Upper Clark Fork River Caged Fish Study:
The Distribution and Timing of Trout Mortality
Annual Report 2011

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Cover Photograph: Study cages (treatment, replicate and replacement) located on the Clark Fork River near Turah, Montana in July 2011.

Table of Contents

Introduction

Trout diversity, health, and survival in the Upper Clark Fork River have been well studied (Phillips and Lipton 1995; Louma et al. 2008). Hillman et al. (1995) documented reduced densities and species of trout in the Clark Fork River as a result of mining. Farag et al. (1995) investigated the bioaccumulation of metals in brown trout, and found copper levels in tissues of trout from two Clark Fork River sites to be higher than those in control sites. Levels of copper were also found to be higher at the contamination source and to decrease downstream. Higher copper concentrations were detrimental to growth and reproduction.

Metals pollution has resulted in low trout species diversity and brown trout dominance throughout most of the Upper Clark Fork River. Comparisons between rainbow trout and brown trout consistently show brown trout to be more tolerant of metals pollution. Rainbow trout were less likely to survive pulse events mimicking thunderstorms (Marr 1995 a, b). Brown trout were shown to have increased metallothionein (metals binding proteins that protect against metals toxicity) in water with elevated metals, whereas rainbow trout did not. Farag et al. (1995) found brown trout from the Clark Fork River possess elevated metallothionein as well. Both rainbow trout and brown trout avoided water with elevated metals, but rainbow trout consistently selected clean water despite acclimation to the elevated metals concentrations (Woodward et al. 1995; Louma 2008).

Diet is a significant avenue for the bioaccumulation of metals (Louma et al. 2008). In laboratory trials, young trout were fed invertebrates collected from the contaminated Clark Fork River. The laboratory specimens exhibited similar lipid peroxidation (i.e., cell damage) to wild trout in the Clark Fork River (Farag et al. 1994; Woodward et al. 1995a). Thus, it is likely fish in the Clark Fork are also exposed to metals through their diet. Laboratory specimens also exhibited reduced growth and survival due to metals exposure, and the same effect is suggested on trout in the Clark Fork River.

Water borne exposure to metals has acute and chronic effects on trout. Laboratory experiments have simulated the effects of pulse and chronic exposure to metals in the water column (Marr et al. 1995a,b). Young trout exhibited significantly reduced survival when exposed to metal concentrations similar to those documented in the Clark Fork River. Sub-lethal metal concentrations can also reduce growth rates (Marr 1995 a,b).

Mayfield and McMahon (2010, 2011) documented high mortality rates for adult trout radio-tagged in the Clark Fork River. Greater than 50% of the trout tagged in April expired by fall. Much of the mortality occurred during high spring discharges and again when water temperatures rose on the descending limb of the hydrograph (Mayfield and McMahon 2010, 2011). Similar results have been shown recently in fish cages at Turah on the Clark Fork River where trout mortality was higher during the low elevation run-off and on the descending limb of the hydrograph as water temperatures rose (D. Schmetterling, Montana Fish, Wildlife and Parks [FWP], personal communication).

Recent population surveys on the Clark Fork River have documented declines in trout abundance and fewer young trout than expected in a section below the Warm Springs Ponds (Lindstrom

2011). Other sections of the Clark Fork and tributaries had stable populations suggesting a localized decline in fish densities below the ponds. The low density of young trout may be indicative of metals pollution as young fish are more susceptible to metals poisoning than larger, older trout (Louma et al. 2008).

Phillips and Spoon (1990) performed caged fish studies in the Clark Fork River during the spring of 1986 through 1989. Mortality was consistently high at Beavertail and consistently low at Clinton, MT below Rock Creek. However, mortality elsewhere varied in space and time, and was not related to metal concentrations. Mortality rates were not consistently higher than controls in cages at Gold Creek and Warm Springs. These results demonstrate the area of the Clark Fork affected by mine wastes, as well as the spatial and temporal variability within that area.

Although metals concentrations (including copper) continue to exceed acute and chronic aquatic toxicity criteria in the Clark Fork River (PBSJ 2010), other conditions have changed. Cleanup work on Silver Bow Creek, and possibly other conditions, may currently affect mortality rates at sites in the Clark Fork River. In addition, assessment of potentially confounding factors that may lessen the response of trout populations to metals cleanup, and maintain high mortality in the mainstem, is warranted. For example, ammonia toxicity and low dissolved oxygen from nutrient loading, effectiveness of the Warm Springs Ponds to manage metals and ammonia contamination, or a synergistic effect of environmental conditions that are made fatal by the addition of metals or ammonia pollution. A more current and complete understanding of mortality rates would aid in planning and monitoring Clark Fork River remediation efforts.

In 2011, Montana Fish, Wildlife and Parks (FWP) received funding from Montana Department of Environmental Quality (DEQ) to implement *in situ* toxicity testing (fish cages) to help assess the effects of current levels of metals contamination in the Clark Fork River on the mortality of fishes, and to use this information as pre-impact monitoring data for the assessment of remediation. The monitoring objectives were to:

1. Determine mortality rates of fingerling brown trout in the upper Clark Fork River at seven sites from Warm Springs Ponds to Turah, three control streams and one handling control site.

2. Identify water quality factors affecting the mortality rates of young trout, including non-metal stressors.

3. Collect data on the pre-remediation condition to allow for adequate before-after, control-impact assessment.

4. Examine the spatial distribution of mortality rates to better understand the influence of Warm Springs Ponds and dilution from tributaries on the mortality of young trout.

5. Provide information to remediation project managers that will aid in the planning and implementation of cleanup efforts.

