall of 2015 and expanded the follow-up data collection (long-term sampling) to 29 sites in the fall of 2016. In 2015, the EPA and contractors collected benthic macroinvertebrate samples at 4 locations from the Animas River within a week following the release (8/12 and 8/13) and at 18 locations in September and October (Table 3.1). The EPA was unable to sample all sites identified in the its follow-up monitoring plan for biology prior to the onset of winter conditions and exceedance of the index period for biological sampling in the fall of 2015. In 2016, EPA and contractors implemented the full biological sampling design with Superfund Technical Assessment and Response Team (START) contractor support and collected benthic macroinvertebrate samples and tissue samples at 29 sites, including 1 site on the Animas River upstream of Cement Creek (A68), 2 tributaries in the upper Animas River watershed (Cement Creek and Mineral Creek), 17 locations on the mainstem of the Animas. San Juan River sampling included 1 location on the mainstream upstream of the Animas confluence (SJAR) and 9 locations on the mainstem of the San Juan River downstream of the confluence. Additional details on EPA GKM field events are found in EPA's Field Activities Report (to be posted at https://www.epa.gov/goldkingmine).

In addition to the response and follow-up biological data collected by EPA, we issued a request for biological data collected by state, tribal, local, and federal partners. Data providers included Colorado Parks and Wildlife (CPW), Colorado Department of Public Health and Environment (CDPHE), New Mexico Environment Department (NMED), New Mexico Department of Game and Fish (NMDGF), Southern Ute Indian Tribe (SUIT), Navajo Nation Environmental Protection Agency (NNEPA), and U.S. Fish and Wildlife Service (USFWS). The type of biological data, sampling dates, and locations of this additional data are included in Table 3.1. When data providers used different location IDs or sampled slightly different locations than those sampled by EPA, we reviewed the sampling coordinates and site descriptions to determine if the locations generally represent a similar section of the river. When locations were similar (e.g., aquatic habitat, no new sources or tributaries), GKM location IDs were assigned to facilitate comparisons of the post-GKM release data with historic sampling sites (Appendix A). Differences in field and analytical methods used by the various data providers are identified in the Section 3.4 and were taken into consideration when developing our approach to the data analysis. Datasets were analyzed collectively when methods were similar and, when possible, results are presented consistent with the state/tribal analysis tools.

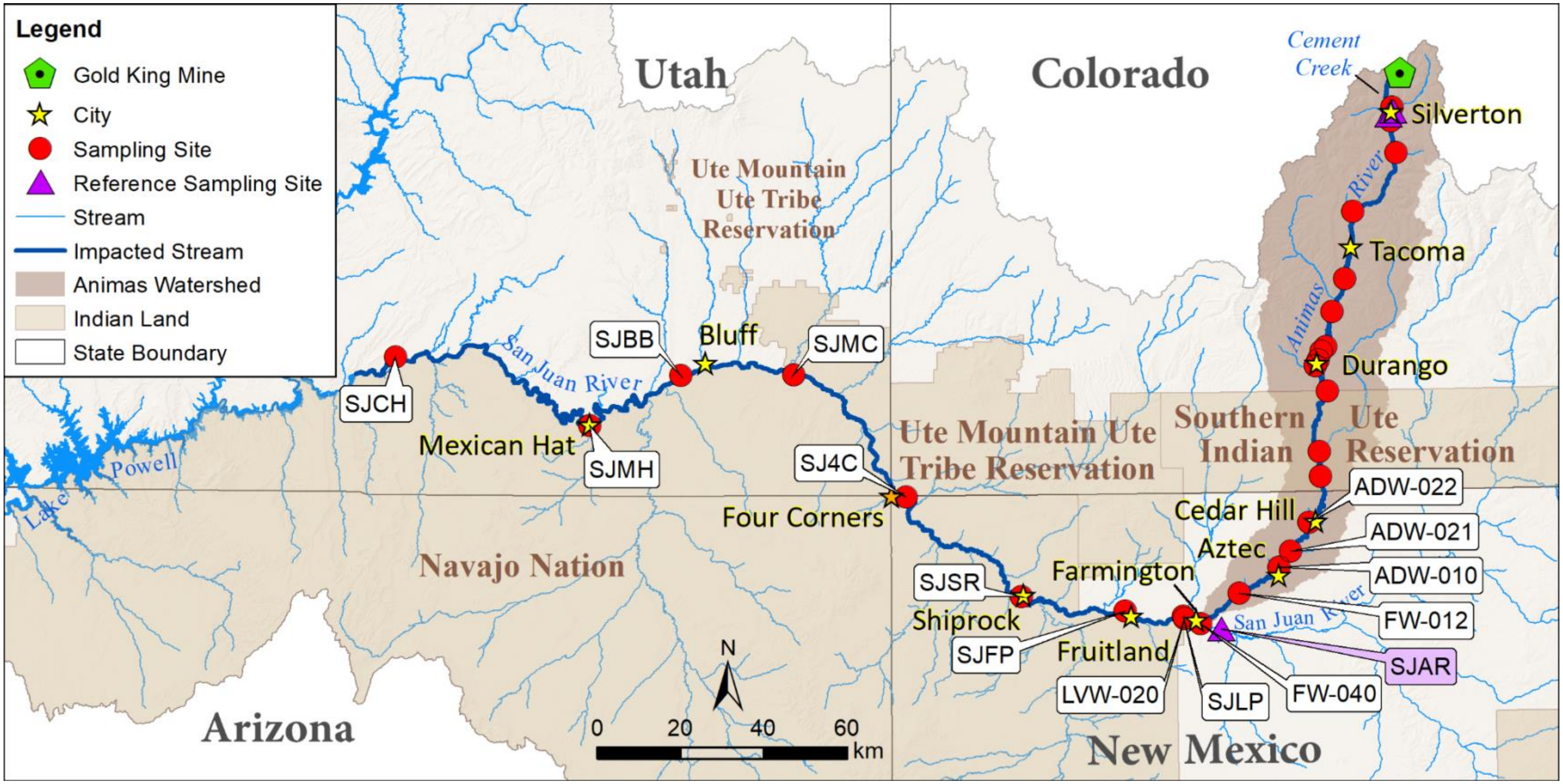

Figure 3.1. Locations sampled by EPA for surface water, sediment, physical habitat and biology in the Animas and San Juan rivers following the GKM release.

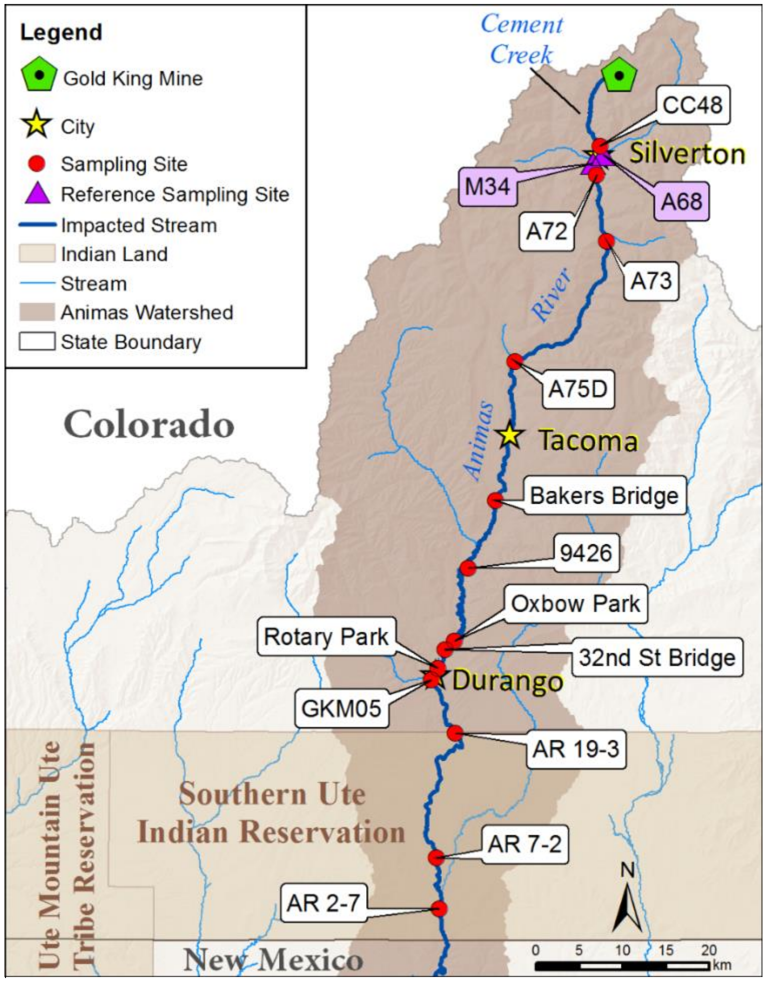

Figure 3.2. EPA sampling locations for biological data in the upper and middle Animas River following the GKM release.

Table 3.1. Sampling locations and dates for biological and physical habitat data collected by the EPA, EPA contractors, states, tribes and federal partners during the GKM-plume through spring 2017. Pre = pre-release data available for this location within the period of record (upper Animas = 2005-8/5/2015; middle and lower Animas and the San Juan = 2000-arrival of the plume). NS = not sampled. * identifies locations that were not impacted by the release and were sampled to characterize background **identifies locations that were only sampled for biology by state and/or tribal partners. See Appendix A of this report for location descriptions and EPA's Field Activities Report for additional details on the EPA's sampling efforts.

	Location	Distance from GKM	Latitude	Longitude	Benthic Macroinvertebrate Assemblage		Benthic Macroinvertebrate Tissue		Fish Tissue		Physical Habitat
		(KM)			Pre		Pre		Pre		
Upper Animas River and Tributaries	CC48	12.54	37.818115	-107.661678	Yes	8/23/16 9/27/16	No	9/27/16	No	NS	Fall 2016
Upper Animas River and Tributaries	A68*	13.9	37.810983	-107.65936	Yes	8/8/15 8/12/15 9/23/15 8/23/16 9/27/16	Yes	9/23/15 9/27/16	No	10/30/16	Fall 2016
Upper Animas River and Tributaries	M34*	15.14	37.802921	-107.672724	Yes	8/23/16 9/27/16	Yes	9/27/16	No	NS	Fall 2016
Upper Animas River and Tributaries	A72	16.4	37.790017	-107.667536	Yes	8/8/15 8/12/15 9/23/15 8/23/16 9/27/16	Yes	9/23/15 9/27/16	No	NS	Fall 2016
Upper Animas River and Tributaries	A73	24.5	37.72215833	-107.6548278	No	10/15/15 8/26/16 10/3/16	Yes	10/15/15 10/3/16	No	10/31/16	NS
Upper Animas River and Tributaries	A75D	45.1	37.59793424	-107.775326	Yes	10/15/15 8/26/16 10/3/16	Yes	10/15/15 10/3/16	No	10/31/16	NS
Upper Animas River and Tributaries	Bakers Bridge	64.0	37.455731	-107.801095	Yes	9/21/15 8/22/16 9/29/16	Yes	9/21/15 9/29/16	No	11/1/16	Fall 2016
Middle Animas	James Ranch**	67.1	37.417822	-107.814819	Yes	9/21/15	No	9/21/15	No	NS	NS
Middle Animas	9426	76.8	37.385148	-107.836946	Yes	10/29/15 8/22/16 9/29/16	No	9/29/16	No	11/1/16	Fall 2016
Middle Animas	Oxbow Park	89.8	37.308898	-107.855793	No	9/18/15	No	9/18/15	No	NS	NS

	Location	Distance from GKM	Latitude	Longitude	Benthic Macroinvertebrate Assemblage		Benthic Macroinvertebrate Tissue		Fish Tissue		Physical Habitat
		(KM)			Pre		Pre		Pre		
Middle Animas River	32nd Street Bridge	91.8	37.294805	-107.870469	Yes	8/6/15 8/7/15 8/13/15 9/22/15 8/22/16 9/28/16	No	9/22/15 9/28/16	No	11/1/16	Fall 2016
	Animas Rotary Park	94.2	37.280534	-107.876622	Yes	8/6/15 8/7/15 8/13/15 9/20/15 8/22/16 9/28/16	No	9/20/15 9/28/16	No	11/3/16	NS
	Above Lightner	96.0	37.26892921	-107.8862952	Yes	9/20/15	No	NS	No	NS	NS
	GKM05	96.5	37.268704	-107.885857	No	10/27/15 8/25/16 9/30/16	No	9/20/15 9/30/16	No	8/14/15 3/18/16 11/3/16	Fall 2016
	AR19-3	104	37.221297	-107.859598	Yes	8/6/15 8/10/15 9/22/15 9/2/16 10/4/16	No	9/22/15 10/4/16	Yes	11/2/16	Fall 2016
	AR16-0**	109	37.187031	-107.869928	Yes	8/6/15 8/10/15 8/22/16	No	NS	No	NS	NS
	AR7-2	123	37.085161	-107.879233	Yes	8/10/15 10/28/15 9/2/16 10/4/16	No	10/4/16	No	11/2/16	Fall 2016
	AR2-7	131	37.032292	-107.875455	Yes	8/10/15 8/22/15 10/28/15 9/2/16 10/4/16	No	10/4/16	Yes	11/2/16	Fall 2016

	Location	Distance from GKM	Latitude	Longitude	Benthic Macroinvertebrate Assemblage		Benthic Macroinvertebrate Tissue		Fish Tissue		Physical Habitat
		(KM)			Pre		Pre		Pre		
Lower Animas River	ADW-022	148	36.933295	-107.909073	No	10/28/15 8/24/16 9/2/16 9/30/16	No	8/2015 3/2016 9/30/16	No	8/2015 3/2016 4/19/17	Fall 2016
Lower Animas River	ADW-021	158	36.872838	-107.960741	No	8/24/16 10/1/16	No	10/1/16	No	4/19/17	Fall 2016
Lower Animas River	ADW-010	163	36.838545	-107.992183	Yes	8/24/16 10/1/16	No	8/2015 3/2016 10/1/16	No	8/2015 3/2016 4/19/17	Fall 2016
Lower Animas River	FW-012	177	36.783635	-108.102111	No	8/27/16 10/2/16	No	10/2/16	No	4/20/17	Fall 2016
Lower Animas River	FW-040	192	36.707467	-108.150813	Yes	8/27/16 10/2/16	No	10/2/16	No	4/20/17	Fall 2016
Upper San Juan River	SJAR*	190	36.719664	-108.207125	Yes	8/29/16 9/27/16	No	8/2015 3/2016 9/27/16	No	8/2015 3/2016 4/18/17	Fall 2016
Upper San Juan River	LVW-020	197	36.730556	-108.251046	No	8/27/16 10/2/16	No	8/2015 3/2016 10/2/16	No	8/2015 3/2016 4/18/17	Fall 2016
Upper San Juan River	SJLP	197	36.73588701	-108.2539868	No	8/29/16 9/27/16	No	9/27/16	No	11/15/16	Fall 2016
Upper San Juan River	SJFP	214	36.74815602	-108.4120157	Yes	8/30/16 9/28/16	No	8/2015 3/2016 9/28/16	No	8/2015 3/2016 11/14/16	NS
Upper San Juan River	SJSR	246	36.78162422	-108.6927838	No	8/30/16 9/28/16	No	9/28/16	No	11/6/16	Fall 2016
Lower San Juan	SJ4C	296	36.99621613	-109.0046838	No	10/26/15 8/31/16 9/29/16	No	9/29/16	No	11/8/16	Fall 2016
Lower San Juan	SJMC	346	37.25822644	-109.3106036	Yes	10/26/15 8/31/16 9/29/16	No	9/29/16	No	11/10/16	Fall 2016

	Location	Distance from GKM	Latitude	Longitude	Benthic Macroinvertebrate Assemblage		Benthic Macroinvertebrate Tissue		Fish Tissue		Physical Habitat
		(KM)			Pre		Pre		Pre		
	SJBB	378	37.25737015	-109.6185856	Yes	10/26/15 9/1/16 9/30/16	No	9/30/16	Yes	11/13/16	Fall 2016
	SJMH	421	37.146948	-109.853672	No	10/26/15 9/1/16 9/30/16	No	9/30/16	No	11/13/16	Fall 2016
	SJCH	511	37.293336	-110.399293	No	10/26/15 8/25/16 10/1/16	No	10/1/16	No	NS	Fall 2016

3.3 Historic biological data

EPA worked with federal, state, tribal and local partners to compile the historic biological data for the Animas and San Juan rivers.[3] Most of the historic data were obtained from online sources, however some were provided through data requests (see Appendix B). For the Animas River, the pre-release period of record was defined as immediately before the GKM release (date varies by sampling location) back to 2005. Data collected prior to 2005 in the upper Animas River were avoided because of changes in the watershed that affected water quality and the aquatic community.[4] In the mid and lower Animas River and San Juan River, the pre-release period of record included data collected back to 2000. The period of record was greater for the San Juan because metal concentrations generally decline with distance from the mining district and any changes in the upper Animas activities in the early 2000's are less likely to be observed in the San Juan River (Chapter 2). Data collected prior to 2000 were avoided since there is a greater likelihood that the study objectives and sampling methods have been modified over the years, reducing the comparability of the datasets.

Additionally, biological communities, particularly benthic macroinvertebrates, display seasonal variability making comparisons difficult when samples were not collected during the same general time of year. Therefore, the pre-release dataset was limited to samples that were collected in late summer and fall to facilitate the comparison with EPA's response data that were mostly collected in the months of August-October. Due to the effect of seasonal variability, we are not presenting historic spring sampling data in this report.

Pre-GKM release benthic macroinvertebrate and fish data were available for a number of the Animas River sampling locations in Colorado and Southern Ute Indian Reservation due to past and continued interest in the effects of proximate mining run-off. The historic benthic macroinvertebrate data for the upper Animas has been funded and collected by several entities and most recent efforts were conducted by EPA Superfund activities with the support of Mountain Studies Institute (MSI 2016). Pre-release and historic macroinvertebrate data were less abundant further downstream on the Animas and San Juan River in New Mexico, Ute Mountain Ute Reservation, the Navajo Nation, and Utah.

Fish population surveys, on the other hand, have been conducted on a regular basis for the last several decades in the Animas River near Durango, CO by Colorado Parks and Wildlife and in the San Juan River, by U.S. Fish and Wildlife Services to support the recovery of listed fish species.

[3] In this report, we use historic, pre-release and background condition to describe when data were collected with respect to the GKM release. Historic data include all data and studies that predate the GKM release. Pre-release data include a subset of the historic data defined by the location specific period of record. Background condition is used to describe the biological condition or concentrations that do not include GKM effects. Background condition can include historic data and data collected after the plume and deposits were removed through the system.

[4] http://animasriverstakeholdersgroup.org/blog/index.php/2015/10/23/gold-king-timeline/

3.4 Sampling methods and laboratory analyses

Existing Colorado Department of Public Health and Environment (CDPHE), Mountain Studies Institute (MSI) and EPA National Rivers and Streams Assessment (NRSA) methods were used to collect the benthic macroinvertebrate and tissue samples in the response and follow-up monitoring. Below is the full list of biological and physical habitat sampling and assessment methods that were used by EPA and other federal, state and tribal partners that provided pre- and post-GKM release data. The data providers, sampling methods and analytical approach for the water and sediment data presented in this report are found in the EPA ORD report (EPA 2016c).

- Macroinvertebrate Collection and Identification
  - Colorado Department of Public Health and Environment Policy Statement 10-1 (CDPHE 2010/2017)
  - Southern Ute Indian Tribe macroinvertebrate sampling protocol (SUIT 2015)
  - New Mexico Environment Department (NMED 2013)
  - EPA Remedial Program method historically used on Animas River described in MSI (2016) and Anderson (2007); identified as the Animas River method in this report
  - EPA National Rivers and Streams Assessment method (EPA 2013a, 2013b)
- Fish Collection and Identification
  - Colorado Parks and Wildlife (CPW 2010, 2015)
  - U.S. Fish and Wildlife Service (USFWS 2012)
- Macroinvertebrate Tissue
  - EPA Remedial Program method historically used on Animas River (MSI 2016)
- Fish Tissue
  - EPA National Rivers and Streams Assessment method (EPA 2013a, 2013b)
- Physical Habitat
  - EPA National Rivers and Streams Assessment method (EPA 2013a, 2013b)

When pre-release data and historic methods were not available for a given location, the EPA defaulted to the NRSA method for follow-up monitoring for that indicator (https://www.epa.gov/sites/production/files/2016-04/documents/nrsa1314_fom_nonwadeable_version1_20130501.pdf). However, many of the sampling locations have abundant pre-release biological data (e.g., benthic macroinvertebrates data in the Animas River). In these situations, the EPA used the method that best matched the pre-release data collection methods to maximize comparability. Below we provide brief descriptions and comparisons of the field and analytical laboratory methods that were considered when determining the comparability of data collected by different, federal, state, and tribal partners.

3.4.1 Benthic macroinvertebrate assemblage

In the upper Animas River from the GKM to Durango, benthic macroinvertebrate samples were collected with a method developed by Chester Anderson and used previously within the Animas River watershed (Anderson 2007). The upper Animas collection method utilizes modified protocols developed by the EPA (Barbour *et al*. 1999) and CDPHE (CDPHE 2010a). In the lower Animas River from Durango to the confluence with the San Juan River, EPA contractors used both CDPHE and EPA NRSA methods depending on the availability of pre-release data at these sites. When the habitat primarily consists of riffle/run, EPA NRSA and CDPHE methods are expected to generate similar results. In the San Juan River, all benthic macroinvertebrate data were collected using EPA NRSA methods. The differences and similarities in the field sampling methods are identified in Table 3.2. Overall, the methods used throughout the basin have a number of common elements thus allowing for comparisons between pre- and post-release benthic macroinvertebrate assemblages. Except for QA field duplicate samples, each sampling event was represented by a single sample per location.

Table 3.2. Comparison of benthic macroinvertebrate sampling methods used by the different data providers. LTU = lowest taxonomic unit. NR = not reported.

Method Comparison	Animas River	CDPHE	EPA NRSA		NMED (EPA EMAP)	SUIT
Method	Wadeable	Wadeable	Wadeable	Non-wadeable	Large rivers	Wadeable
Habitat Selection	riffle	riffle/run	11 multi-habitat transects	11 multi-habitat transects	11 multi-habitat transects	riffle
Sampling Net Type	rectangular dip net w/ dolphin bucket	rectangular kick net w/ dolphin bucket	D-frame	D-frame	D-frame	D-frame w/ dolphin bucket
Sampling Net Size	46 cm X25 cm	8"x18"	12”	12”	12”	18"
Sampling Mesh Size	500 µm	500-600 µm	500 µm	500 µm	500 µm	500 µm
Sampling Area Method	0.115m^2 hand scrubbed rocks	1 m^2 kick	0.093 m^2	1-meter sweep in primary habitat	3 1-meter sweeps (2 in primary habitat; 1 in secondary habitat)	1 m^2 kick
Sample time	90s	60s	30s	NA	not timed	not timed
Reps in composite	5 (diagonally across riffle)	1	11	11	11	1
Total area sampled	0.575 m^2	1 m^2	1 m^2	11m^2	33 m^2	1 m^2
Index Period	not provided	July 1- Oct 1	June 1 - Sept 30	June 1 - Sept 30	NR	NR
Subsampling and Enumeration	500 organisms	300 organisms	500 organisms	500 organisms	NR	300 organisms
Taxonomic Level	LTU	LTU	Genus unless otherwise specified	Genus unless otherwise specified	NR	LTU

3.4.2 Fish populations

The EPA evaluated fish data that were collected by Colorado Parks and Wildlife (CPW) in the upper Animas River and the U.S. Fish and Wildlife Service (USFWS) in the lower Animas River and San Juan River. Both partners collected fish assemblage data with bank and/or raft electrofishing depending on the sampling depth. Although the field sampling methods are somewhat different, the agencies collected from unique river ecosystems that did not spatially overlap allowing for separate data analyses (Table 3.3).

Table 3.3. A comparison of adult fish population sampling methods used by the Colorado Parks and Wildlife and U.S. Fish and Wildlife Service to sample the Animas River and San Juan River, respectively.

	Colorado Parks and Wildlife	US Fish and Wildlife Service
Study area (GKM site ID)	Animas River • Upper Animas (A72) • Lower segments: Animas River #1 (AR19-3) Animas River #2 (32nd St Bridge, Rotary Park, GKM05)	San Juan River Entire river from Navajo Reservoir to Lake Powell; 2 out of every 3 river miles (all San Juan GKM sites)
Method	Two-pass mark and recapture	Single pass
Gear	Upper Animas: bank electrofishing Lower Animas: raft electrofishing	Raft electrofishing (2 rafts)
Index period	NA	Late September to early October
Population data	Count, length, weight, deformities	Count, length, weight, deformities

Colorado Parks and Wildlife

The Colorado Parks and Wildlife (CPW) manages the segment of Animas River downstream of Durango (Animas River #1) as a category 406 "coldwater regulation stocked stream" and Animas River through Durango (Animas River #2) as a category 405 "regularly stocked with fry/fingerling/sub-catchable salmonids" (CPW 2010, 2015). These management practices have led to regular monitoring of the Animas River fishery for the last several decades, including adult (large) fish surveys typically conduced in the spring and fall and fry (small) fish surveys in the summer.

For adult surveys, CPW implements a two-pass mark and recapture method. All fish are marked by punching a small hole in the caudal fin, released back to the river, and resampled two days later. This sampling method generates estimates of species-specific density, biomass and populations demographics. The Animas River surveys target both the introduced trout and native species. Targeted small fish sampling consists of multi-pass depletion surveys along 100m sections of shoreline in shallow water where trout fry and other small fish (e.g., sculpin) would normally be found. Additional details on the CPW fish survey methods are available at http://cpw.state.co.us/thingstodo/Pages/FisheryManagementSurveys.aspx.

U.S. Fish and Wildlife Service

Long-term fish community surveys, including targeted larval and adult surveys, have been conducted in the San Juan River from Navajo Reservoir to Lake Powell by the U.S. FWS with state and tribal partners to support the San Juan River Recovery and Implementation Program (SJRIP). SJRIP was established to support the recovery of the endangered Colorado pikeminnow and razorback sucker, in conjunction with water development projects in the basin. The raft electrofishing collection methods used in sub-adult and adult fish monitoring programs are designed to "quantitatively document trends in fish community population parameters (including relative and absolute population size and size structure) occurring over time among populations of both native and nonnative large-bodied fishes in the San Juan River" (USFWS 2012). The USFWS performs these surveys in September and October each year so between-year comparisons are not seasonally confounded. Typical sampling duration was 20-30 minutes. The data are used to inform management actions that are being implemented by the SJRIP such as mimicry of the natural flow regime, mechanical removal of non-native fishes, removal on in-stream dispersal impediments, or augmentation of endangered fish populations. Additional details on the SJRIP and fish population data are available at https://www.fws.gov/southwest/sjrip/index.cfm.

3.4.3 EPA tissue collection methods

EPA Benthic Macroinvertebrate

A composite, whole body benthic macroinvertebrate (BMI) sample was collected from each site for tissue metal analyses. The samples were collected in a similar manner as the benthic community method used at that site. The near-term response samples were collected by MSI in 2015 and the follow-up sampling was conducted by an EPA START contractor in 2016. Using forceps and a fine mesh net, each specimen was rinsed with deionized water in the field before combining all specimens into a community composite sample for each site. The treatment of the caddisfly casings differed between 2015 and 2016 samples. In 2015, caddisfly larvae were removed from their cases prior to compositing and processing, which was consistent with how the samples were collected in 2014. In 2016, the samples were composited and processed with the caddisfly cases.

To meet laboratory and method analysis requirements, EPA contractors aimed to collect at least 2 grams of wet weight BMI tissue for each site. Analytical methods were modified (i.e., analyzed “as is” or with micro digestion techniques; see Section 3.4.6) for samples that fell short of this minimum tissue requirement because of the limited benthic macroinvertebrate communities at the site. The samples were immediately frozen (not held for gut content purging) and shipped on ice to the contract laboratory for metal analyses.

EPA Fish Tissue

NRSA fish tissue collection methods were implemented at 25 sampling locations (Table 3.1). The NRSA method focuses on fish species common to the region of interest, are sufficiently abundant within a sampling reach, and represent a species and size class that would be consumed by humans. Whole fish samples were frozen and shipped on ice to Physis Environmental Laboratories for analysis. The filet with skin was then dissected from the individual fish in the lab and sample replicates were composited prior to analysis. Tissue samples represent a composite of four to five adult fish of the same species that are similar in size (the smallest individual in the composite is no less than 75% of the total length of the largest individual).

3.4.4 Colorado fish tissue collection methods

The CDPHE and CPW collected fish filets from rainbow and brown trout of catchable size from the Animas River near the Durango area. This stretch of river is designated as gold medal fishery and includes Rotary Park (94 RKM), GKM05 (96.5 RKM), and AR19-3 (104 RKM) sampling locations. Samples were collected immediately following the GKM release in August 2015 and again in March 2016. The results of the metals analyses were used to evaluate potential impacts to human health (CDPHE 2016b).

3.4.5 New Mexico tissue collection methods

The NMDGF collected benthic macroinvertebrate and fish tissue samples immediately following the GKM release in August 2015 and again in March 2016. Sampling locations included 2 sites on the Animas River, (ADW-022;148 RKM and ADW-010; 163 RKM) and three sites on the San Juan River, including one site upstream of the confluence with the Animas River (SJAR; 191 RKM, LV-020; 196 RKM and SJFP; 214 RKM (Figure 3.2)).

NMDGF Benthic Macroinvertebrate Tissue

NMDGF collected benthic macroinvertebrate samples using a 1.0 meter kick-seine. Samples were sorted by taxonomic group in the field prior to metals analysis. The goal was to collect >3.0 grams of tissue in the following taxonomic groups: Plecoptera (stoneflies), Ephemeroptera (mayflies), Trichoptera (caddisflies), and Diptera (true flies). Several orders were either not present or not present in sufficient numbers to collect the minimum tissue requirement. The whole-body samples were placed in a whirl-pak bag, labeled, place in a cold cooler and frozen prior to shipment to the laboratory.

NMDGF Fish Tissue

NMDGF fish tissue samples were collected with a raft electroshocker. Fish samples were dissected prior to metals analysis and were separated into muscle (filets without skin) and liver tissue samples, for all species except speckled dace. Field crews aimed to collect a minimum of 5 grams for each tissue type. Multiple replicates were collected when species were present in sufficient numbers. Each sample represented an individual fish. Given the small mass of the speckled dace, speckled dace muscle samples represent a compost of approximately 5 fish with the head and gut content removed.

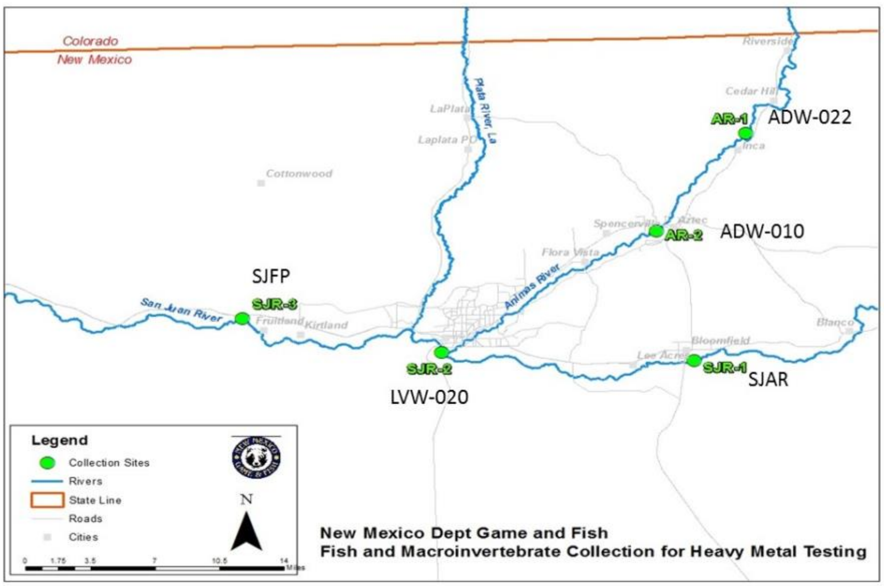

Figure 3.3. New Mexico Department of Game and Fish sampling locations for benthic macroinvertebrate and fish tissue.

3.4.6 Navajo Nation fish tissue methods

Navajo Nation Environmental Protection Agency (NNEPA) analyzed catfish filets without skin collected from the San Juan River at five locations between Farmington, NM and Bluff, UT in June 2017 (NNEPA 2017). Samples consisted of two composites of five fish from each sampling location (50 total fish) and were analyzed for 25 metals.

3.4.7 Physical habitat methods

Physical habitat refers to the structural attributes that influence the biological condition of an aquatic resource. EPA measured the physical habitat characteristics in Table 3.4 to quantify the eight general attributes of physical habitat condition, including: habitat volume/stream size; habitat complexity and cover for aquatic biota; streambed particle size; bed stability and hydraulic conditions; channel-riparian and floodplain interaction; hydrologic regime; riparian vegetation cover and structure; and riparian disturbance.

At each location, physical habitat data were collected from longitudinal profiles and at 11 cross-sectional transects. Streamside riparian plots were evenly spaced along a defined reach at the sampling sites in fall 2016. The length of each sampling reach was determined by the wetted channel width. Main channel and mid-channel substrate were determined by probing the bottom and 11 littoral/riparian plots were spaced systematically, alternating sides along the river sample reach.

Table 3.4 Summary of the components used to characterize physical habitat at wadeable sampling locations. Similar components were measured at non-wadable sites with methods that are modified to allow for sampling from a boat.

Component	Description
Thalweg Profile	Measure maximum depth, classify habitat and check presence of backwaters, side channels and loose, soft deposits of sediment particles at 10 equally spaced intervals between each of 11 transects (100 individual measurements along entire reach) The number of thalweg measurements is specified by the stream's mean wetted width.
Wetted Width / Bar Width	Measure wetted width and bar width (if present) and evaluate substrate particle size classes at 11 cross-section transects and midway between them (21 width measurements and substrate notations along entire reach)
Woody Debris Tally	Between each of the channel cross-sections, tally large woody debris numbers within and above the bankfull channel according to specified length and diameter classes (10 separate tallies).
Channel and Riparian Characterization	At 11 transects placed at equal intervals along reach: • Measure channel cross-section dimensions, bank height, bank undercut distance, bank angle, slope and compass bearing (backsight), and riparian canopy density (with densiometer). • Visually estimate: substrate size class, embeddedness and water depth at five equidistant points on cross-section; areal cover class and type (e.g., woody trees) of riparian vegetation in canopy, understory, and ground cover; areal cover class of fish concealment features, aquatic macrophytes and filamentous algae. • Observe and record: presence and proximity of human disturbances. At 10 cross-sections that are midway between the 11 transects above: • Visually estimate substrate size class at 5 equidistant points on each cross-section
Assessment of Channel Constraint, Debris Torrents, and Major Floods	After completing thalweg and transect measurements and observations, identify features causing channel constraint, estimate the percentage of the channel margin that is constrained for the whole reach, and estimate the bankfull and valley widths. Check for evidence of recent major floods and debris torrent scour or deposition.
Discharge	Measure water depth and velocity at 15 to 20 equally spaced intervals across one carefully chosen channel cross-section. In very small streams, measure discharge by timing the passage of a neutrally buoyant object through a segment whose cross-sectional area has been estimated or by timing the filling of a bucket.

3.4.8 Laboratory analytes and methods

EPA follow-up monitoring fish and benthic macroinvertebrate tissue samples were analyzed for the 16 metals and metalloids on the priority Metal/Cyanide Target Analyte List (TAL) that are most likely to accumulate in biological tissues (see Table 3.5). All tissue samples were processed using inductively coupled plasma (ICP) technologies that use mass spectrometry (MS) instrumentation. Mercury was analyzed using cold vapor atomic fluorescence spectrometry (CVAFS). EPA 2016 tissue samples were analyzed by Physis Environmental Laboratories, Inc., Anaheim, CA. EPA's 2015 benthic macroinvertebrate tissue samples and pre-release samples (2012 and 2014) were analyzed by the Environmental Services Assistance Team (ESAT) contractor in R8 EPA Laboratory, Golden, CO.

Additional data analyzed in this report include samples collected by Colorado Department of Public Health and Environment (CDPHE), Southern Ute Indian Tribe (SUIT), and New Mexico Department of Game and Fish (NMDGF) and Navaho Nation EPA (NNEPA). The CDPHE, NMDGF, NNEPA results were reported as wet weight concentrations, whereas SUIT results were reported as dry weight concentrations. Weights are converted to a common standard when datasets are combined in analyses. Table 3.5 provides a general guide to the laboratory methods used by all data providers.

Table 3.5. Analytical methods, parameters and technology used for the biological tissue samples collected by EPA, CDPHE, SUIT, and NMDGF. BMI = benthic macroinvertebrate; a = dry weight results; b= wet weight result; c = as received result; *indicates a post-GKM release sample.

Data Source	Matrix	Lab	Date (result type)	Methodology	Parameter	Technology
EPA: pre-release & response	BMI	ESAT (R8 Lab) Golden, CO	Oct 2012 (a); Sept-Oct 2014 (c); Sept-Oct 2015 (c)*	EPA Method 200.7	Al, Be, Ca, Fe, Mg, Mn, K, Na, Sr, Zn	ICP/AES
				EPA Method 200.8	Sb, As, Ba, Cd, Cr, Co, Cu, Pb, Mo, Ni, Se, Ag, Tl, V, Zn,	ICP/MS
				EPA Method 245.1	Hg	CVAA
				EPA Method 7473	Hg	TDAAAS
				EPA Method 200.2	Solids, dried at 60ºC	--
EPA: follow-up	BMI & Fish (filet composite w/ skin)	Physis Environmental Lab Anaheim, CA	Sept 2016(a)*; April 2017(a)*	EPA Method 6020	Al, Sb, As, Cd, Cr, Cu, Fe, Pb, Mn, Ni, Se, Ag, Sn, V, Zn,	ICP/MS
				EPA Method 245.7	Hg	CVAFS
				SM 2540B	Solids, dried at 103-105ºC	Gravimetric
CDPHE	Fish (filet)	Laboratory Services Division of CDPHE	Aug 2015 (b)* Mar 2016 (b)*	EPA Method 200.7/200.8	Be, As, Se, Cd, Pb, U, Al, Co, Cu, Mn, Ni, Zn	--
				EPA Method 7473	Hg	TDAAAS
SUIT	Fish (muscle plug)	--	July 2015 (a)	--	As, Be, Se, Cd, Pb, U, Al, Co, Cu, Mn, Ni, Zn, Hg	ICP/MS
				--	Solids	--
NMDGF	BMI & Fish (filet w/o skin; liver)	ALS Environmental	Aug 2016 (b)* Mar 2016 (b)*	EPA Method 6020	Al, As, Cd, Cu, Pb, Mn, Se, Zn	ICP/MS
NNEPA	Fish (filet w/o skin)	TestAmerica	April 2017 (b)*	EPA Method 6020	Al, Sb, As, Ba, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo,Ni, K, Se, Ag, Na, Sr, Ti, Sn, V, Zn,	ICP/MS
				Method 7471B	Hg	CV

Metal concentrations in tissue were converted from wet weight to dry weight and dry weight to wet weight as needed to generate a common unit of concentration using the following equation, which is regularly used to support tissue data analyses (Lusk *et al*. 2005, EPA 2016d):

$$[\text{metal}]\ \text{ppm ww} = [\text{metal}]\ \text{ppm dw} \times (\text{percent solid}/100)$$

3.5 Data QAQC

Biological data collection, processing, and quality review efforts followed quality assurance procedures described in the Quality Assurance Project Plan (QAPP) titled "Sampling and analysis plan/quality assurance plan for Gold King Mine long term monitoring – 2015-2016 sampling events. Version 3" (Appendix in the EPA Gold King Mine Field Activities report; to be posted at https://www.epa.gov/goldkingmine). A comprehensive list of data sources is provided in Appendix B. All data acquired may not have been used in final data products presented in this report but have been archived with project materials. EPA does not make any claims as to the quality or accuracy of the data gathered from state, tribal, and federal partners. The project team applied quality assurance and quality control measures to acquired data to ensure that the analyses performed were properly conducted and that the data used in this report represented the original data obtained from all sources. Acquired data were reviewed and normalized as needed to be able to do a watershed-scale analysis. Inspections occasionally identified errors in the original flies, primarily related to sampling dates. The team corrected the errors in the master file following consultation with source data owners. Edited data were notated in supporting documentation with justification for doing so. Differences in field and analytical methods used by the various data providers were identified, assessed for comparability and considered when developing our approach to the data analysis. Datasets were analyzed collectively when methods were similar, and we present analyses consistent with the state/tribal analysis tools when such tools were available.

3.6 Assessment approach

The report utilized available data to assess the impact of metals released from the Gold King Mine on aquatic communities and their body burden of metals. These characteristics were assessed spatially along the Animas and San Juan rivers that were affected by the release to various levels and temporal duration during and in the 18-months following the event (EPA 2016c). Physical habitat, metal concentrations (pre-release, during the plume and post-release), and the biological community were evaluated with the river distance (km) downstream from the Gold King Mine to determine watershed-wide longitudinal trends. Pre- and post-event data samples were compared where data availability allowed using statistical tests appropriate to available data. Table 3.6 provides a summary of all sources of data that were acquired for this report. Sources that are identified as primary data included multiple sampling locations, dates and sample replicates. The additional data sources represent studies with limited sampling locations and/or sampling dates. Chapters include additional description of data and analytical techniques, statistical tests, and results.

The results of data analysis are organized in 5 chapters:

- Physical habitat in the Animas and San Juan rivers (Chapter 4)
- Benthic macroinvertebrate assemblage (Chapter 5)
- Fish populations (Chapter 6)
- Bioaccumulation of metals in benthic macroinvertebrates (Chapter 7)
- Bioaccumulation of metals in fish (Chapter 8)

Table 3.6. Summary of pre- and post-GKM release biological and physical habitat data collected from the Animas and San Juan rivers presented in this report. Sampling locations are distributed throughout the river unless otherwise noted.

	Data Source		Benthic Macroinvertebrate Assemblage	Benthic Macroinvertebrate Tissue	Fish Population	Fish Tissue	Physical Habitat
Primary Sources of Data	**EPA follow-up: START Contractors**	Post (2016)	Animas & San Juan	Animas & San Juan		Animas & San Juan	Animas & San Juan
	EPA response: Superfund/ Mountain Studies Institute (MSI)	Pre & Post (2015)	Animas (upper & mid)	Animas (upper & mid)			
	CO Parks and Wildlife (CPW)	Pre& Post			Animas (upper & mid)		
	US Fish and Wildlife Service (USFWS)	Pre& Post			San Juan		
	NM Department of Game & Fish (NMDGF)	Post		Animas (lower) San Juan (upper)		Animas (lower) San Juan (upper)	
Additional Data Sources	**Southern Ute Indian Tribe (SUIT)**	Pre& Post	Animas (mid)			Animas (mid)	
	CO Department of Public Health and the Environment (CDPHE)	Pre& Post	Animas			Animas (mid)	
	Animas River Stakeholder Group (ARSG)	Pre	Animas (upper)				
	Animas Watershed Partnership (AWP)	Pre	Animas (upper & mid)				
	EPA: National Rivers and Streams Assessment (NRSA)	Pre	San Juan				San Juan
	NM Environment Department (NMED)	Pre	Animas (lower) San Juan				
	Navajo Nation EPA (NNEPA)	Post (2017)				San Juan	
	Bureau of Reclamation	Pre (1996)				San Juan	

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CHAPTER 4 PHYSICAL HABITAT

The Animas and San Juan river physical habitat analyses focused on four primary indicators of physical habitat conditions in rivers and streams: relative bed stability and excess fines, in-stream fish habitat complexity, riparian (streamside) vegetation, and riparian disturbance (EPA 2016b). Table 4.1 provides details on each indicator and accounts for several individual physical habitat metrics. The primary indicators help document the impact of our human footprint across the landscape as well as the progress made through widespread protection and mitigation efforts. When pre-release NRSA data were available, the 2016 habitat condition was compared to pre-release results.

Table 4.1. EPA National Rivers and Streams Assessment physical habitat indices used to describe the aquatic habitat condition.

Habitat Index	Description
Relative bed stability and excess fines (LRBS)	streambed stability defined by relative substrate and particle size
Instream habitat complexity (XFC_NAT)	areal cover of woody debris, brush, undercut banks, overhanging vegetation, boulders, and rock ledges
Riparian vegetation (XCMGW)	areal cover and type of streamside vegetation found in the ground layer (<0.5m), mid-vertical layer (0.5-5.0 m) and upper vertical layer (>5.0m)
Riparian human disturbance (W1_HALL)	relative measure of 11 human activities: walls, dikes, revetment or dams, buildings, pavement or cleared lots, roads or railroads, influent or effluent pipes, landfills or trash, parks or lawns, row crop agriculture, pasture or rangeland, logging and mining.

In addition to the four primary habitat indicators, the longitudinal patterns of percent fine sediment, channel slope, and elevation throughout the Animas and San Juan rivers were evaluated to help interpret the spatial differences in biological communities. Longitudinal patterns were measured with the river distance (km) downstream from the Gold King Mine.

The baseline physical and chemical characteristics within the study area establishes the foundation for the aquatic biota that are expected to occur at the different sampling locations. The physical habitat characteristics include naturally occurring conditions (e.g., elevation, temperature, substrate) as well as anthropogenic alterations to these conditions (e.g., riparian disturbance, channelization, watershed erosion). The habitat within the effected river length ranges from an elevation 8,500 ft in the Animas at the confluence with Cement Creek to 3,400 ft at the furthest downstream location on the San Juan River. With the elevation change, the aquatic habitat transitions from a mountain stream that is characterized by narrow channels, steep gradient, and substrate dominated by cobble/boulder in the upper Animas River to a wide channel, low gradient, frequently braided channels dominated by sand and finer substrates in the mainstem of the San Juan River (Figure 4.1.a). The channel slope in the Animas River is greatest at sampling locations closest to GKM (A68 = 1.5%, A72 = 0.7%) and in Mineral Creek (M34 = 1.6%). The median slope in the Animas River sampling locations is 0.4 %. The slope of the San Juan River, on the other hand, is similar from the confluence with the Animas to the most downstream locations, with values ranges from 0.07-0.16%.