Methods

Cage Construction

Thirty-six wooden cages were constructed in late winter 2011. The cages resembled those used by FWP on the Middle Clark Fork River, but were 34% larger to accommodate the brown trout used in this study (Figure 1). The internal volume of the cages was 0.75 ft^3 (actual volume of water available). Knotless nylon seine material (1/16 inch bar mesh) was used for the netting on the sides and bottom of the cages. Cages were also fitted with floats to provide buoyancy.

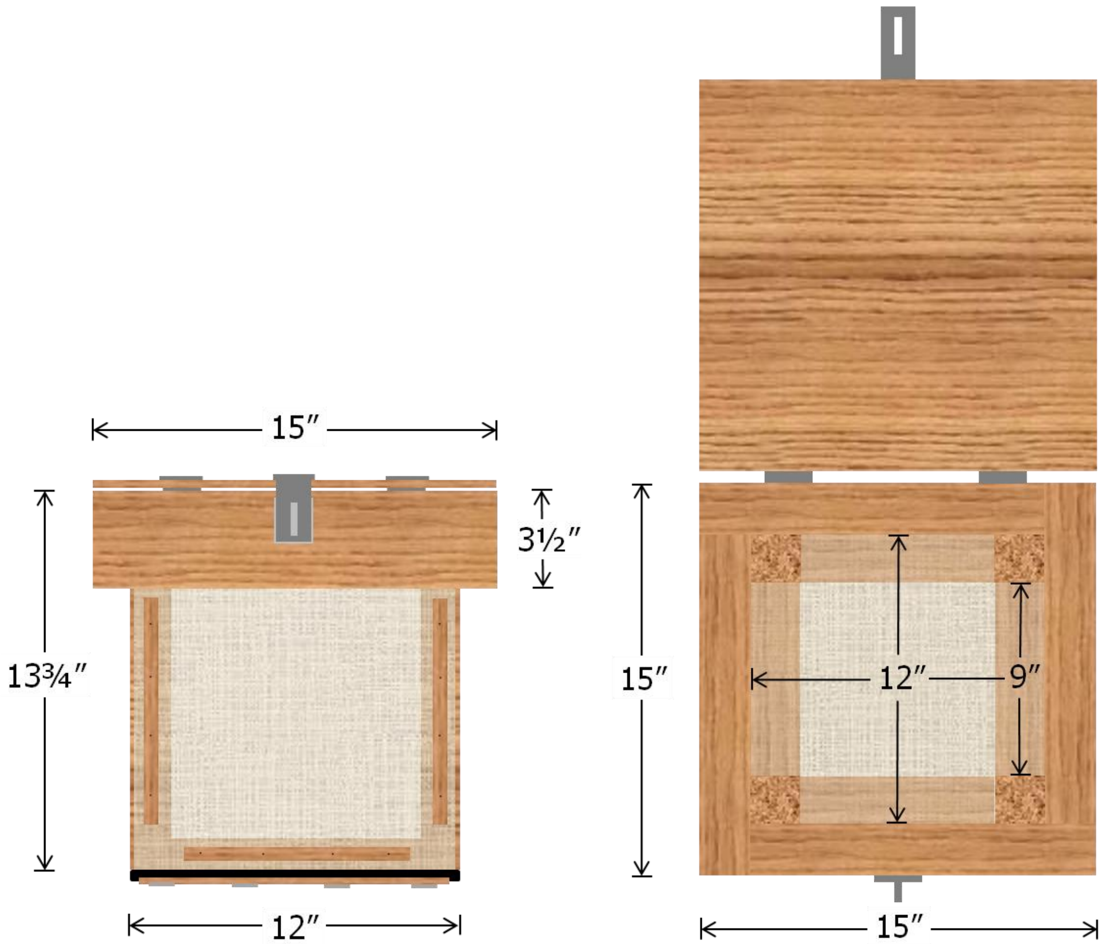

Figure 1. Dimensions of the cages constructed for the study.

Study Sites

Cages were deployed at eleven locations in the Upper Clark Fork River Drainage in late March 2011 (Figure 2). Seven treatment sites were located on the Clark Fork River:

1) Downstream of the Warm Springs Ponds (upstream of Warm Springs Creek)
2) Galen, MT
3) Deer Lodge, MT
4) Upstream of the Little Blackfoot River
5) Downstream of Gold Creek

6) Bearmouth, MT
7) Turah, MT

Three control sites were located on tributaries:

8) Lower Little Blackfoot River
9) Lower Flint Creek
10) Lower Rock Creek

One handling control site was located in a spring-fed channel:

11) Clinton, MT

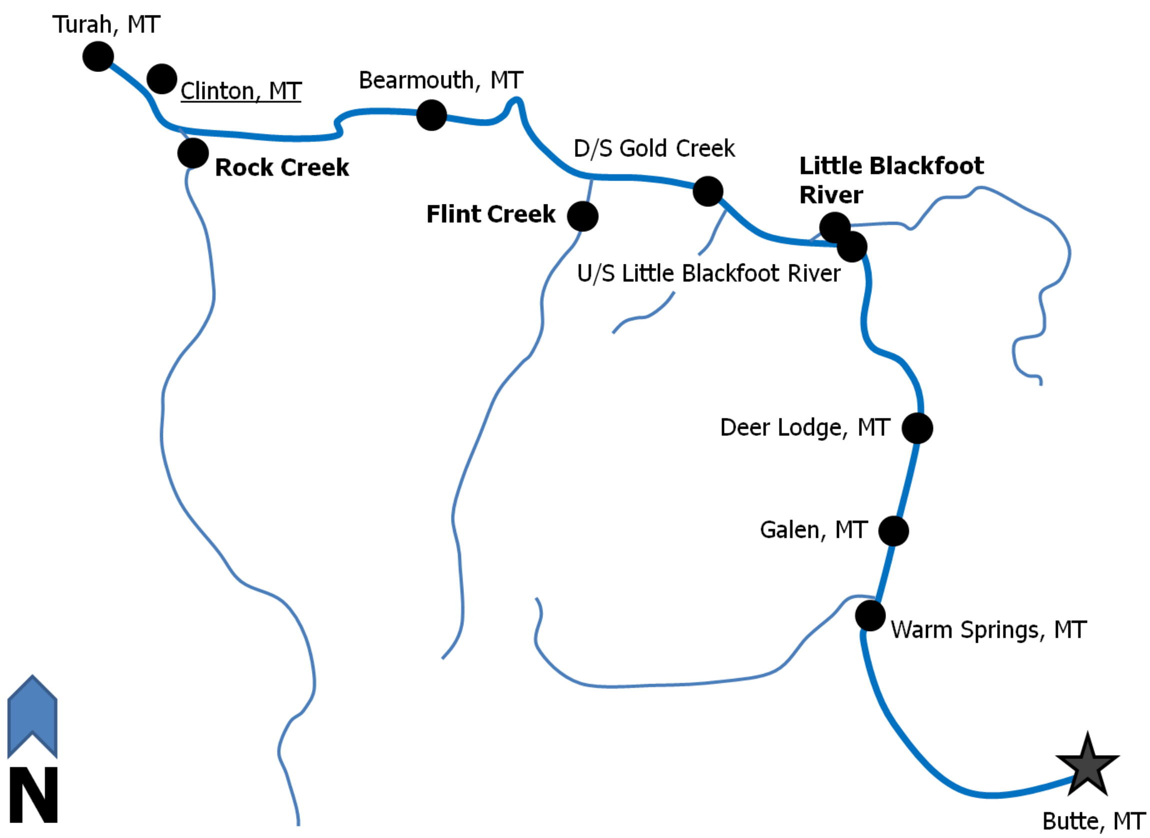

Figure 2. Distribution of the eleven study sites in the Upper Clark Fork River Drainage. Control sites are shown in bold and the handling control is underlined.

All sites except the spring channel near Clinton, MT (handling control), were located near U.S. Geological Survey (USGS) gauging stations equipped to measure discharge four times per hour. The handling control served as a reference to adjust mortality rates if cage checks (e.g., cleaning and relocating) negatively impacted survival, independent of water quality.

Deployment

Exact locations of the cages were dependent on the availability of low velocity habitats with access to refuge during periods of high runoff. Cages were positioned in velocities less than 0.75 ft/s. Three cages were deployed at each site. Two served as treatment cages (i.e., one replicate) and the third held fish for histology specimens and replacement of individuals in the treatment cages. The study began with 25 brown trout per cage and these densities were maintained in the treatment cages as long as possible by replacing them with individuals from the replacement cage. Cages were secured with sections of reinforcing bar (rebar) driven into the substrate, as well as sash weights and tether lines (Figure 3). The sash weights provided additional anchoring during rising water levels, and tether ropes insured the cages were not completely lost should a flood event occur.

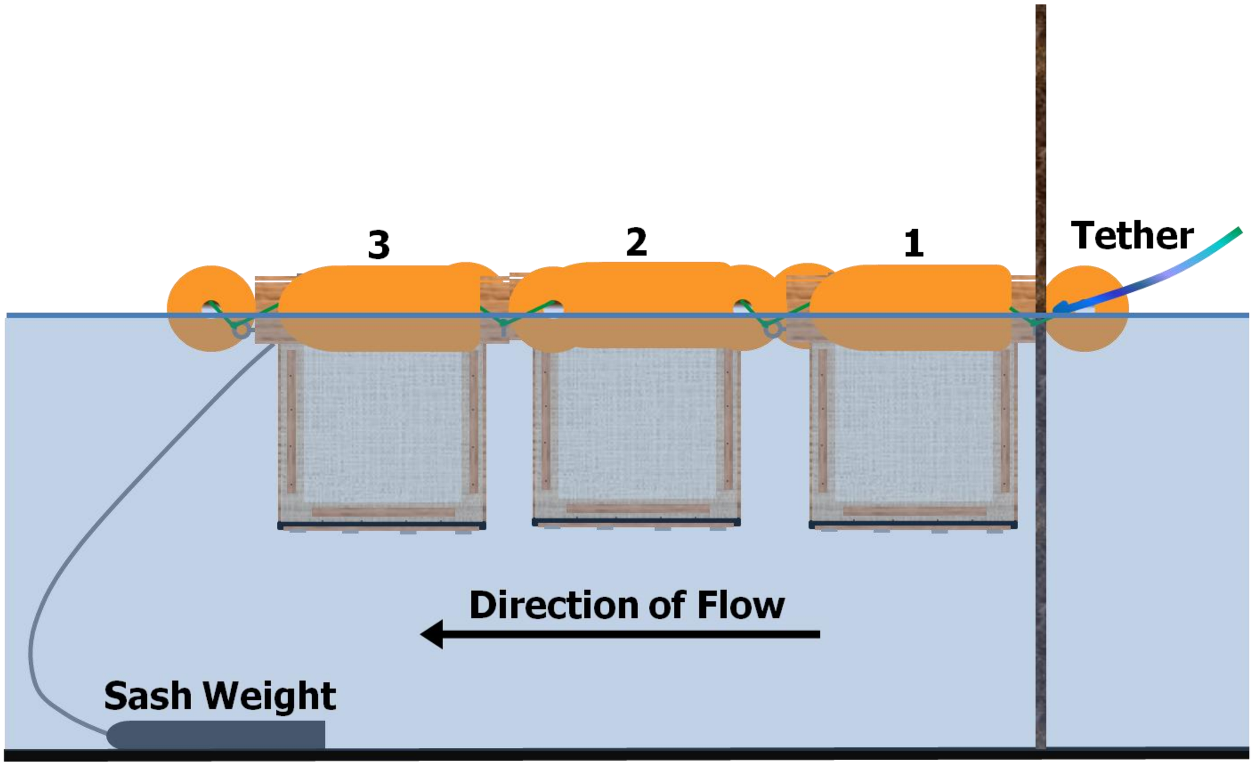

Figure 3. Representation of cage deployment (arrangement of cages differed by site, and cages often drifted together).