One measure of anthropogenic impacts in the immediate aquatic habitat is the level of disturbance in the riparian zone. A wide range of human disturbance in the riparian area is observed in the Animas River watershed with all sampling locations exhibiting metrics with medium to high levels of disturbance. Riparian disturbance generally decreases downstream in the San Juan River, with the most disturbed sites near the confluence with the Animas (Figure 4.1.b).

Three sampling locations in the San Juan River have pre-release habitat data that were collected as part of the National Rivers and Stream Assessment. Two sites, SJMC and SJBB are located on the San Juan River below the confluence with the Animas River. The third site, SJAR is located on the San Juan River above the confluence with the Animas River. The condition of riparian vegetation and habitat complexity has scored consistently good over the years at all three locations (Table 4.2). The relative bed stability and habitat complexity has also remained consistent at SJMC and SJBB, yet changed to a more degraded condition at the upstream location SJAR in 2016. Riparian human disturbance scored medium and high at both downstream locations over time, suggesting that human activities near the mainstem have been consistently present at these sites.

a)

b)

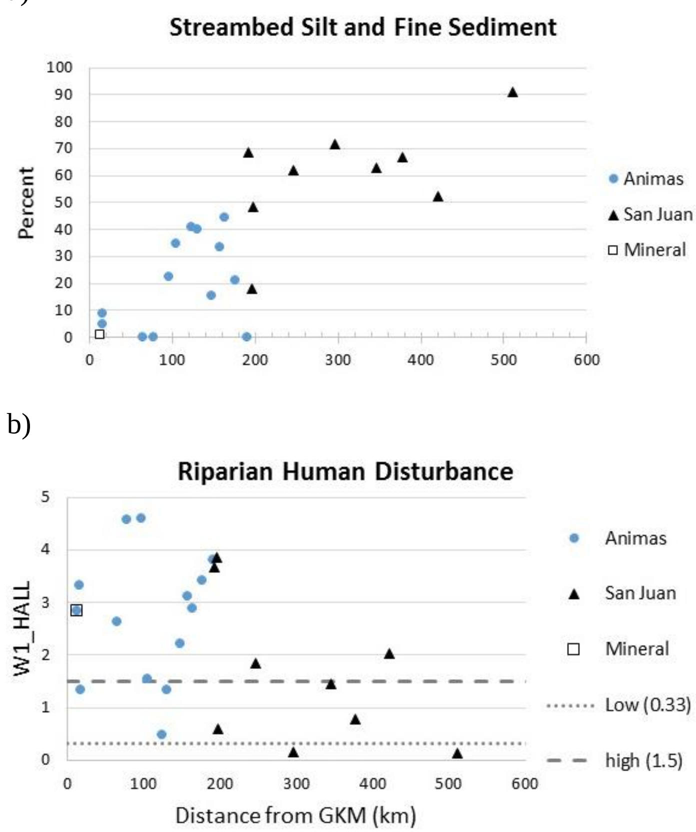

Figure 4.1. Longitudinal change in a) streambed silt and fine sediment and b) riparian human disturbance physical habitat characteristics for the Animas River, San Juan River, and Mineral Creek.

Table 4.2. Physical habitat condition for sampling locations on the San Juan River with pre-release NRSA physical habitat data including 1 site on the San Juan River located upstream of the confluence with the Animas (SJAR), and two downstream locations SJMC and SJBB.

SITE	Year	Relative Bed Stability	Riparian Vegetation	Habitat Complexity	Human Riparian Disturbance
SJAR (upstream)	2016	Poor	Good	Fair	High
	2013	Fair	Good	Good	Medium
	2009 (May)	Poor	Good	Good	Medium
	2009 (June)	Fair	Good	Good	Low
SJMC	2016	Fair	Good	Good	Medium
	2013	Fair	Good	Good	High
	2009	Fair	Good	Good	Medium
SJBB	2016	Fair	Good	Good	Medium
	2013	Fair	Good	Good	High

CHAPTER 5 BENTHIC MACROINVERTEBRATE ASSEMBLAGES

As discussed earlier in this report there are two overarching study questions about the potential impacts due to the GKM release to biology in the Animas and San Juan rivers. First, did the GKM event add to biological degradation in the already contaminated upper Animas river; and second did the GKM event degrade biological communities in other downstream segments of the Animas and San Juan rivers that had no previously known metal contamination? Chapter 5 reports on the results from the benthic macroinvertebrate assemblage analysis, with an assessment of both pre- and post-release condition, and longitudinal condition moving downstream from the confluence of Cement creek. This analysis found no conclusive evidence of changes in the already degrade upper Animas river when comparing GKM pre- and post-release benthic macroinvertebrate assemblage data, using both a multi-metric index or individual assemblage metrics. When comparing samples throughout the Animas and San Juan Rivers, there was no significant difference between per- and post-release samples and that benthic macroinvertebrate assessable condition generally improved moving downstream.

5.1 Benthic macroinvertebrate data

Benthic macroinvertebrate assemblage data collected in the Animas and San Juan rivers between June 2008 to October 2016 were obtained from state, tribal and federal agencies as described in Chapter 3 and listed in Table 3.5. Characteristics of the macroinvertebrate community composition were used to assess the health of aquatic benthos in relation to time and location within the Animas and San Juan rivers. Time was expressed in periods relative to the GKM release including pre-event, the event period defined as August 5 to August 13, 2015 when water quality effects were greatest, and post-event, from August 14, 2015 to April 2017 when the last samples were collected. Location was expressed as distance measured along the river centerline length starting at the Gold King Mine in the headwaters of the Animas River in Cement Creek. Samples of 300 to 500 organisms were collected from multiple habitats or targeted riffle/run locations using kick-nets of varying size. Since most samples were processed using a fixed subsample approach, only relative abundance of organisms and not density could be used during analysis. Most taxa were identified to genus or species. Assessment of seasonal differences of the benthic macroinvertebrates assemblages between pre- and post-GKM release was limited due to inadequate sampling in seasons other than summer. Spring sampling with pre- and post-GKM release results were limited to only two sites (AR16-0 and AR2-7), thus only samples collected during an index period of June through October were used in order to limit the confounding effects of seasonal variation.

There are various metrics that assess the health of aquatic communities using population sampling to characterize presence and relative abundance of macroinvertebrates and/or fish species. Some metrics target specific members of the macroinvertebrate community (e.g. taxonomic or functional feeding group), while others combine multiple assemblage metrics into multi-metric indices (MMI) that provide a more holistic assessment of condition of the entire community. This assessment applied both individual assemblage and multi-metric indices to assess the general health of macroinvertebrate communities in the Animas and San Juan rivers before and following the GKM release. Additional analyses of pre- and post-GKM release benthic macroinvertebrate data collected from the upper and middle Animas River were presented in MSI (2016, 2017).

5.2 Benthic macroinvertebrate assemblage assessment tools

Two individual benthic macroinvertebrate metrics known to be responsive to elevated metal concentrations were included in analysis of benthic macroinvertebrate species. ***Taxa richness*** is the total number of distinct taxa units (TotalTaxa). ***Percent EPT*** (%EPT) is the percent of the total number of individuals that represent Ephemeroptera, Plecoptera and Trichoptera taxa. These taxa favor habitats in cool, clear water and rocky substrate that are most characteristic of the Animas River. Within the EPT taxa, four additional taxonomic groups that have been identified as sensitive to metals pollution were also assessed. These included three Ephemeroptera taxa (Percent Ephemerellidae, Percent Heptageniidae, Percent Baetis), and one Plecoptera taxa (Percent Taeniopterygidae).

MMIs have also been used throughout the United States to assess aquatic condition based on fish and macroinvertebrate assemblage data (*e.g.*, Karr and Chu 2000; Barbour *et al.* 1999; Barbour *et al.* 1996). The multi-metric approach is an analytical process that combines metrics to define a locally or regionally relevant index. The process involves summarizing various assemblage attributes (*e.g.*, composition, tolerance to disturbance, trophic and habitat preferences) as individual "metrics" or measures of the biological community. Candidate metrics are evaluated for aspects of performance for each waterbody and a subset of the best performing metrics are then combined into a locally-defined MMI. EPA and Colorado CDPHE have each developed MMI methods; both were applied to the benthic macroinvertebrate data in an attempt to gain better resolution and to be representative of the Animas River and San Juan River habitats.

EPA's MMI methodology was first developed in the Wadeable Stream Assessment (WSA) of 2004 and later refined and used in the National Rivers and Streams Assessment (NRSA) of 2008/09 (EPA/841/R-16/008). Additional details on the development of EPA's MMI is found in Stoddard *et al.* (2008) and Herlihy *et al.* (2008). EPA's NRSA MMI is composed of 6 benthic macroinvertebrate metrics that are specific to aggregated Level III ecoregions. Ecoregions are areas where ecosystems, and the type, quality, and quantity of environmental resources are generally similar. The ecoregions relevant to the EPA MMI assessment included the Western Mountain (WMT) and Xeric (XER) ecoregions.

EPA's ecoregion MMIs were developed for each ecoregion in the study area by summing the six metrics that performed best in each (Table 5.1). Note that the 6 best-performing metrics varied somewhat between the two ecoregions. The calculated MMI at a site is compared to two condition thresholds that generally represent the 25th and 5th percentile of reference sites in the ecoregion. If the MMI is: greater than or equal to the upper threshold, the condition of the community is considered good; between the upper and lower threshold, conditions are fair; or less than the lower threshold, conditions are poor.

Table 5.1. EPA's NRSA benthic macroinvertebrate multi-metric index (MMI) characteristics for ecoregions applicable to the Animas and San Juan rivers.

	Ecoregion	
	Western Mountain (WMT)	**Xeric (XER)**
Metrics	• EPT % taxa richness • % individuals in top 5 taxa • Scraper taxa richness • Clinger % taxa richness • EPT taxa richness • Tolerant % taxa richness	• Non-insect % individuals • % individuals in top 5 taxa • Scraper taxa richness • Clinger % taxa richness • EPT taxa richness • Tolerant % taxa richness
Good threshold	≥54	≥53
Poor threshold	<40	<40

Colorado's MMI assigned 3 biotypes to the Animas and San Juan rivers. Colorado's approach defines a biotype as an aggregation of benthic macroinvertebrate sites that have similar community composition. The biotype of a sampling location is defined by the ecoregion, elevation, and stream slope. The Mountain and Transition biotypes were applied to the upper and middle Animas River. All of the Mountain and a portion of the Transition biotypes coincided with EPA's WMT ecoregion. The lower Animas and San Juan River sites are located within the Plains and Xeric biotypes.

The CDPHE MMI also uses the 5-6 best-performing benthic macroinvertebrate metrics (CDPHE 2017). Metrics selected for the 3 biotypes assigned to the Animas and San Juan are listed in Table 5.2. Using Colorado's approach, the biological condition of a waterbody is determined by comparing the calculated MMI to an upper and lower threshold that indicates attainment or impairment of ecological function, respectively. When the MMI score falls between the attainment and impairment thresholds, additional metrics are applied to determine if a waterbody supports the biological use.

A value for the NRSA and the Colorado MMIs was calculated for each sample dependent on the ecoregion/biotype of the site. EPA and Colorado MMI's developed for the Animas and San Juan rivers differ somewhat in ecoregion delineation, selected community metrics, and the attainment and impairment thresholds (Tables 5.1 and 5.2). Both MMI scores range from 0 to 100, with higher scores indicating healthier benthic macroinvertebrate assemblages.

Table 5.2. Colorado benthic macroinvertebrate MMI characteristics for Animas River biotypes. A full description of the multimeric development can be found in CO DPHE 2017. Description of each metric is located in Appendix C.

	Biotypes		
	Mountain	**Transition**	**Plains and Xeric**
Metrics	• TotalTax • EPTTax^ • pEPTnoB • ClngrTax • IntolTax • pi_DecrMtnTrn • PredTaxFAC • ScrapPctFAC	• EPTTax* • NonInPct • pEPTnoB • ColeoPct • pt_Intol* • pi_IncrMidElev • ClingrTax* • PredShrTaxFAC	• TotalTax • pt_noninsect • pEPTnoB • SprwlTax^ • IntolTax • pi_IncrPlains • PredTaxFAC • ScrapPctFAC
Attainment threshold	**48**	**45**	**42**
Impairment threshold	**40**	**34**	**29**

^ Metric has been adjusted based on Julian Day of sample collection.
* Metric has been adjusted based on average summer temperature

5.3 Trends in macroinvertebrate communities

5.3.1 Longitudinal trends within the river system

Individual biological community metrics including the percentage of Ephemeroptera, Plecoptera, and Trichoptera individuals relative to the entire sample assemblage (%EPT) and the total number of taxa are shown in Figures 5.1 and 5.2, respectively. Site values were plotted longitudinally within the Animas and San Juan rivers as a function of river distance. Data were identified by collection period relative to the GKM release. A few samples were collected during the GKM event at selected locations within the upper Animas River, although the Animas and San Juan rivers were broadly sampled a number of times in the following 2-year post-GKM release period. A narrower set of sites also had pre-event data.

Individual community metrics can be influenced by differences in water quality stressors as well as availability of suitable habitat for a species within a river system of this length. Lower %EPT or number of taxa can indicate more highly polluted or degraded water, or it may simply indicate less available suitable habitat for particular species. The species within the EPT taxa tend to prefer higher gradient, coarse substrate habitats and would be expected to be observed in high abundance in the mountainous headwaters of the Animas. There is a strong longitudinal habitat gradient within the Animas River as the river descends from the steep mountainous headwaters into the relatively low gradient valley in Hermosa Springs/Durango area at river distance 65 km (Figure 4.1). Habitat conditions continue to transition to low gradient, fine substrate channels in the lower Animas through the length of the San Juan River (Chapter 4).

A longitudinal trend in macroinvertebrate communities was evident to some extent in the study area. The %EPT was generally highest in the upper Animas and similar in all sampling periods (Figure 5.1). However, variability was as pronounced at similar distances as it was along the length of both rivers. The main exception to this pattern is low %EPT observed in Cement Creek at river distance 14 km. Cement Creek is one of three major tributaries in the Animas River headwaters that directly receives large amounts of natural and mining-caused AMD producing water with low pH and high metal concentrations.

The total number of taxa is also a commonly used aquatic community metric, where fewer taxa than expected for a given habitat within an ecoregion can indicate degraded water quality or poorer habitat. The number of taxa observed was lowest in headwaters streams within the mining district and was similar in all sampling periods (Figure 5.2). While the %EPT declined with distance in the Animas River, total taxa increased along the length of the Animas River. Total taxa peaked in the lower Animas near where it joins the San Juan River at river distance 193 km. Higher numbers of taxa persist for a few kilometers in the San Juan River and then decline through the remaining length of the San Juan River, probably reflecting the homogenous fine-grained river bed that characterizes much of its length. There was almost the same variability in total taxa observed among sites at similar distances as there was observed along the length of the affected rivers.

EPA NRSA and Colorado MMI scores are shown below in Figures 5.3 and 5.4, respectively. The lowest MMI scores were observed in segments persistently impacted by acid mine drainage within Cement Creek and immediately downstream of its confluence with the Animas River at Silverton, CO, presumably reflecting the poor water quality that is routinely documented in this area. MMI scores remained generally low and below attainment levels in the Animas River for some distance downstream of the mining district. NRSA and Colorado MMI scores improved and achieved attainment at river distances of ~80 and 50 km, respectively near Durango, CO. The Colorado MMI tended to cluster at lower MMI values within the 80-100 km distance during the 5-day GKM event period, but were within the pre-release range of variability (Figure 5.4). The zone of impact of acid mine drainage in the upper Animas River is consistent with EPA ecological risk assessments conducted in support of mine remediation efforts (EPA 2015).

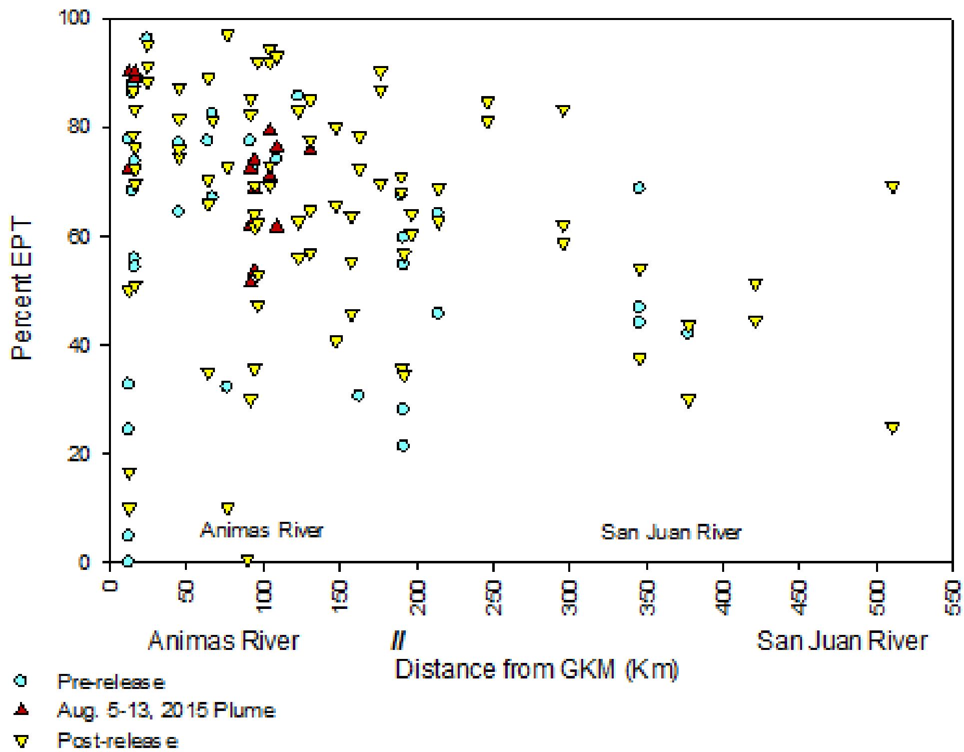

Figure 5.1. Percent of benthic macroinvertebrate assemblage composed of Ephemeroptera, Plecoptera, and Trichoptera (%EPT).

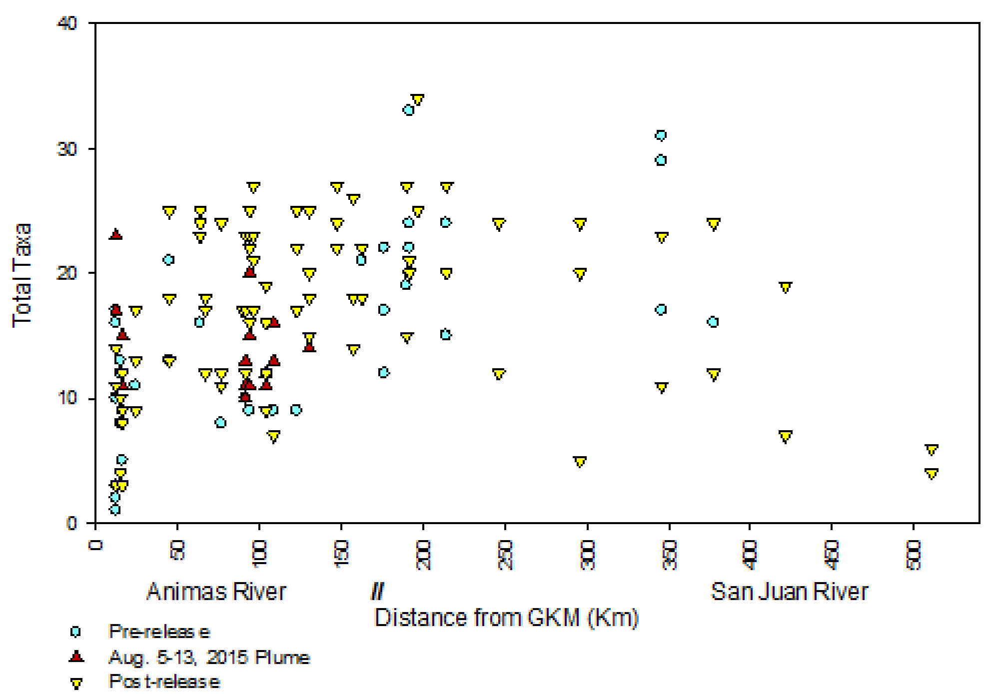

Figure 5.2. Total number of taxa collected during each sampling event.

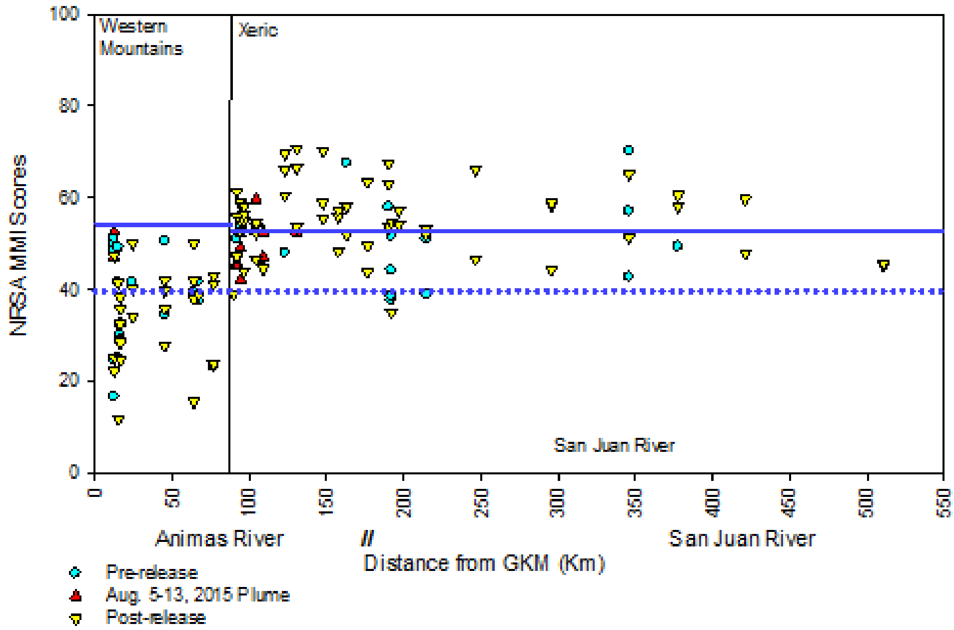

Figure 5.3. EPA's NRSA MMI scores for samples collected through the Animas and San Juan rivers. Solid blue line = threshold between good and fair condition for each of the two ecoregions; Dashed blue line = threshold between fair and poor condition for each of the two ecoregions.

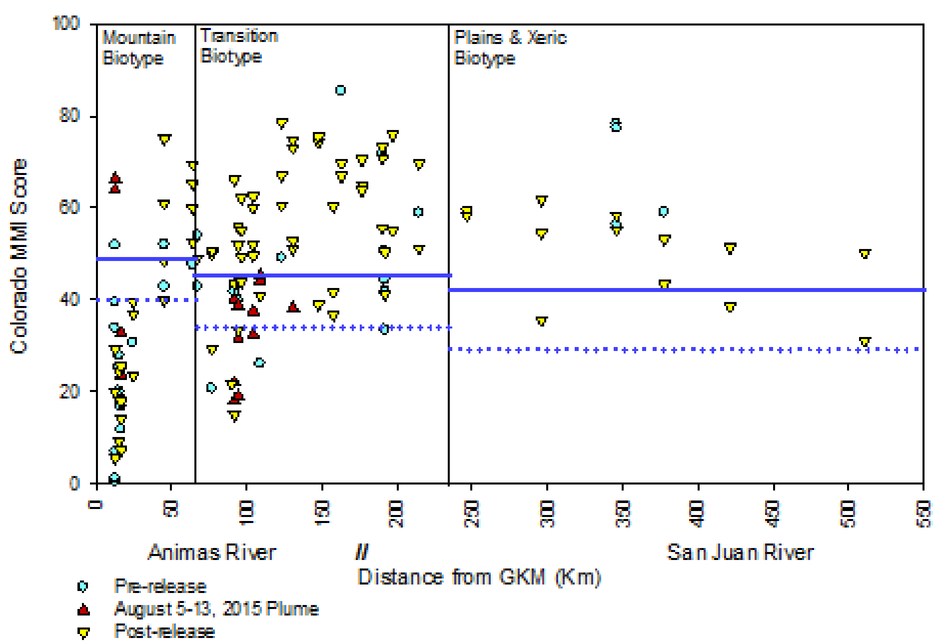

Figure 5.4. Colorado MMI scores for samples collected through the Animas and San Juan rivers. Solid blue line = attainment threshold for each of the three biotype regions; Dashed blue line = impairment threshold for each of the three biotype regions.

5.3.2 Pre- and post-GKM release comparisons of benthic macroinvertebrate data

In addition to understanding the general condition of the benthic macroinvertebrate assemblage throughout the Animas and San Juan rivers, it is also important to understand if the assemblage observed prior to the GKM release changed after the event. Table 5.3 shows the pre- and post- event median MMI and metric values from a subset of sites that were sampled for benthic macroinvertebrates during pre- and post- event. These sites were distributed along the length of the Animas and San Juan rivers, as shown in Figures 5.1 through 5.4.

Due to the limited number of sites with pre- and post-event data, a non-parametric Wilcoxon Signed Rank test was used to determine whether the median values of the post-event samples of each of the assemblage metrics were statistically different than the median values of the pre-event samples (Table 5.3). The Wilcoxon test found no significant difference between the pre- and post-GKM NRSA MMI or the Colorado MMI. The individual metrics, %EPT and total taxa were statistically compared in the same way. There was no significant difference in %EPT between the pre- and post-GKM release time periods. Total taxa were significantly greater in the post-GKM release samples than those collected prior to the release. Appendix C shows the site specific median values for the pre- and post- event periods. While the Wilcox Signed Rank test showed no significant differences between the pre- and post-GKM release time periods, the number of signed-rank scores greater than 10 was substantially higher than the sign-rank scores less than -10 within the middle Animas (Figure 5.5), suggesting that benthic macroinvertebrates communities showed little impact from the GKM release.

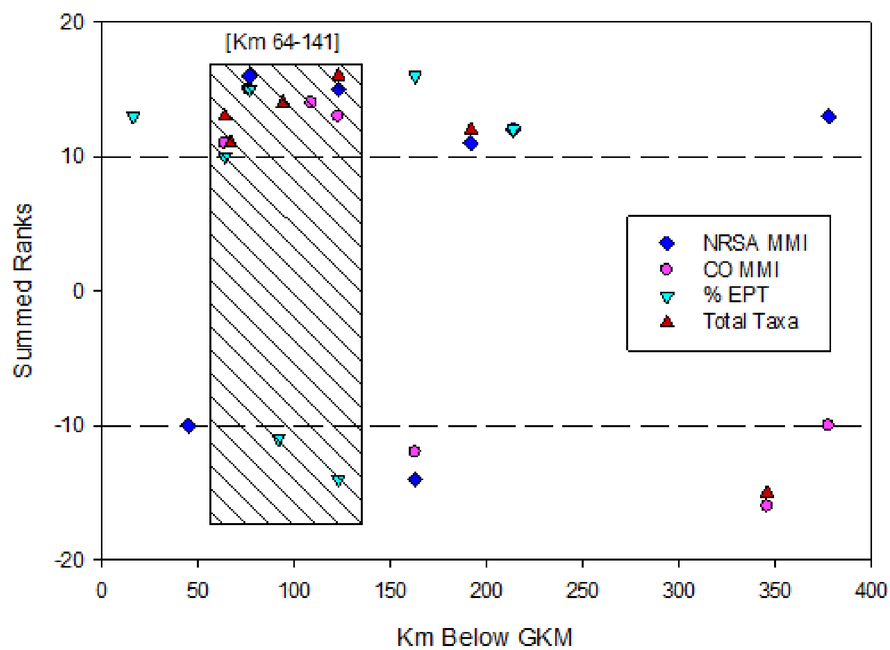

Figure 5.5. Changes in macroinvertebrate community metrics for sites with both pre- and post-release data following the GKM release in August 2015. Changes in each metric are expressed as summed ranks from Appendices C.2 through C.5, screened to show only positive or negative changes greater than 10.

Table 5.3. Comparison benthic macroinvertebrate assemblage indices and metrics for sites that have pre- and post-GKM release data. Statistical analysis used for the comparison was a Wilcoxon's signed-ranks test. * Significant at $p < 0.05$. [Appendix C shows site specific median values for pre- and post- event periods]

	Pre-GKM release Median (Range of Median Values Across Sites)	Post-GKM release Median (Range of Median Values Across Sites)	z-value	*p*-value
% EPT	69.1 (30.5 -96.1)	68.0 (36.8 – 91.3)	-2.5854	0.0821
Total Taxa	15.3 (8 – 29)	18.0 (9 – 25.4)	-2.3049	0.01779*
NRSA MMI	45.8 (23.2 – 67.7)	47.3 (32.6 – 66.1)	-1.0859	0.2979
Colorado MMI	47.5 (16.8 – 85.4)	48.6 (17.9 – 70.8)	-0.7756	0.4637

Previous research within Colorado has identified a number of taxa groups that are sensitive to metal pollution (Clements 1994, Clements *et al.* 2000). Due to the relative low number of these sensitive taxa throughout the watershed, no statistical analysis was conducted to assess changes in relative abundance between pre- and post-GKM release time periods. Of these sensitive taxa, *Baetis spp.* showed the greatest distribution throughout the watershed (Figures 5.6-5.9). *Baetis spp.* showed similar relative abundance between the pre- and post-GKM release time periods, with the middle Animas showing the greatest relative abundances regardless of time periods (Figure 5.7). Clements *et al.* (2000) did find differences in sensitivity of specific species of *Baetis*, and since taxa were not consistently identified to species in the samples used in this study, those differences may have limited our ability to observe any differences between the two time periods. Three other metal pollution sensitive taxa groups, Heptageniidae, Ephemerellidae, and Taeniopterygidae had substantially lower relative abundance throughout the watershed compared to *Baetis spp*. regardless of pre- or post-GKM release (Appendix C). In fact, Taeniopterygidae was limited to only the upper Animas, and was found in very low abundance. Some of these taxa are more prevalent during the winter/spring season due to life history and the seasonal abundance of more developed instars. Thus, they may be underrepresented during the summer/fall collections.

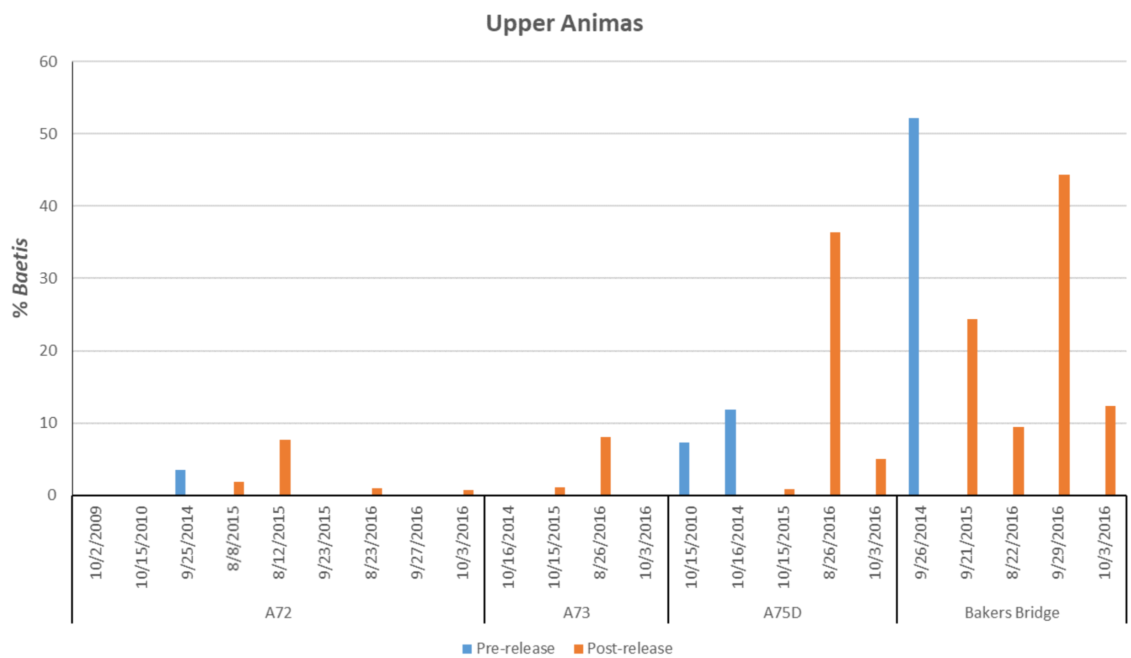

Figure 5.6. Relative abundance of *Baetis spp.* within the upper Animas from pre- and post-release sampling events. The lack of a bar for a given sampling date indicates that no organisms were sampled.

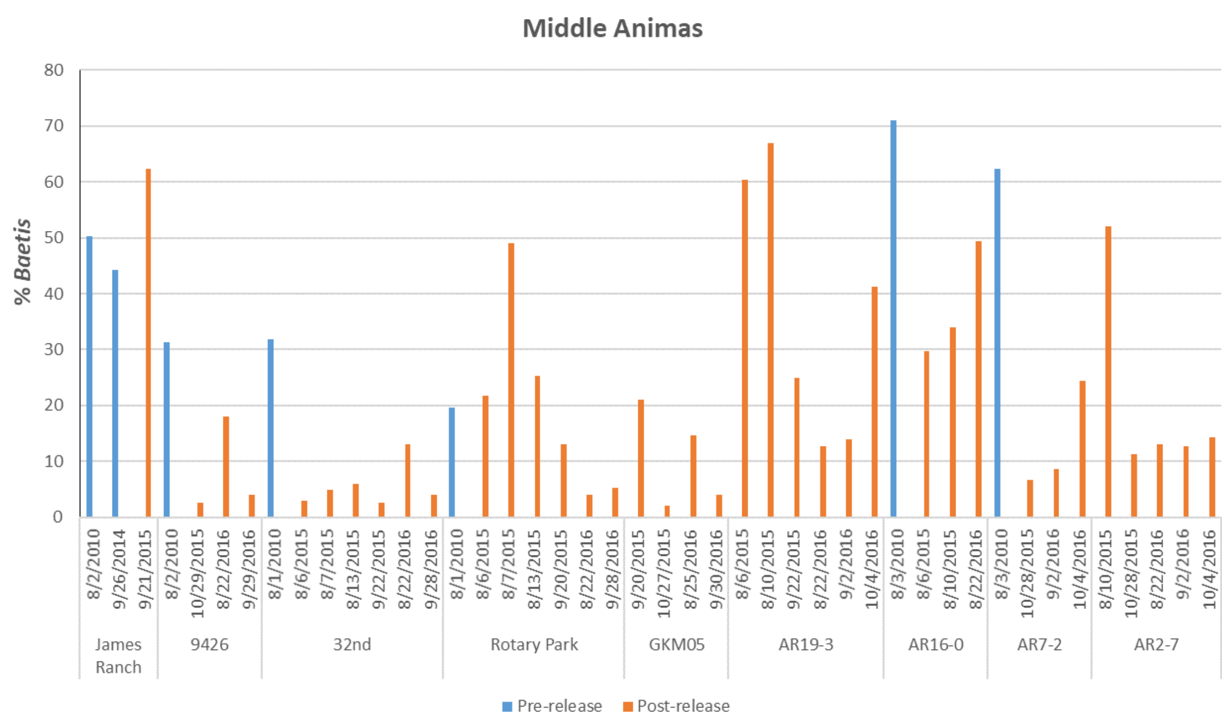

Figure 5.7. Relative abundance of *Baetis spp.* within the Middle Animas from pre- and post-release sampling events.

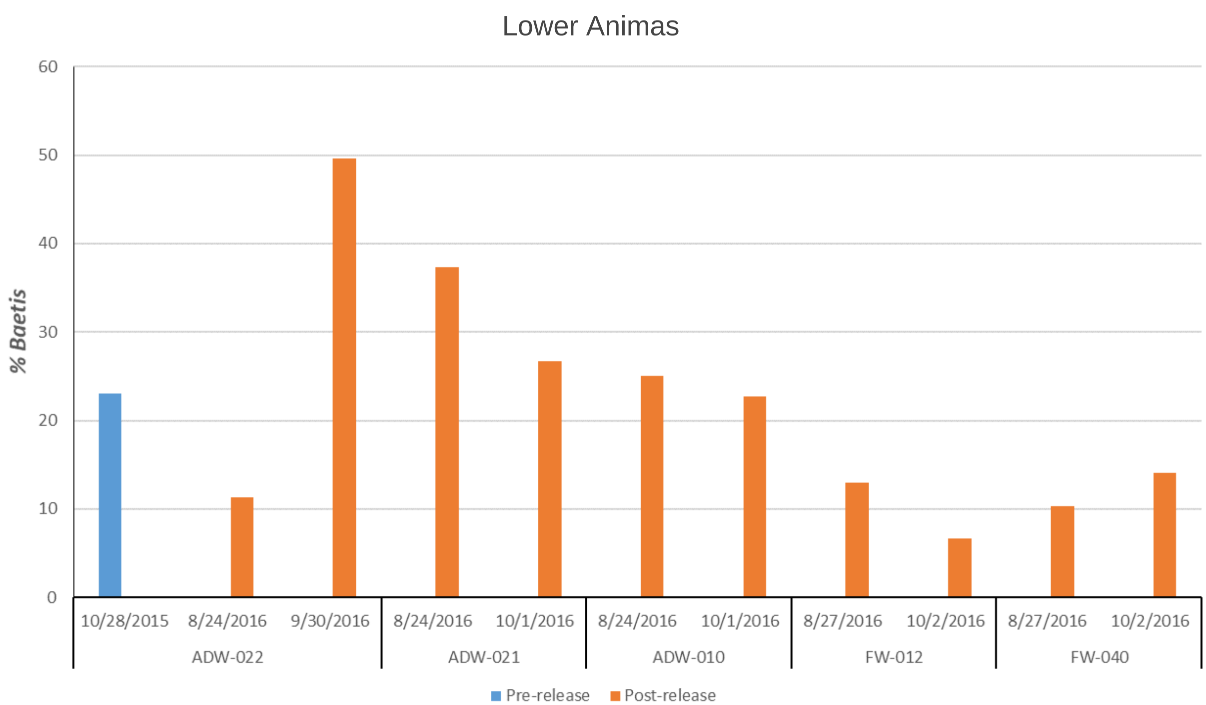

Figure 5.8. Relative abundance of *Baetis spp.* within the Lower Animas from pre- and post-release sampling events.

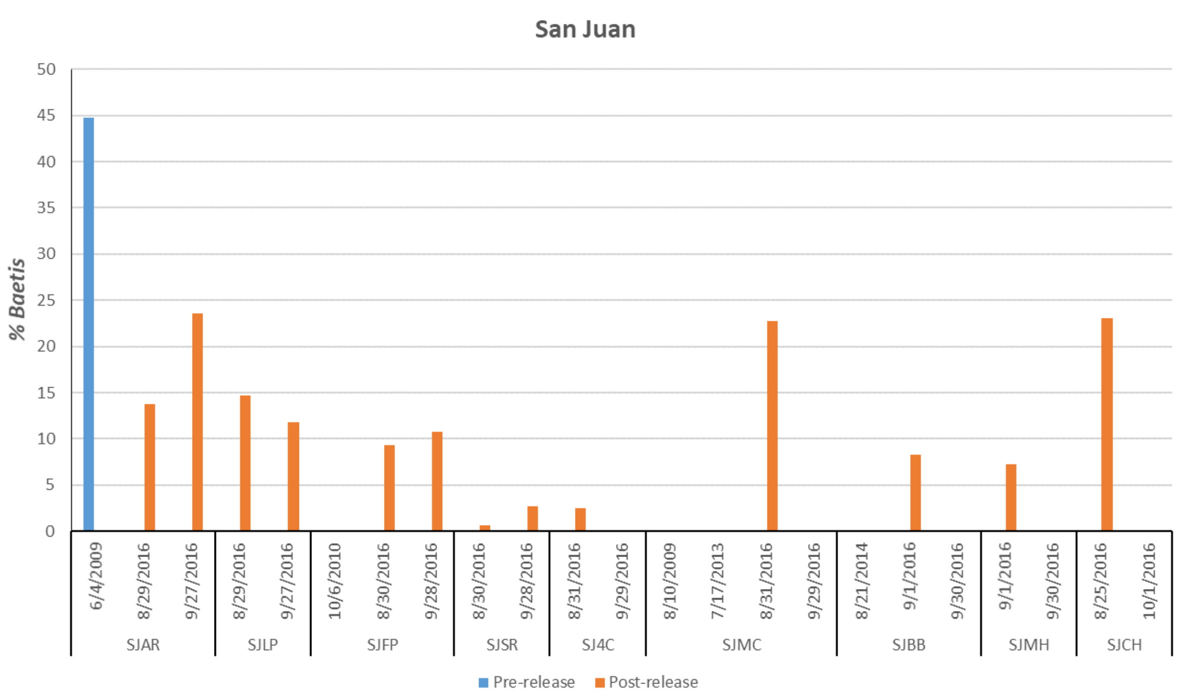

Figure 5.9. Relative abundance of *Baetis spp.* within the San Juan River from pre- and post-release sampling events. The lack of a bar on a given sampling date indicates no organisms were sampled.

5.4 Summary of benthic macroinvertebrate assemblage data

Macroinvertebrate population indices show that aquatic communities have experienced substantial degradation in the headwaters of the Animas River due to historical mining activities in the upper Animas watershed. Water and sediment quality conditions improve with distance from the headwaters as cleaner incoming tributary flows moderate impacts. Previous studies have documented historic and ongoing mining impacts on water and sediment quality and aquatic communities in this area (USGS 2007; EPA 2015; EPA 2016c). Mining impacts are most prevalent and persistent in the upper Animas River segment from Silverton to Durango, CO. The aquatic communities in the lower Animas and San Juan rivers, when applying the Colorado and NRSA MMIs, generally show attainment of the aquatic life use and fair-good conditions, respectively.

Habitat differences as well as water quality appear to influence individual community metrics such as the Percent of Ephemeroptera, Plecoptera, and Trichoptera taxa (%EPT) and total taxa. The 550-kilometers of the Animas and San Juan rivers affected by the GKM release have diverse habitats that affect the distribution and abundance of macroinvertebrate and fish species along their length. Additionally, great variability was observed among samples collected at the same local. This variability likely reflects the natural fluctuation inherent in riverine benthic communities but also likely reflects differing sampling methodologies.

This analysis focused on samples collected during an index period of June through October. No significant changes to the summer/fall benthic macroinvertebrate assemblages due to the GKM release were observed when comparing post-release and pre-release samples in the Animas or San Juan Rivers. These results are consistent with analyses presented in MSI (2016, 2017). Potential changes in spring benthic macroinvertebrate communities are also of interest since some studies have reported that effects of metals are often greatest in spring during periods of elevated flow and increased metal concentrations (Clements 1994). MSI (2017) presented the analysis of the limited spring benthic macroinvertebrates data collected from two middle Animas River sampling locations (James Ranch and 32nd Street Bridge) and concluded that the post-GKM release community was consistent with historic conditions.

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CHAPTER 6 FISH POPULATIONS

Fish population characteristics in the post-GKM release data (2015 and 2016) were compared to long term trends in fish population surveys conducted in the Animas and San Juan rivers to determine how different fish species and age classes responded to the GKM release. More specifically, were acute impacts to the fish populations observed during the initial GKM release when metals concentrations were highest? Were long-term changes in fish populations observed a year after the GKM release? Chapter 6 presents spatial and temporal trends observed in large (adult) and small (small adults and fry) fish surveys. Our analyses showed there were no significant impacts on adult fish population abundance in the Animas River after the GKM release, potential impact to juvenile bluehead suckers near Durango, and inconclusive results in the San Juan River.

6.1 Fish and wildlife studies in the Animas River

The most robust fish population and wildlife response data for the Animas River were collected by the Colorado Parks and Wildlife (CPW), which included a sentinel fish toxicity study, intensified fish population surveys, wildlife mortality survey, and partnering with CDPHE to measure metals in fish tissue. Large fish surveys were conducted in the Animas River (Figure 6.1) near Durango (Reaches 1 and 2) and four locations in the upper Animas: Teft Spur, Elk Park, A72 (Silverton, CO) and upstream of the Cement Creek confluence near Howardsville, CO. The Howardsville site is a “control” site not affected by the GKM release. Small fish/fry surveys were conducted at seven locations on the Animas River. Two dead beavers were collected and submitted to Colorado State University for necropsy. The results of the laboratory analyses did not suggest that the beavers died of exposure to toxic concentrations of minerals or metals. The cause of death was inconclusive. Results of the sentinel fish study and fish surveys follow. The metals in fish tissue results are presented in Chapter 8.

Table 6.1 Summary of the GKM response sampling and data collected by Colorado Parks and Wildlife.