Brown trout were selected for this study given their dominance in the Upper Clark Fork River. Due to low densities of young trout in the upper river, study specimens were obtained from a state hatchery. The fingerlings ranged from 50-85mm and were feed-trained on pelleted food upon delivery.

On March 30, approximately 900 fingerling brown trout were obtained from Big Springs Hatchery in Lewistown, MT. The trout were transported from the hatchery to Helena, MT in a hauling truck and from Helena to the sites in an aerated cooler. At each site trout were anesthetized with clove oil, measured to total length, and divided into one of the three cages. Prior to being anesthetized, fish were acclimated to the site's water temperature with the addition of onsite water. At the first site stocked, the hatchery water was 8.2°C and water temperatures at the sites ranged from 4.8°C to 8.6°C. Mean length of trout stocked in cages was 68mm.

On March 31, a large rain event in the upper Little Blackfoot River drainage caused the Little Blackfoot to rise dramatically, washing the cages up on the bank. The cages were re-stocked with brown trout from Big Springs Fish Hatchery on April 4 (mean length was again 68mm).

Mortality Monitoring

Beginning the week of April 4, trout mortality was monitored twice per week. At each visit trout were fed one tablespoon of pelleted trout feed. During the first three months trout were fed 1.0 mm sinking feed (Silver Cup Extruded Salmon). The remaining months, trout were fed slightly larger No. 3 sinking feed (Silver Cup Crumbled Salmon/Trout). As discharges varied at the sites, cages were repositioned to seams and eddies with reduced velocities. Velocities around the cages were measured periodically to insure velocities did not exceed 0.75 ft/s. The exterior of the cages were brushed clean as needed to insure exchange of water in the cages. Mortalities were replaced with individuals from the replacement cage (Cage 3). Mortalities were measured to total length and frozen. All specimens were labeled and archived in a freezer at the Region 2 FWP headquarters.

Water Quality

Water samples were collected three times at each of the eleven sites. Collections roughly coincided with low-elevation runoff (ascending limb of the hydrograph), peak runoff and the descending limb of the hydrograph (Figure 4). Grab samples were collected for the caged fish study on May 10, June 30 and August 1, using the techniques outlined by the DEQ Field Procedures Manual for Water Quality Assessment Monitoring (DEQ 2005). All samples were delivered to Energy Laboratories Inc. in Helena, MT, within 6 hours of collection. Samples were analyzed for dissolved and total recoverable metals including copper, arsenic, lead, cadmium, and zinc, as well as total ammonia nitrogen (NH_3-N). Atkins collected additional water data under a contract for DEQ during the quarterly monitoring of the Clark Fork River Operating Unit (CFROU), and this data is available in a comprehensive report published by Atkin's (Figure 4; Atkins 2012).

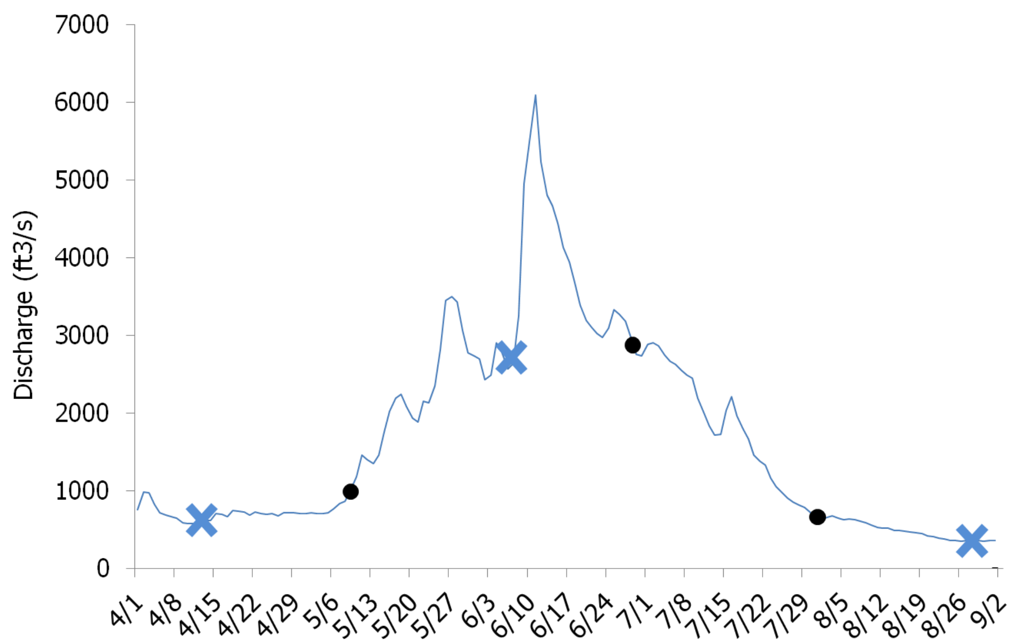

Figure 4. Clark Fork River hydrograph at the Gold Creek gauging station (roughly the midpoint of the study section). Black dots represent FWP and blue crosses represent Atkins collections.

Freshwater Aquatic Life Standards (ALS) were obtained from the table of standards for Montana waters or calculated using the hardness relationships described by DEQ (2010). The standards presented in the results are the mean ALS values across sites by date. The Acute ALS values were calculated as:

$$\text{Acute} = exp.\{ma[ln(hardness)]+ba\}$$

where *ma* and *ba* = values listed by DEQ (2010). Similarly, the Chronic ALS values were calculated as:

$$\text{Chronic} = exp.\{mc[ln(hardness)]+bc\}$$

where *mc* and *bc* = values listed by DEQ (2010).

Maximum daily water temperatures were obtained for each site with water temperature data loggers (HOBO ® U22 Pro v2). Loggers were attached to the rebar securing the cages in the channel and the units were most often set 6-12 inches above the substrate. During mid to late summer however, rapidly falling water levels left loggers near the surface at a few sites. Thus, higher water temperatures recorded at sites during these instances may not be representative of the temperatures within the cages. Loggers found near the surface were repositioned on the rebar and the cages were often moved to deeper water. Due to logger malfunction at the Rock Creek control site, the first half of the 2011 temperature data was lost. A portion of this data was substituted with data available from the adjacent USGS station.

Discharge

Discharge data presented in this report were obtained from USGS gauge stations, recording measurements four times per hour. Estimates of mean daily discharge were downloaded from the USGS National Water Information System: Web Interface. All estimates presented in this report were reviewed and approved for publication. Gaps in the Rock Creek dataset during June and July were the result of equipment malfunctions. No station exists at the site near Clinton, MT.

Histology

Histology specimens were preserved to help link fish condition to observed mortality patterns and metals concentrations. Specimens were collected twice in 2011, once in early August after mortality rates rose in mid- to late-July, and once in late August 2011 at the completion of the study season. Live specimens were placed in Davidson's solution, a combination of glacial acetic acid (100 ml), 95% ethyl alcohol (300ml), 10% neutral buffered formalin (200 ml), and distilled water (300 ml). A slit was made in the belly of most specimens to insure all organs were adequately preserved. After 72 hrs, specimens were transferred into alcohol. Specimens were submitted to the Bozeman Fish Health Center in January 2012. The samples will be examined for cellular changes, physical irritants, bacteria levels and copper accumulations, as well as the general condition of the gills, kidney, liver and skin.

Analysis

Statistical analyses of trout survival consisted of chi-square comparisons between observed and expected survival. Survival was used in place of mortality since three sites experienced zero mortality and chi-square calculations do not accommodate zero values. Survival was expressed as mean counts instead of mean percentages (number dead/25), since input data is standardized during the calculation of the chi-square statistic.

Relative contributions of tributaries were calculated with daily discharge data from ten USGS gauges. These estimations were used to adjust the predicted survival values used in the analyses. Ratios of contribution were used to adjust expected values and the potential influence on trout survival. The predicted survival at Warm Springs was set at 25 (i.e., 100%) because no recent work provides an empirical value.

Results

Mortality Monitoring

Although cages were in place from April 1 to August 31, losses at the Galen site by June 20 yielded fewer than 25 fish in the replicate cage. Thus, survival was analyzed until mid-June when all replicate cages contained 25 individuals. Mean survival (cumulative until June 20) observed from the Warm Springs site downstream was as follows; Warm Springs (100%), Galen (70%), Deer Lodge (98%), upstream of Little Blackfoot River (98%), within the Little Blackfoot River (90%), downstream Gold Creek (98%), Flint Creek (94%), Bearmouth (100%), Rock Creek (86%), Clinton (100%) and Turah (92%; Figure 5). No mortality had been observed at the Clinton handling control by June 20, and thus no adjustment was made to the cumulative survival observed at other sites.

Survival until June 20 displayed interesting trends. Survival remained high (100%) at the Warm Springs site, but fell to 70% at the Galen site only 1.5 miles downstream (Figure 5). By the Deer Lodge site, approximately 11.5 miles downstream survival recovered to 98%. The relatively high survival at the remaining mainstem sites indicated the need to split sites into groups for analyses. Thus, the sites upstream of the Little Blackfoot River (upper) were analyzed separately from the remaining sites downstream (lower). Relative contributions were quantified by mean discharge and used to adjust expected survival values. Adjustments were made for the three sites below tributaries and included Gold Creek, Bearmouth and Turah. Chi-square analyses indicated expected survival until June 20 differed significantly from observed survival at the upper sites ($P = 0.05$), but did not differ significantly at the lower sites ($P = 0.99$), at alpha levels of 0.05. These results suggest a source of mortality exists at or above the Galen site and is diluted downstream.