Study	Dates	Description
Sentinel fish/water quality data	8/6-8/10/2015	1.5-inch rainbow trout fry were placed in cages at three sites in the Animas River and one control site for four-days. Water-quality samples were periodically collected at cage sites to measure metals exposure.
Fish surveys	8/24-8/27/2015	Large fish surveys near Durango (Reaches 1 & 2)
	September 2015	Small fish surveys (7 Animas River sites)
	9/8-9/10/2015	Large fish surveys (Howardsville, A72, Elk Park, Teft Spur)
	3/18/2016	Large fish surveys near Durango (Reaches 1 & 2)
	7/19-7/20/2016	Small fish surveys (7 Animas River sites)
	9/14-9/16/2016	Large fish surveys near Durango (Reaches 1 & 2)
Survey for fish and wildlife mortality	8/16/2015	CPW staff rafted sections of the Animas to survey for fish kill or other impacts. Two dead beavers were collected and submitted for necropsy. Six dead fish were collected.
Metals in fish tissue	8/14/2015	Five brown trout and five rainbow trout were collected from the Animas near Durango. Filets were submitted to CPDHE for testing.
	3/18/2016	

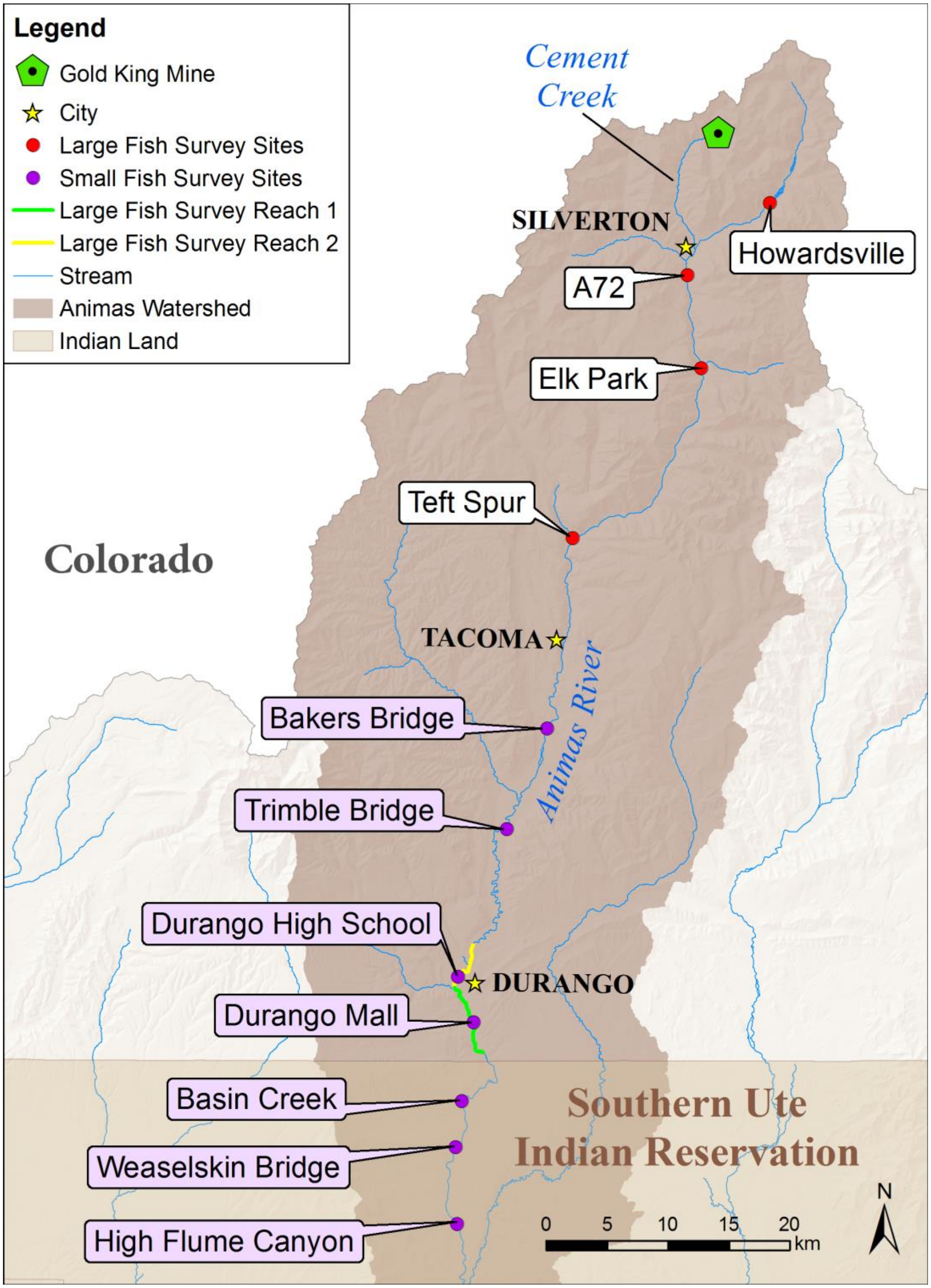


Figure 6.1. Map depicting CPW large and small fish survey locations on the Animas River.

6.1.1 Sentinel fish study

CPW placed three cages that each contained 12 trout fry (1.5 inches) at three locations in the Animas River (32nd St. Bridge, the Hatchery, and High Bridge) and five cages at a control site (Junction Creek) as the plume moved through Durango, CO from 8/6-8/11/15. The 96-hr exposure to the GKM plume would be considered similar to the duration of acute toxicity tests typically used in the derivation of water quality criteria. Cages were checked three times a day for the condition of the fish (e.g., responsiveness) and mortality. Water samples were collected in the morning and afternoon and more frequently at hatchery site during plume's passing and analyzed for a suite of total/dissolved metal concentrations. Although metal concentrations rose and pH fell at the Animas River locations as the plume passed (e.g., from almost 8 to 7.3 at the hatchery), only limited fish mortality was observed. Of the 108 fish that were deployed in the GKM plume, 2 mortalities were observed at the most downstream location. Death of the fry appeared to be related to handling of the fish rather than the GKM release (Appendix D). In addition, no widespread fish kills were reported on the Animas River during or after the GKM release. The low trout fry mortality is consistent with the water quality criteria exceedance analyses that indicated the peak metal concentrations observed in the Durango area were not acutely toxic to fish (see Section 2.3.3; Figure 2.12).

6.1.2 Fish population data

Pre- and post-GKM fish population data were collected by the Colorado Parks and Wildlife (CPW) agency using electrofishing in the fall at various locations on the Animas River (Figure 6.1), as described in Section 6.1. For Reaches 1 and 2 near Durango, the original dataset included samples taken as early as August 1912. The pre-release conditions included on surveys done every few years during the period 2002-2016. For the other four large fish survey sites, we examined data from sampling in 1992, 1998, 2005, 2010, 2014, and 2015 (after the GKM release). These data represent either the total count of fish caught during sampling events (Reaches 1 and 2), or the density of fish (fish/mile). For the former count data, the level of effort (total stream length sampled) was consistent from year to year, allowing for annual comparisons to be made. For Reaches 1 and 2, we focused our examination on native species and the recreationally-important salmonids: bluehead sucker, flannelmouth sucker, mottled sculpin, brown trout and rainbow trout. We plotted count data (summed across both reaches) through time to assess whether post-GKM release samples collected in 2015 and 2016 indicate a departure from pre-GKM release conditions. Brook trout were the only targeted species at the four other survey sites. Data indicated virtually no fish caught at A72 or Elk Park since 2005, so only data from Teft Spur and Howardsville were examined.

We also looked at data from CPW small fish electrofishing surveys at seven different locations on the Animas River (Figure 6.1). These were multi-pass depletion surveys along 100 meter sections of shoreline in shallow water where trout fry would normally be found. The most upstream location is near Baker's Bridge, and the most downstream location is about ten miles from the Colorado/New Mexico border on the Animas (High Flume Canyon). These surveys have been done periodically since 1996, and are typically done in July. An extra collection effort was made in September 2015, about a month after the GKM release.

6.1.3 Temporal patterns of fish populations in the Animas River

Sampling at Teft Spur indicates that brook trout populations dropped between 2005 and 2010 (Figure 6.2). This may be related to the cessation of remediation activities of the Gladstone treatment plant on Cement Creek in 2004 (CPW 2015, MSI 2017). The number of brook trout caught at Teft Spur in 2015 (post-GKM release) was slightly greater than in 2014 and 2010. At Howardsville, brook trout density was slightly greater in 2015 compared to 2014, but about half the density seen in 2005 and 2010.

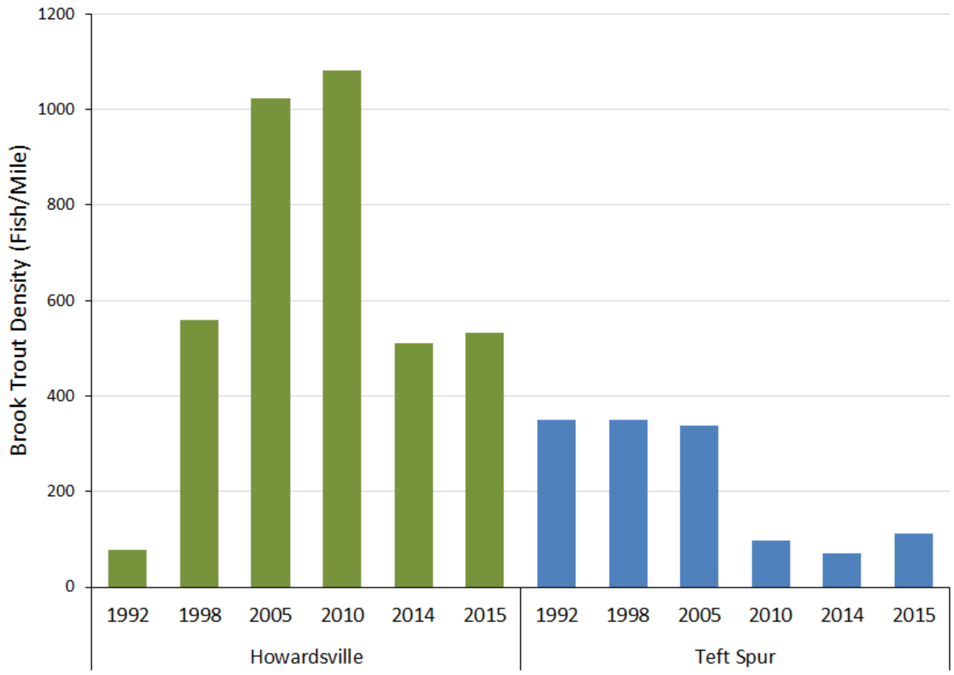


Figure 6.2. Density of brook trout caught near Howardsville, CO and Teft Spur in surveys conducted by the Colorado Department of Parks and Wildlife.

In Figure 6.3 (and later in Figures 6.8 and 6.9), dashed blue boxes denote pre-release "norms" of species abundance. The upper and lower bounds of these rectangles depend on the number of pre-release samples. With less than 10 samples, the largest and smallest pre-release values define the upper and lower rectangle boundaries. For 10-20 samples, the highest and lowest pre-release values were discarded and the 2nd highest and 2nd lowest values define the bounds. Using this methodology, we then determined if the number of individuals caught during 2015 and 2016 (shown on the plots using red triangles) were outside the range defined by pre-release data.

Fish counts were generally within the pre-release ranges, and no counts were low except for rainbow trout in 2016 (Figure 6.3), which was slightly less than the pre-release low in 2010 (93 versus 98). However, the 2016 count is well within two standard deviations of the average number caught between 2002-2014, so statistical significance was minimal. Also, effects of the GKM release on trout populations in the Durango area were confounded with the annual autumn stocking of rainbow and brown trout. Rainbow trout stocking in the fall of 2016 was low due to a shortage of hatchery fish (Appendix D). In 2015, nearly all trout stocking was completed prior to the GKM-release, so the 2015 survey results for trout reflected typical stocking rates. Overall, adult fish within these populations appear not to have been affected by the GKM release.

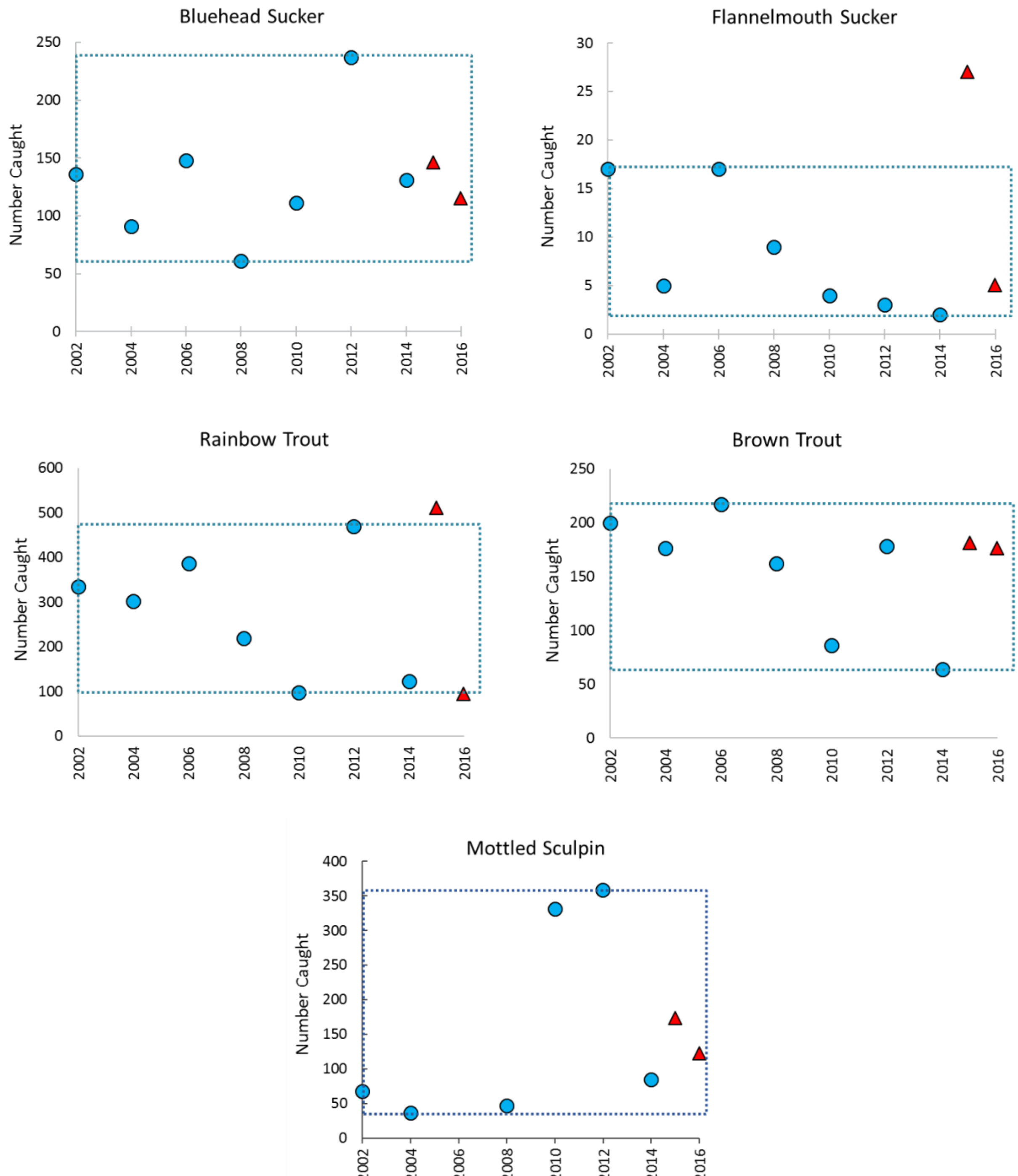
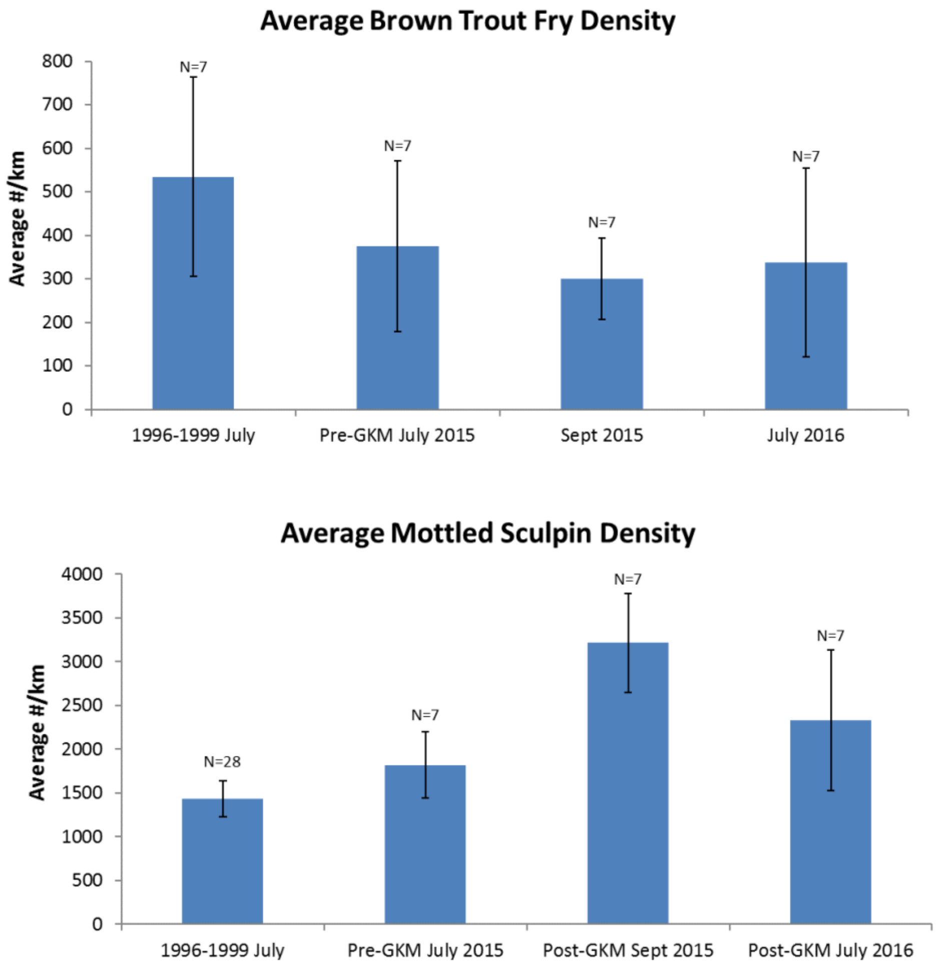


Figure 6.3. The number of common native fishes and important salmonids caught from the Animas River near Durango, CO (Reach 1 & 2 in Figure 6.1) in surveys conducted by the Colorado Department of Parks and Wildlife. Blue dots are pre-GKM release samples. Red triangles are post-GKM release samples. The blue dashed rectangles in each figure denotes pre-GKM release survey ranges.

Comparisons of the pre- and post-release small fish survey results provided insight into potential impacts to metal sensitive taxa (i.e., mottled sculpin) and life stages. Figure 6.4 shows the brown trout fry and mottled sculpin density (#/km) before and after the GKM release. The survey in September 2015, weeks after the GKM release, captured 20% less trout fry than in July 2015, and 11% less than July 2016. This decrease in trout fry density could be related to the GKM release, but is more likely explained by the seasonal movements of fry out of shoreline areas and into deeper pools in September and the lower flow in September (CPW 2015), given the lack of mortality in the sentinel fish study. Figure 6.4 also shows that the density of mottled sculpin seen in the small fish surveys does not indicate impact from the GKM release. The shallow, graveled, shoreline habitat sampled in the small fish surveys is considered a more accurate representation of mottled sculpin densities than the large fish surveys, which target larger fish in deeper water (Personal communication, Jim White, CPW Aquatic Biologist).



6.2

Figure 6.4. Top: the average density (#/km) of brown trout fry caught at seven Animas River sampling areas by CPW in the late 90's compared to surveys in 2015 and 2016. Error bars are +/- one standard error of the mean. Bottom: the density of mottled sculpin caught in those same shoreline small fish surveys.

Relative abundance of bluehead sucker in the Animas River near Durango, plotted by total fish length and averaged from 2002-2014, 2015 (post-GKM release) and 2016 is shown in Figure 6.5. The relative abundance of adult fish in 2015 was similar to the pre-GKM release conditions, but an absence of fish less than 200 mm in the 2016 data was apparent.

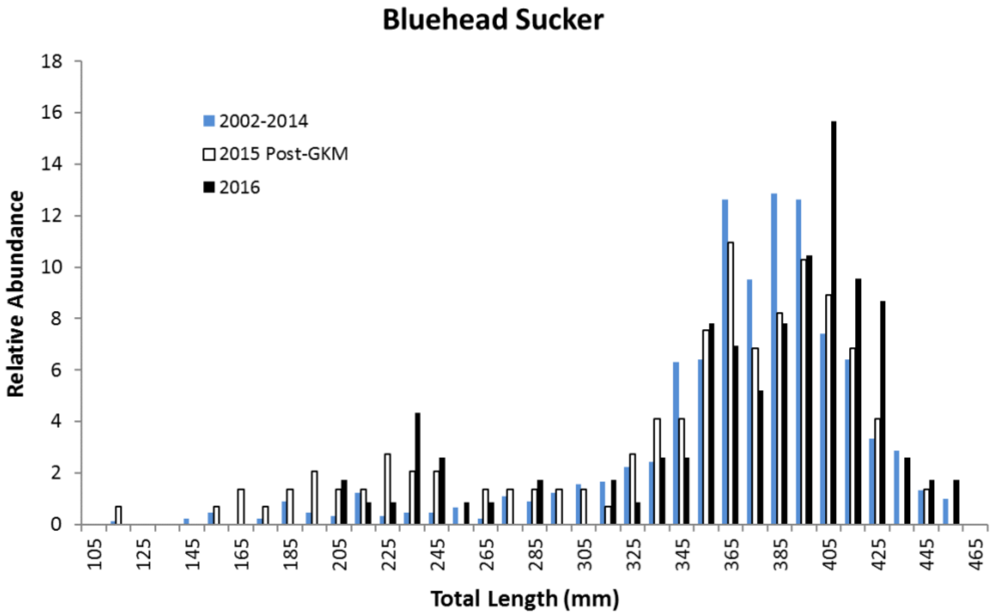


Figure 6.5. Relative abundance of bluehead sucker in the Animas River near Durango (Reach 1 and 2 in Figure 6.1) for pre-release (2002-2014) compared to the fall of 2015 and 2016.

6.2.1 Summary of CPW survey data

No widespread fish kills were reported on the Animas River during or after the GKM release. The CPW sentinel fish cage study confirmed that the GKM release was not acutely toxic to fish. Additionally, we did not find any evidence of declines in the number of adults sampled from the Animas River in 2015 and 2016 for any of the species we reviewed (Figure 6.2 and 6.3).

The lack of evidence of a negative response on adult populations the year following the GKM release does not address potential longer-term impacts. The GKM release may have been more stressful on larval and juvenile fish (Figures 6.4 and 6.5); however, CPW saw evidence of reproduction by native species (mottled sculpin, bluehead sucker) in the months following the release, suggesting that the GKM release was not acutely toxic to younger life stages (CPW 2015). Our review of the bluehead sucker data, which includes an additional year of monitoring (2016), shows a lack of juvenile bluehead sucker (< 200 mm)(Figure 6.5) while population abundance has remained within the range of pre-GKM release conditions (Figure 6.3). For trout species, impacts on younger life stages were challenging to identify since population levels are primarily determined by stocking and harvest rates established by the state of Colorado (Appendix D) and not the recruitment of larvae and juveniles.

6.3 Fish abundance in the San Juan River

A long running dataset on fish abundance collected by the US Fish and Wildlife Service on the San Juan River from Blanco, NM to the Clay Hills boat ramp in Utah (Figure 6.6) was analyzed to compare the post-GKM release samples in 2015 and 2016 to data from 2000-2014. Fish were captured using raft electrofishing, and the duration of the sampling period (in seconds) was used to standardize the number of fish caught into a catch-per-unit-effort (CPUE) metric. The USFWS performs these surveys in September and October each year so between-year comparisons are seasonally comparable. Typical sampling duration was 20-30 minutes. We focused our analyses on four native species frequently seen across time and sites: flannelmouth sucker (FMS), bluehead sucker (BHS), razorback sucker (RZS) and speckled dace (SPD). The theoretical foundation for changes in fish abundance would be that the increased levels of dissolved metals (primarily aluminum and iron) seen in the San Juan River in the fall of 2015 through the spring snowmelt period of 2016 could be an irritant, causing fish to emigrate from these areas. There is a weir and low-head dam in the few miles downstream of Fruitland on the San Juan (SJFP), and these features could serve to inhibit the movement of fish from this area to the area upstream (LVW-020).

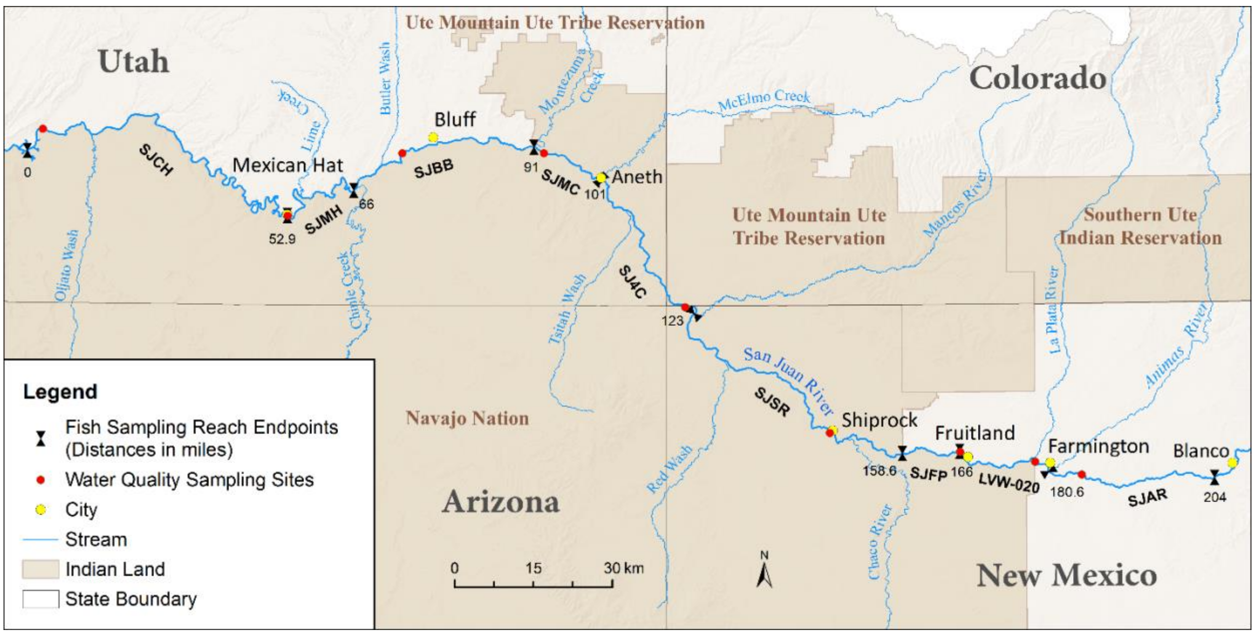


Figure 6.6. Nine segments of the San Juan River that have been historically sampled to assess fish abundance by the USFWS. Mile markers are given below each reach endpoint. Only one region, SJAR, is upstream of the confluence of the San Juan and Animas River.

6.3.1 Longitudinal patterns of fish populations in the San Juan River

Three species (BHS, FMS, SPD) showed the same longitudinal pattern of abundance in the 2000-2016 population sampling. Figure 6.7A shows the average catch per hour of sampling effort (CPUE) of FMS. As with the other two species, FMS are most abundant at sites immediately downstream of the confluence with the Animas River (LWV-020 and SJFP) and decline longitudinally along the length of the San Juan. However, these species are still present in low numbers as far downstream as Clay Hills more than 300 km from Farmington. These species are also found in much lower abundance upstream of the confluence with the Animas River (SJAR). These species are likely responding to temperature gradients within the San Juan, with the sites closer to the Animas River confluence being warmer at the time of sampling than either sites upstream or farther downstream of the confluence. Upstream of the Animas confluence (~55km), the San Juan receives the cool hypolimnetic waters discharged from Navajo Lake.

Razorback sucker is listed as federally endangered, and a formal management plan exists for assisting its recovery to historic levels. The plan (fully implemented in 2009) includes a decades-long stocking program that introduces fish at a variety of sites in the middle to lower San Juan. The average CPUE for razorback sucker are shown in Figure 6.7B peaks at Fruitland, suggesting that the weir and dam downstream of Fruitland may limit upstream migration of razorback sucker to the LVW-020 segment in Farmington.

Channel catfish, a competitor of the other species, is found in increasing abundance moving downstream from Fruitland to Bluff, UT, and then declines moving farther down to Mexican Hat and Clay Hills (Figure 6.7C). The Colorado pikeminnow (not shown) is observed occasionally at low abundance at various locations along the length of the San Juan River.

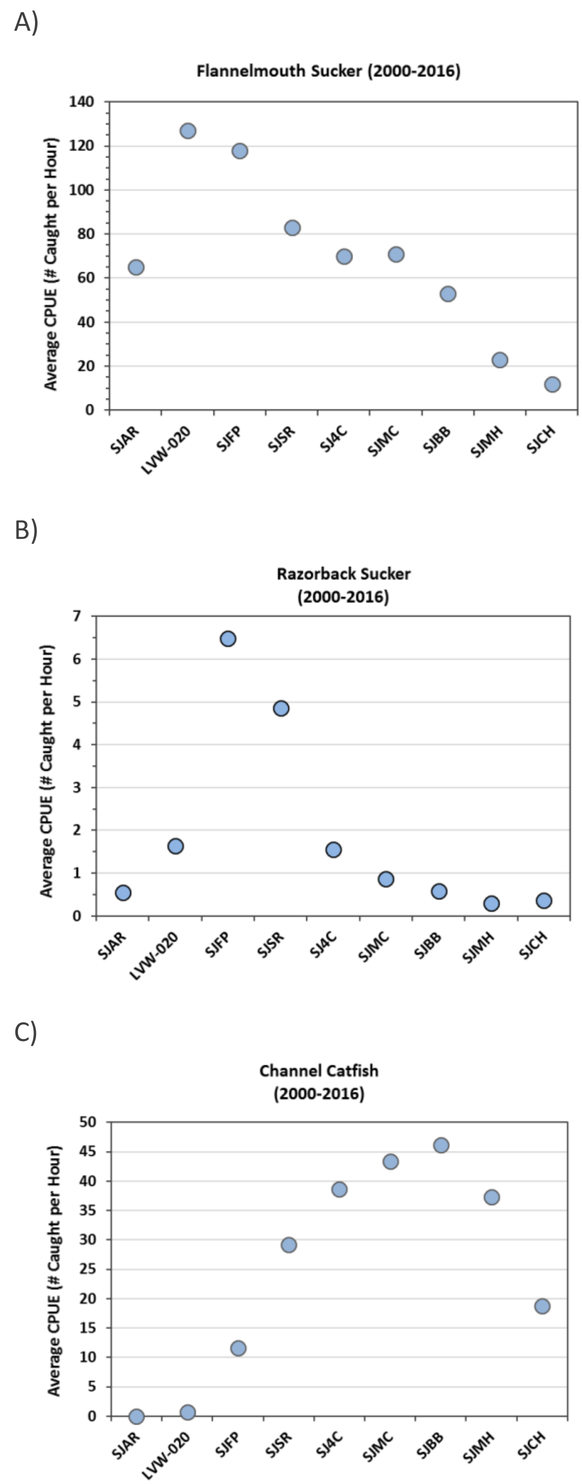


Figure 6.7. The average CPU for A) flannelmouth sucker, B) razorback sucker, and C) channel catfish at sampled reaches of the San Juan River averaged from 2000-2016.

6.3.2 Temporal patterns of fish populations in the San Juan River 2000-2016

BHS, FMS, and SPD were commonly observed throughout most of the San Juan River but varied in abundance longitudinally along the river (Figure 6.7). The temporal patterns of the average catch per unit effort (CPUE) of these three species is shown in Figure 6.8, for three groupings of sites with similar species abundance. Figures 6.8A, B, and C (top row) include sites located in the upper San Juan where these three species were found at highest abundance (LVW-020 and SJFP). Data for sites where these species were observed at intermediate abundance in the middle reaches of the San Juan River (SJSR, SJ4C, SJMC and SJBB) are shown in Figure 6.8D, E, and F (middle row). Data at low abundance sites in the lower San Juan River (SJMH and SJCH) are shown in Figure 6.8G, H, and I (bottom row). The reference site (SJAR), located upstream of the GKM influence, is not included. As in Figure 6.3, the dashed blue rectangles define “norms” based on pre-release data. Due to a larger historic sample size, the largest and smallest pre-release observations were excluded from defining the pre-release range (see section 6.1.2 for further explanation).

The temporal trends in population estimates for RZS are shown grouped by high abundance (SJFP and SJSR) in Figure 6.9A and low abundance sites (SJ4C, SJMC, SJBB, SJMH and SJCH) in Figure 6.9B. The blue rectangle denotes norms for pre-release data (2000-2014) to give context to the CPUE measures seen in 2015 and 2016. RZS populations were not examined at LVW-020 or at SJAR, which is upstream of the Animas River. Temporal trends in abundance of channel catfish and Colorado pikeminnow are shown in Figure 6.9C and D.

The average CPUE in surveys at San Juan River sites in 2015/2016 (Figures 6.8 and 6.9) were generally within pre-release norms. However, abundance of BHS and FMS were low, especially at intermediate abundance sites (Figure 6.8D and E). Longer terms trends of CPUE reductions in BHS, FMS, and SPD are apparent in a number of these plots. Low 2015/2016 CPUE for these species is concurrent with high abundance for the species shown in Figure 6.9. RZS CPUE in 2015/2016 was at or near pre-GKM release highs in the San Juan (Figure 6.9A and B), channel catfish CPUE in 2015/2016 was above the pre-GKM release median of 30 (Figure 6.9C), and Colorado pikeminnow CPUE in 2015/2016 was also well above the pre-GKM release median of 0.07 (Figure 6.9D). Ecological interactions (these species are either competitors with or predators on BHS/FMS/SPD) thus play a confounding role. As stated earlier, if changes in water quality and sediments were more stressful/impactful on age-0 and juveniles of these species, reductions in adult populations may not be evident for several more years. Both RZS and Colorado pikeminnow are currently under conservation management plans, and appreciable numbers of both species are annually stocked into the San Juan River.

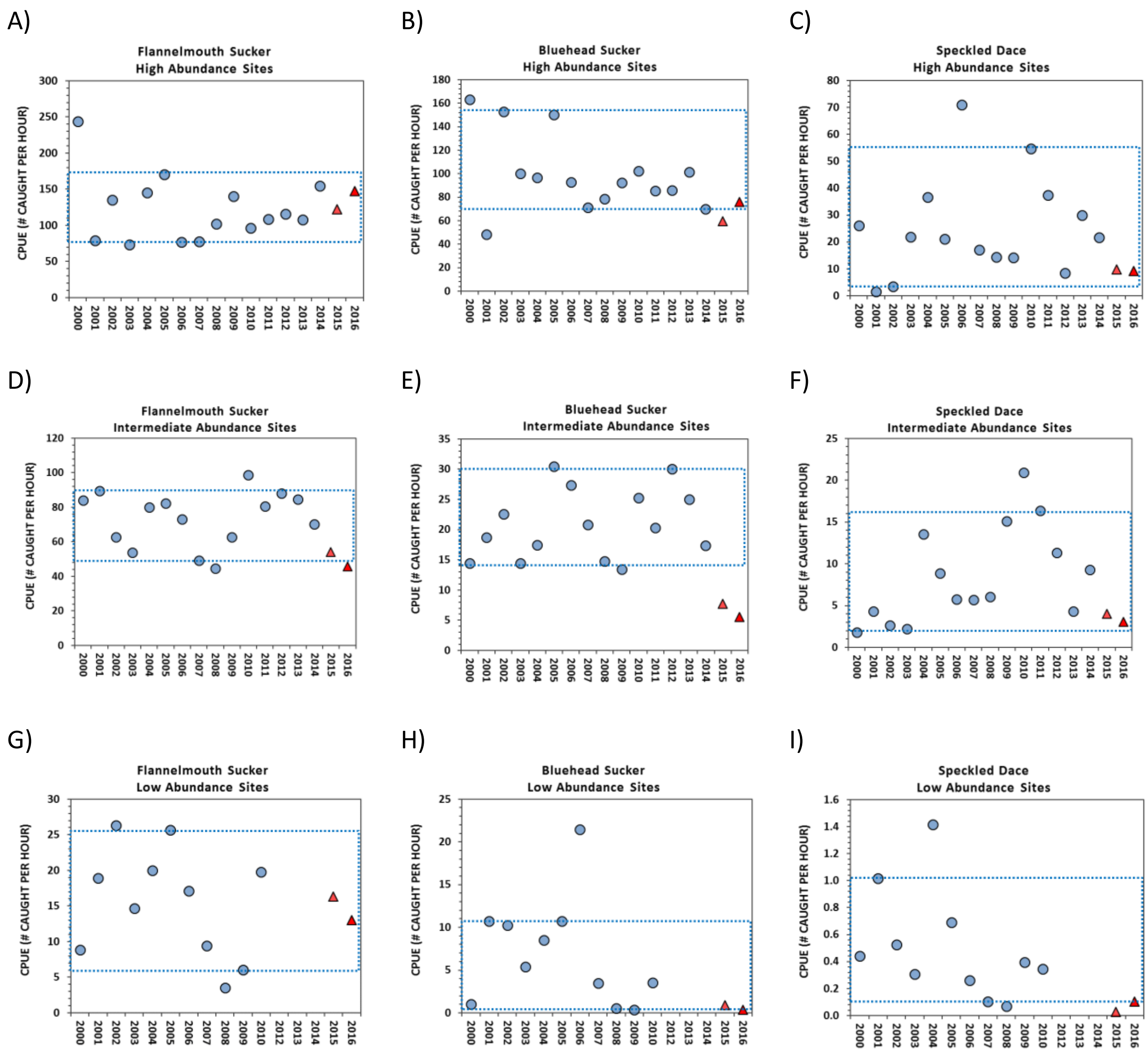


Figure 6.8. Average CPUE for flannelmouth sucker, bluehead sucker, and speckled dace at A-C) high abundance sites (LVW-020 and SJFP), D-F) intermediate abundance sites (SJSR, SJ4C, SJMC, and SJBB), and G-I) low abundance sites (SJMH and SJCH) during the years 2000-2016. Values are the average CPUE across sites in the abundance category. Red triangles highlight samples from 2015 and 2016. Dashed blue boxes indicate pre-GKM release ranges of CPUE estimates.

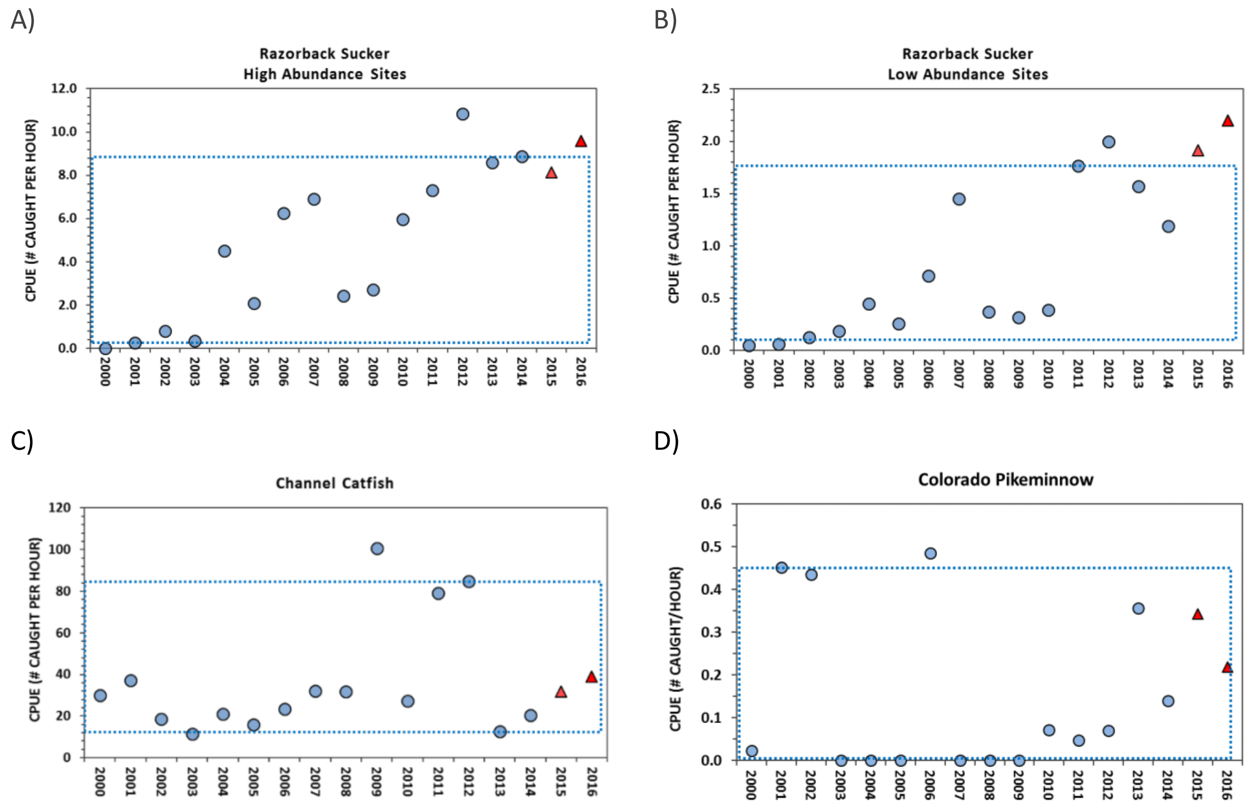


Figure 6.9. Average CPUE for 2000-2016 for A) razorback suckers at high abundance sites (SJFP and SJSR), B) razorback sucker at low abundance sites (SJ4C, SJMC, SJBB, SJMH, and SJCH), C) channel catfish at all sites, and D) Colorado pikeminnow at all sites. Values are the average CPUE at sites in the abundance category. Red triangles highlight samples in 2015 and 2016 after the GKM release. Dashed blue boxes indicate pre-GKM release ranges of CPUE estimates.

CHAPTER 7 METALS IN BENTHIC MACROINVERTEBRATE TISSUE

Bioaccumulation of metals in benthic macroinvertebrate tissue post-GKM release was assessed with samples collected by the EPA and the NMDGF. In Chapter 7, we explore the spatial and annual variability in benthic tissue metal concentrations and differences in benthic tissue concentrations among taxonomic groups as they relate to the passing of the GKM plume, the presence of GKM metal deposits, and pre-GKM release tissue concentrations. Analyses that could conducted with these data were limited by the availability of pre-release data, collection dates, and differences in sampling methodology, analytical methods, and laboratories between years. For example, EPA benthic macroinvertebrate tissue samples were a composite of all taxonomic groups and were collected with the fall assemblage sample. NMDGF samples were sorted and analyzed by taxonomic group and represent two seasons (fall and spring). Statistical analyses were limited to locations that were sampled with the same methods. Additionally, all samples were frozen following collection and likely included substantial quantities of organic detritus and/or mineral sediment in their gut contents. These samples are most relevant to estimating metal exposure to higher-trophic level organisms for ecological risk assessments since invertebrates are ingested whole. The inclusion of gut content and in some situations caddisfly casings, in the tissue sample made it difficult to quantify differences in benthic macroinvertebrate exposure to bioavailable metals. Additional information on the ecological risk associated with metals in tissue sampled from the Animas River is available in the *Upper Animas Mining District: Draft Baseline Ecological Risk Assessment* (EPA 2015).

Our analyses showed benthic tissue samples collected from the upper Animas after the GKM release in September 2015 had significantly greater copper and lower manganese concentrations when compared to pre-release concentrations. All other changes in metal concentrations from 2014 were not statistically significant and were within the range of potential sample variability. In the lower Animas and upper San Juan rivers, some metals were elevated at the lower Animas sites in August when compared to the San Juan and samples collected the following spring.

7.1 EPA benthic macroinvertebrate tissue data

EPA contractors collected benthic macroinvertebrate tissue samples post-GKM release in 2015 and the following fall in 2016. In 2015, samples were collected from 9 sites in the upper and middle Animas River, including the upstream location A68 down to AR19-3 (104 RKM). In 2016, EPA contractors sampled a total of 29 sites in the Animas and San Juan rivers, which included three upstream/tributary locations (A68, M34, and SJAR). Most metals were measured at concentrations that were detectable with the analytical method used, except for mercury, silver and vanadium (Table 7.1).

Table 7.1 Total number of benthic macroinvertebrate tissue samples (pre- and post-GKM) and percent detection by analyte.

All Data	N	% detected
Percent Solids	33	100
Aluminum	46	96
Antimony	46	70
Arsenic	50	88
Cadmium	50	98
Chromium	50	98
Copper	50	100
Iron	46	96
Lead	50	100
Manganese	46	100
Mercury	50	68
Nickel	46	87
Selenium	50	80
Silver	46	70
Tin	32	100
Vanadium	46	70
Zinc	50	100

In the upper Animas River, pre-release data were available for a limited number of sites to conduct a pre- and post-GKM release comparison of metals in benthic macroinvertebrate tissue. Four locations were sampled in 2012 (A68, M34, A72 and Baker's Bridge) and five locations were sampled in 2014 (A68, A72, A73, A75D, Baker's Bridge). All benthic macroinvertebrate data were normalized to a common unit of expression prior to analysis. The 2016 EPA samples were reported as µg of metal per gram of dry tissue, or µg/g dry weight (dw) or ppm dw. The August post response samples collected in 2015 and pre-release samples collected in 2014 from the upper Animas River were reported at µg of metal of tissue “as received”, which is equivalent to a wet weight (ww) concentration since samples were not dried prior to analysis.[5] The 2014 and 2015 samples had insufficient biomass to analyze for percent solids given the overall low numbers of invertebrates found at the site. For these samples, the dw concentrations were estimated using the percent solids measured at that location in 2016 or the mean percent solids when more than one sample was available, including the 2012 and 2016 duplicate samples. No percent solid data were available for the James Ranch and Oxbow Park sampling locations. Therefore, the dry weight concentrations for the 2015 samples collected from James Ranch and Oxbow Park were estimated using the mean percent solids of all Animas River sampling locations (Animas River mean = 23.4%, range = 15.9%-33.5%; San Juan River mean percent solid = 14%, range = 8.5%-22.7%). Results that were reported between the method reporting limit (MRL) and the method detection limit (MDL) were set to the estimated detected value. Results with concentrations less than the MDL are presented at the MDL.

Field duplicate samples were collected at two locations in the Animas River (Rotary Park and FW-020) and one location in the San Juan River (SJCH). The collection of duplicate samples at a sub-set of the sampling locations is a standard QA/QC practice to document the precision of the sampling process. Field duplicates are used to assess homogenization of the samples in the field, reproducibility of sample preparation and analysis and, heterogeneity of the matrix by calculating the relative percent difference (RPD), which was calculated as:

RPD = 100*([field duplicate]-[sample])/mean[field duplicate, sample])

Since site-replicate samples were not collected, the RPD was also used in this analysis to evaluate potential within site variability (see Chapter 7.3). Comparisons of the RPDs by site and metal showed high inter- and intra-site variability of metals in benthic macroinvertebrate tissue. The greatest intra-site variability was observed in the SJCH and FW-012 samples. Some of the variability is likely attributed to the difference in the % solids measured in FW-012 and SJCH samples (Figure 7.1) and the low tissue mass collected for analysis, which was especially true at SJCH where habitat transitions from lotic to lentic. The total mass of tissue in the SJCH sample and the field duplicate was 1.771g and 0.884g, respectively. High intra-site variability in the EPA benthic macroinvertebrate tissue data highlights the complexity of metal bioaccumulation, challenges with quantifying changes in tissue concentrations over time, and sample characteristics that limit our ability to detect statistical differences.

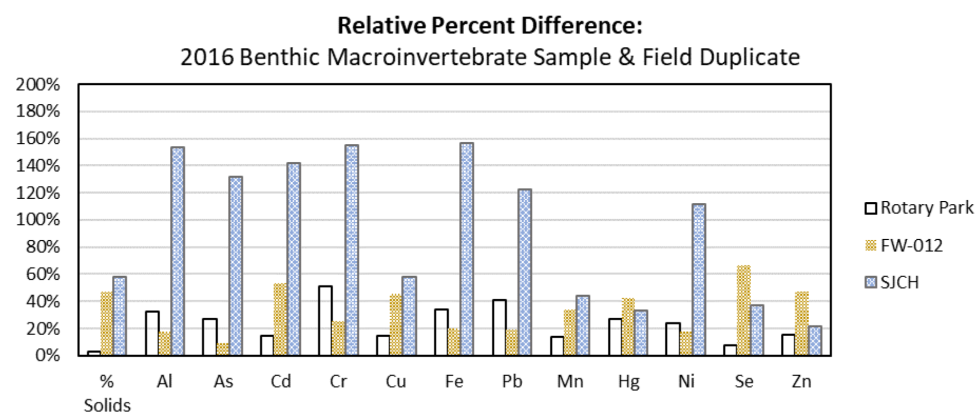


Figure 7.1 Comparison of the absolute value of the relative percent difference in benthic macroinvertebrate tissue by metal and sampling location. Relative percent difference was calculated as the difference between the sample result and the duplicate results divided by the mean of the sample and duplicate result.

[5] Expressing the result “as received” accounts for the wet weight measured in the lab. It acknowledges that there was likely some water loss from the sample resulting from freezing and transportation to the lab prior to analysis.

7.2 Pre- and post-GKM release comparisons of upper Animas benthic tissue data

Pre-release benthic macroinvertebrate tissue data were available for six sites on the upper Animas River, allowing for a pre- and post-GKM release comparison of metals in benthic tissue. All benthic tissue samples were collected between late September and early October, which minimizes potential variability due to seasonal differences in metal bioaccumulation. The 2014 data were first presented in the *Baseline Ecological Risk Assessment* (EPA 2015) and an analysis of the 2014 pre- and 2015 post-GKM release data was prepared by MSI for EPA following the GKM release (MSI 2016). Differences in sample processing and laboratory analyses between years limited our ability to quantify differences observed between the pre (2012 and 2014) and post (2015 and 2016) samples, and the statistical analyses that can be performed (see Section 3.4.3 and Table 3.4). However, similarity in the 2014 and 2015 field and laboratory methods allowed for statistical comparison of those results, which are presented below and in MSI (2016).

Table 7.2. Mean metal concentrations in benthic macroinvertebrate tissue samples collected from the upper Animas River (A68, A72, A73, A75D and Baker's Bridge). Non-detects samples were set to the MDL. * indicates when all samples were below the MDL. MDLs can change over time, when different analytical methods are used, and between laboratories.

	Mean Concentration (ppm dw)			
	Pre-GKM		Post-GKM	
	2012	**2014**	**2015**	**2016**
Aluminum	NA	874	997	9430
Arsenic	7.1	1.5	0.8	7.6
Cadmium	9.4	2.3	1.7	6.5
Copper	173.3	47	102	119
Iron	NA	2983	3217	19715
Lead	198	13	11	170
Manganese	NA	242	134	3292
Mercury	0.06	0.37*	0.13*	0.03
Nickel	NA	1.34	0.75	6.26
Selenium	1.17	1.86	1.45	1.30
Zinc	3376	504	440	1660

Figures 7.2 and 7.3 present benthic macroinvertebrate tissue concentrations sampled from the upper Animas River though the middle Animas RKM 150. Pre-GKM release data were limited to the upper Animas sites of A68, A72, A73, A75D and Baker's Bridge, and the tributary site M34. Results at RKM 0 in the figures represent the upstream sampling location on the Animas River (A68) and Mineral Creek (M34). In general, when differences in concentration are observed between years, the 2014 and 2015 results are more similar than the 2012 and 2016 (Table 7.2).

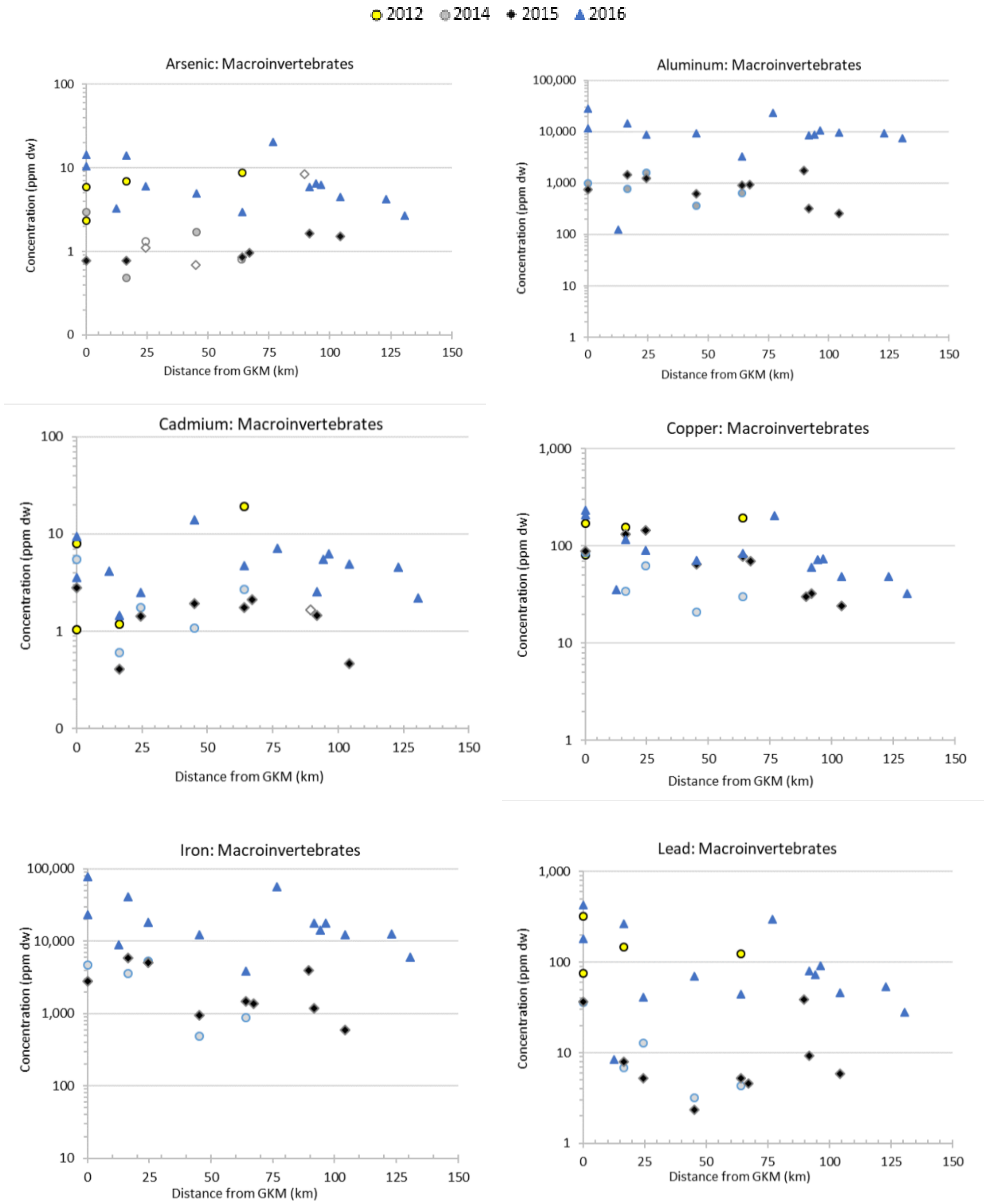


Figure 7.2. Comparison of pre (2012&2014) and post (2015 &2016) GKM release arsenic, aluminum, cadmium, copper, iron and lead concentrations in benthic macroinvertebrate tissue samples collected from the upper and middle Animas River. Pre-release data were limited to sites sampled upstream of 65 RKM. Sites located at 0 RKM represent the upstream and tributary sites, A68 and M34, respectively. Open symbols = MDL. The 2012 samples were not analyzed for aluminum and iron.

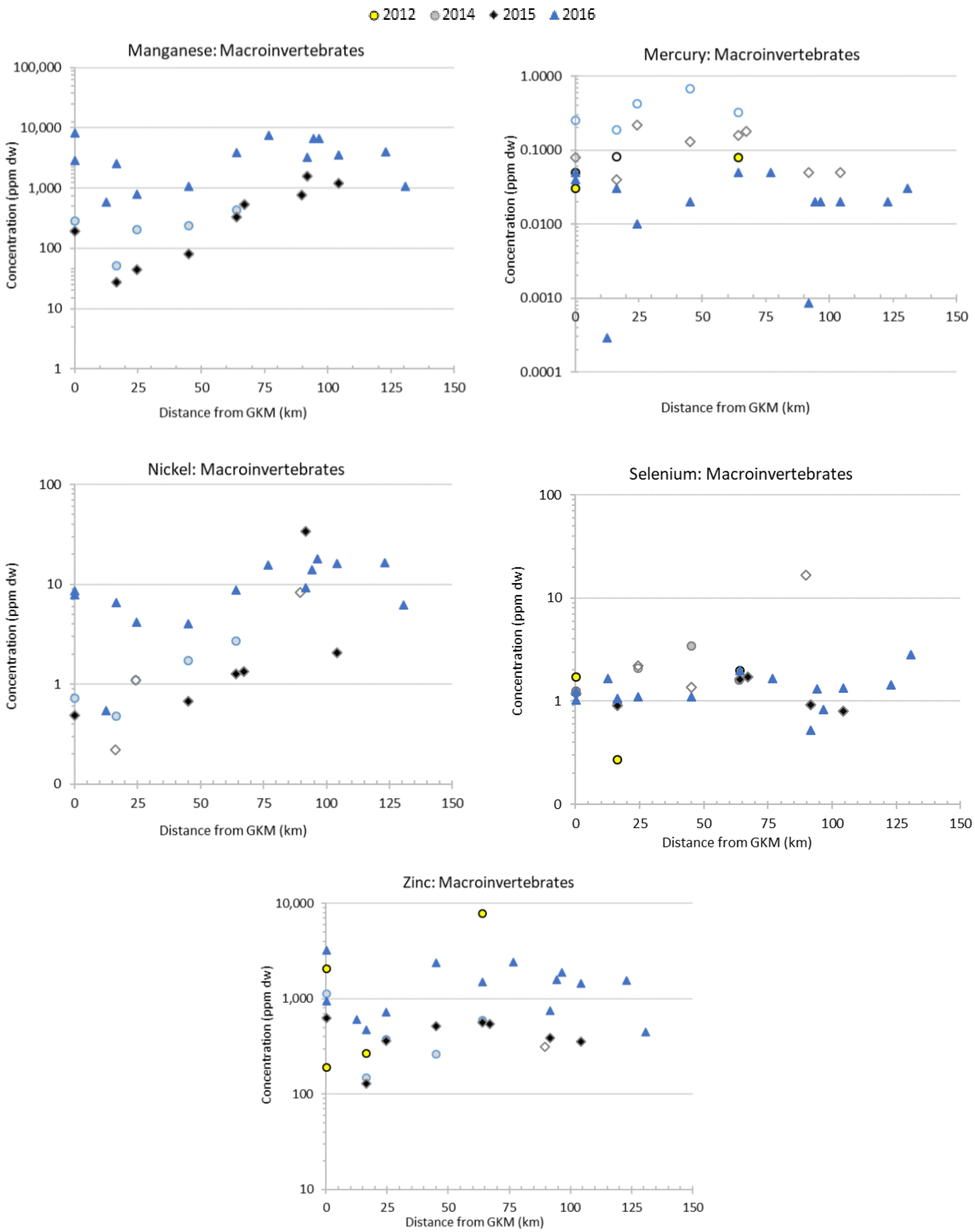


Figure 7.3 Comparison of pre (2012&2014) and post (2015 &2016) GKM release manganese, mercury, nickel, selenium, and zinc concentrations in benthic macroinvertebrate tissue samples collected from the upper and middle Animas River. Pre-release data were limited to sites sampled upstream of 65 RKM. Sites located at 0 RKM represent the upstream and tributary sites, A68 and M34, respectively. Open symbols = MDL. The 2012 samples were not analyzed for manganese and nickel.

A comparison of concentrations at sampling location immediately up and downstream of a given location showed localized elevated tissue concentrations in the upper Animas. Elevated Al, Ba, Fe, K, and Pb were measured in benthic tissue collected in 2015 from the depositional habitat at Oxbow Park (90 RKM) (MSI 2016). Oxbow Park was not sampled in 2016 to determine if the high concentrations continued the following year; however, the 3 nearest downstream locations (32nd St. Bridge;91.8 RKM, Rotary Park; 94.2 RKM, and GKM05; 96.5 RKM) generally had similarly high metal concentrations in benthic tissue. In 2016, the greatest concentrations of As, Al, Cu, Fe, Pb, Ni, and Zn in benthic tissue were observed further upstream at site 9426 (76.8 RKM). Pre-GKM release data were not available at any of these sites for a comparison to post-release concentrations.

The 2015 benthic macroinvertebrate tissue samples were collected within the closest timeframe of the GKM plume and when GKM sediment deposits were still present in the upper Animas (EPA 2016c). Five sites in the upper Animas River had 2014 and 2015 samples suitable for statistical comparisons (A68, A72, A73, A75D and Baker's Bridge). Given the limited sample size (i.e., n = 4; a single sample from each location and date) and the lack of sample replicates, percent differences between 2014 and 2015 metal concentrations were evaluated with both a non-parametric Wilcoxon signed-rank test and a two-tailed t-test. Eight metals deemed most biologically significant in the GKM release were examined: aluminum, arsenic, cadmium, copper, lead, iron, manganese, and zinc.

$$\text{Percent Difference} = 100*(C_{2015}-C_{2014})/C_{2014}$$

Where C is the natural logarithm of the metal concentration, with a value of 1.0 added to the concentration prior to logging so 2014 concentrations near zero will not cause the fraction to dramatically increase. The mean percent difference across the four sites was also compared to the mean relative percent difference calculated from the natural log+1 transformed 2016 Animas River sites from duplicate samples (see Section 7.1), as well as the change in concentration that was observed at the upstream location A68. The percent differences calculated for each metal and sampling location are presented in Figure 7.4.

The mean percent difference in benthic macroinvertebrate tissue concentrations was not significantly different for most metals, except for copper and manganese (Table 7.3). Results of the t-test indicate that copper was significantly greater ($p=0.005$) and manganese was significantly lower in 2015 ($p = 0.04$). Both copper and manganese were borderline significant when evaluating the differences with the non-parametric test ($p=0.07$). A comparison of the change in copper concentration by location showed copper increases ranging from 20-37% at the four sites downstream of GKM and a smaller, 1% increase was observed at the upstream location A68 (Figure 7.4). This trend was also observed with manganese, except concentrations were lower in 2015. The absolute value of the percent difference in copper and manganese was also greater than the within-site sample variability (last column of Table 7.3). These multiple lines of evidence suggest that the changes in copper and manganese concentrations were associated with the GKM-release. MSI (2016) also identified a statistically significant increase in copper in benthic macroinvertebrates when evaluating the non-transformed mean 2015 and 2014 concentrations (paired t-test; $p = 0.0339$).

With respect to the other metals with non-significant differences (Al, As, Cd, Fe, Pb, Zn), the four downstream locations experienced a mix of increasing and decreasing concentrations that canceled each other out, leading to the non-significant results (Figure 7.4). A different pattern of changing metal concentrations was observed at the upstream location A68. These metals remained the same or declined; however, the absolute value of the mean difference was less than or similar to the potential sample variability, suggesting the differences in concentration may be within the range of variability expected at the site.

Table 7.3. The mean difference in metal benthic macroinvertebrate concentration between the pre-release (2014) and post-GKM (2015) samples collected from the upper Animas River (A72, A73, A75D and Baker's Bridge), results of the statistical comparisons of the percent change, and potential sample variability. P-values less than 0.05 indicate a result significantly different from zero. Potential sample variability is equal to the mean relative percent difference calculated from the natural log -transformed 2016 Animas River sites with duplicate samples (see Section 7.1).

Analyte	Mean % Difference of Ln(1+Concentrations) (2015-2014)/2014	Wilcoxon Signed-Rank p-value	T-Test p-value	Potential Sample Variability
Aluminum (Al)	5%	0.14	0.18	3%
Arsenic (As)	-1.5%	0.72	0.94	8%
Cadmium (Cd)	-4.7%	0.72	0.80	25%
Copper (Cu)	30%	0.07	0.005	9%
Iron (Fe)	6%	0.14	0.09	3%
Lead (Pb)	-8%	0.47	0.48	11%
Manganese (Mn)	-17%	0.07	0.04	3%
Zinc (Zn)	2%	0.72	0.60	6%

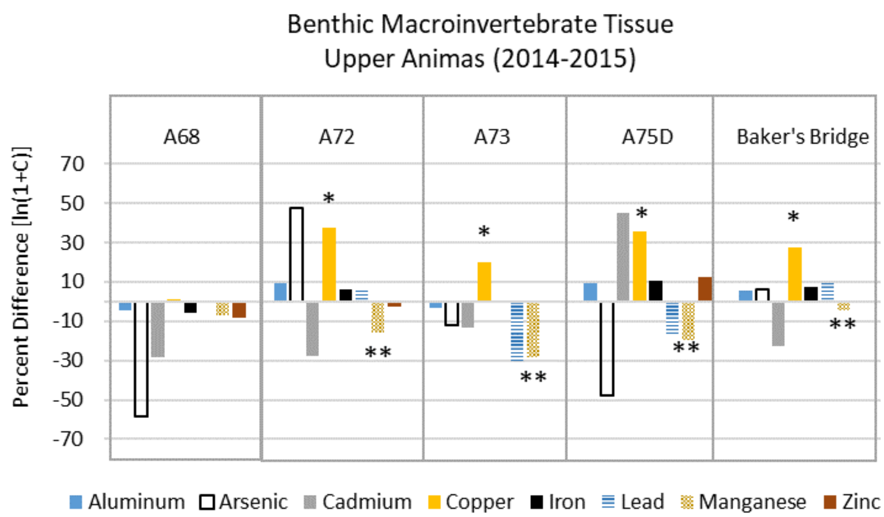


Figure 7.4 Percent difference calculated from the natural log transformed pre-GKM release (2014) and post-GKM release (2015) data collected from the upper Animas River. * identifies a statistically significant increase in copper (p = 0.005) and ** identifies a statistically significant decrease in manganese (p = 0.04).

Metal concentrations in benthic macroinvertebrate samples collected from the upper Animas River in 2016 were generally elevated when compared to 2014 and 2015 results (Table 7.2). The elevated concentrations in 2016 were observed throughout the watershed, including the upstream and tributary sites, suggesting that something other than the GKM release contributed to the high metal concentrations (Figures 7.2 and 7.3). A comparison of metal concentration in benthic tissue by sampling year showed the highest concentration of cadmium, copper, lead, and zinc were measured in the pre-release samples collected in 2012. The greatest concentrations of aluminum, arsenic, iron, manganese, and nickel were measured in the post-GKM release samples in 2016; note that of these five parameters, only arsenic was measured in 2012. The highest mean selenium concentration in benthic macroinvertebrate tissue was observed in the pre-release samples collected in 2014 (Table 7.2). We were unable to compare mercury concentrations between years due to differences in the method detection limits.

7.3 Lower Animas and upper San Juan NMDGF post-release benthic macroinvertebrate data

Lower Animas River and upper San Juan River locations sampled by the New Mexico Department of Game and Fish are presented in Figure 3.2. The benthic macroinvertebrate samples were sorted by taxa prior to metals analysis. The benthic macroinvertebrate taxa collected varied by location and sampling date (August 2015 and March 2016, both post-GKM). Mayflies and caddisflies were the most frequently collected taxonomic group (Table 7.4). True flies were only sampled at sufficient mass for analysis at the San Juan location upstream of the confluence SJAR. Stoneflies were only sampled from ADW-010 and SJFP. Only the August 2015 samples were analyzed for mercury and concentrations were generally less than the reporting limit.

Analyzing the results by taxonomic group shows that metal bioaccumulation in benthic macroinvertebrates is highly variable. Consistent trends in bioaccumulation between taxonomic groups were not observed. For example, the greatest concentration of lead was measured in caddisflies. Mayflies and stoneflies accumulated the greatest concentrations of cadmium and copper, respectively (Figures 7.5 and 7.6). At sampling location ADW-022, cadmium concentrations in mayflies were approximately 6 times the concentration measured in caddisflies. Similarly, copper concentration measured in stoneflies were approximately 5 times the concentration measured in mayflies and caddisflies. Since the sampling did not include replicates and metals in benthic invertebrate tissue can be highly variable within a site, it is not clear if the differences observed between the taxa represent differences in bioaccumulation or the spatial variability of metals, and hence exposure that could occur at a given sampling location.

Table 7.4. The total number and percent detection of samples that exceed the laboratory reporting limit of the NMDGF benthic macroinvertebrate tissue metals data by taxonomic group. Mercury was only analyzed in the August 2016 sampling event.

Metal	Mayflies (Ephemerpotera)		Stoneflies (Plecoptera)		Caddisflies (Trichoptera)		True flies (Diptera)	
	N	% detect	N	% detect	N	% detect	N	% detect
Aluminum	10	100%	3	67%	7	100%	2	100%
Arsenic	10	100%	3	67%	7	86%	2	100%
Cadmium	10	50%	3	67%	7	57%	2	50%
Copper	10	90%	3	67%	7	71%	2	100%
Lead	10	90%	3	67%	7	86%	2	100%
Manganese	10	100%	3	100%	7	100%	2	100%
Mercury	5	20%	2	0%	4	50%	1	100%
Selenium	10	50%	3	67%	7	57%	2	50%

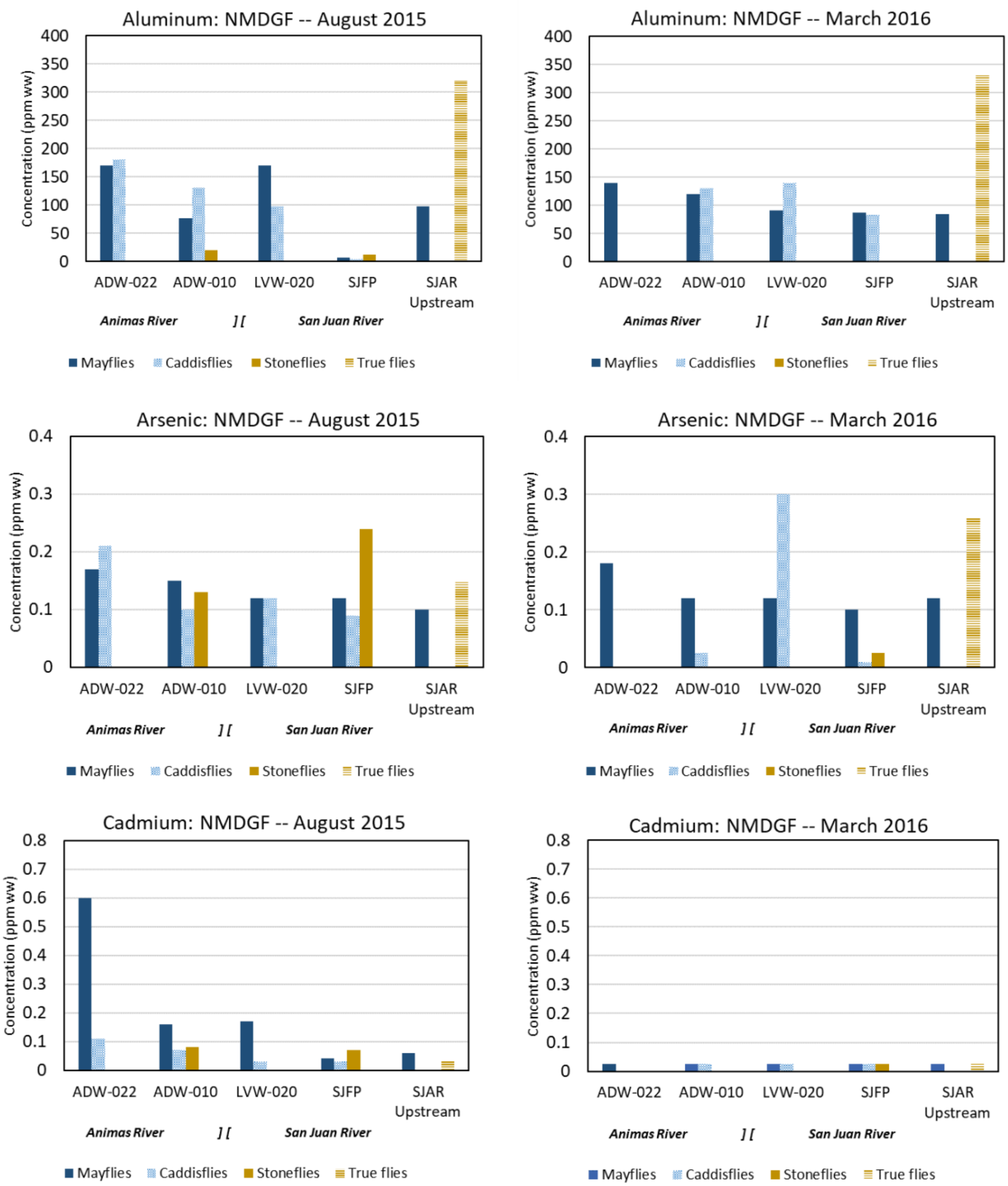


Figure 7.5. The concentration of aluminum, arsenic, and cadmium measured in benthic macroinvertebrate tissue samples collected by the NMDGF in the lower Animas and upper San Juan rivers in August 2015 and March 2016. The August 2015 samples were collected after the GKM release when deposits were present (see Section 2.3.2, Figure 2.11).

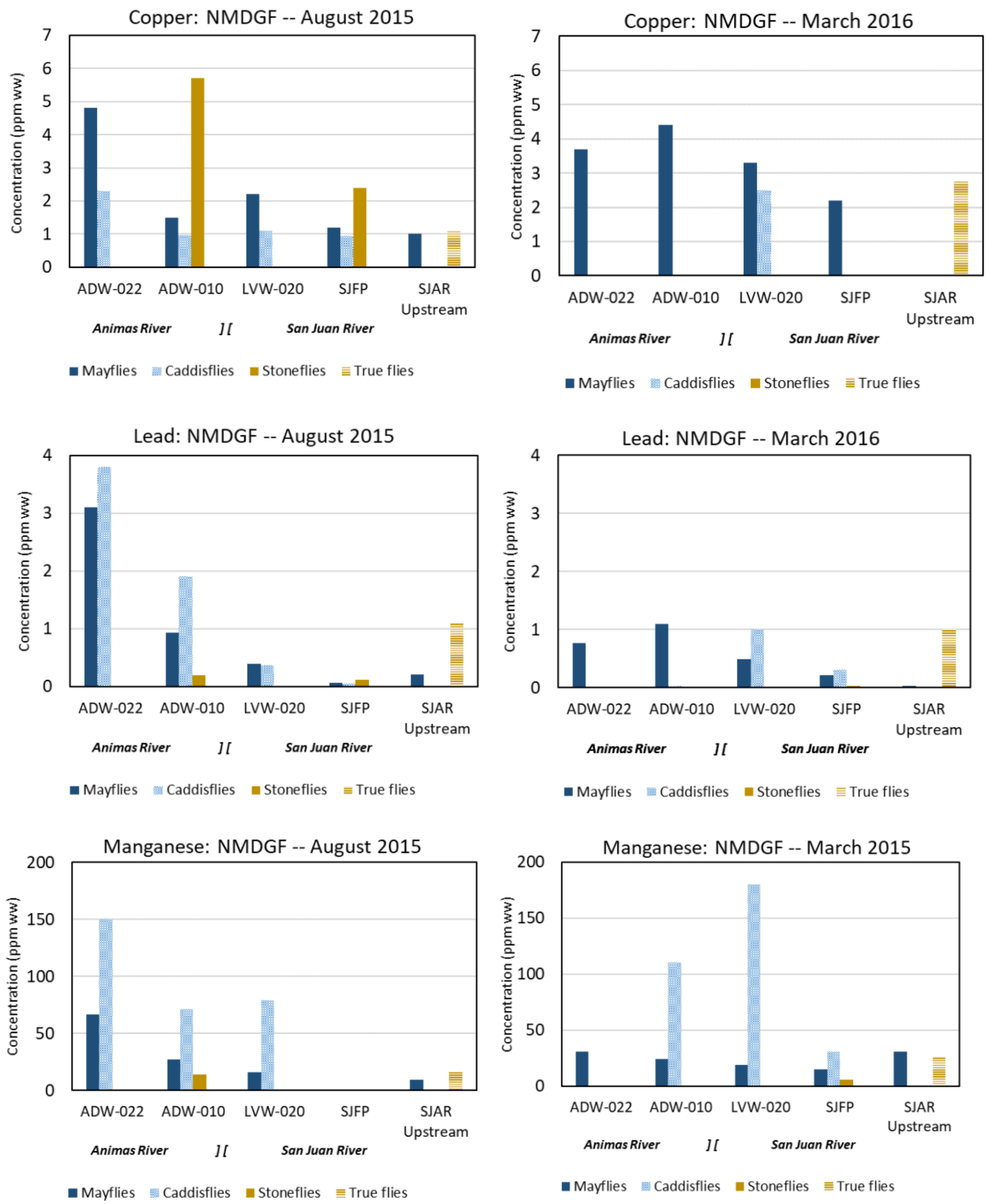


Figure 7.6 Concentration of copper, lead, and manganese measured in benthic macroinvertebrate tissue samples collected by the NMDGF in the lower Animas and upper San Juan rivers in August 2015 and March 2016. The August 2015 samples were collected after the GKM release when deposits were present (see Section 2.3.2, Figure 2.11).

The mean metal concentration in benthic tissue measured by location and sampling date was used to assess the spatial and temporal differences in the NMDGF data. When calculating the mean concentration, qualified results that were less than the reporting limit were set to a method detection limit given the high variability of reporting limits that were observed among the sampling locations and dates. No pre-release data were available from these sampling locations for analysis. Additionally, mean concentration results obtained by NMDGF are not directly comparable to EPA macroinvertebrate tissue results due to methodology differences. NMDGF samples were sorted and analyzed by taxonomic group whereas EPA samples were a composite of all taxonomic groups. To test for differences in the mean concentration of metals in the weeks following the GKM release (August 2015) and the following spring (March 2016), a non-parametric test was used that considers the sign and magnitude of the observed differences (Wilcoxon Signed Rank test). Site SJAR, upstream of the Animas-San Juan confluence, was not included in our analysis. Since the differences between each pair were evaluated across locations, the results of the test assess for a statistical difference throughout the study area rather than by location.

Spatial patterns in the NMDGF dataset were consistent with the longitudinal patterns observed in the EPA 2016 benthic tissue dataset (see Chapter 7.4). For arsenic and aluminum, the mean concentrations were relatively similar at all sampling locations and dates, with the greatest concentrations observed at the upstream location SJAR (Figure 7.7). The greatest mean concentration of cadmium and lead were observed in the August samples collected from the lower Animas River sites (ADW-022 and ADW-010). Cadmium and lead concentrations decreased at these sites the following spring to what appears to be the background condition determined by concentrations measured at SJAR. It is reasonable to interpret the high concentration of these metals in the August benthic invertebrate samples as a response to the GKM sediments that were deposited in this area for three weeks after the event, although the differences in the August 2015 and March 2016 concentrations were not statistically significant (Figure 7.7; all p-values > 0.09). An alternative hypothesis is that differences in the mean concentrations reflected seasonal changes in the taxonomic composition of benthic macroinvertebrate and life stages occurring at the sites. Samples were not collected in the summer/fall of 2016 so seasonal changes in benthic macroinvertebrate tissue concentrations cannot be determined.

Copper and manganese concentrations measured in the lower Animas (ADW-022 and ADW-010) and San Juan at LVW-020 (immediately downstream of the confluence with the Animas River) were greater than SJAR on both sampling dates. The mean concentration of metals at SJFP (17 km downstream of LVW-020), on the other hand, were similar to or less than what is observed at SJAR. Locations with the greatest metal concentrations also exhibited the greatest variability among the taxonomic groups collected from the site.

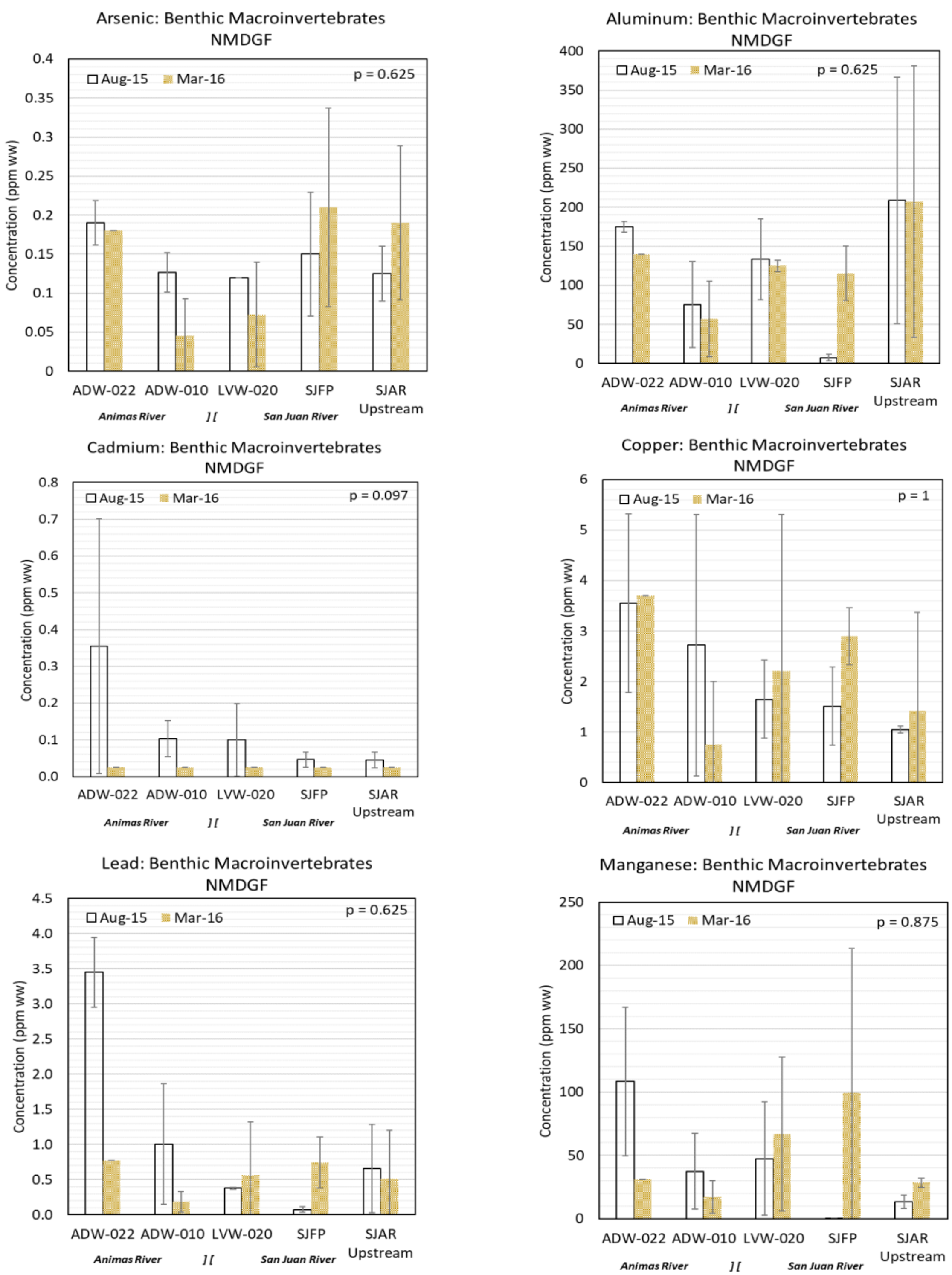


Figure 7.7. The mean concentration of metals ± 1SD (ppm ww) in all benthic macroinvertebrate taxonomic groups by location in August 2015 and March 2016. N varied by sampling location and date and ranged from 1-3. Both sampling dates represent post-GKM release conditions. Error bars represent 1 standard deviation and the variability of metals among taxonomic groups at that location. The p-values are the results of Wilcoxon signed rank test to compare concentrations by sample date.

7.4 Summary of metals in benthic macroinvertebrates tissue

The accumulation of metals in benthic macroinvertebrates collected from the Animas and San Juan rivers following the GKM release was spatially and temporally variable. Samples collected from the upper Animas after the GKM release that represent near-term sampling (September 2015) had greater copper and lower manganese concentrations when compared to pre-release concentrations. All other changes in metal concentrations from 2014 were not statistically significant and were within the range of potential sample variability. The lack of a consistent response by metal sheds light on the complexity of metal bioaccumulation in benthic organisms.

In the lower Animas and upper San Juan rivers, historic concentrations were not available for a pre- and post-GKM release analysis. Concentrations of cadmium and lead measured in the near-term samples (August 2015) were elevated at the lower Animas site ADW-022 when compared to the following spring (March 2016). Benthic tissue samples collected from ADW-022 also generally had greater metal concentrations than the immediate up and downstream locations in the fall 2016. Many metals were elevated in the fall 2016 samples when compared to the pre-release (2014) and the near-term post-release (2015) concentrations. The 2016 samples were similar to concentrations observed in 2012. These high concentrations were also observed in the upstream and tributary samples after the GKM deposits were flushed through the river system during the spring snow melt in 2016 (EPA 2016c), suggesting something other than the GKM release contributed to the high concentrations. Differences in field collection methods, analytical methods and the benthic community composition likely contributed to the differences observed between years (e.g., estimating percent solids vs. measuring percent solids, the removal of caddisflies from their casings prior to analysis).

High intra- and inter-site variability and longitudinal patterns in the recent benthic macroinvertebrate tissue data are consistent with the results presented by Besser *et al.* (2001). The authors characterized concentrations of cadmium, copper, lead and zinc in specific macroinvertebrate taxa (*Rhithrogena,* mayfly; *Arctopsyche,* caddisfly; *Megarcys,* stonefly; *Zapada,* stonefly) collected from the upper Animas and tributaries in 1996. The Besser study showed significantly different metal concentrations in benthic macroinvertebrates among taxa and among sampling locations. Metal concentration trends observed in their data were partly explained by organism size and differences in feeding groups, with smaller taxa generally accumulating more metals. Concentration differences between sampling location in the Besser study were limited by the presence and/or absence of specific taxa at a given sampling location. Longitudinal changes in the benthic macroinvertebrate community also confounded the post-release GKM benthic tissue data and limited the inferences we could make from the analyses.

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CHAPTER 8 METALS IN FISH TISSUE

Numerous factors determine the likely uptake of metals in individual fish, including species, size of fish, and the environmental conditions in which they live. Fish rapidly take-in metals through their gills or food and expel them rapidly (on the order of hours to days), thus the internal body burden reflected in tissue concentrations should reflect the ambient water or feeding (sediment) environmental concentrations close to the time they are sampled. Chapter 8 explores the bioaccumulation of metals in individual fish and populations, by location and time with respect to the passing of the GKM plume, the presence of GKM deposits, and pre-release tissue concentrations. These analyses address questions relating to long-term changes in biological communities observed a year after the GKM release. Pre-GKM release fish tissue data were limited to a single sampling event on the Animas River by Southern Ute Indian Tribe, therefore analyses primarily focus on the characteristics and differences in the post-release tissue concentrations. We also summarize the state and tribal findings with respect to human health risk associated with consumption of fish post-GKM.

Our analyses showed that some fish accumulated metals in the weeks after the GKM event in the lower Animas River that received GKM metals deposits. Metals were significantly elevated in bluehead and flannelmouth sucker liver and speckled dace muscle tissue. The degree of metal accumulation in liver differed by species, sampling location, and among the metals, with aluminum, cadmium, lead and manganese exhibiting the greatest concentrations. For the most part, elevated liver concentration did not translate to high muscle concentrations. Metal concentrations in fish declined to background conditions when samples were collected again the following spring and never triggered human health consumption advisories.

8.1 Fish tissue data

Metals in fish tissue data collected prior to and after the GKM release were assembled to characterize the body burden of metals in fish in the Animas and San Juan rivers affected by ongoing acid mine drainage in the headwaters mining district and the GKM release. This analysis used pre-GKM fish tissue data collected by the Southern Ute Indian Tribe (SUIT) and post-GKM event data collected by the New Mexico Department of Game and Fish (NMDGF), the Colorado Department of Public Health and Environment (CDPHE), Navajo Nation EPA (NNEPA) and the EPA. Differences in sampling methods limited the data that could be combined for pre-and post-GKM event comparisons. See Chapter 3 for additional details about pre- and post-GKM event fish sampling and analytical methods.

CDPHE and NMDGF collected fish tissue samples when the maximum effect of the GKM release was observed (August 2015) and the following spring (March 2016). These datasets were used to assess the short-term effect of the GKM deposits left as the plume migrated through the river system. The presence of deposits and relative change in metal concentration (i.e., change from background) varied with sampling date and river location. CDPHE's sampling focused on trout muscle samples in the Durango area. The NMDGF sampled metal concentrations in fish muscle and livers in several species the lower Animas and upper San Juan Rivers.

SUIT sampled fish in July 2015, prior to the GKM event. These data were compared to EPA, CDPHE and NMDGF post event data collected near the same locations to assess whether tissue concentrations observed at various times after the 2016 snowmelt period were similar to pre-event levels.

8.2 Analysis of NMDGF post-GKM release fish tissue data

The most robust data available to directly assess the effects of the GKM release were collected in the New Mexico portion of the Animas and San Juan rivers after the GKM release by the New Mexico Department of Game and Fish (NMDGF).

8.2.1 About NMDGF fish data

NMDGF collected fish tissue samples for metal analyses twice after the GKM release at 2 locations in the lower Animas River and at 3 locations in the upper San Juan River. (See Figure 3.2 for site locations.) Approximately 60 km of river length within New Mexico is bracketed between the upper and lowermost sampling locations near the Colorado/New Mexico border, including 40 km of the lower Animas from Cedar Hill to the confluence with the San Juan River in Farmington and 20 km of the San Juan River near Farmington. A reference site was sampled on the San Juan River above the confluence with the Animas.

NMDGF conducted the first sampling in the weeks following the GKM release in August and a second in March 2016 prior to snowmelt runoff. The NMDFG sampling design provided a meaningful statistical comparison of metal bioaccumulation in four species of fish when the maximum effect of the GKM release was observed relative to background environmental concentrations of metals.

Table 8.1 summarizes the general status of metals in sediment and water in the Animas and San Juan rivers during the two fish sampling events. During the August 2015 fish sampling, GKM sediment deposits were present in significant amounts in the lower Animas, declined in the downstream direction, and were negligible in the San Juan River. Sediment concentrations in the lower Animas returned to background after the monsoonal storm at all locations and were low when fish tissue was sampled in March 2016. Metal concentrations in the water were elevated in both segments of the river in the post-event period (August 2015) and were at low background levels in both segments during the March 2016 sampling (Chapter 2).

Table 8.1. Summary of general metals effects from the GKM release in the Animas and San Juan rivers during fish sampling in August 2015 (post-release) and March 2016. March 2016 sample were used to estimate background condition given the limited pre-release data tissue data from these sampling locations.

Sampling Date	Lower Animas (2 locations)	San Juan River (2 locations)
August 2015	• Significant GKM deposits in sediments • Elevated water concentrations	• Little, if any, GKM deposits • Elevated water concentrations
March 2016	• No GKM deposits in sediments • Low water concentrations	• No GKM deposits in sediments • Low water concentrations

Characteristics of the Fish Community

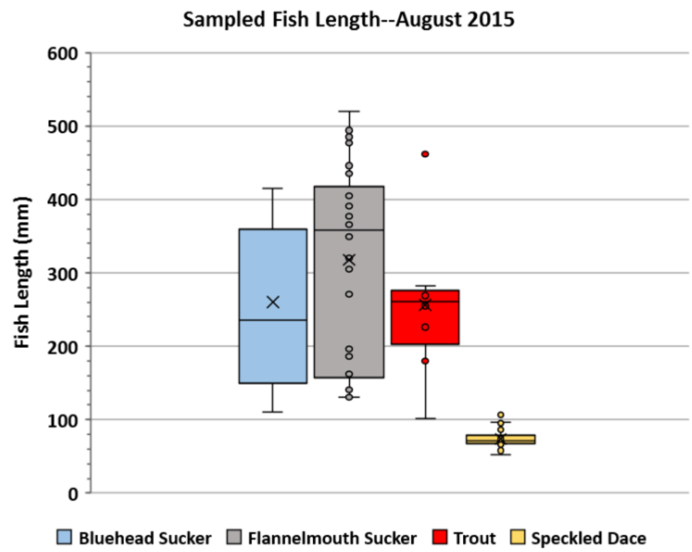


Figure 8.1. Body size distribution of fish by species sampled at all sites in August 2015. Boxplots show mean, median, and quartiles.