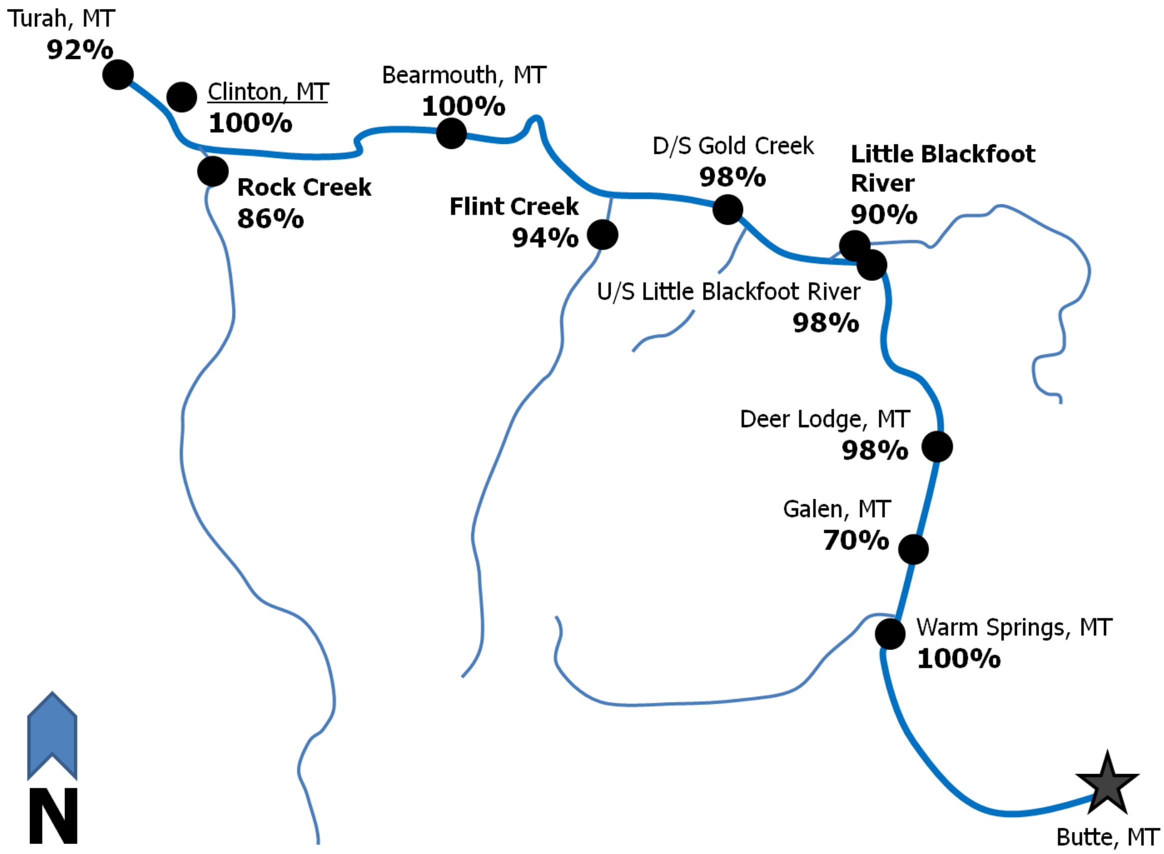

Figure 5. Mean survival observed until June 20, 2011. Control sites are shown in bold and the handling control is underlined.

Figures 6-15 illustrate the mortalities, maximum water temperatures and discharges observed during 2011. The dashed horizontal lines delineate 20°C and highlight instances where water temperatures may have become stressful. The black dots positioned along the hydrographs signify water sample collections and are useful in the water quality section of the report. Although mortalities were analyzed as means, they are plotted by cage (red and black) in the figures.

At the majority of sites, most mortality followed peak runoff as water temperatures rose. Sites deviating from this trend included the Galen, Little Blackfoot River and Flint Creek sites, which exhibited more consistent mortality throughout the study season. Survival at Rock Creek was unexpectedly low during 2011 (Figure 14). By June 20, Rock Creek was the second lowest in survival and beginning in mid-August survival fell dramatically. The cages at Rock Creek were located in a side-channel with some spring influence.

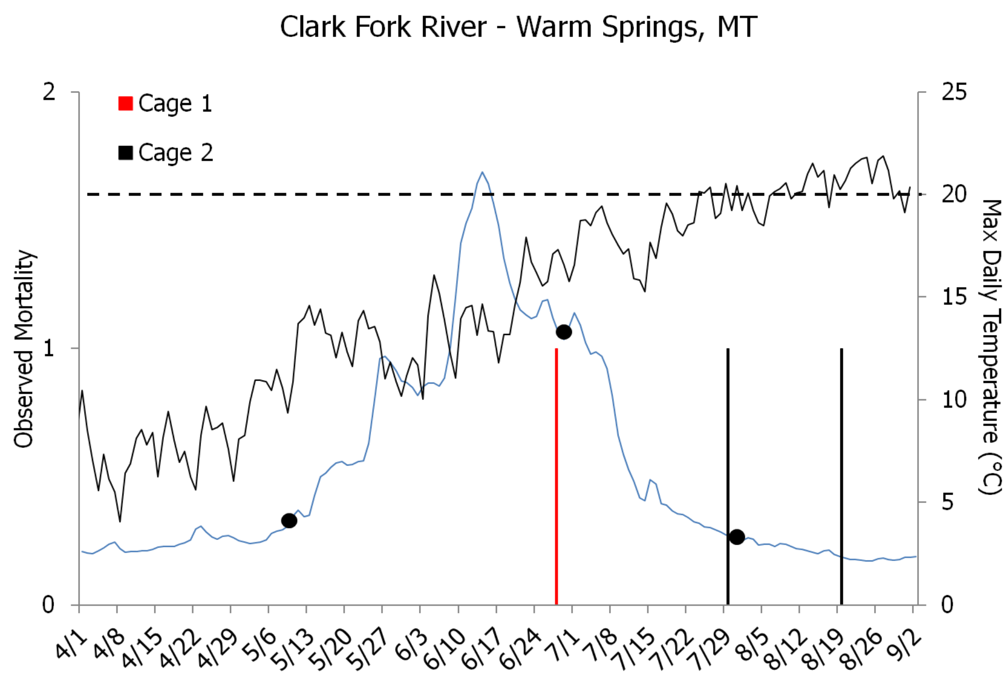

Figure 6. Mortalities, water temperatures (black) and discharges (blue) observed at the Warm Springs site located upstream of Warm Springs Creek.