Multiple species were present through the 60-km length of rivers, although not all species were sampled at all sites. Up to 10 individuals from each species were collected for analysis of metal concentrations in their tissue (Table 8.2). Skinless filets (muscle) and liver samples were collected from bluehead sucker, flannelmouth sucker, rainbow trout, brown trout and channel catfish. Speckled dace muscle samples represented a composite of 5 fish with the head and gut content removed, and therefore include skin, scales and fins. All tissue samples were processed for 6 metals (Al, As, Cd, Cu, Hg, Pb, Mn, Se, and Zn). Table 6.2 provides the count of sampled fish by species at each site during each of the two sampling events. Brown trout, bluehead sucker, flannelmouth sucker, and speckled dace were sampled from all 5 sampling locations in both rivers. Rainbow trout were sampled the Animas River, and channel catfish were found in the San Juan River. The range of fish size expressed as body length for each species grouping all sites is shown in Figure 8.1. Given the limited number of sites where catfish were sampled, limited results are presented for this species.

Multiple factors contribute to the accumulation of metals in body tissue, including the specific environmental concentrations at sampling locations, fish characteristics such as species and individual fish size, and the type of tissue sampled. The individual factors are explored graphically in Figures 8.2 through 8.9 to develop a general understanding of the accumulation of metals in fish following the GKM release. A general linear modeling (GLM) approach was applied to statistically evaluate the NDDFG dataset. Results of the GLM are presented in Appendix E.

Table 8.2. Count of fish by location, sampling event, tissue type and species during fish tissue sampling conducted by the NMDGF after the GKM release (August 2015) and in March 2016. Livers were collected from the same fish as muscle tissue and were not collected from speckled dace. If a species or tissue was not sampled at a location, the count is indicated as --. **speckled dace muscle samples represent a composite of 5 fish with the head and gut content removed.

Location	ADW-022	ADW-010	LVW-020	SJFP	SJAR	Total
River	Lower Animas	Lower Animas	San Juan	San Juan	San Juan	
Distance from GKM (RKM)	147	163	196	214	Upstream	
Liver	**53**	**35**	**41**	**44**	**27**	**200**
August 2015	**19**	**9**	**20**	**15**	**Not Sampled**	**63**
Bluehead sucker	10	8	9	0	--	27
Flannelmouth sucker	0	0	10	10	--	20
White sucker	0	1	--	0	--	1
Brown trout	7	0	1	0	--	8
Rainbow trout	2	0	0	0	--	2
Channel catfish	--	--	--	5	--	5
March 2016	**34**	**26**	**21**	**29**	**27**	**137**
Bluehead sucker	11	9	10	5	7	42
Flannelmouth sucker	10	10	10	10	9	49
White sucker	1	0	--	1	--	2
Brown trout	10	2	1	5	10	28
Rainbow trout	2	5	0	0	1	8
Channel catfish	--	--	--	8	--	8
Muscle (filet without skin)	**74**	**56**	**61**	**62**	**63**	**316**
August 2015	**30**	**20**	**30**	**25**	**25**	**130**
Bluehead sucker	10	8	9	0	5	32
Flannelmouth sucker	0	0	10	10	10	30
White sucker	0	1	--	0	--	1
Brown trout	8	0	1	0	0	9
Rainbow trout	2	1	0	0	0	3
Speckled dace**	10	10	10	10	10	50
Channel catfish	--	--	--	5	--	5
March 2016	**44**	**36**	**31**	**37**	**38**	**186**
Bluehead sucker	11	9	10	5	7	42
Flannelmouth sucker	10	10	10	10	10	50
White sucker	1	0	--	1	--	2
Brown trout	10	2	1	5	10	28
Rainbow trout	2	5	0	0	1	8
Speckled dace**	10	10	10	8	10	48
Channel catfish	--	--	--	8	--	8

8.2.2 Individual fish, population and tissue-specific responses

The general bioaccumulation of metals in individual fish is illustrated for liver tissue in Figure 8.2 and muscle tissue in Figure 8.3. The liver sequesters and regulates metals in fish and is an indicator of exposure. Liver concentrations do not translate directly to muscle concentrations. Metal concentrations are shown for individual fish, identified by species and plotted by body length grouping all sites and both sampling events. These figures highlight several key characteristics of how metal bioaccumulation can vary within a fish community and the complexity of the response.

Only some individuals within each species accumulated metals. Many liver and most muscle samples were less than detection limits, indicated by the flat line that represents the non-detection limit of the laboratory test for each metal. The effect of sample time will be assessed later in this section, but most of the high tissue concentrations were observed in the August 2015 samples.

Metal concentrations in livers were generally greater than those in muscle, consistent with the scientific literature that identifies sequestration of metals in liver tissue. Muscle samples with detected concentrations were an order of magnitude lower than those in the liver (Figures 8.2 and 8.3). Concentrations in the liver of some individuals were more than three orders of magnitude greater than the average of the population.

Bioaccumulation varied by species and metal. Most species and many individuals had detectable concentrations of copper and manganese in liver tissue, while species reacted differently to the other metals (Figure 8.2). Bluehead suckers had detectable liver concentrations for most of the six metals, while trout and flannelmouth sucker livers primarily accumulated copper, lead, manganese and cadmium. Only the muscle tissue of speckled dace was tested (Figure 8.3). More individuals in the speckled dace population had high metal concentrations in muscle compared to other species.

Selenium and mercury concentrations in muscle and liver are shown in Figure 8.4. Unlike other metals, concentrations of mercury in muscle were similar to or greater than in the liver. As with other metals, speckled dace accumulated greater mercury and selenium concentrations in muscle than other species while trout livers had greater selenium concentrations than other species.

Fish size played a small role in metal bioaccumulation within the species groups. There was a slight positive trend in copper concentrations in trout liver with fish size. For the most part, however, the smaller fish in each population tended to have higher concentrations than the larger fish. While species vary physiologically in how they take up metals, this pattern could also result from the habitat niche that individuals occupied. Gold King Mine release deposits varied laterally across the channel as well as longitudinally along the rivers. Metal concentrations left by the GKM release were probably greater along the channel edges than in the main current.

There was clear accumulation of metals in liver and to a lesser extent muscle in some individuals within the population of fish, although there was high variability between species, metals, tissue type, and among individuals within the population.

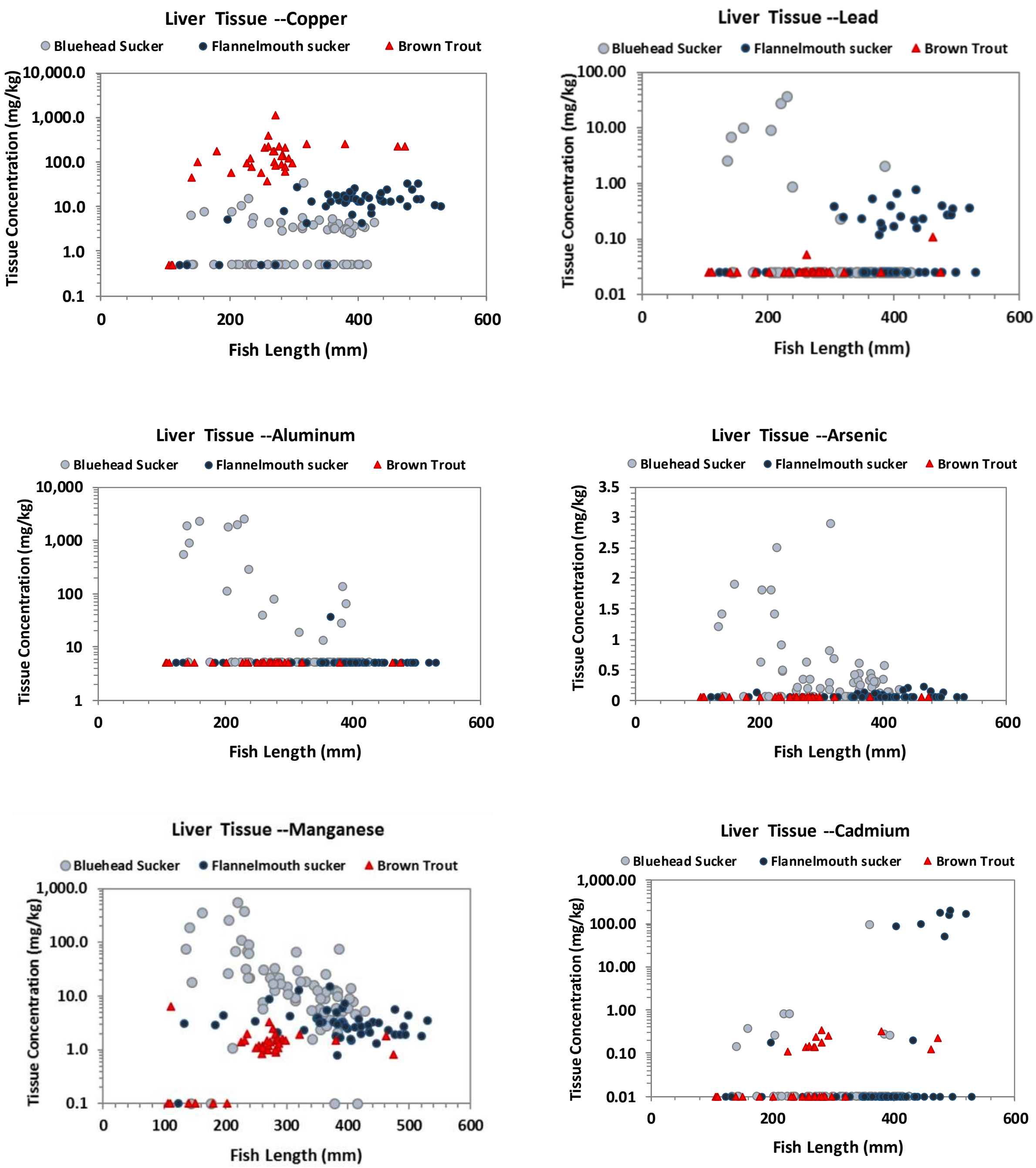


Figure 8.2. Liver tissue concentration of copper, lead, aluminum, arsenic, manganese and cadmium (mg/kg ww) in individual fish identified by species. Data collected at all sites in the two sampling events are grouped in these figures.

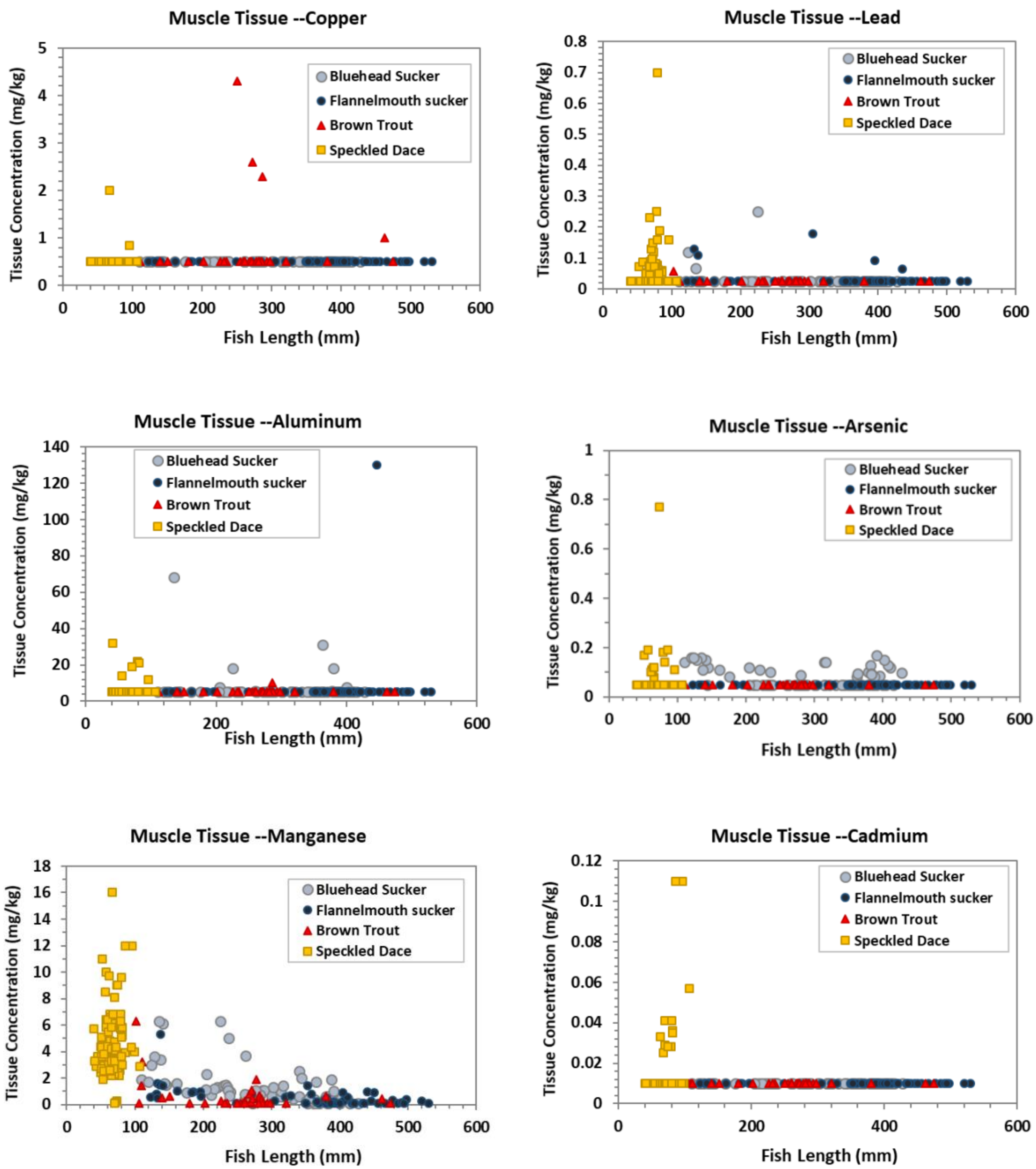


Figure 8.3. Muscle tissue concentration of copper, lead, aluminum, arsenic, manganese and cadmium (mg/kg ww) in individual fish identified by species. Data collected at all sites in the two sampling events are grouped in these figures.

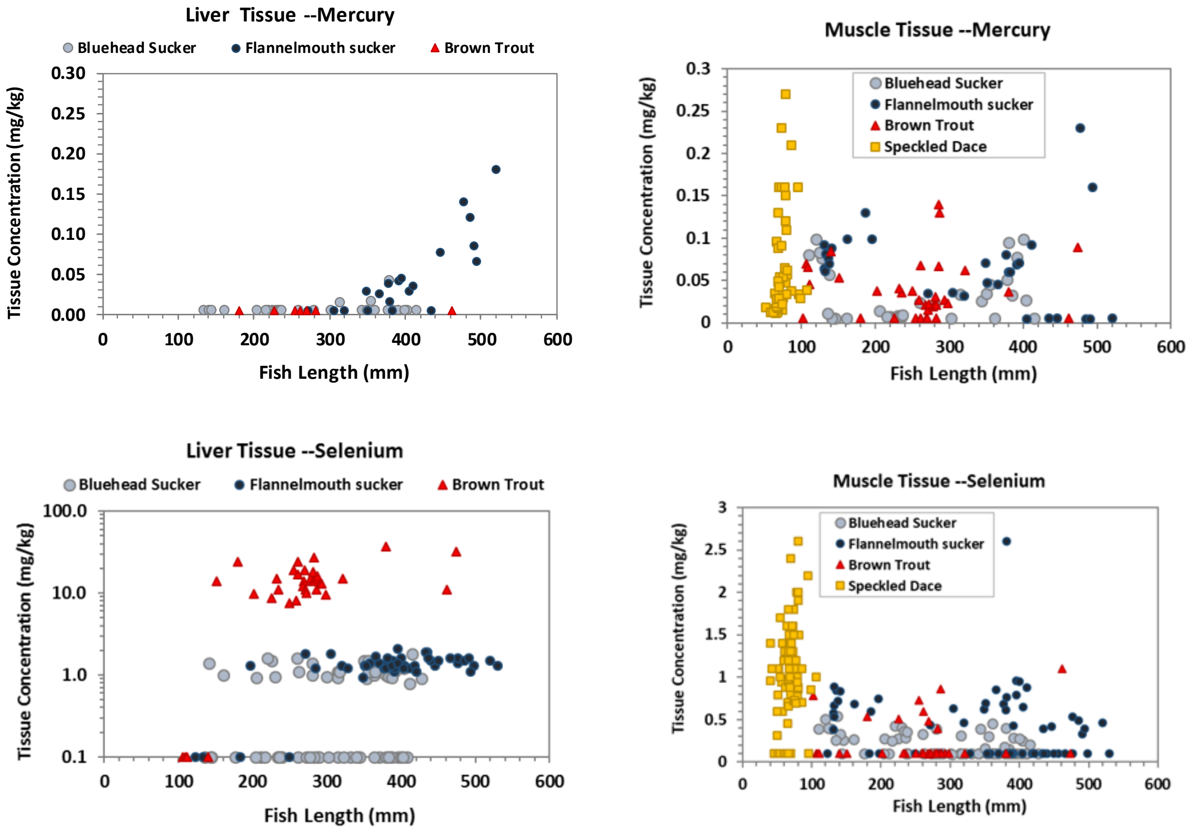


Figure 8.4. Tissue concentrations of mercury and selenium (mg/kg ww) in liver and muscle samples of individual fish identified by species. Data collected at all sites in the two sampling events are grouped.

A two-sample t-test was used to statistically compare the species-specific mean metal concentration in liver and muscle by sampling date. Results showed that bluehead sucker liver tissue had statically greater aluminum, lead, manganese and selenium concentrations in August when compared to the mean concentration the following March (; p<0.0;5 Table 8.3). Statically significant differences were also observed in the mean cadmium and lead concentrations measured in flannelmouth sucker liver tissue. Trout liver concentrations, on the other hand, did not differ between sampling dates. Differences in the mean concentration of metals in muscle in August and March were not generally significant for trout, bluehead sucker and flannelmouth sucker, the exception being selenium. Mean cadmium, lead and selenium in speckled dace muscle were significantly greater in August when compared to March. These results suggest that many suckers and dace responded to the increased exposure and accumulated metals associated with the GKM-deposits in the lower Animas and upper San Juan rivers; however, given the seasonal differences in the sampling dates, it is challenging to identify how much of the difference in concentration was due to the presence of the GKM deposits verses the expected seasonal variability in metal bioaccumulation that has been shown to be temperature dependent. When compared to water temperatures in March, the greater temperatures in August would be expected to increase fish metabolic rates, thus promote increased metal uptake.

Table 8.3. Mean metal concentration measured in liver and muscle tissue samples collected by NMDGF in August 2016 and March 2017. Highlighted cells identify statistically significant differences in the species mean concentration by sampling date (p<0.05).

	Trout		Bluehead sucker		Flannelmouth sucker		Speckled dace	
	August	**March**	**August**	**March**	**August**	**March**	**August**	**March**
LIVER								
Aluminum	5	5	438.30	18.91	6.55	5.00		
Arsenic	0.05	0.05	0.53	0.35	0.05	0.08		
Cadmium	0.12	0.10	3.47	0.02	47.41	0.02		
Copper	169.63	211.19	2.55	3.15	16.43	14.12		
Lead	0.04	0.03	3.58	0.05	0.29	0.06		
Manganese	1.10	1.45	90.70	13.87	3.93	3.28		
Selenium	18.33	15.44	0.86	0.43	1.457	1.390	No Data	
MUSCLE								
Aluminum	5.00	5.00	8.54	5.74	11.25	5.00	5.18	7.18
Arsenic	0.05	0.05	0.07	0.07	0.05	0.05	0.40	0.35
Cadmium	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Copper	0.56	0.80	0.50	0.50	0.50	0.50	0.51	0.54
Lead	0.03	0.03	0.03	0.03	0.04	0.03	0.05	0.03
Manganese	0.94	0.46	1.62	0.86	0.26	0.37	5.42	4.39
Selenium	0.58	0.10	0.30	0.10	0.58	0.31	1.10	0.79

8.2.3 Multiple metals in fish tissue considerations

Did some individuals or species accumulate significant amounts of multiple metals? To evaluate multi-metal accumulation, we summed the concentrations of 7 metals in each fish (Al, As, Cd, Cu, Mn, Pb, and Se). Because the metals have large differences in concentration within the body, we first converted the concentration by $\log_{10}$, and then normalized the distribution for each metal within the population using the z-score formula:

$$z= (x-m/SD)$$

Where z is the scaled value of the observation x, m is the mean of x, and SD is the standard deviation of x. The scaling put each metal on the same relative scale and gave it equal weight when the metals were summed. This formulation centers the distribution mean at zero.

The summed metal values in liver and muscle for each species, combining both sampling periods, are shown in Figure 8.5.A and B, respectively. The summed metal values in liver and muscle in the two sampling periods, combining all species, are shown in Figure 8.5.C and D, respectively. Given the normalization, each of the tissue groups had a minimum value of -4 to -6 when all seven metals were at non-detect levels. Copper and manganese are generally detected in tissue, as they are regulated by the body, and therefore the minimum values are not generally observed. The summed metal values spanned from a minimum of -6 to values greater than 15 in each tissue type for a few individuals. We identified individuals with high overall metal body burden as those with a value greater than +1 standard deviation of the mean (liver> 3.76 and muscle > 3.497). Three or more metals had to be present in relatively high concentrations in the individual to reach this value.

Of 151 liver samples, 18 individual fish had high overall metal body burden. Flannelmouth (n=7) and bluehead suckers (n=10) made up most of this group. It also included 1 trout. Most high values were observed in August (89%) as opposed to March. The individual fish with high liver body burdens were somewhat more likely to occur in the San Juan River (61%) than the Animas (39%). The majority of those in the San Juan River were taken in Farmington at LVW020.

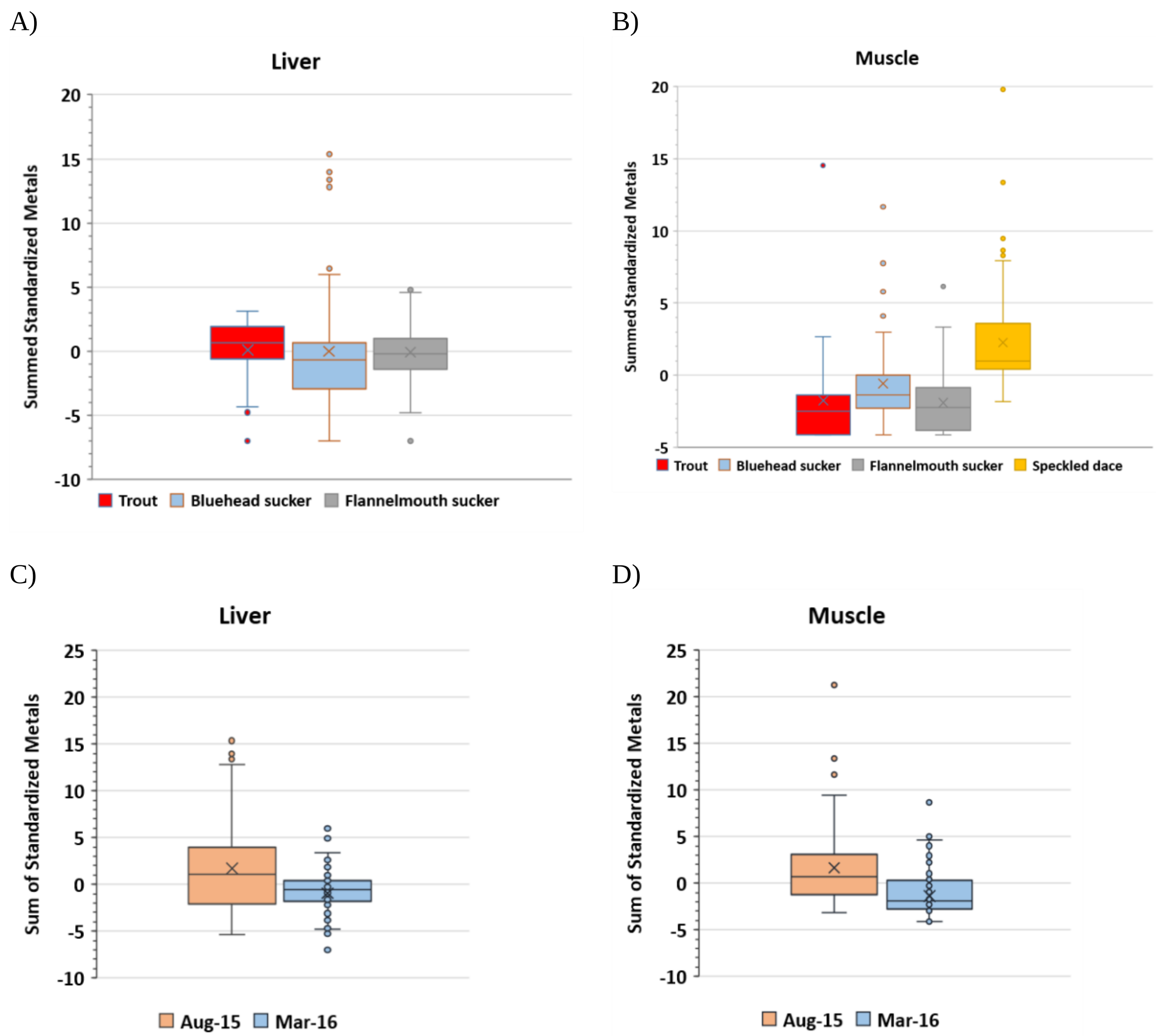


Figure 8.5. Comparison of the cumulative metal values in A) liver by species, B) muscle by species, C) liver by sampling date and D) muscle by sampling date. The concentration of each metal was transformed to $\log_{10}$ and standardized to the mean and standard deviation of the population prior summing.

Of 268 muscle samples, 32 fish had significant concentrations of multiple metals. Most of these fish (78%) were speckled dace (Figure 8.5.B). This was also apparent in the individual metal concentrations in Figures 8.3 and 8.4. Five bluehead suckers and 1 each of flannelmouth sucker and trout were also in this group. Nearly half (16 fish) of the high summed values s in muscle occurred in the San Juan River, where they were evenly distributed between the 3 sampling locations This included the reference reach located upstream of the Animas. and were almost as likely to occur in March as in August. The other half (17 fish) of the high summed values were observed in the Animas River, with the majority sampled in August (88%).

The analysis of summed metals suggests that speckled dace accumulated relatively more metals in muscle when compared to other species. Only a few individual trout, bluehead suckers and flannelmouth suckers accumulated multiple metals at high concentrations in either liver or muscle tissue. High cumulative metal burden was observed in some individuals in March, but generally it was greater in the August when metal concentrations in sediment were greater than background.

8.2.4 Sampling location trends

The range of tissue metal concentrations observed at the individual sites varied, especially in August 2015. Figures 8.6 and 8.7 show the average metal concentrations in liver and muscle tissue by site, grouping all fish species. There was a longitudinal gradient in tissue concentrations of many of the metals during the August sampling after the GKM release that generally corresponded to the increase in sediment metals documented in the lower Animas River.

Livers had much higher average concentrations of lead, aluminum, arsenic, copper, and manganese in August at the two Animas River locations than at the two San Juan River sites. Muscle samples also tended to have a gradient from upstream to downstream in August, but differences in concentrations among sites were much smaller. Lead was elevated at all locations in the August sampling, with the highest levels observed at the San Juan River reference site. Lead in muscle tissue was low at all sites in March.

In the San Juan, August 2015 fish muscle samples collected from the San Juan sites had metal concentrations that were generally similar to the March 2016 levels. Several metals, including selenium, arsenic and manganese were also higher during August at the San Juan upstream site than other San Juan sites. Tissue concentrations were significantly reduced at the Animas River sites in March relative to August and were similar to the San Juan River sites.

These patterns suggest that fish tissue concentrations were generally elevated in response to the high sediment concentrations in the Animas River that persisted for approximately 3 weeks after the GKM release (see Chapter 2; Figure 2.11). Bioaccumulation of metals in fish collected from the San Juan River was low, consistent with the low August sediment concentrations. Sediments returned to background sediment conditions in the Animas and San Juan River after August 27, 2015, due to a monsoonal storm that removed the deposited material (EPA 2016c). Tissue concentrations at all Animas and San Juan sites were at background conditions in March 2016.

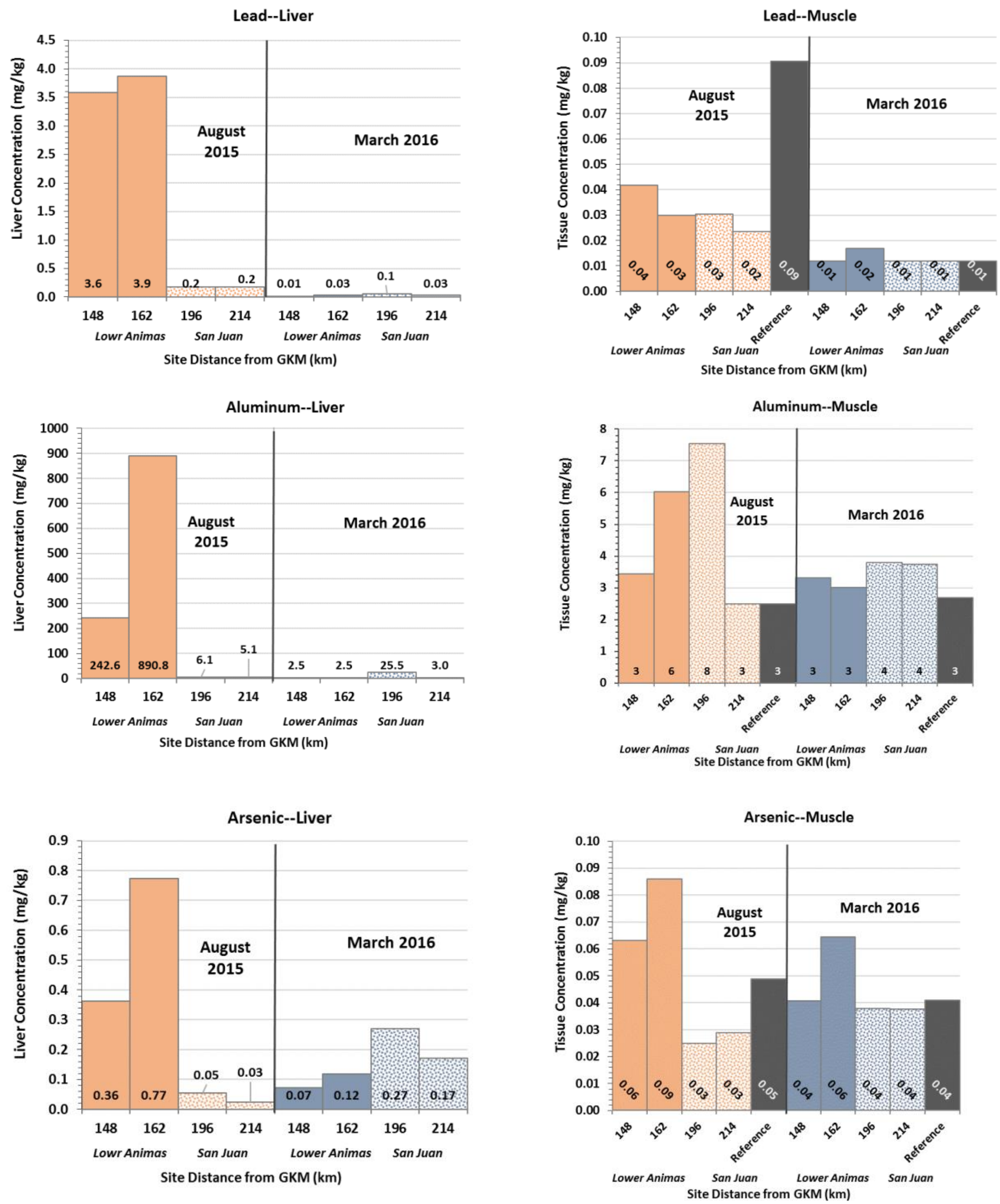


Figure 8.6. Mean concentration of lead, aluminum, and arsenic in liver and muscle (mg/kg ww) of all fish sampled at each location in each sampling period. Sites are organized by distance from GKM from left to right with lower Animas sites identified by solid bars and San Juan sites identified by textured bars. Muscle concentrations at the reference site on the San Juan River are shown as the darkest bar. Liver samples collected at this location in March are not presented.

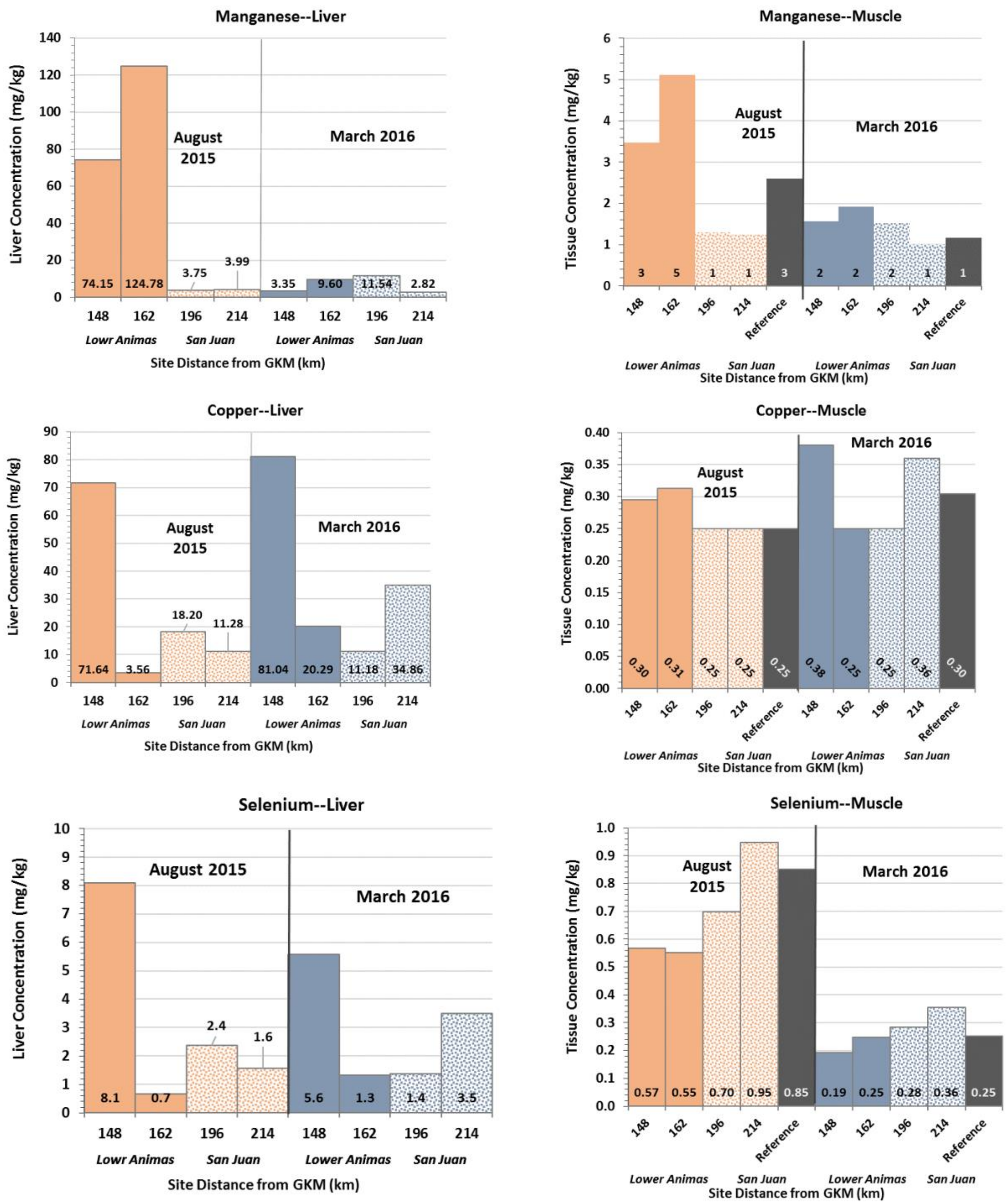


Figure 8.7. Mean concentration of manganese, copper, and selenium in liver and muscle (mg/kg ww) of all fish sampled at each location in each sampling period. Sites are organized by distance from GGKM from left to right with lower Animas sites identified by solid bars and San Juan sites identified by textured bars. Muscle concentrations at the reference site on the San Juan River are shown as the darkest bar. Liver samples collected at this location in March are not presented.

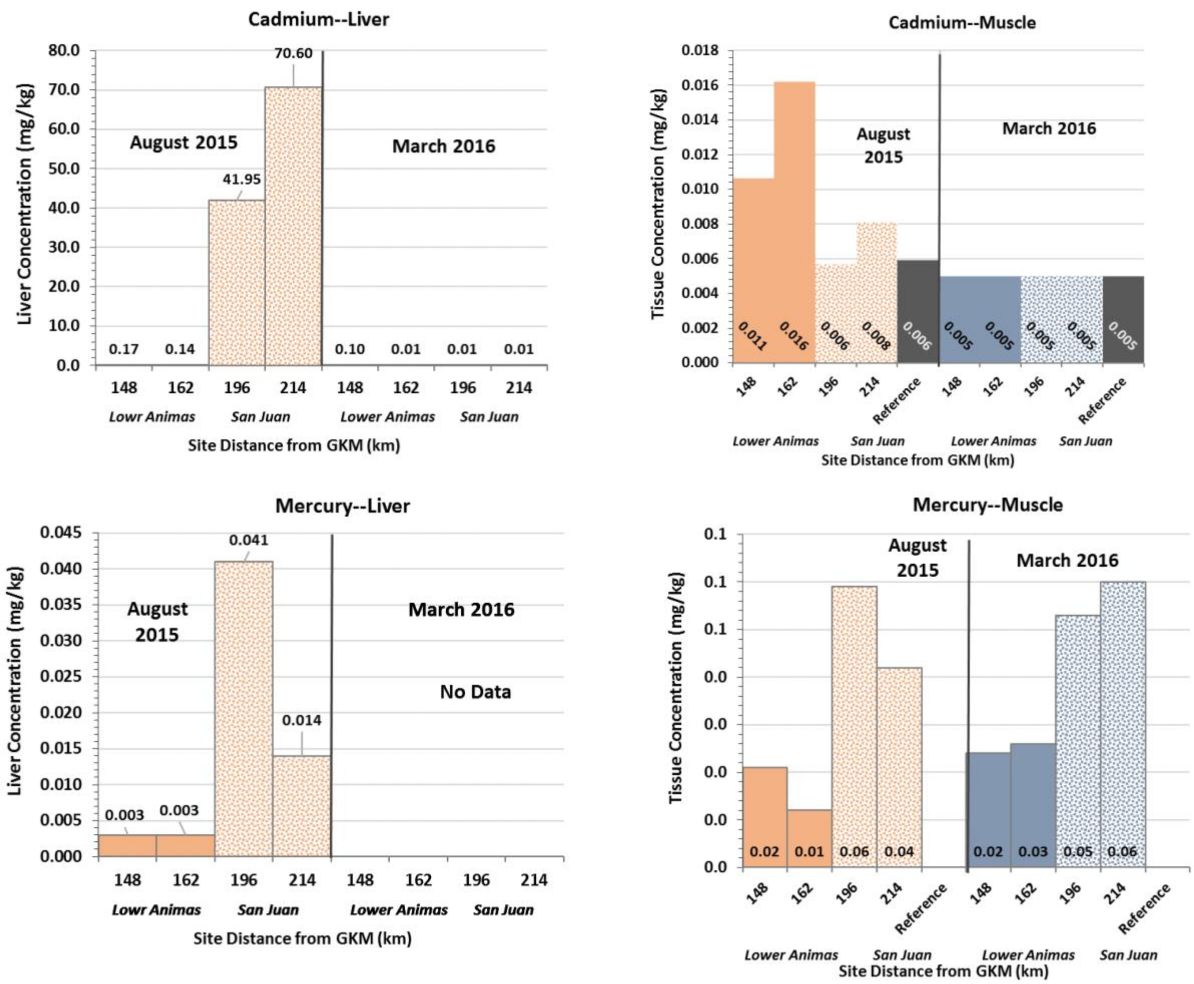


Figure 8.8. Mean concentration of selenium and mercury in liver and muscle (mg/kg ww) of all fish sampled at each location in each sampling period. Sites are organized by distance from GKM from left to right with lower Animas sites identified by solid bars and San Juan sites identified by textured bars. Muscle concentrations at the reference site on the San Juan River are shown as the darkest bar. Liver samples or mercury samples collected at this location in March are not presented

There were exceptions to the general patterns observed for many of the metals in that some tissue metals were higher in the San Juan River than the Animas River. Notably, aluminum in muscle tissue, was highest in the San Juan River at 196 RKM (Figure 8.6). Cadmium in liver tissue was much greater in the San Juan River than the Animas River in August. Copper concentrations in liver and sediment followed a longitudinal pattern through the river segments in both the August 2015 and March 2016 samplings and were similar in both periods suggesting copper is more persistent in fish tissue that the other metals. Lead was much higher at the reference site in the August 2015 sampling than any site directly impacted by the GKM release (Figure 8.6).

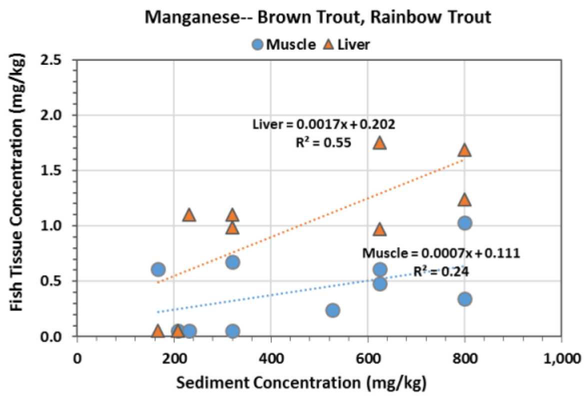
Mercury and selenium concentrations tended to be greater in the San Juan River than the Animas River in both sampling events and concentrations were comparable to the San Juan River reference site (Figures 8.7 and 8.8). Similar to cadmium liver concentrations, the greatest mercury in liver concentrations were measured in flannelmouth suckers that were not sampled from the lower Animas (Figure 8.4).

We note that spatial and temporal differences and trends observed in the NMDGF data were influenced by differences in the species that were sampled during each event (Table 8.2), and expected seasonal changes in fish use and bioaccumulation rates. For example, flannelmouth suckers were not sampled at either of the Animas River sites during August 2015. This partly explains differences between sampling locations in August 2015 (e.g. high Cd in liver in the San Juan River) since the greatest cadmium concentrations were observed in the larger flannelmouth sucker and one bluehead sucker (> 380 mm; Figure 8.2). Brown trout were not sampled at ADW-010 (163 RKM) during August although they were found there in low numbers in March. It is not clear whether the GKM release may have played a role in the presence or absence of species, however no clear changes in fish populations in response to the GKM release were observed (Chapter 6).

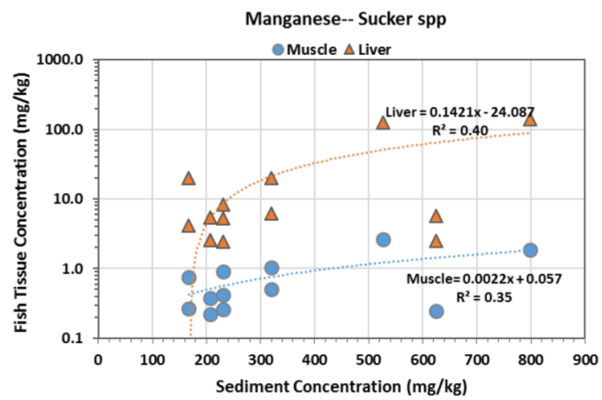
To illustrate the complexity of the relationship between the body burden of metals in the fish community in response to environmental conditions, the tissue concentration of brook trout grouped with rainbow trout and bluenose sucker grouped with flannelmouth sucker are shown in relation to the range of sediment concentration observed in the August and March samplings at all sites in Figure 8.9. Manganese and copper are internally regulated metals required for life functions. These metals would be expected to be accumulated by fish and should tend to reach a saturation point. Lead is not a regulated metal and its concentration in the body is likely to reflect exposure through diet.

The concentration of manganese in tissue showed increasing trends with sediment concentrations especially in the liver. However, sucker species accumulated greater concentrations of manganese than the brown and rainbow trout. The greatest body burden of copper was observed in the brown and rainbow trout and reached relatively high levels even at relatively low sediment concentrations. Trout did not accumulate much lead but suckers appeared to accumulate larger amounts when sediment concentrations exceeded 50 mg/kg. Although there was some influence of environmental metal concentrations on fish body burden, these complexities preclude any broad generalizations about fish response to the levels of metals observed at the lower Animas and San Juan rivers after the GKM release.