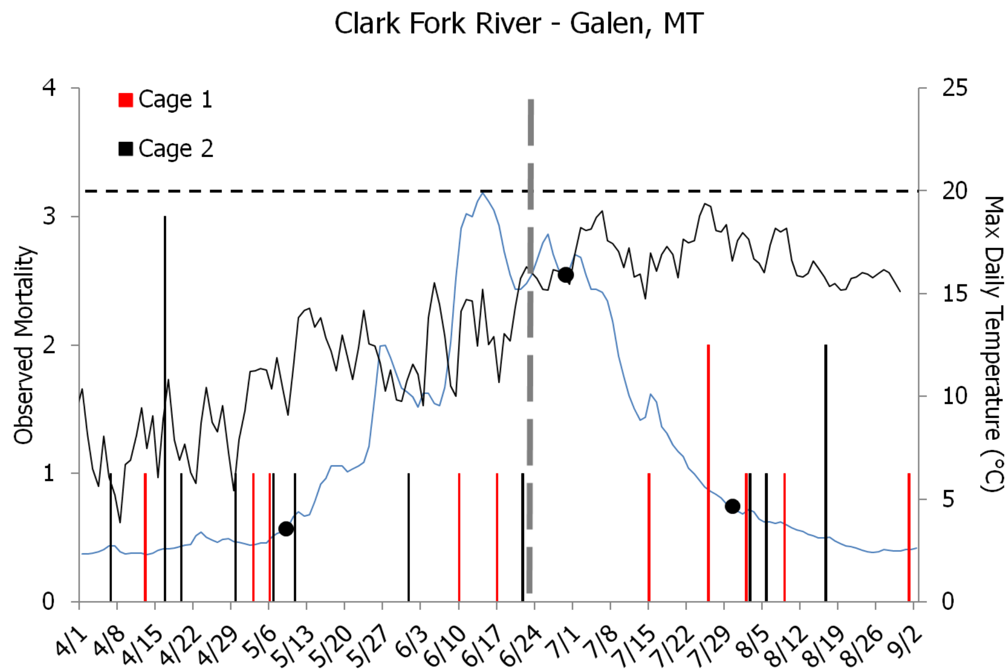

Figure 7. Mortalities, water temperatures (black) and discharges (blue) observed at the Galen site near Perkins Lane. The vertical dashed line on June 23 represents the date when Cage 3 was emptied.

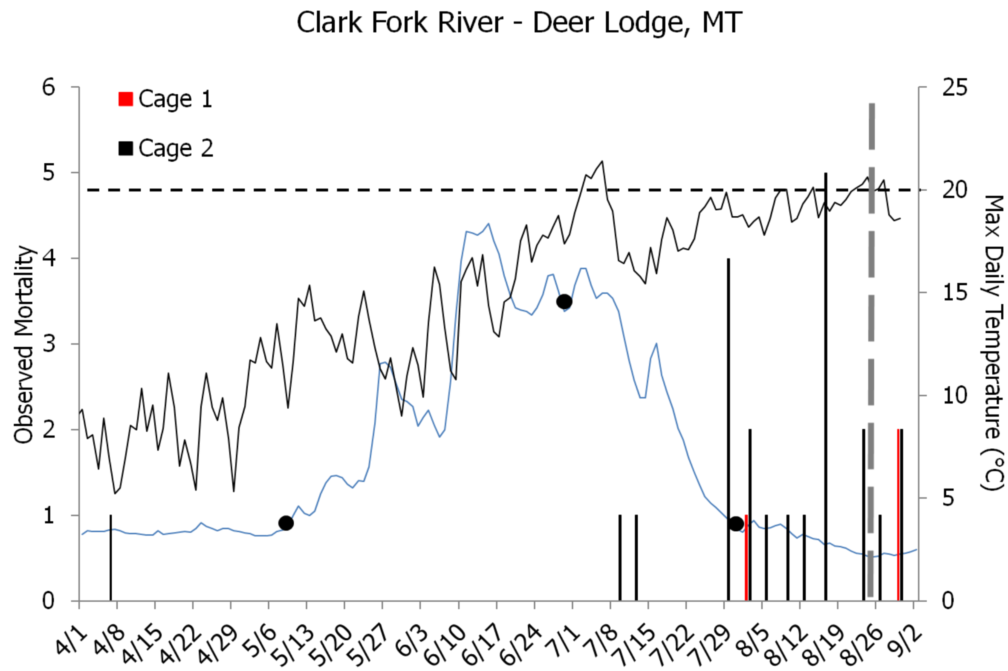

Figure 8. Mortalities, water temperatures (black) and discharges (blue) observed at the site upstream of Deer Lodge, MT. The vertical dashed line on August 25 represents the date when Cage 3 was emptied.