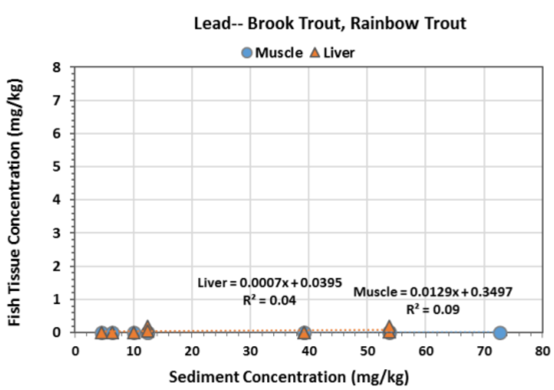
Manganese, Sediment Concentration



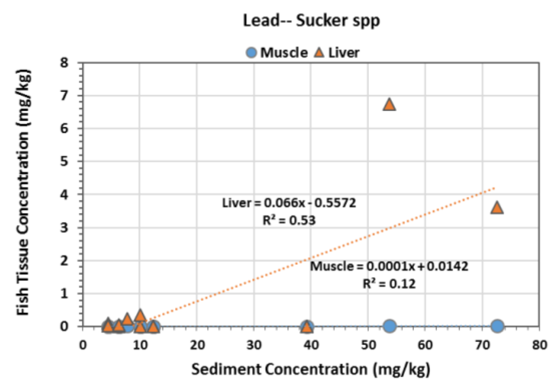
Manganese, Sediment Concentration



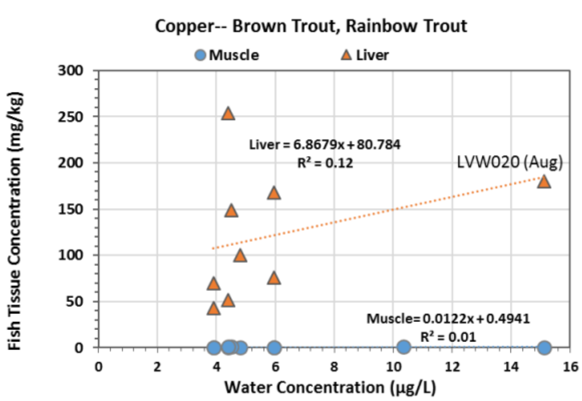
Lead, Sediment Concentration



Lead, Sediment Concentration



Copper, Water Concentration



Copper, Water Concentration

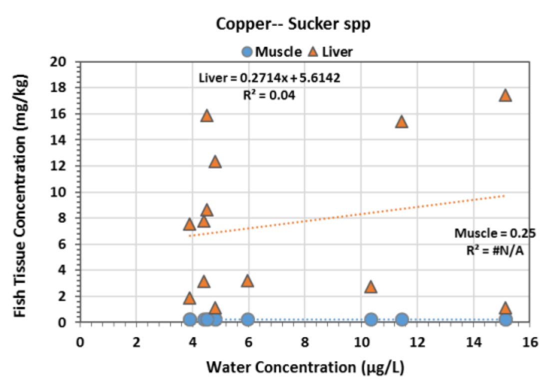


Figure 8.9. Relationship of fish tissue concentration to environmental metal concentrations for brook trout and rainbow trout grouped and bluenose and flannelmouth suckers grouped for 3 metals. The best predictor of fish tissue concentration is shown: sediment concentration for manganese and lead and water concentrations for copper.

8.2.5 Summary of NMDGF fish data

The fish tissue concentrations collected by the NMDGF in the Animas and San Juan rivers in August immediately after the GKM release and again in March 2016 show general patterns of fish response as illustrated in Figures 8.2 through 8.9. These figures explore the influence of specific environmental or biological factors by averaging over the other possible influential factors. These generalized analyses demonstrate that:

- Fish in the Animas River accumulated metals during the period after the GKM release when GKM deposits were documented to be present in sediments and water (approximately 3 weeks);
- Fish in the San Juan River did not generally accumulate metals after the GKM release relative to background;
- Some individuals in the population accumulated metals while many did not;
- Species varied in the amount and type of metals accumulated by individual cohorts;
- Liver tissue had greater concentrations of metals than muscle tissue;
- Some species accumulated more metals than others: the muscle of speckled dace and the livers of bluehead sucker tended to have higher concentrations of metals than other species;
- Tissue concentrations of mercury and selenium were higher in the San Juan River than the Animas

8.3 Comparison of pre- and post-GKM fish tissue metal concentrations in the Animas River among data providers

Fish filet data collected by multiple parties were compared to determine if fish tissue concentrations measured in 2016 changed from the pre- release conditions. Pre-release data were limited to the Southern Ute Indian Tribe fish tissue samples collected from the Animas River on their reservation between RKM 110 and RKM 130 in July 2015. EPA contractors sampled the same locations in Fall 2016 well after GKM release deposits had been mobilized and removed from the river during the previous spring snowmelt (EPA 2016c). EPA contractor sampled filet + skin and SUIT sampled muscle plugs, so some differences due to methods could occur. Fish tissue filets were also sampled from the Animas River in March 2016 at relatively nearby locations soon after the GKM release in Colorado by CPW (see Table 6.1) and in New Mexico by NMDGF, as discussed in section 8.2. The March 2016 sampling of muscle tissue by CPW in Durango at RKM 94 and NMDGF at RKM 147 were added to this analysis to illustrate the general status of metals in fish in March 2016 relative to pre-release conditions documented by the SUIT. Fish tissue muscle concentrations averaging brown and rainbow trout in four datasets are shown in Figure 8.10.

The body burden of 8 metals in the SUIT and EPA trout data from the Animas River were low and within a narrow range in March 2016, except for aluminum. CDPHE documented much higher concentrations of aluminum in muscle than observed in other data sets in March. Metal concentrations were not assumed to be at background conditions in Durango in March 2016 as GKM deposits were still in place in the middle Animas, whereas they had been removed by monsoonal storms from the lower Animas by this time. Aluminum was higher in Colorado while other metal concentrations were similar in CO and NM data given detection limits. The similarity of SUIT and EPA data supports the conclusion that metal concentrations were at background conditions in Fall 2016 after snowmelt in 2016. The NMDGF data at the downstream location were also similar to the SUIT and EPA data.

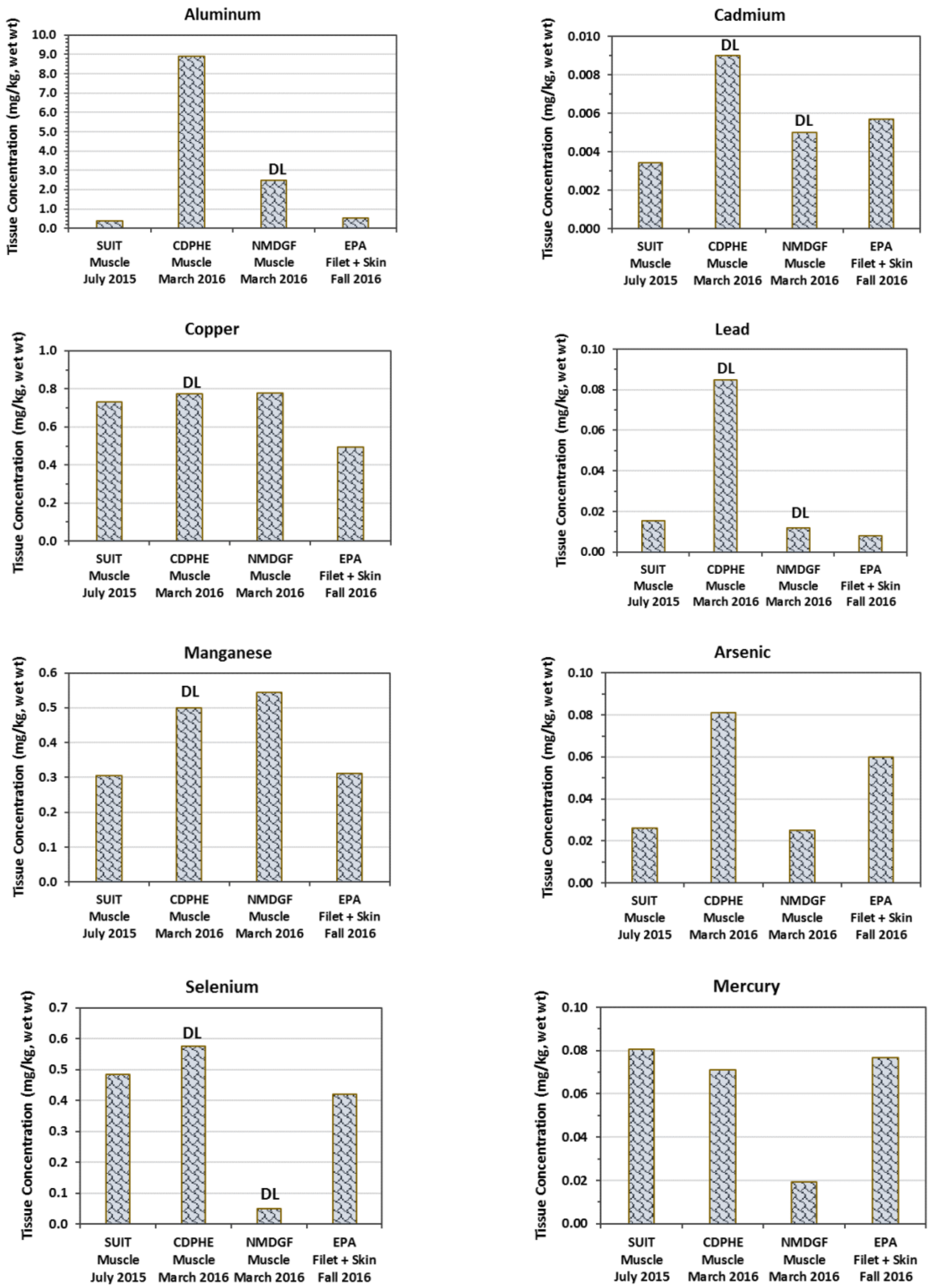


Figure 8.10. Comparison of pre-GKM brown trout and rainbow trout muscle tissue data collected at the Southern Ute Indian Reservation to post-GKM data collected by EPA contractors (Fall 2016), Colorado Department of Public Health (March 2016), and Environment and New Mexico Department of Game and Fish (March 2016). The SUIT and EPA data were collected at the same locations and the CDPHE and NMDFG were sampled approximately 25 km (16 mi) up and downstream, respectively. SUIT data = muscle plugs; NMDGF and CDPHE data = skinless filet; and EPA data = filet + skin. DL indicates samples were below the laboratory detection limit, which are shown as 50% reported detection limit.

8.4 Comparison of post-GKM fish tissue metals concentrations in the San Juan River among data providers

Channel catfish (skinless filet) were sampled in two reaches of the San Juan River by the Navajo Nation EPA (NNEPA) in June 2017. These data were compared to channel catfish collected by EPA contractors (filet + skin) at several locations in the San Juan River in fall 2016 and by NMDGF in the upper San Juan River near Fruitland and Farmington in March 2016 and August 2015 after the GKM event. No channel catfish were collected at the San Juan upstream location at Archuleta or in Farmington at LVW 020 downstream of the Animas confluence. The concentrations of aluminum and trace metals are shown in Figure 8.11 and selenium and mercury are shown in Figure 8.12.

Metals in tissue were generally very low and often less than detection limits. Results were similar among datasets considering differences in detection limits. Metal concentrations in fish tissue were generally lower in the San Juan than the Animas (more discussion on this in Chapter 9). Tissue concentrations collected by NMDGF in the San Juan River in August 2015 after the GKM release were similar to those in fall 2016.

Levels of metals in fish tissue tend to increase slightly from upstream to downstream in the San Juan, including copper, arsenic, selenium, mercury, manganese, and zinc. Other metals are similar along the length of the San Juan River.

8.5 Fish tissue concentrations relative to fish consumption advisory levels

The primary purpose of the CPW, NMDGF, and NNEPA samplings was to report on the status of fish tissue metal concentrations relative to consumption advisories in order to inform recreational and subsistence fishers. Because there is a potential for fish to concentrate metals in their tissue over time, Colorado and New Mexico collected fish again in the spring of 2016.

Colorado detected some metals in the muscles of brown and rainbow trout sampled from the middle Animas River in August 2015 after the GKM release and in March 2016. CPW concluded that all tissue samples fell below risk screening levels for all metals and fish could be consumed without risk. CPW concluded that all tissue samples fell below risk screening levels for all metals and could be consumed without risk. CPW also concluded that all tissue concentrations were within the range of concentrations observed in other Colorado fish datasets and likely represented background levels (CPW 2016).

NNEPA (2017) reported that metals in the tissue of catfish in the San Juan River were below human health consumption screening advisory levels.

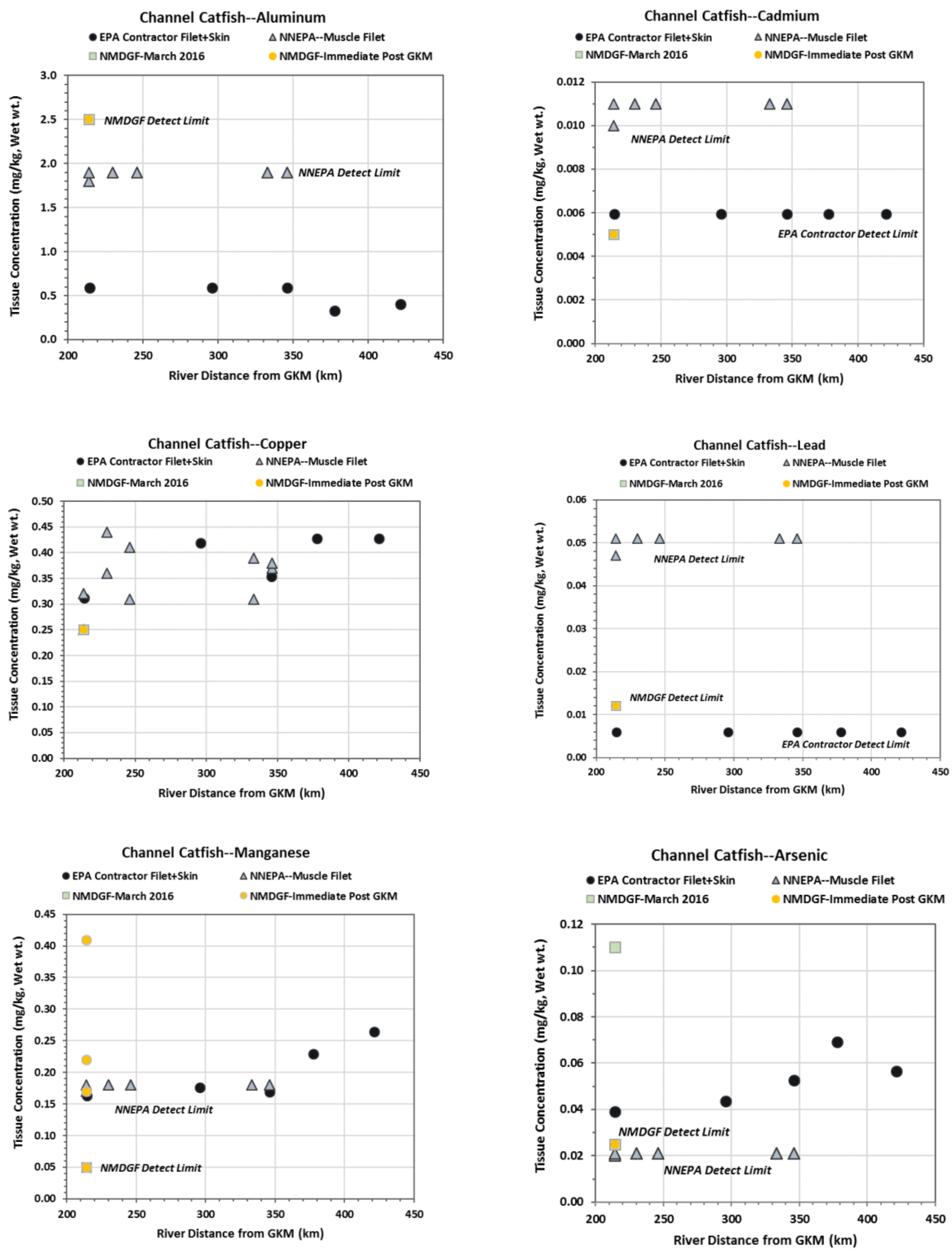


Figure 8.11. Tissue concentration of aluminum and trace metals in channel catfish at multiple locations in the San Juan River. Data were collected by NNEPA and EPA contractors after 2016 snowmelt had returned conditions to background. NMDGF data were collected in August 2015 immediately after the GKM release and in March 2016. DL indicates samples were below the laboratory detection limit, which are shown as 50% reported detection limit.

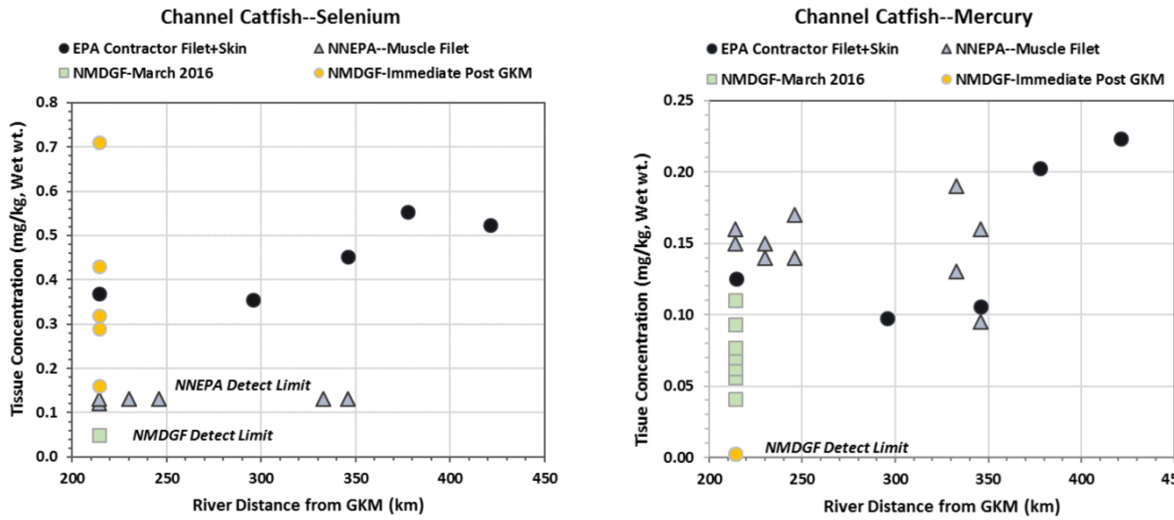


Figure 8.12. Tissue concentration of selenium and mercury in channel catfish at multiple locations in the San Juan River. Data were collected by NNEPA and EPA contractors after 2016 snowmelt had returned conditions to background. NMDGF data were collected in August 2015 immediately after the GKM release and in March 2016. DL indicates samples were below the laboratory detection limit, which are shown as 50% reported detection limit.

8.6 Summary of metals in fish tissue

Fish take up metals from sediment and water through their diet and across their gills. Many metals are essential for growth and survival and are non-toxic at low concentrations; however, some metals are nonessential and toxic and even essential metals are toxic at high concentrations. Mining activities in the headwaters of the Animas and ore processing facilities near population centers have left persistent environmental contamination of metals in the upper Animas River. Watershed scale fish tissue sampling has shown historic and ongoing elevated concentrations of many metals in fish that live in the upper Animas River. Metals contamination affects fish survival and reproductive success in the most impacted reaches within this area.

The GKM release caused a short-term spike in water concentrations and additional loading of metals into the streambed within the already contaminated portions of the upper Animas River. The GKM-release also elevated metals to levels not routinely observed in the lower Animas and San Juan rivers for some period of time after the GKM plume passed through the system. Metals in sediment in the lower Animas River were elevated for three weeks following the release; however, concentrations were much less than those observed in the upper Animas.

Bioaccumulation of metals within the fish community was complex varying by species, metal, and individual characteristics and exposure. Our analysis of fish tissue data collected from lower Animas and upper San Juan rivers showed metals were significantly elevated in bluehead and flannelmouth sucker liver and speckled dace muscle tissue within weeks after the GKM release in the section of the lower Animas River that received GKM metals deposits. The degree of metal accumulation in liver differed by species, sampling location, and among the metals, with aluminum, cadmium, lead and manganese exhibiting the greatest concentrations. Cadmium and mercury in liver tissue and selenium in muscle were greater in the San Juan than the Animas. Tissue samples collected after GKM deposits were cleared from the system the following spring showed liver concentrations had declined to background levels. For the most part, the elevated liver concentrations in 2015 did not translate to elevated muscle concentrations. No fish mortality is known to have occurred because of metals contamination in the Animas and San Juan rivers. There were no fish population data available from the lower Animas River to help us understand if the metal concentrations in fish tissue were sufficiently high to adversely affect the fish populations.

When fish were sampled the following spring and fall in 2016, the concentration of metals in muscle/filet samples were similar to pre-release concentrations and were low throughout both rivers. Metal concentrations in muscle tissue did not exceed human health consumption recommendations.

CHAPTER 9 ONE-YEAR POST-GKM RELEASE: WATERSHED-SCALE LONGITUDINAL TRENDS IN METAL BIOACCUMULATION

EPA benthic macroinvertebrate and fish tissue (filet + skin) sampling produced the only commonly collected and laboratory processed data that characterized metal concentrations in biota through the entire length of the Animas and San Juan rivers. These data were collected by EPA contractors in Fall 2016 and April 2017[6] as part of the EPA's CMP (EPA 2016a). Sampling occurred after June 2016 when GKM deposits were mobilized from the rivers and transported to Lake Powell during snowmelt runoff (EPA 2016c). Thus, the 2016 EPA results do not characterize the full bioaccumulation potential that may have occurred immediately after the GKM release but rather are a better characterization of background conditions. Results from this sampling event can be used to inform future tissue sampling study objectives.

Table 9.1. Geometric mean metal concentration +/-1SD in benthic macroinvertebrate composite samples collected in fall 2016 and fish filet samples (ppm dw) collected from the Animas and San Juan rivers in fall 2016 and spring 2017.

	Animas River		San Juan River	
	geometric mean (± 1SD)		geometric mean (± 1SD)	
Benthic Macroinvertebrates				
Aluminum	7,919	± 4754	5,198	± 2,900
Arsenic	4.8	± 5.1	3.2	± 1.7
Cadmium	2.1	± 3.6	0.4	± 0.4
Copper	60	± 51.5	21.1	± 3.9
Iron	10,997	± 14,369	4,058	± 2,366
Lead	49	± 184.9	3.6	± 2.0
Mercury	0.021	± 0.01	0.11	± 0.05
Manganese	1,924	± 2,389	223	± 187
Nickle	6.6	± 5.7	3.5	± 1.7
Selenium	1.4	± 0.6	2.7	± 0.7
Zinc	760	± 822	112	± 25
Fish Filet				
Aluminum	2.1	± 0.6	2.9	± 5.1
Arsenic	2.1	± 1.0	2.0	± 0.6
Cadmium	0.02	± 0.04	0.02	± 0.01
Copper	1.9	± 0.8	1.8	± 1.0
Iron	21.4	± 8.0	24.3	± 20.0
Lead	0.03	± 0.06	0.03	± 0.02
Mercury	1.4	± 1.2	1.1	± 0.9
Manganese	0.2	± 0.3	0.7	± 0.2
Nickle	0.05	± 0.01	0.05	± 0.03
Selenium	1.8	± 0.4	1.8	± 0.4
Zinc	36.0	± 29.9	29.8	± 5.7

Monitoring one-year post-GKM demonstrated a continued strong longitudinal gradient of many metals in sediment, water, and biota within the Animas River, extending from the mining district to the confluence with the San Juan River. Metals measured in benthic macroinvertebrate composite samples and fish filet from the EPA 2016/17 sampling are shown in Figures 9.1 and 9.2 with sediment and water concentrations from samples collected in 2016 (February through November 2016). These longitudinal trends are consistent with the pre-release data presented in Chapter 2.

Sediment and water metal concentrations generally decline from high values observed in the impacted mining district by two to three orders of magnitude by the time the Animas joins the San Juan River. In the Animas River, sediment metal concentrations were frequently greater than concentrations in water. Metal concentrations in the San Juan River are typically less than the Animas and are similar through the length of the river. In the San Juan River, metals in sediment were frequently equal to or less than total water concentrations, which are strongly influenced by episodic stormflow and sediment loads (EPA 2016c).

Like metals in sediment and water, EPA 2016/17 benthic macroinvertebrate and fish tissue data in the Animas River showed a systematically declining pattern for many metals, including arsenic, lead, copper, manganese, and zinc, as seen in earlier datasets (Figure 9.1; Chapter 2). Fish and benthic macroinvertebrates living in the upper Animas River experience persistently higher metal concentrations in

[6] EPA was unable to collect fish tissue samples from the lower Animas River and upper San Juan river sites during the fall sampling (October and November 2016) and therefore collected remaining fish tissue samples the following spring in April 2017.

their environment and generally have higher metal body burdens (Figures 7.8 and 7.9). Metals in benthic macroinvertebrate tissue had concentrations more similar to sediment than water. This trend is logical since the benthic composite samples were analyzed with the gut content and casings, when applicable, and therefore also contained some sediment. Metal concentrations in fish filet were consistently less than benthic macroinvertebrate concentrations (Table 9.1). Measuring metals in fish filet provide a meaningful measure of exposure to human consumption of fish, but do not reflect the total body burden given the sequestration of metals to different organs that are not typically consumed (e.g., liver; see Chapter 8).

Metals that did not follow the general declining spatial gradient include aluminum, selenium, mercury, and nickel (Figures 9.2). Aluminum in benthic macroinvertebrates and sediments was consistently high throughout the Animas and San Juan rivers. Total aluminum in water was variable with the lowest total aluminum concentrations in water measured near 100 RKM and the highest concentrations were measured in the San Juan River. Selenium concentrations in benthic macroinvertebrates slightly increase with the distance traveled downstream (Figure 9.2). The mean selenium concentration in benthic tissue collected from the Animas River downstream of GKM was less than the San Juan (Table 9.1). The greatest nickel concentrations in benthic macroinvertebrate tissue, on the other hand, were sampled from the middle Animas locations, starting just upstream of Durango and into the Southern Ute reservation (71 through 123 RKM). Mercury in benthic macroinvertebrate and fish tissue clearly increased with distance downstream of GKM, with greater concentrations observed in the San Juan than the Animas River (Table 9.1). Similar to the middle Animas sampling locations 9426 (76.8 RKM) and Oxbow Park (90 RKM; see Chapter 7.2), benthic macroinvertebrates collected from lower Animas site ADW-022 (148 RKM) had elevated concentrations of many metals (As, Cu, Fe, Pb, Zn) when compared to the nearest upstream and downstream sampling locations.

At a site scale, there was also wide variation in metal concentrations in fish tissue. This variation may be partially due to annual variation in water and sediment concentrations or differences among species-specific bioaccumulation rates. Fish tissue concentrations of the more soluble metals such as zinc, and cadmium show affiliation with both sediment and water (Figure 9.1). The historic Bureau of Reclamation La Plata Project reported fish tissue concentrations with the same general longitudinal pattern as the EPA filet samples (Figures 2.5 – 2.8).

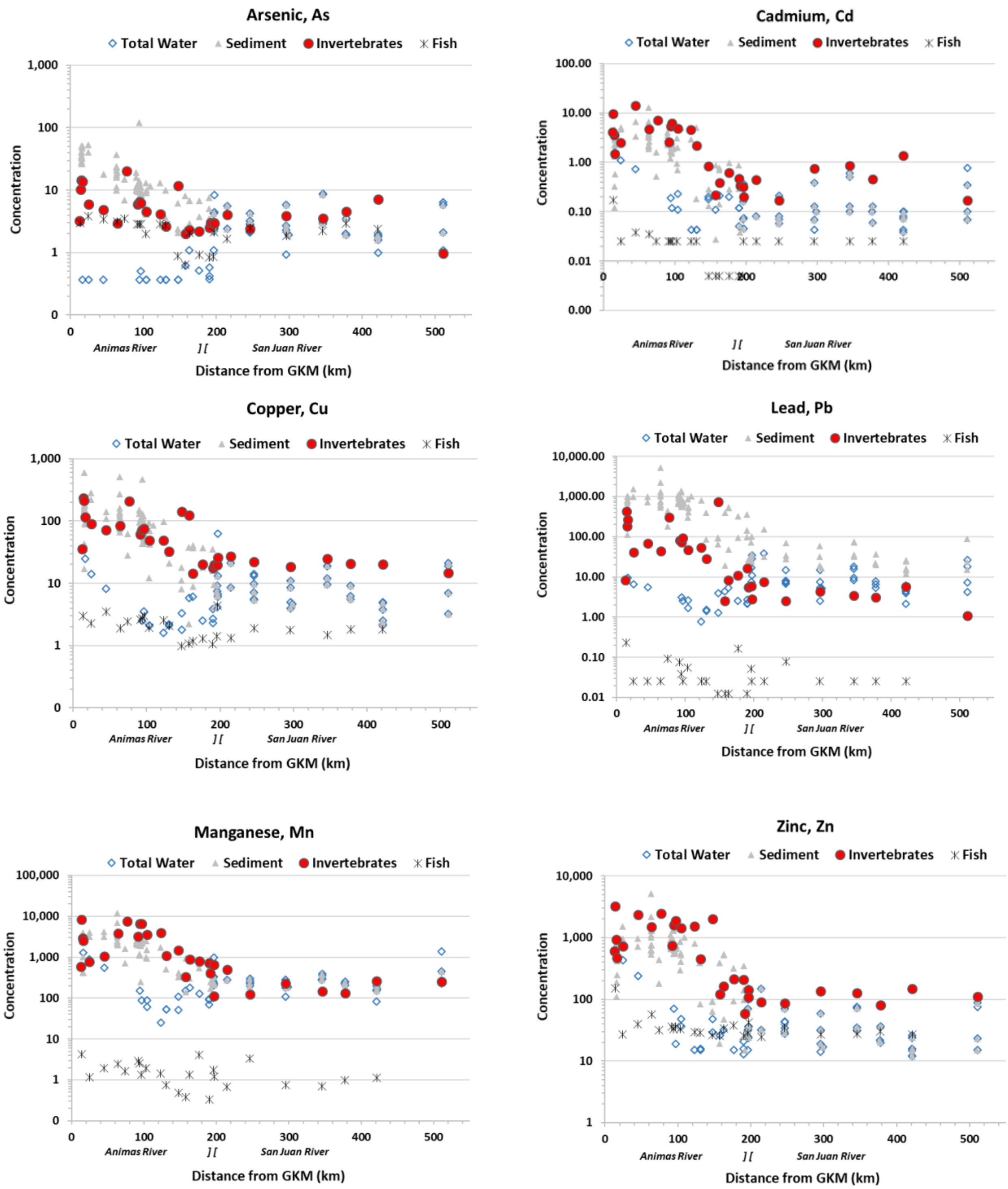


Figure 9.1 Concentration of 2016 arsenic, lead cadmium, copper, lead, manganese and zinc in benthic macroinvertebrate and fish filet (ppm dw), sediment (ppm dw), and dissolved water (ppb) with distance from GKM (km). Fish filet data points are the average of the individual fish collected at the site.

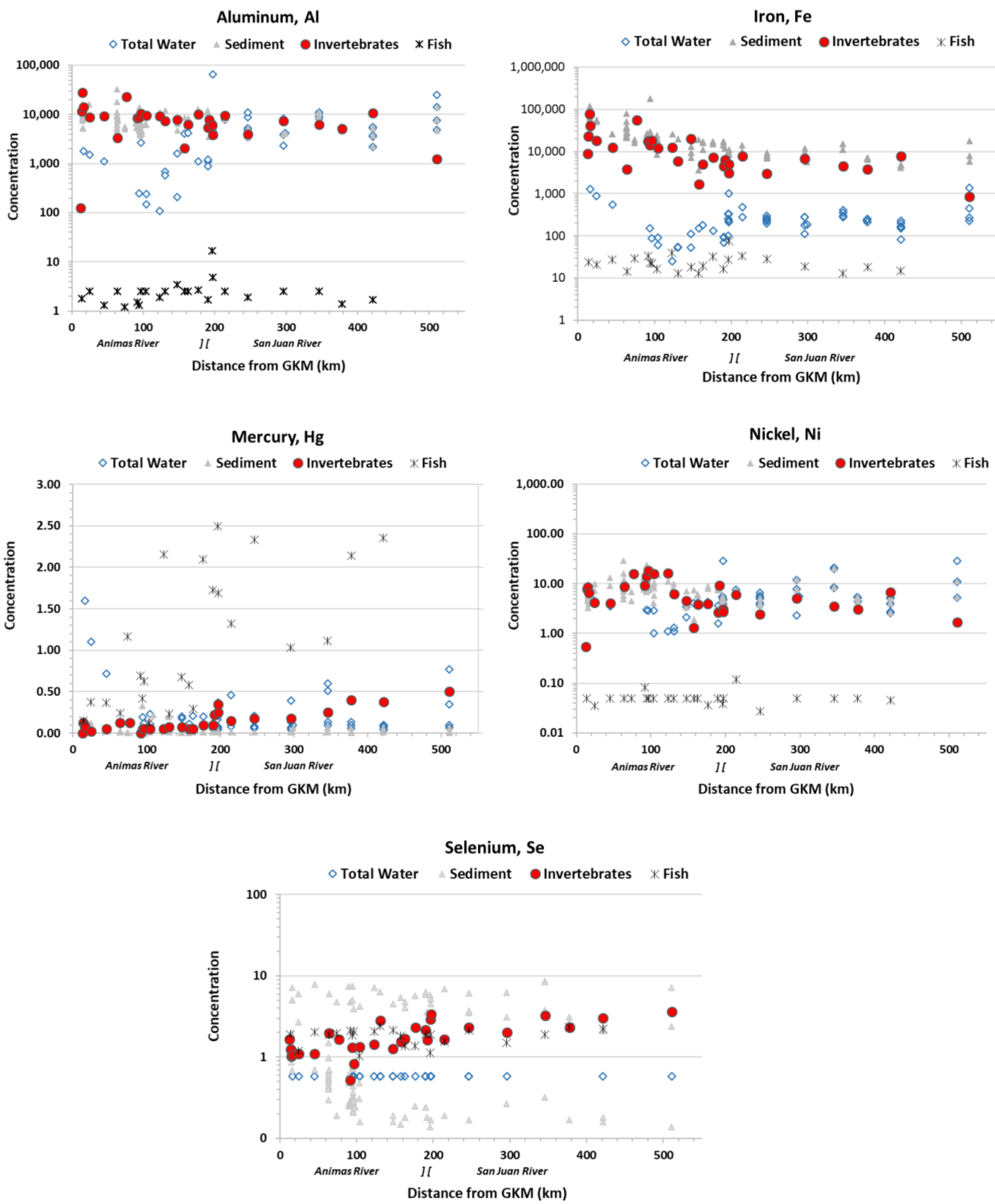


Figure 9.2 Concentration of 2016 aluminum, iron, mercury, nickel, and selenium in benthic macroinvertebrate and fish filet (ppm dw), sediment (ppm dw), and dissolved water (ppb) with distance from GKM (km). Fish filet data points are the average of the individual fish collected at the site.

CHAPTER 10 SYNTHESIS AND DISCUSSION

In this report, EPA complied biological data from multiple sources to evaluate how the aquatic community, populations, and metal sequestration in tissue responded to the GKM release. The sampling and analysis approach were designed to address the following main questions:

- Were there changes in the abundance and diversity of macroinvertebrates and fish after the GKM release?
- Did the biota take up metals associated with the release? If so, have concentrations returned to background conditions?

Historic monitoring and assessment efforts have identified pre-existing adverse impacts to water quality, sediment quality and biological communities in this watershed. New data collected post-GKM release were compared to pre-release biological data and were evaluated with respect to the near-term biological conditions (days to weeks following the release when GKM deposits were still present) and the long-term biological conditions a year after the release (after deposits moved through the system).

10.1 Animas River aquatic community response

The Animas River is one of many rivers in the western US that is impacted by historic mining and ongoing acid mine drainage. There is a longitudinal gradient in metal concentrations and biological condition from the headwaters downriver reflecting the persistent contamination in the upper Animas River. The upper Animas River (Silverton to Baker's Bridge) experienced the highest metal concentrations during the GKM plume, the greatest number of water quality criteria excursions, and the greatest deposition of sediment immediately following the plume. Although the majority of the release material was initially deposited in the upper Animas, the deposits were present for a short duration (approximately 8 months), the quantity was not large compared to legacy contamination, and concentrations were similar to what they had been before the release (EPA 2016c, Rodriguez-Freire 2016).

For the upper Animas River, metal concentrations in benthic macroinvertebrate tissue were high in the pre-release dataset and continue to be high following the GKM release (Chapter 7). A significant increase in copper and decrease in manganese concentrations in benthic macroinvertebrate tissue were observed in post release samples in 2015. A year later in fall 2016, most metals were elevated in benthic macroinvertebrate tissue when compared to the 2014 pre-release and the 2015 post-release concentrations, yet the 2016 concentrations were similar to concentrations observed in 2012. The high concentrations in benthic macroinvertebrate tissue in 2016 were also observed in the upstream and tributary samples suggesting that something other than the GKM release contributed to the concentration change. Differences in field collection methods, analytical methods, and the benthic community composition likely contributed to the variability observed between the 2012/2016 and 2014/15 sampling years.

Historic biological monitoring conducted in this portion of the watershed over the last several decades has established that the upper Animas downstream of the confluence with Cement Creek, Cement Creek and several tributaries with historic mining impacts support limited benthic macroinvertebrate and fish communities because of the poor water quality. Sensitive species that would be expected to respond to the GKM release were historically extirpated from this section of the upper Animas leaving only some of the most metal tolerant aquatic life and life stages present when the release occurred. Therefore, no clear differences in the aquatic community structure were observed in the pre- and post-GKM release biological data in the upper Animas River.

The benthic macroinvertebrate and fish populations in the Animas River generally improve in the middle reach (below Baker's Bridge to the Southern Ute Indian Tribe-New Mexico border) and lower Animas river (New Mexico reach). These reaches support more sensitive taxa that are not found in the upper

Animas. As the GKM release moved through the river and approached the middle Animas river, both dissolved and colloidal/particulate metal concentrations declined rapidly as chemical reactions and hydraulic processes diluted, transformed, and deposited material. This resulted in fewer excursions of water quality criteria and concentrations that are less likely to adversely affect aquatic biota. Therefore, we did not observe a loss of or change in the more sensitive invertebrate and fish taxa at the downstream locations, and for some metrics, a slight improvement was observed a year following the event. With regard to the individual benthic macroinvertebrate metrics, %EPT was not significantly different between the pre- and post-GKM release time periods; however, total taxa were significantly different, with the post-GKM release time period showing greater overall total taxa compared to the pre-GKM release (Chapter 5). Additionally, localized high metal concentrations in post-release benthic tissue were observed in the middle Animas; however, these sites were not consistent among years (Oxbow Park in 2015 and 9426 in 2016) and did not have pre-release data to be able to understand if the high concentrations were the result of the GKM-release (Chapters 7 and 9).

Our analysis of the 2015 post-GKM release fish data collected by CPW from the Animas River near Durango (Chapter 6) agrees with existing state analyses, reports, and press announcements that conclude fish were not exposed to acutely toxic concentrations (CPW 2015, NMDGF 2015). Fish populations near Durango, including stocked trout and native species, were at historic highs one month following the GKM release. Trout biomass, density, quality and population demographics were similar or increased relative to those in the previous year, a result that may have been influenced by weather and water conditions that year as well as reduced angling after the GKM event (CPW 2015).

CDPHE and NMDGF collected fish tissue samples when the maximum effect of the GKM release was observed (August 2015) and the following spring (March 2016). CDPHE's sampling focused on trout filet samples in the Durango area. CDPHE (2016b) reported that tissue concentrations of Al, As, Cu, Mn, Ni, and Zn were detected in brown and rainbow trout in August 2015, although levels were well below fish consumption screening levels. GKM deposits remained in the middle Animas river through the winter. In March 2016, most metals were less than the detection limit in both species, except for aluminum. Aluminum concentrations were greater in March 2016 than August 2015 (immediately following the release), yet continued to be well below consumption screening levels.

Questions have remained about the potential long-term impacts to fish reproduction and larval fish that were exposed to the plume since larval fish are typically more sensitive to toxics, fish shocking techniques do not assess larval life stages, and larval fish mortality may not be as visually apparent as an adult fish kill. Our review of the 2016 fish abundance data for naturally reproducing species in the middle Animas River (suckers and sculpin) shows that populations are within the normal range; however, it can be challenging to determine if a specific life stage is impacted when the species has a long-life expectancy. The maximum life expectancy for the bluehead and flannelmouth suckers is relatively long (25 years) compared to the mottled sculpin (6 years; Page and Burr 2011). Additional monitoring would be needed to address questions related to potential adverse effects to younger life stages and the reproductive output of the naturally reproducing populations.

The lower Animas River (New Mexico segment) had limited historic biological data to support a pre- and post-GKM release analysis. No fish population data were identified and the pre-release benthic macroinvertebrate data was limited to one event in 2009 at ADW-010 (163 RKM). Furthermore, pre-release concentrations of metals were well characterized in the water, sediment, and biota in this downstream segment of the river. Due to these data limitations, we were unable to determine if the aquatic community changed in response to the release.

Although the pre-release data were limited for this segment of the river, the NMDFG fish tissue sampling design for the lower Animas and upper San Juan rivers provided meaningful statistical comparisons of near-term release and long-term metal bioaccumulation following the release by including multiple species, replicates, tissue types (muscle and liver) and sampling dates. Samples were collected in August 2015 and the following spring (March 2016) after the sediments deposited in the lower Animas were removed by a monsoonal storm event. Monitoring showed that sediment concentrations declined to background levels after this storm and remained generally low through the winter months. Metals were significantly elevated in bluehead and flannelmouth sucker liver and to a lesser extent in muscle tissue during August 2015 in the lower Animas River at locations with elevated GKM metals in water and sediment. For the most part, however, high concentrations in the liver did not translate to high concentrations in the muscle. When fish were sampled the following spring in March 2016, the concentration of metals were low throughout both rivers. Additionally, the body burdens of 8 metals in the pre-release (SUIT) and post-release EPA fish data taken at the same locations were similar despite differences in methods, supporting the conclusion that biological conditions were at background conditions in fall 2016. The CDPHE and NMDGF and data at the up and downstream locations were also very similar to the SUIT and EPA data.

NMDGF (2015) expressed particular concern about acute effects from copper, due to the importance of this metal in contributing to toxicity in the upper Animas (Besser *et al.* 2001; Besser and Leib, 2007; EPA 2015). Copper concentrations in sediment were significantly elevated in the lower Animas immediately after the GKM event, but were less than historic concentrations measured in the upper Animas. Copper and cadmium fish tissue concentrations were similar between fall 2015 and spring 2016 in the lower Animas, and always had higher concentrations in the liver than in the muscle. Copper concentrations in the livers of brown trout were comparable to those reported for brook trout in the upper Animas River in Besser *et al.* (2001) and Besser *et al.* (2007). Copper concentrations were also elevated in the livers of flannelmouth and bluehead suckers (Chapter 8). Copper was not generally detected at high levels in macroinvertebrates collected from the lower Animas in August following the release in 2015 and the following spring in 2016. However, concentrations observed at two lower Animas locations in 2016 were similar to concentrations measured in the upper Animas River (Chapter 9). The NMDGF data highlighted that metals are not uniformly taken up within a fish population and the macroinvertebrate community when metal concentrations increase over a short duration. There were no fish population data available from this section of the Animas River to help us understand if the metal concentrations in fish tissue were sufficiently high to adversely affect the fish populations.

10.2 San Juan River aquatic community response

The GKM release had the potential to affect the San Juan aquatic community through two different avenues. There were potential direct toxic and physical effects resulting from the metals in the plume itself (e.g., mortality and metal avoidance), as well as indirect physical changes due to the closure of the irrigation canals and the additional water release from the Navajo Dam (e.g., increase in flow, temperature changes, increased suspended sediment). With respect to direct effects, by the time the GKM plume reached the confluence with the San Juan River, total metal concentrations had declined by three orders of magnitude from what they were when the plume entered the Animas because of the combined effects of the dilution, chemical reactions, and deposition. The excursions of aquatic life water quality criteria in the San Juan River were limited to metals that are naturally high in the sediment and water based on monitoring data upstream of the confluence, making direct GKM related toxic effects unlikely. With respect to indirect effects, the dam release was intended to mitigate the impact of the GKM plume and contributed to the lower metal concentrations. This release increased flow and suspended sediment for several days, yet intermittent high flow events in response to precipitation are normal in the San Juan. The temperature changes in the San Juan upstream of the Animas River confluence associated with the dam release was not normal. USFWS (2016) suggested this may have contributed to fish movement downstream.

Metals in fish tissue samples collected from the San Juan River by all data sources (EPA, NMDGF and NNEPA) were generally very low and often less than detection limits, consistent with the concentrations observed in the water and sediment. Results were similar among datasets considering differences in detection limits. Metal concentrations in fish tissue were generally lower in the San Juan than the Animas. Tissue concentrations collected by NMDGF in the San Juan River immediately after the GKM release were similar to those in fall 2016.

The robust fish population dataset collected by the FWS in the San Juan River showed that fish abundance in 2015 and 2016 was generally within pre-release norms. The exception to this was the abundance of bluehead sucker, flannelmouth sucker and speckled dace in the middle reaches of the San Juan in both 2015 and 2016. There are many possible explanations for the suppressed abundance of these species. Their low post-event abundance coincided with historically high populations of predator/competitor species (i.e., razorback sucker, Colorado pikeminnow and channel catfish). The San Juan River downstream of Navajo reservoir is managed for the recovery of the razorback sucker and Colorado pikeminnow, including annual stocking and removal of non-native fish.

We cannot say if the combined changes in the physical and chemical conditions that occurred as the plume move through the San Juan River contributed to abundance changes in certain fish species; however, the aquatic life water quality criteria excursions during the GKM event were limited and the sediment suspended in the river due to the increased flow was similar to a moderate-sized storm event. It is more plausible that a combination of ecological and physical interactions, and/or fisheries management actions contributed to the observed changes, rather than a result of the GKM release.

10.3 Watershed scale bioaccumulation of metals

The EPA 2016 biological sampling was the first effort to obtain biological data that covered the entire Animas and San Juan rivers in a single sampling event with consistent sampling methods, which allowed us to evaluate watershed-scale longitudinal patterns in bioaccumulation. The wide range and systematic declining pattern of background metals in the Animas River establishes an underlying stressor gradient on biological communities. Metals measured in benthic macroinvertebrates and fish tissue samples generally followed the systematic declining pattern observed in water and sediment collected after the GKM release. These results suggest that tissue concentration of many of metals are in an equilibrium with their prevailing environmental concentrations. The highest metal concentrations in tissue were observed in the upper Animas and the lowest concentrations were observed in the San Juan. The exceptions to this pattern include aluminum, selenium, mercury and nickel. Aluminum concentrations are elevated in sediment and benthic macroinvertebrate tissue throughout the Animas and San Juan rivers and are consistent in fish tissue. Greater selenium concentrations were measured in benthic macroinvertebrates sampled from the San Juan River when compared to the Animas River. We were unable to determine whether metal concentrations in tissue were predominately influenced by water concentrations, sediment concentrations, or dietary exposure.

10.4 Future monitoring considerations

10.4.1 Sampling and analytical considerations

Our ability to conduct a watershed-scale analysis with data collected by all data providers was limited by the different sampling and analytical methods and revealed the need for a consistent sampling approach. This was especially true for studies focusing on bioaccumulation of metals. Different study objectives (e.g., human health vs. ecological risk) will define the parameters measured and type of tissue sample that were collected, resulting in datasets that are challenging to compare. A fish tissue study evaluating human health risks will target fish tissue samples that represent the portion of the fish that is typically consumed. Although this sounds straight forward, sampling efforts to address this objective have included three different types of tissue samples: filet, filet with skin and muscle plugs. There is limited literature that address how the retention of the skin in the laboratory analysis will influence the result, but there is reason to believe that the two samples are not directly comparable. The small amount of tissue collected with a muscle plug can limit the analytes and types of analyses creating data gaps when compared to other dataset, yet this sampling approach represents the only non-lethal technique available.

Measuring metals in the whole fish and/or liver provides meaningful data to evaluate ecological risk. Our review of the fish liver data collected by NMDGF showed that liver samples provide more information on metal bioaccumulation than muscle samples since it is more responsive to environmental conditions (Chapter 8); however, liver data are not directly comparable to or useful for studies that are focused on human health concerns. Additionally, the bluehead and flannelmouth suckers were generally more responsive to metals than trout and accumulated Pb and Al in their livers. These fish species are not typically targeted for tissue monitoring given the human consumption study objectives. If ecological risk, either to the fish or wildlife continues to be a question of concern, researchers should consider expanding the target species and the collection of additional tissue types (i.e., liver or the remaining carcass to estimate whole body concentrations).

Benthic macroinvertebrate tissue studies have equally variable sampling techniques making it challenging to compare data from different sources. The collection of sufficient benthic macroinvertebrate tissue sample for metal analysis can be labor intensive, requiring several hours from the field crew members to collect 2 or more grams of tissue typically required by the lab. This is especially true when the macroinvertebrate community is limited to begin with or dominated by small individuals (e.g., early instars, chironomids). Although laboratories are able to accommodate small tissue samples with micro digestion techniques, this introduces method variability into results and can increase method detection limits.

When caddisflies are present at a site, the study objectives will determine if the field crew should remove the external casings prior to sample shipment to the lab. There are reasons to support both sampling methods. Fish will typically consume the caddisfly larvae with their casings, which support the retention of the case in the analysis; however most of the casing materials are not biologically available and will pass through the fish without accumulating in the tissue. The potential for the case to increase the exposure to the fish is not well understood. The acid digestion techniques for a tissue sample are weaker than those used to digest a sediment sample. If sediments are present, in the form of casings or the gut of the organism, this will typically result in greater metal concentration because of partial digestion of the sediments. If the concentration of metal in the tissue is a future area of focus, one would want to consider incorporating a gut content purge prior to analysis so the measurement represents metal assimilation (Sola and Prat 2006).

We received a mix of tissue metals data that were expressed as wet weight (ww), dry weight (dw) and "as received", which was treated equivalent to a wet weight. It is common to report fish tissue results as a ww concentration when evaluating human health concerns and dw when evaluating ecological risk. It is simple to convert a sample from ww to dw or dw to ww if the sample moisture content is reported (Chapter 3.4.6). Moisture content is measured by oven drying the sample at 60°C until constant weight is recorded. It typically requires additional tissue sample for analysis and must be planned for when determining the total mass of tissue needed for laboratory analyses. When the moisture content is not reported by the lab, it is common to estimate the moisture content in fish filet samples since they typically range from 70-80% moisture (30-20% solids), regardless of size and fish species (Lusk 2005, EPA 2016d).

The moisture content of benthic macroinvertebrate samples, on the other hand, is more variable and challenging to estimate. This is especially true when significant differences in taxa are observed between sampling locations. A benthic assemblage dominated by soft bodied organisms (e.g., tipulids, nematodes, chironomids) will have a different moisture content than an EPT dominated assemblage. This change in benthic assemblage composition likely contributed to the wide range of percent solid measurements observed in the study area (Animas River mean percent solid = 23.4%, range = 15.9%-33.5%; San Juan River mean percent solid = 14%, range = 8.5%-22.7%). We minimized the uncertainty in introduced into our analysis by using the percent solids measured from a sample collected from the same location on a different date, or the mean percent solids observed in the watershed, depending on data availability.

In summary, the accumulation of metals in benthic macroinvertebrates and fish is notoriously variable as noted in this report, the many historic studies in the Animas River (Besser *et al.* 2001; Anderson 2007, Besser *et al.* 2007, Besser and Leib 2007; EPA 2015) and in other mining impacted streams (Mebane *et al.* 2012, Cadmus *et al.* 2016, Mebane *et al.* 2017). Complexity results from many aspects of metals exposure and response, including spatial distribution of metals, differences among macroinvertebrate and fish species in reacting to metals, and differences among individual uptake within a species. The NMDGF data highlighted that metal bioaccumulation is not consistent within a fish population and a macroinvertebrate community when metal concentrations increase over a short duration. Some individuals and taxa accumulated metals while many did not.

10.4.2 Opportunities for future watershed-scale monitoring and analysis

The extensive post-GKM monitoring program conducted throughout the Animas and San Juan rivers by EPA, states and tribes revealed patterns of metal concentrations that were not comprehensively studied. EPA (2016c) highlighted the importance of seasonal monsoonal storms that typically occur in late summer in the lower Animas and San Juan rivers. These storms can generate high concentrations of dissolved and particulate metals. Relative metal concentrations are consistent with the local lithology of the contributing watersheds and the mass of metal is consistent with sediment loads (EPA 2016c). Aquatic life water quality criteria are exceeded at times during snowmelt and monsoonal rain events. The relative role of these events on the condition of the aquatic community compared to other stressors in the watershed is not well understood. Future monitoring of these segments may improve the understanding of the sources and importance of metals to aquatic biota during these events.

Aluminum is one of the metals measured at high concentrations during snowmelt and storm events. It was also a major component of the GKM release and contributed to the majority of aquatic life water quality criteria excursions that were observed during the plume (EPA 2016c). Excursion frequency differed by location in the river and by the state or tribal water quality criteria used in the analysis. EPA published draft updated aluminum aquatic life ambient water quality criteria for freshwaters in July 2017 that takes into account the latest scientific knowledge regarding aluminum toxicity to aquatic life (EPA-822-P-17-001). The draft criteria are modified by the ambient water quality parameters that are known to influence metal bioavailability. The more bioavailable the aluminum is, the more likely it is to cause a toxic effect. The water quality parameters that have the greatest impact on aluminum's bioavailability are pH, DOC, and hardness.

- pH: a low pH generally makes it easier for aluminum to be dissolved, and therefore more bioavailable. At higher pH, aluminum speciation changes make it more bioavailable.
- DOC: higher dissolved organic carbon reduces the bioavailability of aluminum because it binds to form aluminum complexes.
- Hardness: higher hardness values mean there are more ions present that compete with aluminum. This makes aluminum less bioavailable.

Longitudinal analyses presented in Chapter 9 identify that aluminum is consistently high in the water, sediment and benthic macroinvertebrate tissue throughout the Animas and San Juan rivers. States and tribes may want to consider reviewing and updating their aluminum aquatic life criteria following the publication of a final criteria recommendation to aid in the analysis of future aluminum data.

This report identified a number of metals and biological datasets relevant to the biological condition of aquatic communities in the Animas and San Juan rivers. These data include extensive research and studies on metals in the headwaters of the Animas River conducted by multiple organizations to establish resource status and inform management decisions and the extensive water, sediment, and biological monitoring conducted over the two years following the GKM release in August 2015. These studies have established a general pattern of persistent metals impacts on biota in the rivers, especially in the upper Animas River, and identified general response to the relatively short-term GKM release. The robustness of pre- and post-event analyses of biological datasets collected by different organizations for different purposes was limited due to documented and undocumented differences in methods.

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APPENDIX A: SAMPLING LOCATIONS AND ASSOICATED SAMPLING IDENTIFICATIONS

Appendix A. Sampling Sites: the description, location and additional sampling locations sampled by other organizations that are identified under the GKM_ID as sites that are similar for pre- and post-GKM release comparisons of biological data. * identifies sites that act as background/reference for the release.

Location (GKM_ID)	Associated Location(s)	Site Organization	Latitude	Longitude	Description/Location	USGS gage available?
CC48	CC48	EPA - superfund	37.818115	-107.661678	Cement Creek upstream from Animas at gage	Y
	09358550 (gage)	USGS	37.819984	-107.663275		
	323	CORW				
	CC49	CDPHE	37.80999	-107.66069	Cement Creek at confluence	N
	CC0001	ARSG				
A68*	A68	EPA - superfund	37.810983	-107.65936	Animas River updtream of Cement Creek: 14th Street Gauge @ 13th Street Bridge	Y
	09358000 (gage)	USGS	37.811202	-107.659167		
	103	CORW				
M34*	M34	EPA	37.802921	-107.672724	Mineral Creek just upstream of the Animas River	Y
	09359010 (gage)	USGS	37.8028	-107.6722		
A72	A72	EPA	37.790017	-107.667536	Animas River access from Road 31 in Silverton, CO	Y
	09359020 (gage)	USGS	37.79027	-107.667578		
	82	CDPHE			Animas River at gauge below Silverton	
	3611	CORW				
	3517	CPW			upstream end of Animas River #3	
A73	A73	EPA	37.72215833	-107.6548278	Animas River upstream of Elk Creek; access from railcar B	N
	3442	CORW				
	3516	CPW	37.72643	-107.65517	Animas River at Elk Park, approximately 200 m upstream of Elk Creek; middle of Animas River #3	N

Location (GKM_ID)	Associated Location(s)	Site Organization	Latitude	Longitude	Description/Location	USGS gage available?
A75D	A75D	EPA	37.59793424	-107.7753268	Animas River upstream of Cascade Creek; access from railcar B	Y
	3438	CORW			Animas upstream of Cascade Creek	
	09359500 (gage)	USGS			Animas River at Tall Timbers	
	3515	CPW	37.59996	-107.77032	Animas River, below Crazy Women Gulch	
	Teft Spur	CPW			downstream site on Animas River #3	
Bakers Bridge	Bakers Bridge	EPA	37.455731	-107.801095	Animas River at Bakers Bridge 20 miles south of Silverton	N
	GKM02	EPA	37.454134	-107.801601		
	88	CORW				
	81	CDPHE	37.45871	-107.79915		
James Ranch	James Ranch	MSI	37.417822	-107.814819	Animas River at James Ranch	
9426		EPA	37.385148	-107.836946		
	9426	CDPHE	37.38506	-107.83686	Animas River near Trimble Bridge downstream of Hermosa Creek	N
	89	CORW				
Oxbow Park	Oxbow Park *sediment only*	EPA	37.309037	-107.855714	Animas River at Oxbow Park	N
		MSI				
32nd St Bridge	32nd St Bridge	EPA	37.294805	-107.870469	Animas River near Bridge at 32nd Street in Durango	N
	371759107520601	USGS	37.299991	-107.868199		
	3577	CORW				
Animas-Rotary Park	Animas-Rotary Park	EPA	37.280534	-107.876622	Animas River at Rotary Park	Y
	09361500 (gage)	USGS	37.280718	-107.876927		
	91	CORW				
	3576	CORW				
	CORIVWCH_WQX-91	RCWWN	37.27932	-107.87966	ANIDURCO	
Above Lightner	Above Lightner	MSI	37.26892921	-107.8862952	Animas River upstream of Lightner Creek	
GKM05	12150	CPW	37.274429	-107.88454	Animas River at DHS pedestrian bridge to 9th Street, approximately 350 m downstream of Rotary Park (this could go with GKM05)	
	GKM05	EPA	37.268704	-107.885857	Animas River under bridge at corner of US 550 and US 160	Y
	09361500 (gage)	USGS				
	9418	CDPHE				
	9423A	CDPHE	37.2745	-107.8843		
AR19-3	AR19-3	SUIT	37.2213842	-107.854161	Animas River at the Southern Ute Boundary	Y
	Purple Cliffs	EPA			Animas upstream of the Southern Ute Boundary	

Location (GKM_ID)	Associated Location(s)	Site Organization	Latitude	Longitude	Description/Location	USGS gage available?
	09363500 (gage)	USGS			Animas River at Cedar Hill	
	GKM01	EPA	37.221297	-107.859598	Boat launch under River Rd. Bridge	
	3.71319E+14	USGS			Animas upstream of the Southern Ute Boundary	
	3590	CORW			Animas upstream of the Southern Ute Boundary	
	92	CDPHE	37.221542	-107.859455	Animas upstream of the Southern Ute Boundary	
	NAR1	SUIT			Animas River at the Southern Ute Boundary	
	10245	CPW			Animas River at Purple Cliffs	
AR16-0	AR16-0		37.187031	-107.869928		
	Animas 1		37.187051	-107.878685		
	Animas @ Basin Creek		37.185	-107.87833		
AR7-2	AR7-2	SUIT	37.084992	-107.878383	Animas River upstream of Florida River	N
	NAR4	SUIT				
		EPA	37.085161	-107.879233	Animas River south of Durango - access via Road 213	
AR2-7	AR2-7	SUIT	37.04431	-107.872392	Animas River downstream of Florida River confluence	
	AR2-7a	EPA	37.032292	-107.875455	Animas River - access near Heaven on Earth Rd	
	NAR6	SUIT	37.024806	-107.8738		N
	AR0028	SUIT	37.025833	-107.872778	Animas @ Twin Crossings	
	Animas 2	SUIT	37.027275	-107.874365		
ADW-022	ADW-022	EPA	36.920559	-107.909909	Animas River at the Aztec Domestic Water System Intake, near Cedar Hill	N
			36.933295	-107.909073		
	AR-1	NMDGF				
ADW-021	ADW-021	EPA	36.872838	-107.960741	Animas River at Intake Sampling Location	N
ADW-010	ADW-010	EPA	36.838545	-107.992183	Animas River at Hwy 550 Bridge below Aztec	Y
	09364010 (gage)	USGS	36.837463	-107.991684		
	66Animas028.1	NM				
	66NM078.1 (NM0020168)	NM				
	AR-2	NMDGF				
FW-012	FW-012	EPA	36.783635	-108.102111	Animas River north of Farmington, NM	N
	66Animas017.4	NM			Animas River at Intake Sampling Location	
	4136	CORW				
FW-040	FW-040	EPA			Animas River upstream of the San Juan River	Y

Location (GKM_ID)	Associated Location(s)	Site Organization	Latitude	Longitude	Description/Location	USGS gage available?
SJAR*	09364500 (gage)	USGS	36.719664	-108.207125		N
	66Animas001.7	NM				
	SJAR	EPA	36.707467	-108.150813	San Juan River just upstream of the Animas River	
	66SanJua101.6	NM				
	SJR-1	NMDGF	36.706709	-108.19835	San Juan River upstream of the Animas River near Bloomfield	
	NMR9-0905	EPA-NRSA	36.70792574	-108.2114498		
	FW08NM022	EPA-NRSA				
LVW-020	LVW-020	EPA	36.730556	-108.251046	San Juan River at Intake Sampling Location	N
	09365000 (gage)	USGS	36.73588701	-108.2539868	San Juan River downstream of the confluence with the Animas	
	66SanJua100.2	NM	36.7217	-108.224		
	SJR-2					
SJLP	SJLP	EPA	36.73588701	-108.2539868	San Juan River downstream of the confluence with the Animas near Northern Edge Casino	Y
	09365000 (gage)	USGS				
	67SanJua096.3	NM			San Juan River in Farmington, NM	
SJFP	SJFP	EPA	36.74815602	-108.4120157	San Juan River near Fruitland, NM	N
	NMRM-1005	EPA-NRSA	36.75051779	-108.4181808		
	9367540	USGS				
	67SanJuan082.6	NM				
	SJR-3	NMDGF				
SJSR	SJSR	EPA	36.78162422	-108.6927838	San Juan River near Shiprock, NM	Y
	09368000 (gage)	USGS				
SJ4C	SJ4C	EPA	36.99621613	-109.0046838	San Juan River near Four Corners (CO/NM border)	Y
	4954000	Utah	37.002775	-109.03177	San Juan River near Four Corners (near Hwy 161 in CO/UteMtnUte)	
	09371010 (gage)	USGS			San Juan River near Four Corners	
SJMC	SJMC	EPA	37.25822644	-109.3106036	San Juan River upstream of Montezuma Creek	N
	4953990	Utah	37.258226	-109.310604		
	FW08UT014	EPA-NRSA	37.22371769	-109.2086935		
	UTR9-0901	EPA-NRSA	37.22371769	-109.2086935		
SJBB	SJBB	EPA	37.25737015	-109.6185856	San Juan River near Bluff	N

Location (GKM_ID)	Associated Location(s)	Site Organization	Latitude	Longitude	Description/Location	USGS gage available?
	4953250	Utah	37.260279	-109.613734	San Juan River near Bluff - San Island	
	UTRM-1009	EPA - NRSA	37.25537255	-109.6217678	San Juan River near Bluff	
SJMH	SJMH 4953000 09379500 (gage)	EPA Utah USGS	37.146948	-109.853672	San Juan River in Mexican Hat	Y
SJCH	SJCH 4952942 3.71248E+14	EPA Utah USGS	37.293336 37.293008	-110.399293 -110.399621	San Juan River / Lake Powell at Clay Hills boat ramp	N

*Background/reference sites – data to be used to characterize background loading to Animas and San Juan unrelated to Gold King Mine Influence.

APPENDIX B: DATA SOURCES

Links to access biological data collected from the Animas and San Juan rivers. BMI = benthic macroinvertebrate.

	Data Source	Information Obtained
Federal	**EPA follow-up sampling** https://www.epa.gov/goldkingmine	BMI assemblage (2015, 2016) BMI tissue (2016) Fish tissue (2016, 2017) Physical habitat (2016)
	WQX https://www.epa.gov/waterdata/water-quality-data-wqx	BMI assemblage • NMED (2010) **Stations:** https://www.waterqualitydata.us/Station/search?organization=21NMEX_WQX&sampleMedia=Biological&startDateLo=01-01-2010&mimeType=csv&zip=yes&sorted=no **Results:** https://www.waterqualitydata.us/Result/search?organization=21NMEX_WQX&sampleMedia=Biological&startDateLo=01-01-2010&mimeType=csv&zip=yes&sorted=no • Southern Ute Indian Tribe (2013, 2014) **Stations**: https://www.waterqualitydata.us/Station/search?organization=SOUTHUTE&sampleMedia=Biological&startDateLo=01-01-2013&startDateHi=12-31-2014&mimeType=csv&zip=yes&sorted=no **Results**: https://www.waterqualitydata.us/Result/search?organization=SOUTHUTE&sampleMedia=Biological&startDateLo=01-01-2013&startDateHi=12-31-2014&mimeType=csv&zip=yes&sorted=no&dataProfile=biological • The Rivers of Colorado Water Watch Network (RiverWatch) (2012)

Data Source	Information Obtained
	Stations: https://www.waterqualitydata.us/Station/search?organization=CORIVWCH_WQX&sampleMedia=Biological&startDateLo=01-01-2012&startDateHi=12-31-2012&mimeType=csv&zip=yes&sorted=no **Results**: https://www.waterqualitydata.us/Result/search?organization=CORIVWCH_WQX&sampleMedia=Biological&startDateLo=01-01-2012&startDateHi=12-31-2012&mimeType=csv&zip=yes&sorted=no&dataProfile=biological
EPA Superfund SCRIBE database Data are available through site contacts identified on the Bonita Peak Superfund website: https://cumulis.epa.gov/supercpad/cursites/csitinfo.cfm?id=0802497	EPA collected/funded • BMI assemblage (2014; 2015 post-GKM release) • Metals BMI tissue data • (2012, 2014; 2015 post-release) BMI assemblage data • SUIT (2008, 2009) • ARSG (2010) • AWP (2010)
US Fish and Wildlife https://www.fws.gov/southwest/sjrip/ Data were acquired through a request.	San Juan River fish population data (1991-2016)
EPA National River and Streams 2009 data: https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys 2013 data: Data available through a request	BMI assemblage (2009,2013) Physical habitat (2009, 2013)
U.S. Bureau of Reclamation Data were obtained from the pdf: Animas-La Plata Project. Final Supplement to the Final EIS. Appendix B. April 1996	Metals in fish tissue (1996)

	Data Source	Information Obtained
State	**NM Environment Department** **NM Department of Game and Fish** https://www.env.nm.gov/river-water-safety/animas-river-data-documents-page/#wildlife	Metals in fish and BMI tissue (August 2015, March 2016)
	Colorado Parks and Wildlife www.cpw.state.co.us Data were acquired through a request.	Animas River fish population data near Durango and Silverton (1912-2016) GKM response sampling including fish population surveys, sentinel caged fish data, and field datasheets. Beaver necropsy report.
	Colorado Department of Public Health and Environment BMI assemblage data were acquired through a request. Metals in fish tissue: https://www.colorado.gov/pacific/cdphe/animas-river-water-quality-sampling-and-data	BMI assemblage (2008, 2014, 2016) Metals in fish tissue (August 2015, March 2016)
Tribal	**Southern Ute Indian Tribe** Data were acquired through a request.	Metals in fish tissue data (July 2015) BMI assemblage (2015 pre- and post-GKM release, 2016)
	Navajo Nation Environmental Protection Agency Data were presented in the final report dated November 2017.	Metals in fish tissue data (June 2017)

	Data Source	Information Obtained
NGO	**Animas River Stakeholder Group** http://animasriverstakeholdersgroup.org/blog/index.php/data/	BMI assemblage

APPENDIX C: BENTHIC MACROINVERTEBRATE ASSEMBLAGE SUPPORTING INFORMATION

Table C.1 . Benthic macroinvertebrate metric descriptions.

Metric Code	*Metric Description*
Taxa Composition	*Percentages of taxa groups*
pEPTnoB	% EPT excluding Baetidae of all individuals
NonInPct	% Non-Insect individuals of all individuals
ColeoPct	% Coleoptera individuals of all individuals
Habit	*Mode of locomotion or attachment*
ClngrTax	Number of Clinger Taxa
SprwlTax	Number of Sprawler Taxa
Pollution Tolerance	*Sensitivity to stressors*
IntolTax	Number of Intolerant Taxa
pt_Intol	Intolerant taxa as a percentage of all taxa
pi_DecrMtnTrn	% decreaser indicator individuals in Biotypes 1 and 2
pi_IncrMidElev	% increaser indicator individuals in Biotypes 1
pi_IncrPlains	% increaser indicator individuals in Biotypes 3
Taxa Richness	*Counts of taxa, percentage of taxa*
TotalTax	Total number of taxa in the sample
pt_noninsect	Non-insect taxa as a percentage of all taxa
EPTTax	Number of EPT taxa in the sample
Functional Feeding Group	*Mechanism for obtaining food*
PredPctFAC	% Facultative Predator individuals of all individuals
ScrapPctFAC	% Facultative Scraper individuals of all individuals
PredShrTaxFAC	Number of Facultative Predator or Shredder Taxa

Table C.2 NRSA MMI median values, with median difference per site and Wilcoxon signed-rank.

Site	Pre-event Period Median	Post-event Period Median	Difference	Signed Rank
A72	29.98	32.58	-2.60	-7
A73	41.53	40.15	1.38	6
A75D	42.59	37.80	4.79	10
Bakers Bridge	39.45	39.90	-0.45	-1
James Ranch	39.64	38.85	0.79	3
9426	23.20	41.32	-18.12	-16
32nd Street	51.00	47.50	3.50	9
Rotary Park	53.92	52.78	1.14	5
AR16-0	46.58	47.17	-0.59	-2
AR7-2	48.07	66.10	-18.03	-15
ADW-010	67.65	55.05	12.60	14
FW-040	58.00	63.13	-5.13	-11
SJAR	41.44	44.86	-3.42	-8
SJFP	45.04	52.65	-7.62	-12
SJMC	57.13	58.20	-1.07	-4
SJBB	49.43	59.32	-9.89	-13

Table C.3 Colorado MMI median values, with median difference per site and Wilcoxon signed-rank.

Site	Pre-event Period Median	Post-event Period Median	Difference	Signed Rank
A72	16.80	17.90	-1.10	-3
A73	30.70	36.90	-6.20	-7
A75D	47.45	54.75	-7.30	-8
Bakers Bridge	47.60	62.55	-14.95	-11
James Ranch	48.45	49.00	-0.55	-1
9426	20.60	39.70	-19.10	-15
32nd Street	41.80	31.05	10.75	9
Rotary Park	39.90	38.70	1.20	4
AR16-0	26.10	44.22	-18.10	-14
AR7-2	49.10	67.00	-17.90	-13
ADW-010	85.40	68.30	17.10	12
FW-040	71.50	70.80	0.70	2
SJAR	44.20	45.80	-1.60	-6
SJFP	58.90	60.45	-1.55	-5
SJMC	77.30	56.80	20.50	16
SJBB	59.00	48.20	10.80	10

Table C.4 % EPT median values, with median difference per site and Wilcoxon signed-rank.

Site	Pre-event Period Median	Post-event Period Median	Difference	Signed Rank
A72	55.86	76.33	-20.47	-13
A73	96.12	91.33	4.79	4
A75D	70.71	78.75	-8.04	-7
Bakers Bridge	77.48	68.12	-9.36	-10
James Ranch	74.67	81.33	-6.66	-6
9426	32.17	72.69	-40.52	-15
32nd Street	77.41	67.17	10.24	11
Rotary Park	73.00	64.00	9.00	8
AR16-0	74.00	76.33	-2.33	-3
AR7-2	85.67	62.67	23.00	14
ADW-010	30.53	75.33	-44.80	-16
FW-040	67.45	68.18	-0.73	-1
SJAR	54.67	45.59	9.08	9
SJFP	54.88	65.75	-10.87	-12
SJMC	46.81	45.77	1.04	2
SJBB	42.02	36.84	5.18	5

Table C.5 Total Taxa median values, with median difference per site and Wilcoxon signed-rank.

Site	Pre-event Period Median	Post-event Period Median	Difference	Signed Rank
A72	8.0	9.0	-1.0	-1
A73	11.0	13.0	-2.0	-4
A75D	17.0	18.0	-1.0	-1
Bakers Bridge	16.0	24.5	-8.5	-13
James Ranch	12.0	17.5	-5.5	-11
9426	8.0	12.0	-4.0	-8
32nd Street	10.0	12.5	-2.5	-6
Rotary Park	9.0	20.0	-11.0	-14
AR16-0	9.0	13.0	-4.0	-8
AR7-2	9.0	22.0	-13.0	-16
ADW-010	21.0	20.0	1.0	1
FW-040	19.0	27.0	-8.0	-12
SJAR	23.0	20.5	2.5	6
SJFP	19.5	23.5	-4.0	-8
SJMC	29.0	17.0	12	15
SJBB	16.0	18.0	-2.0	-4

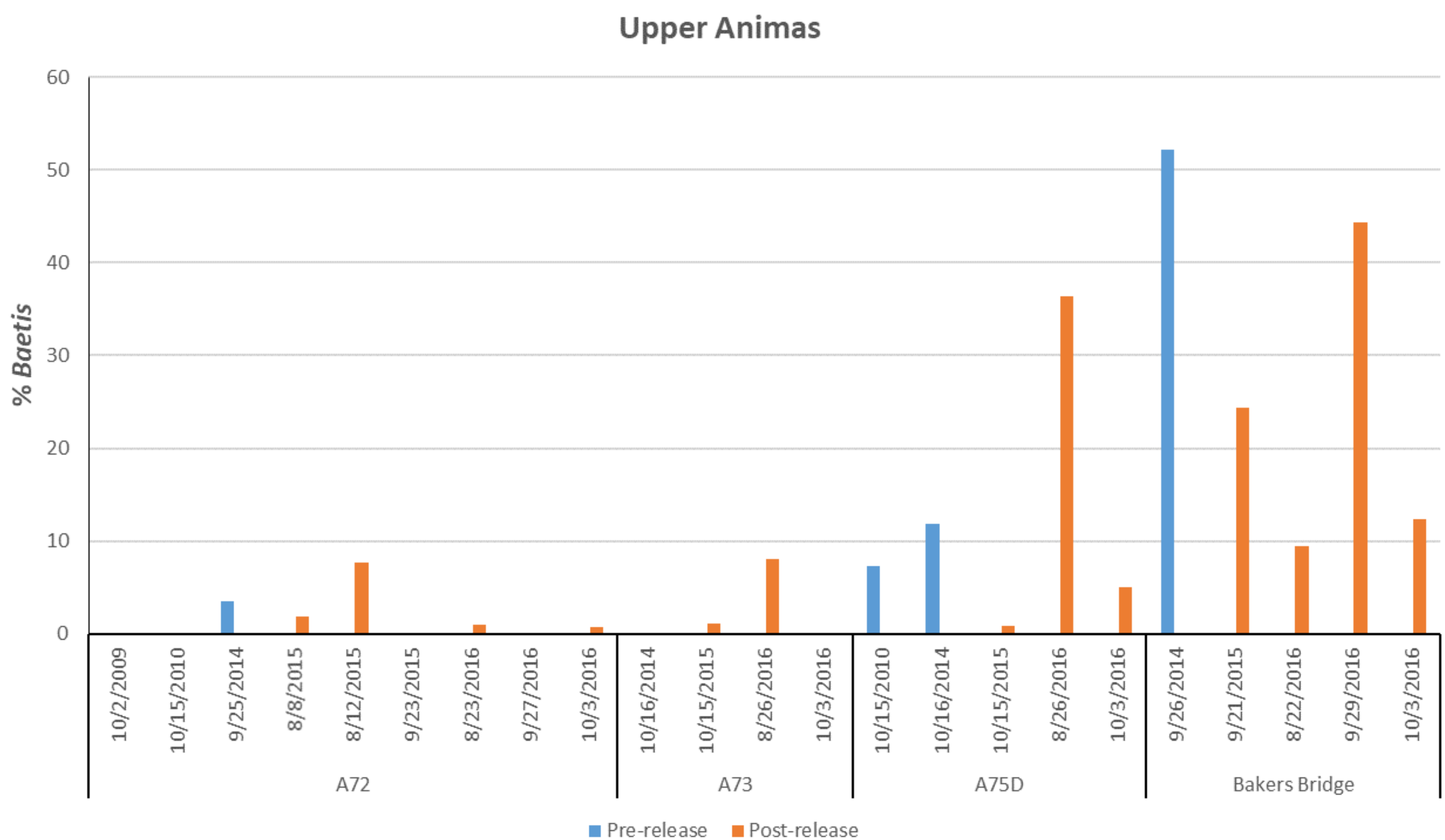

Figure C.1. Relative abundance of *Baetis spp.* within the upper Animas from pre- and post-release sampling events.

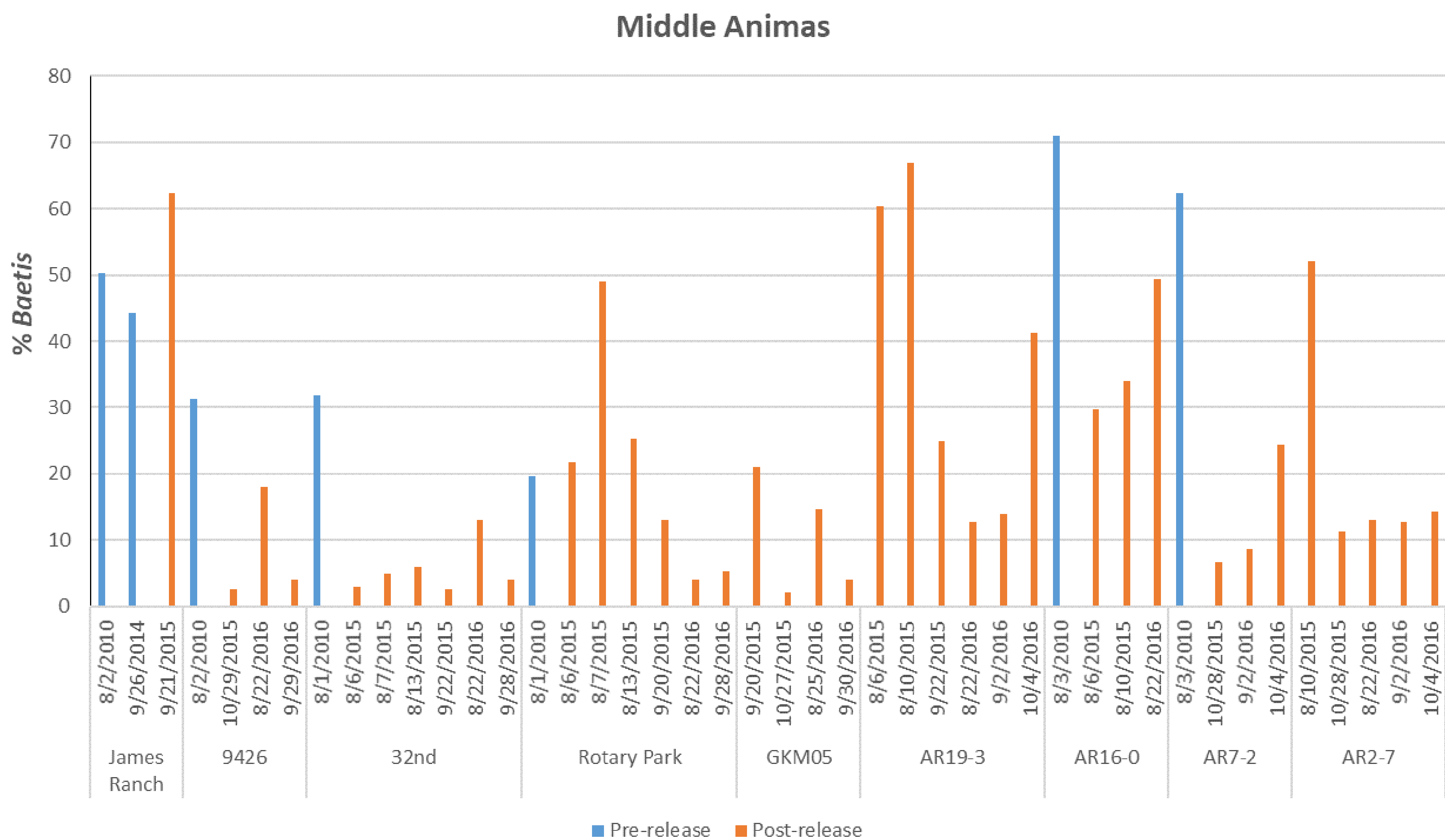

Figure C.2 Relative abundance of *Baetis spp.* within the Middle Animas from pre- and post-release sampling events.

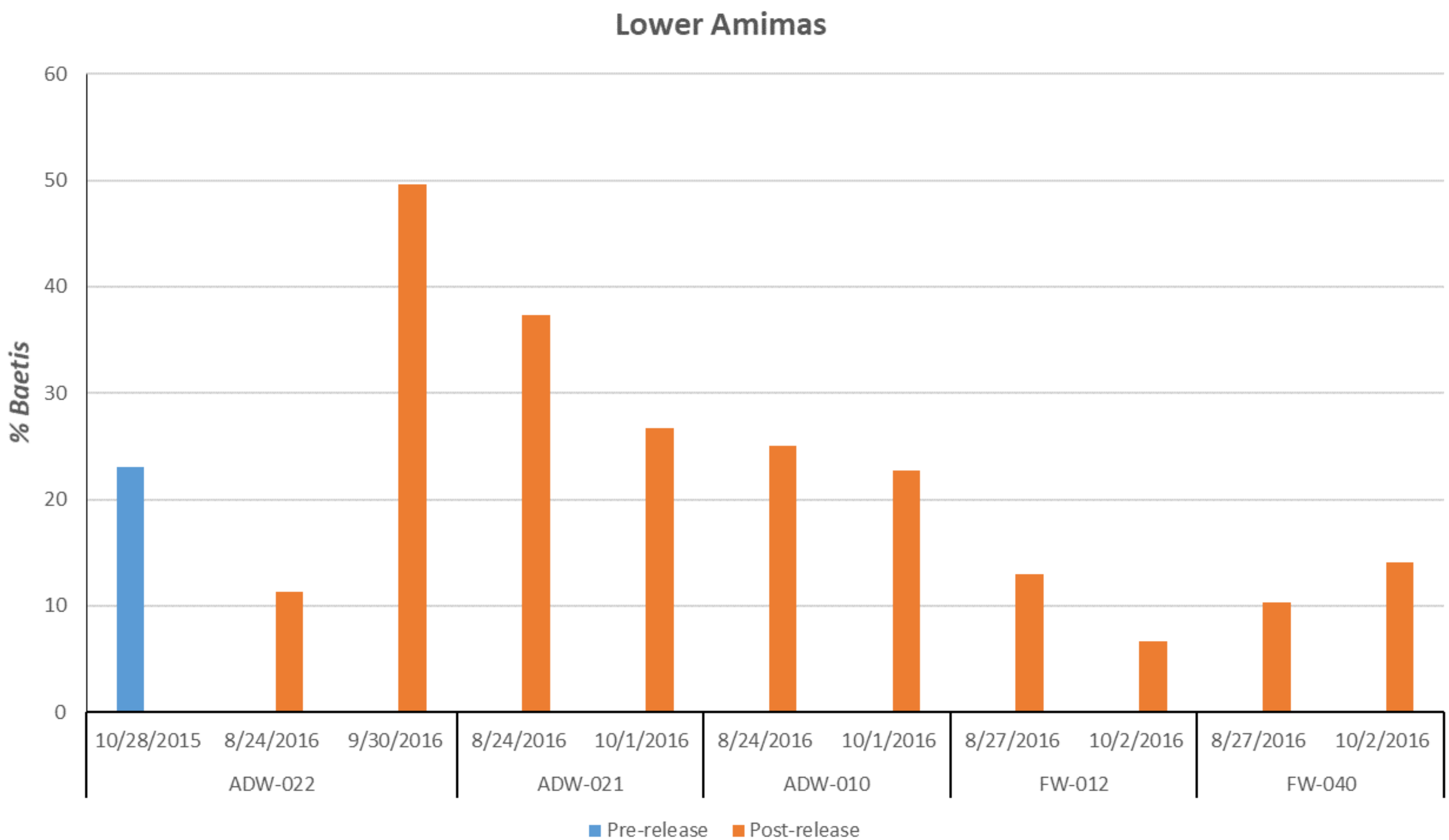

Figure C.3. Relative abundance of *Baetis spp.* within the Lower Animas from pre- and post-release sampling events.

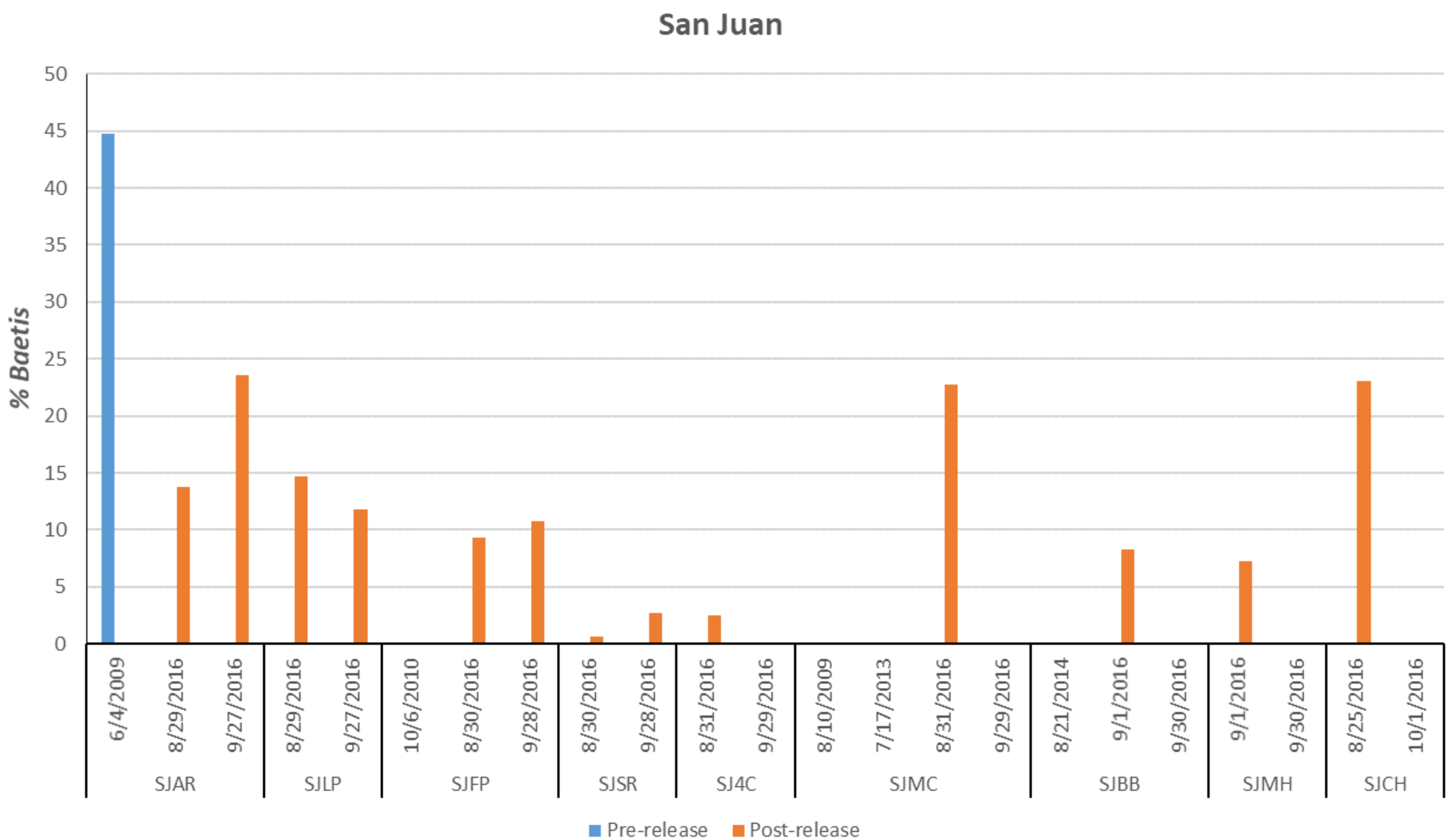

Figure C.4 Relative abundance of *Baetis spp.* within the San Juan River from pre- and post-release sampling events.

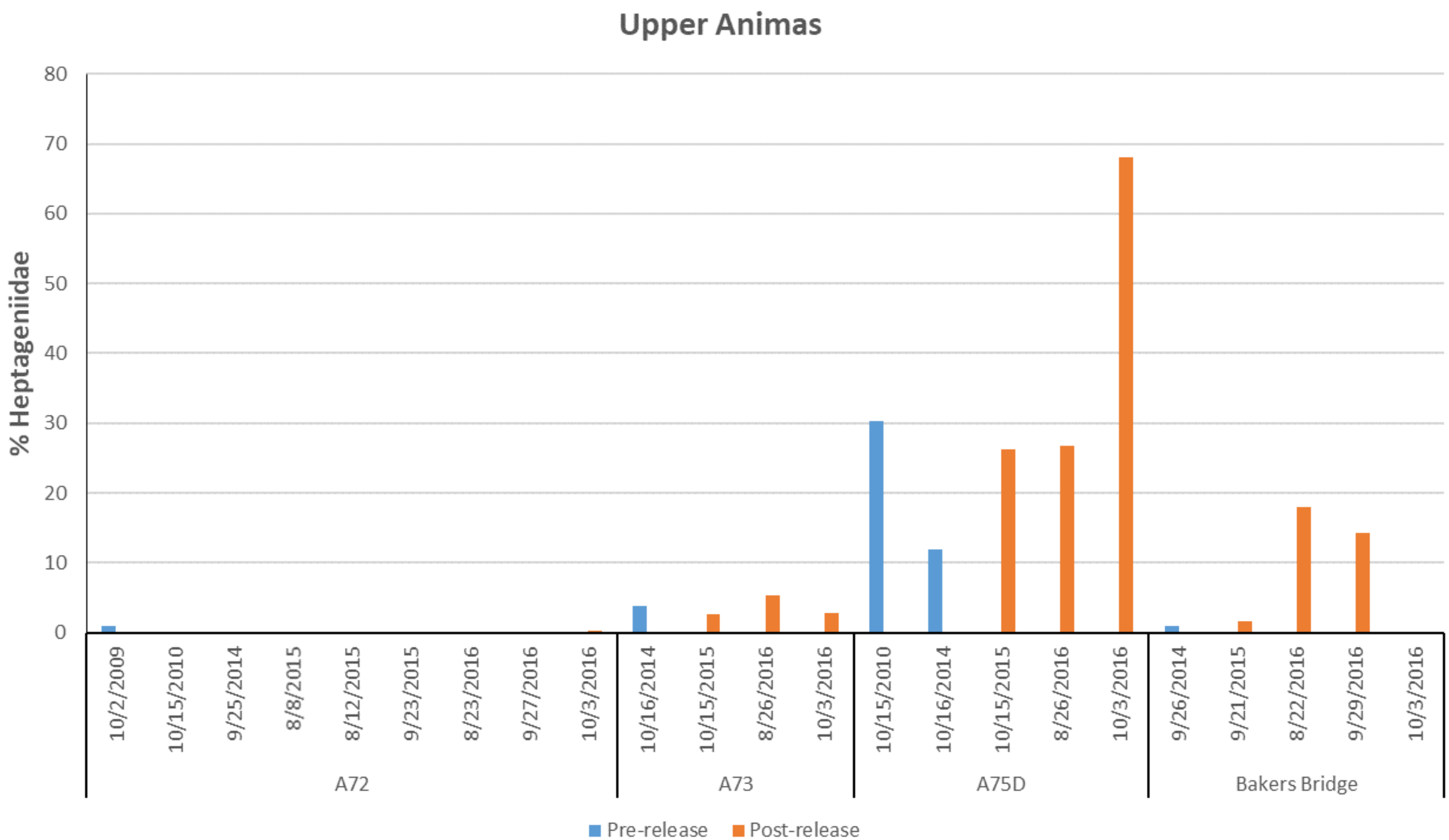

Figure C.5. Relative abundance of Heptageniidae within the upper Animas from pre- and post-release sampling events.

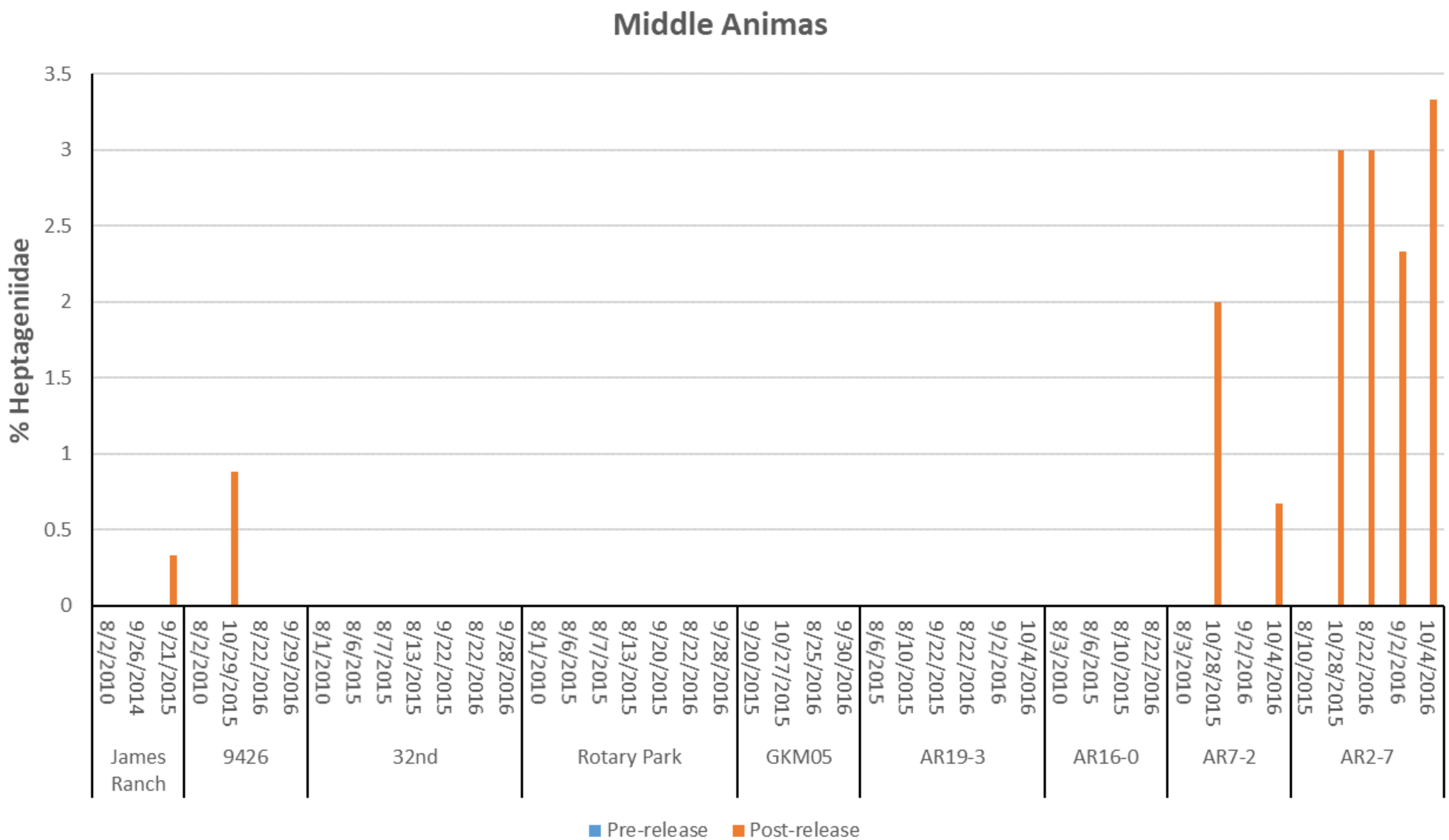

Figure C.6. Relative abundance of Heptageniidae within the Middle Animas from pre- and post-release sampling events.

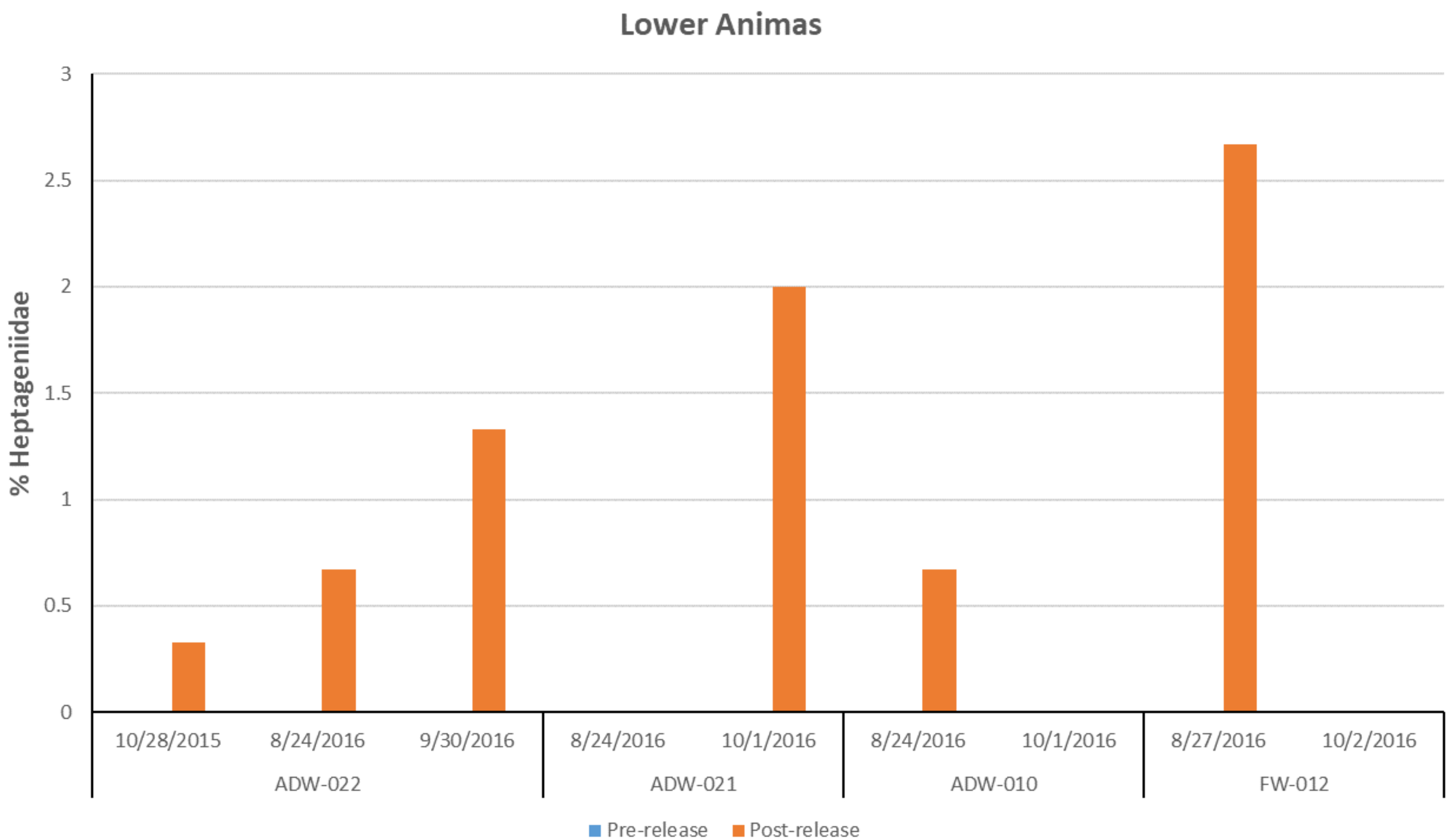

Figure C.7. Relative abundance of Heptageniidae within the Lower Animas from pre- and post-release sampling events.

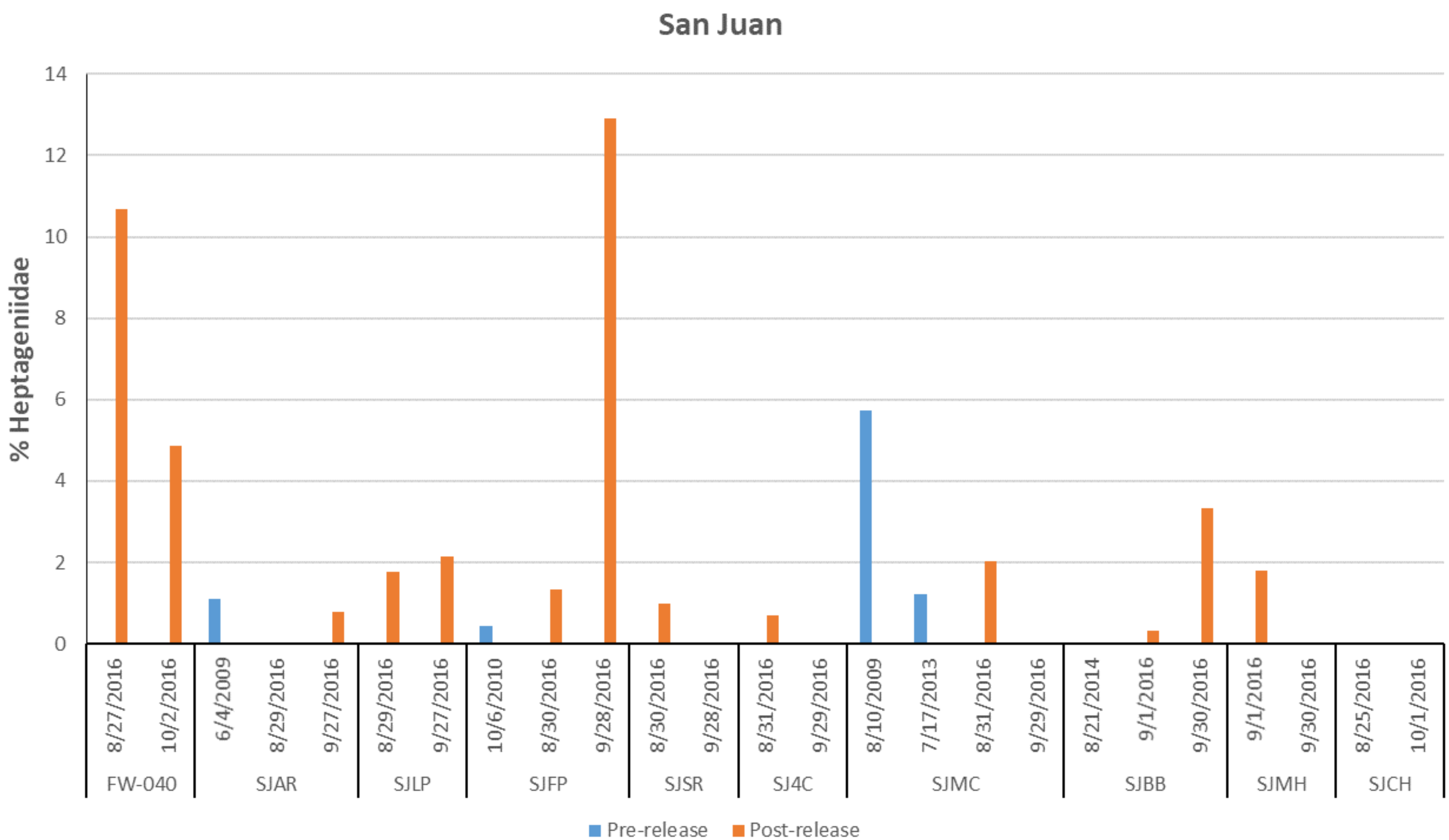

Figure C.8. Relative abundance of Heptageniidae within the San Juan River from pre- and post-release sampling events.

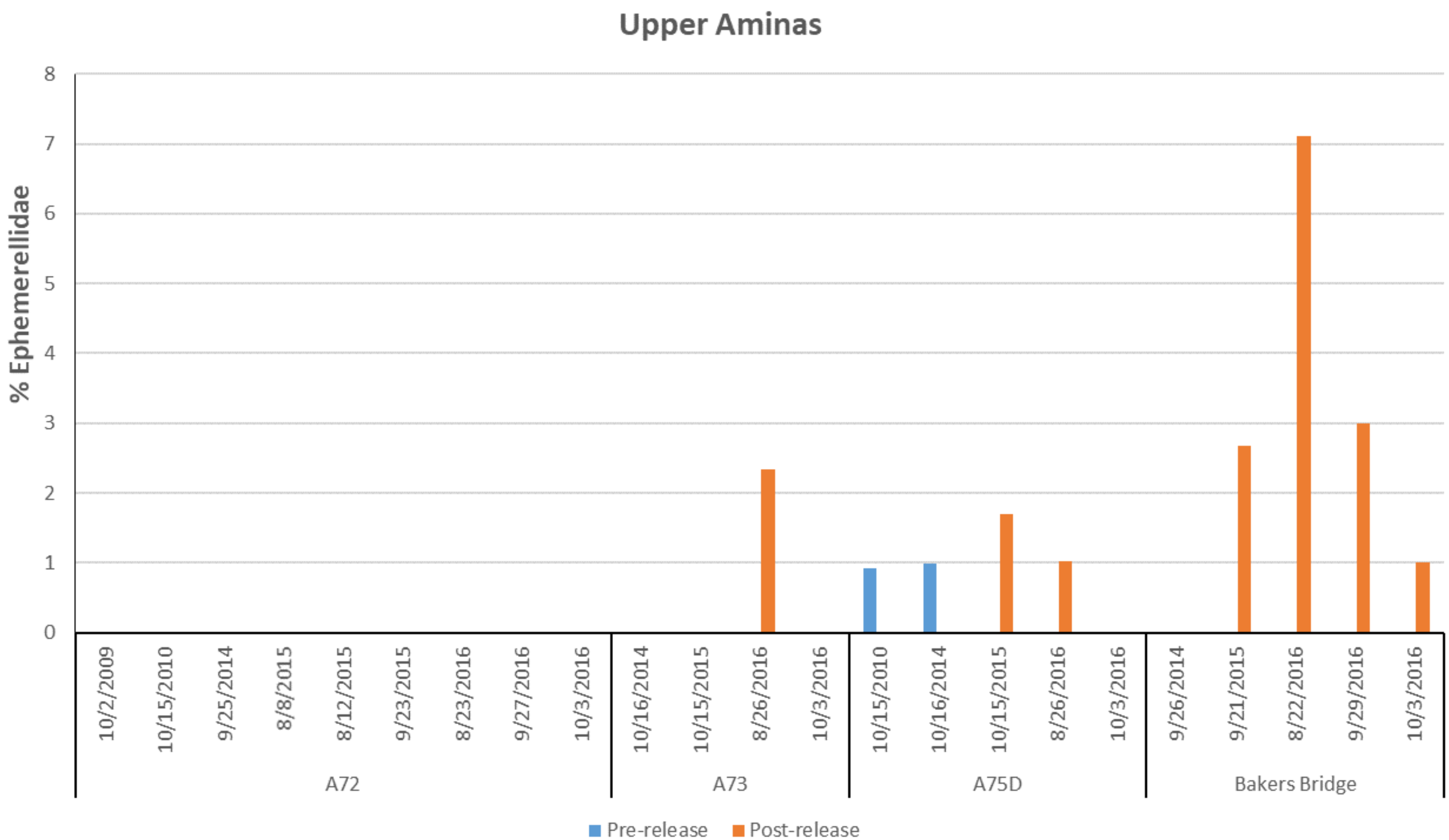

Figure C.9. Relative abundance of Ephemerellidae within the upper Animas from pre- and post-release sampling events.

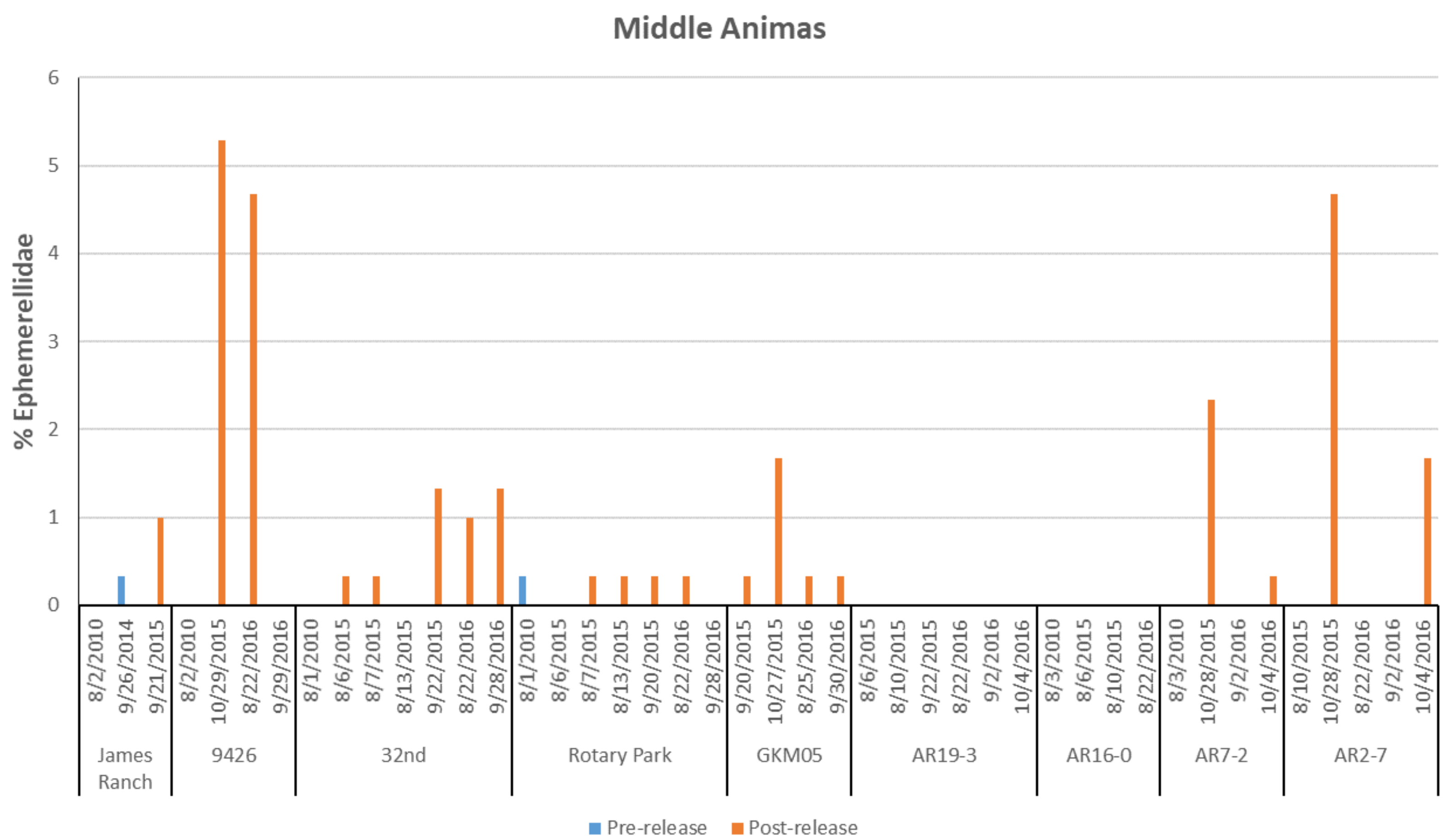

Figure C.10. Relative abundance of Ephemerellidae within the Middle Animas from pre- and post-release sampling events.

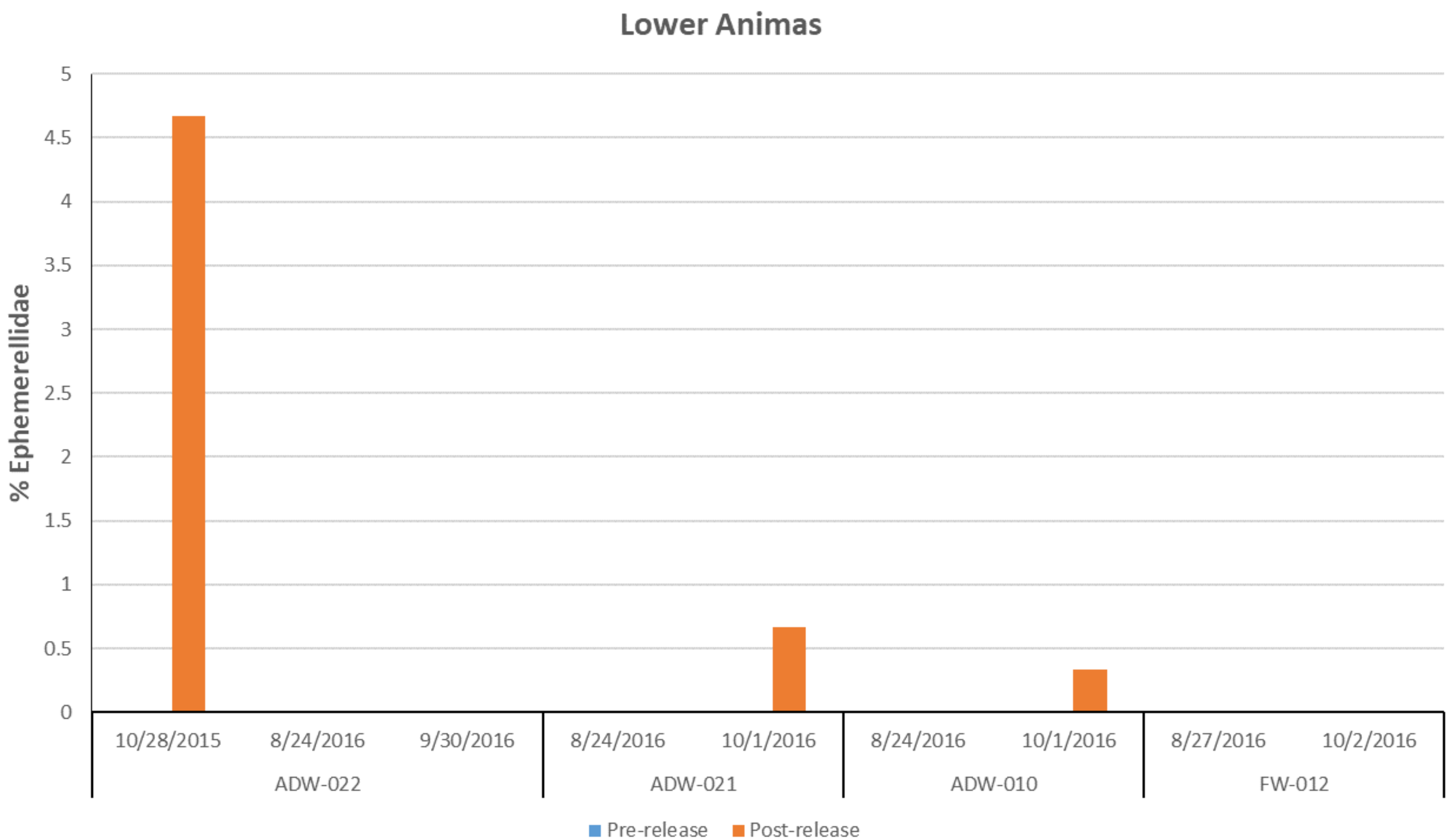

Figure C.11. Relative abundance of Ephemerellidae within the Lower Animas from pre- and post-release sampling events.

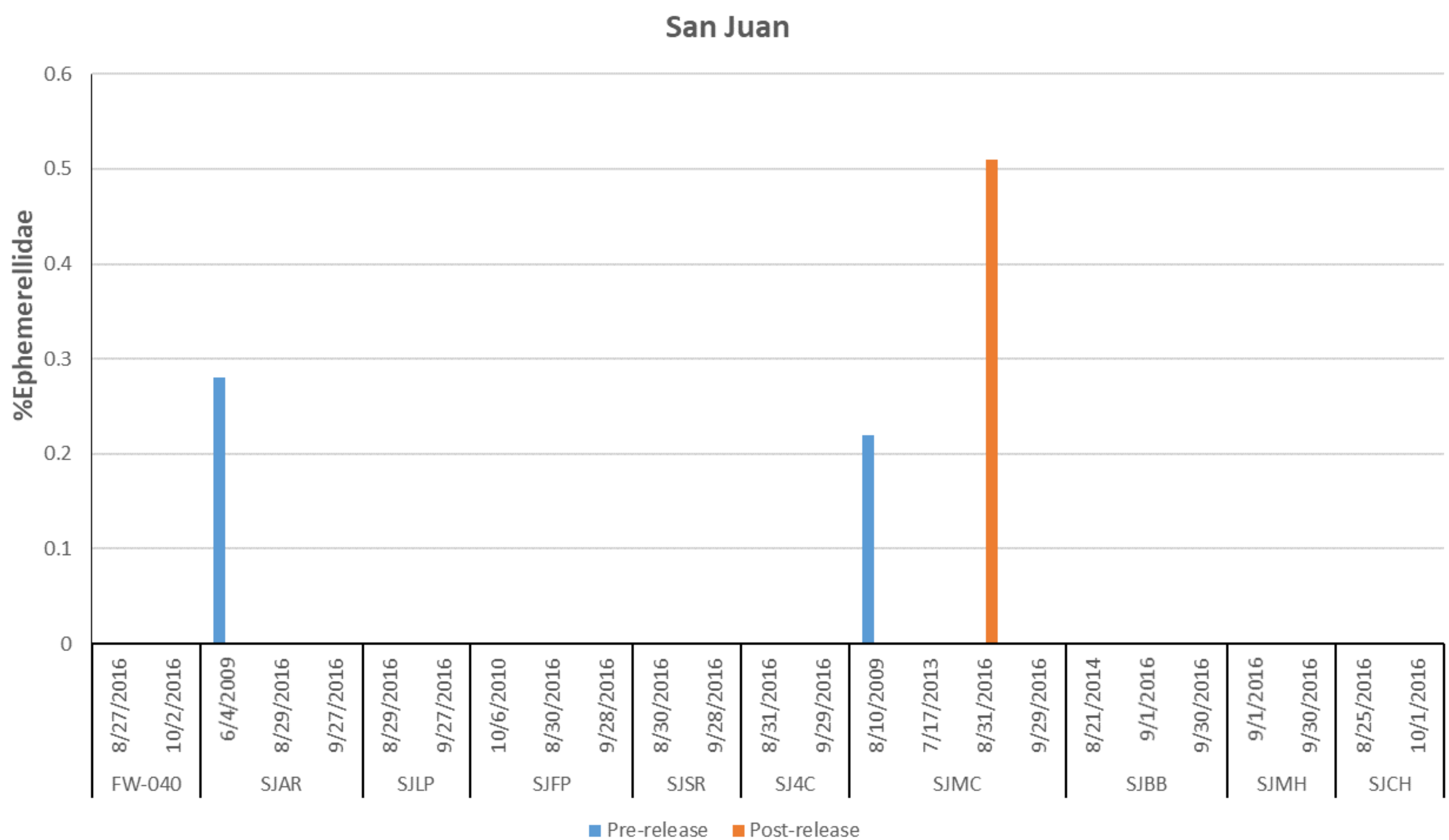

Figure C.12. Relative abundance of Ephemerellidae within the San Juan River from pre- and post-release sampling events.

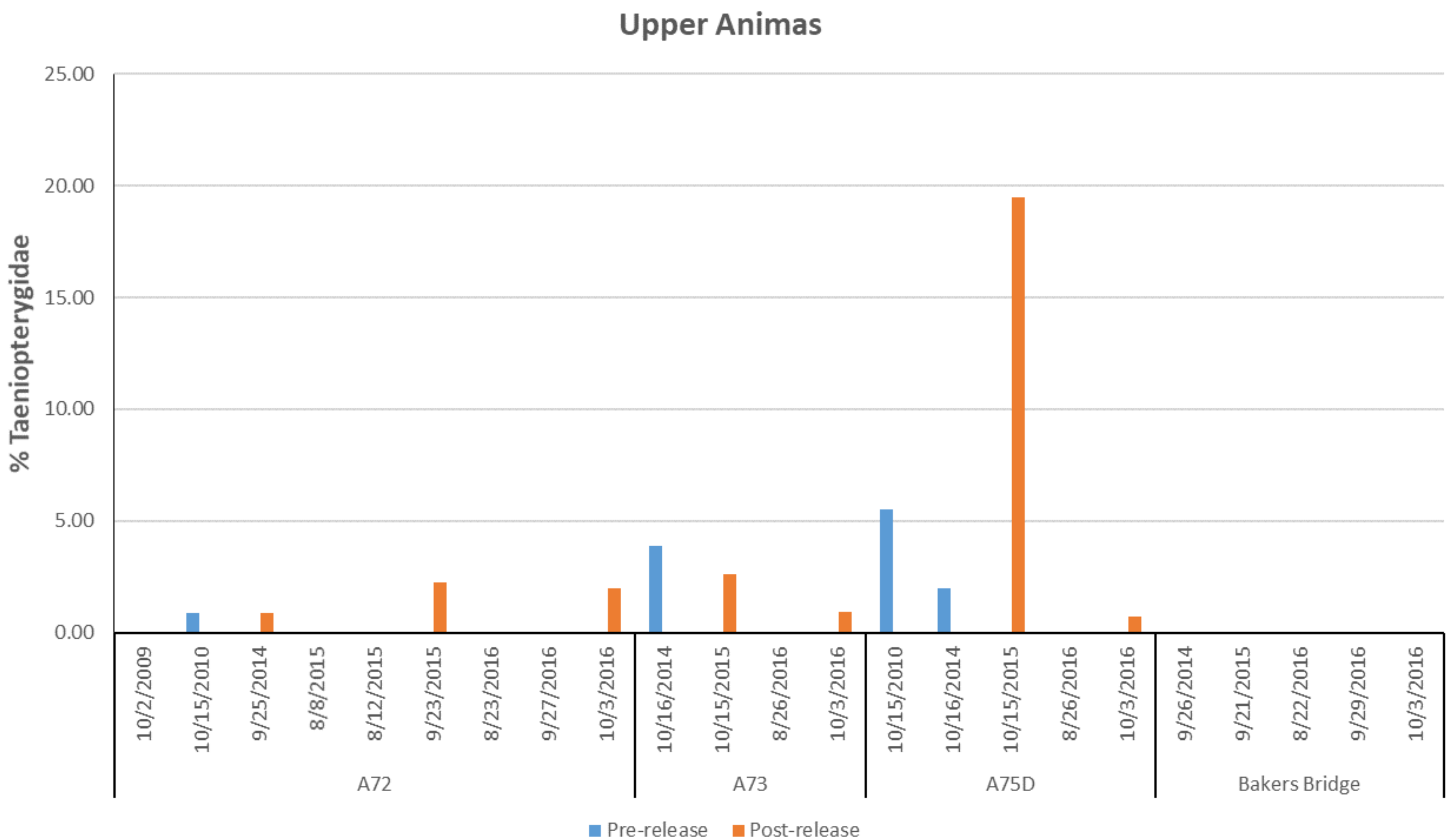

Figure C.13. Relative abundance of Taeniopterygidae within the upper Animas from pre- and post-release sampling events.

APPENDIX D: COLORADO PARKS AND WILDLIFE SENTINEL FISH STUDY NOTES AND FISH STOCKING RECORDS

Table D.1 Records for 96-hour sentinel fish study.

Location	Number of fish	Dates times checked, notes	mortality
Junction Creek (Control site, not affected by GKM)	60 (5 cages/12 fish per cage)	8/6 19:26, all look good	0
		8/7 6:21, all look good	0
		8/7 13:27, less turbid than this am	0
		8/7 19:00, look good	0
		8/8 7:28, fish look good	0
		8/8 12:35, fish look good	0
		8/8 20:00	0
		8/9 7:12	0
		8/9 12:49	0
		8/9 19:00	0
		8/10 8:22, all good	0
		8/10 14:07, fish look good	0
		8/10 18:48, fish look good	0
		8/11 11:20	0
Animas River at 32nd Street	36 (3 cages/12 fish per cage)	8/6 19:15, all look good	0
		8/7 6:35, all look good	0
		8/7 13:46, less turbid than this am	0
		8/7 18:48, fish look good	0
		8/8 6:53, fish look good	0
		8/8 12:24, fish look good	0
		8/8 20:18	0
		8/9 7:26	0
		8/9 12:32, water looking more muddy than orange	0
		8/9 18:43, water looking more muddy than orange	0
		8/10 7:55, all good	0
		8/10 13:46, fish look good	0
		8/10 18:22, fish look good	0
		8/11 10:50	0
Animas River at Hatchery	36 (3 cages/12 fish per cage)	8/6 19:58, all look good	0
		8/7 6:53, could not find 3c	0
		8/7 14:20, less turbid than this am	0
		8/7 19:18, look good	0
		8/8 7:45, fish look good	0
		8/8 13:15, fish look good	0
		8/8 19:45, 1 stressed in 2c	0
		8/9 8:02	0
		8/9 13:01, water looking more muddy than orange	0
		8/9 19:17, water looking more muddy than orange	0
		8/10 8:33, all good	0
		8/10 14:30, fish look good	0
		8/10 19:00, good fright response in cage	0
		8/11 12:00	0

Table D.1 Records for 96-hour sentinel fish study.			
Location	Number of fish	Dates times checked, notes	mortality
Animas River at High Bridge	36 (3 cages/12 fish per cage)	8/6 19:41, all look good	0
		8/7 7:17, all but 1 look good	1
		8/7 15:02, less turbid than this am	0
		8/7 19:38, all but 1 look good	0
		8/8 8:00, fish look good	0
		8/8 12:53, fish look good	0
		8/8 19:18, look good	0
		8/9 8:28	0
		8/9 13:40, water looking more muddy than orange	0
		8/9 19:38, all good!	0
		8/10 9:00, fish look good	0
		8/10 14:50, fish look good	0
		8/10 19:24, good fright response in cage	0
		8/11 12:35	1

Table D.2 Fish stocking records for Animas Reaches 1 and 2.				
Date stocked	Reach	Species	number	Length (in)
8/10/2016	1	Brown trout	9,997	4.49
8/10/2016	2	Hofer x Harrison rainbow trout	9,079	3.09
8/10/2016	2	Brown trout	10,088	4.61
Total 2016	1+2	Brown	20,085	
Total 2016	2	Rainbow	9,079	
9/3/2015	1	Brown trout	14,052	3.55
8/5/2015	**GKM release**			
7/28/2015	2	Hofer x Harrison X Snake R rainbow	1,500	10.44
7/21/2015	2	Brown trout	793	3.62
7/20/2015	1	Brown trout	11,835	3.93
7/20/2015	2	Brown trout	11,835	3.93
7/7/2015	2	Hofer x Snake R rainbow trout	1,000	10.19
Total 2015	1+2	Brown	38,515	
Total 2015	2	Rainbow	25,000	
8/12/2014	1	Hofer X Colo R rainbow trout	10,000	2.6

Table D.2 Fish stocking records for Animas Reaches 1 and 2.

Date stocked	Reach	Species	number	Length (in)
8/12/2014	2	Hofer X Colo R rainbow trout	10,000	2.6
7/31/2014	2	Bel-aire rainbow trout	380	10.57
7/18/2014	2	Hofer x Harrison rainbow trout	760	10.61
7/3/2014	2	Bel-aire rainbow trout	760	10.6
6/23/2014	1	Brown trout	10,003	3.15
6/23/2014	2	Brown trout	10,002	3.15
6/23/2014	2	Rainbow trout	25,686	3.98
Total 2014	1+2	Brown	20,005	
Total 2014	1+2	Rainbow	47,586	

APPENDIX E: METAL IN FISH TISSUE SUPPORTING INFORMATION

NMDGF Generalized Linear Model

To examine the simultaneous influence of factors that influence metal bioaccumulation, we applied a generalized linear model (GLM) to the NMDFG data. The GLM is a flexible generalization of ordinary linear regression that allows for categorical or continuous response variables that have error distribution models other than a normal distribution. Analysis was performed using a GLM routine in the base R package (R-3/3/2, R Core Team 2016). The GLM evaluates the relationship between independent parameters and the response variable without relying on limiting assumptions of model error distributions.

For this investigation, the response variable was tissue concentration. The covariates included categorical variables of site, sampling date, fish species and tissue type. Table E.1 lists the covariates and the levels of categorical variables. To perform the test with categorical variables, one level within each categorical covariate must be designated as the "reference," meaning the influence at other levels of that covariate are relative to the influence of the reference level. Gray-shaded cells in Table 8.3 indicate the reference level of each categorical factor. Body length was also included as a continuous independent variable. A separate model was constructed for each metal. Tissue concentrations were first $\log_{10}$ transformed to reduce the influence of large outliers in the data, especially in the liver (see Chapter 8).

Table E.1. Independent variables examined in the generalized linear model for metal concentrations in fish tissues in the lower Animas and San Juan rivers collected in 2015 and 2016. The generalized linear model specifies a reference within each parameter. The selected reference is highlighted by shading.

Site	Tissue	Collection Date	Species	Fish Length (mm)
Animas: ADW-022 (148 RKM)	Muscle	August 2015	Bluehead sucker	Continuous
Animas: ADW-010 (163 RKM)	Liver	March 2016	Brown trout	
San Juan: SJLP (196 RKM)			Flannelmouth sucker	
San Juan: SJFP (214 RKM)			Speckled dace	
San Juan: SJAR (Reference)				

GLM model results are presented in Table E.2, which provides the model intercept, the coefficients for each level of the categorical covariates, and the statistical significance (via cell color coding) of each factor in contributing to tissue concentration. In this analysis, the importance of each factor is measured independent of confounding relationships to other factors, so that the model coefficients truly represent the isolated influence of that single factor on tissue concentrations. The units of the coefficients in Table 8.6 are $\log_{10}$ (mg/kg), and cell shading denotes the significance of each term in the model. Blue-shaded cells indicate significantly lower metal concentrations than the reference level of the factor, and yellow-shaded cells indicate significantly higher metal concentrations than the reference level. The significance level for shaded cells is $p<0.05$, but the raw output from R indicates various levels of significance to $p<0.0001$. Many of the significance values were at $p<0.001$.

The coefficients in the table provide the independent contribution of each of the covariates to tissue concentration. The GLM found that the aluminum concentration in fish collected in August 2015 (averaged across sites, species, tissues, fish size) was 0.09 $\log_{10}$ units greater than fish collected in March 2016 (Table E.2). This difference was statistically significant. Brown trout had, on average, 0.934 $\log_{10}$ units higher copper concentrations than bluehead sucker. This value was statistically significant. The coefficients in the table can be used to calculate an estimate of tissue concentration at any combination of site, date, tissue, species and fish size. Starting with the intercept for a specific metal, add the coefficient of the selected level (if that level is significantly different from the reference level) for each of the categorical covariates, and then multiply fish length (mm) by its coefficient (if significant) and add that to the sum to attain the model estimate of tissue concentration for that metal after converting to standard units from $\log_{10}$.

Generally, the GLM results suggest the same interpretations of the NMDFG data as indicated in the analyses as discussed in Section 8.2. However, when accounting for multiple influential factors at once, the statistical significance of some comparisons was lessened (e.g. the effect of location) while the significance of others was enhanced (e.g. the effect of species for individual metals). GLM results presented in Table E.2 are briefly summarized by factor.

Location. Fish collected at the two Animas River sites had significantly higher manganese concentrations than the San Juan reference site. Fish collected on the Animas River at ADW-010 (163 RKM near Aztec, NM) had statistically higher aluminum concentrations than the reference site. Other metals cadmium, copper and lead also tended to be higher at this site (positive coefficient) but were not statistically significant.

The two San Juan sampling sites below the confluence with the Animas River generally showed no difference in metal concentrations relative to the San Juan reference site with the exception of mercury and aluminum. Significantly higher mercury concentrations were found in fish collected at the San Juan reference site relative to the other four sites (i.e., the entire mercury column within the Location factor is blue). Significantly higher aluminum concentrations were found at kilometer 196, just downstream of the confluence with the Animas River.

Table E.2. Generalized linear model results for metal concentrations in fish. Cell values are estimated model coefficients in $\log_{10}$ concentrations in mg/kg. Blue-shaded cells indicate a statistically significant model coefficient that is less than the reference level of the factor (given in parentheses in the gray "Factor" heading). Yellow-shaded cells indicate a statistically significant model coefficient that is greater than the reference level of the factor. For the continuous variate Total Length, blue-shaded cells indicate a significant negative relationship between body length and metal concentration, while yellow-shaded cells represents a significant positive relationship between those variables.

	$\log_{10}$ Metal Concentrations (mg/kg)							
Factor	**Aluminum**	**Arsenic**	**Cadmium**	**Copper**	**Lead**	**Mercury**	**Manganese**	**Selenium**
Intercept	**1.12**	**-0.714**	**-2.29**	**0.09**	**-1.38**	**-1.26**	**1.29**	**-0.673**
Location (SJ Reference)								
River KM 148	0.091	-0.014	0.117	0.118	0.039	-0.786	0.315	-0.001
River KM 163	0.181	-0.008	0.128	0.069	0.076	-0.743	0.382	-0.042
River KM 196	0.108	-0.042	0.078	0.011	-0.038	-0.553	-0.041	-0.034
River KM 214	0.088	0.005	-0.122	0.106	-0.019	-0.557	0.006	0.142
Collection Date (March 2016)								
August 2015	0.09	-0.022	0.314	0.014	0.309	-0.476	0.107	0.447
Tissue (Liver)								
Muscle	-0.162	-0.238	-0.405	-1.07	-0.272	0.267	-1.023	-0.707
Species (Bluehead Sucker)								
Brown Trout	-0.193	-0.387	0.271	0.934	-0.095	-0.228	-0.802	0.764
Flannelmouth Sucker	-0.102	-0.298	0.196	0.34	0.114	0.189	-0.209	0.28
Speckled Dace	-0.304	-0.246	0.476	0.549	0.081	0.583	0.248	0.911
Fish Size								
Total Length (mm)	-0.0009	-0.0003	0.0013	0.001	-0.0002	0.0011	-0.0018	0.0007

Significantly less than reference parameter at pr < .05

Significantly more than reference parameter at pr < .05

Table E.3. Generalized linear model results for metal concentrations in fish evaluating species and environmental concentrations of metals. Cell values are estimated model coefficients in $\log_{10}$ concentrations in mg/kg. Blue-shaded cells indicate a statistically significant model coefficient that is less than the reference level of the factor (given in parentheses in the gray "Factor" heading). Yellow-shaded cells indicate a statistically significant model coefficient that is greater than the reference level of the factor. For the continuous variates of Total Length, and environmental concentrations, the blue-shaded cells indicate a significant negative relationship between the variable and metal concentration, while yellow-shaded cells represent a significant positive relationship between those variables.

	$\log_{10}$ Metal Concentrations (mg/kg)						
Factor	**Aluminum**	**Arsenic**	**Cadmium**	**Copper**	**Lead**	**Manganese**	**Selenium**
Intercept	**1.21**	**-0.856**	**-2.7**	**-1.15**	**-1.55**	**-0.4**	**-1.865**
Tissue (Muscle)							
Liver	0.146	0.247	0.411	1.097	0.256	1.043	0.748
Species (Bluehead Sucker)							
Brown Trout	-0.26	-0.4	0.22	0.991	-0.121	-0.722	0.835
Flannelmouth Sucker	-0.104	-0.277	0.181	0.316	0.158	-0.228	0.29
Speckled Dace	-0.39	-0.195	0.439	0.712	-0.032	0.425	1.029
Fish Size							
Total Length (mm)	-0.0011	-0.00016	0.001	0.0016	-0.0005	-0.0012	0.00094
Environmental Concentration							
Water Concentration (ug/l)	0.00000114	-0.058	2.36	-0.0014	0.0067	-0.00054	0.736
Sediment Concentration (mg/kg)	0.000001	0.0033	0.0424	0.0011	0.0025	0.0019	0.179
MacroInvert Concentration (ug/g)	-0.00066	-0.46	-0.269	0.009	-0.052	0.0038	-0.0087

Significantly less than reference parameter at $p < .05$

Significantly more than reference parameter at $p < .05$

Collection Date. For most metals tested, August 2015 concentrations after the GKM release were significantly greater than observed in March 2016. Mercury, however, was lower in August than March.

Tissue. For all metals except mercury, significantly greater metal concentrations were found in liver samples relative to muscle tissue. Most metals bind readily to compounds found in the liver (metallothioneins), while mercury also binds to thiols/sulfhydryls commonly found in muscle tissue. Higher concentrations of metals in liver than muscle tissues are well supported in scientific literature.

Species: A mixture of results were seen for this factor, as would be expected based on the variability in tissue concentrations displayed by species in Figures 8.7, 8.8 and 8.9. The bluehead suckers had significantly higher concentrations of aluminum and arsenic than the other species, but significantly lower concentrations of cadmium, copper and selenium. Speckled dace showed the highest concentrations of manganese, selenium, mercury and cadmium; Brown trout had the highest concentrations of copper; Flannelmouth sucker had the highest concentrations of lead.

Fish Size. Metal concentrations increased with fish size for cadmium, copper, mercury and selenium, and decreased with fish size for aluminum and manganese.

We ran a second GLM model on the NMDGF data to evaluate the direct relationship of environmental variables and eliminated the categorical variables of location and date that reflected the sampling design. Across the sampling dates and locations, there was a range of water and sediment concentrations reflected in the sampling that allowed direct analysis of the effect of environmental metal concentrations on fish tissue concentrations (e.g. e.g. Figures 8.4 and 8.5). The range of environmental metal concentrations within the NMDGF data was much narrower than the Animas River as a whole, but concentrations varied sufficiently to detect their influence on fish tissue concentrations. The concentration of metals averaged for all macroinvertebrates at each sampling location was included as an indicator of potential dietary exposure. The environmental conditions at the sites are provided in Figure 8.5 and macroinvertebrate concentrations are discussed in Chapter 7. Tissue type and species were included as variables in the environmental concentration oriented model because of their importance in determining response to metals within the entire fish community.

The regression coefficients and their statistical significance are provided in Table 8.5. There were some statistically strong relationships between fish tissue concentrations and environmental concentrations of some metals, but there were no general relationships between the accumulation of metals in fish and the concentrations of metals in sediment, water, or macroinvertebrates. The variability in metal accumulation between species and among individual fish within these populations was a stronger influence than the pervasive environmental concentrations.