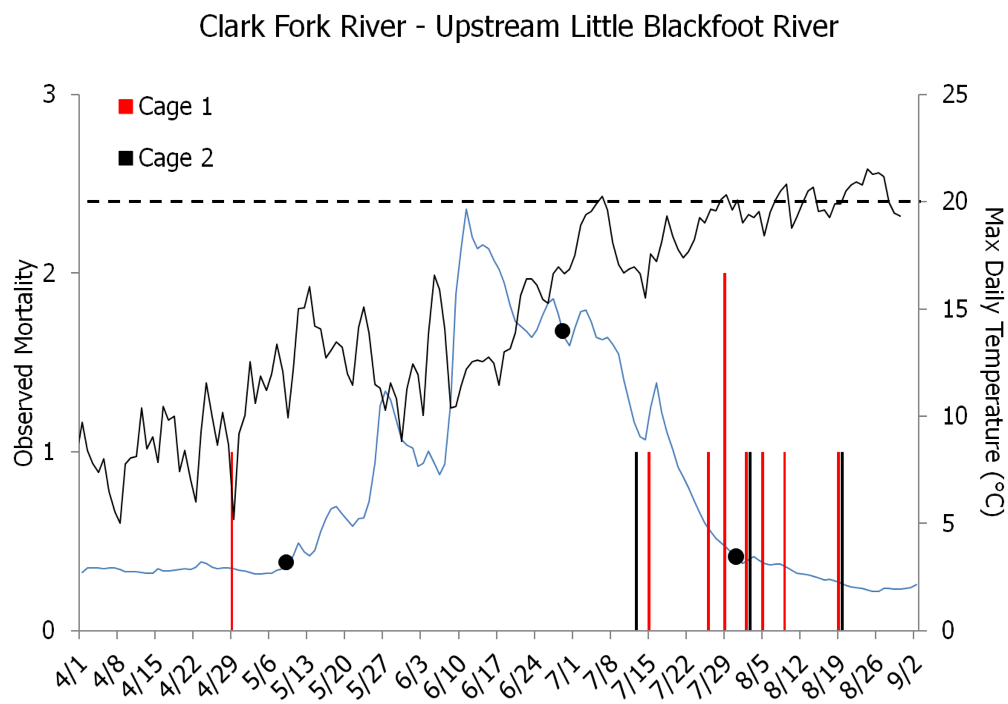

Figure 9. Mortalities, water temperatures (black) and discharges (blue) observed at the site upstream of the Little Blackfoot River.

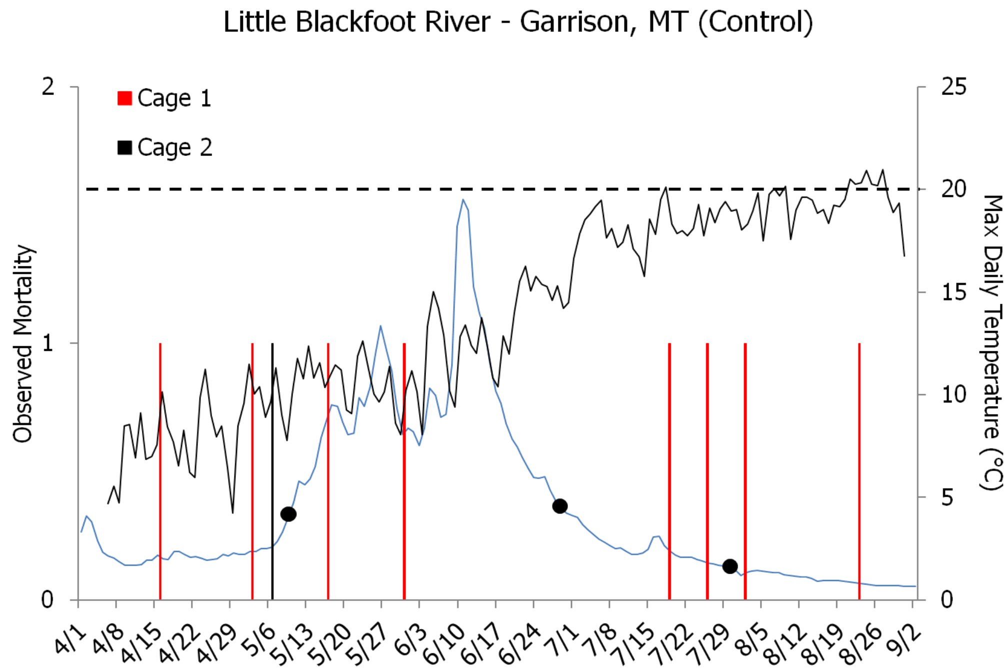

Figure 10. Mortalities, water temperatures (black) and discharges (blue) observed in the Little Blackfoot River.

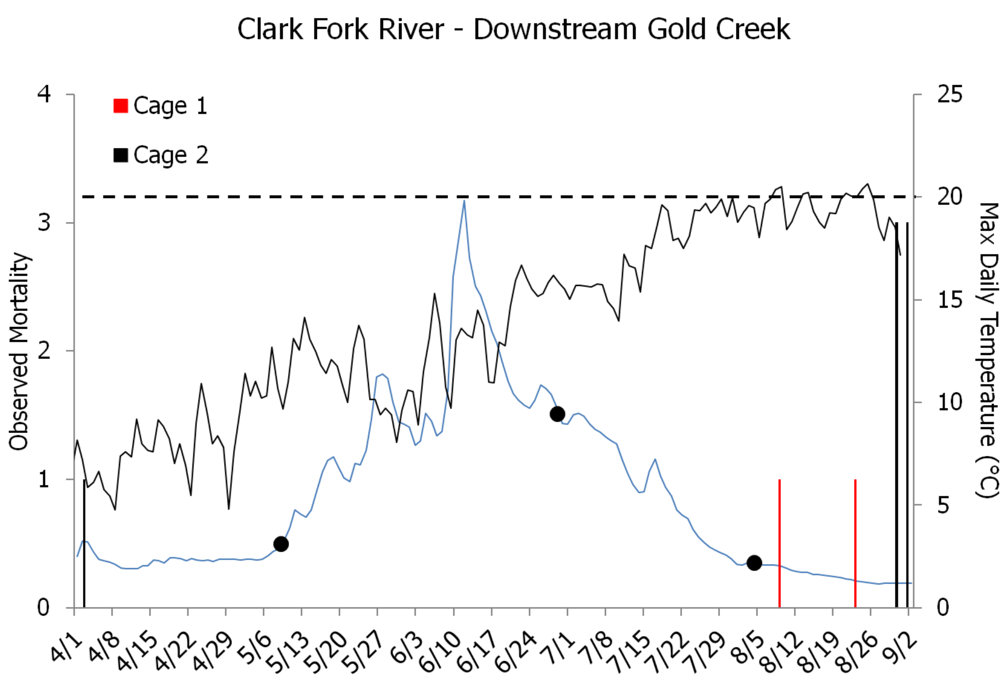

Figure 11. Mortalities, water temperatures (black) and discharges (blue) observed in the Clark Fork River downstream of Gold Creek.

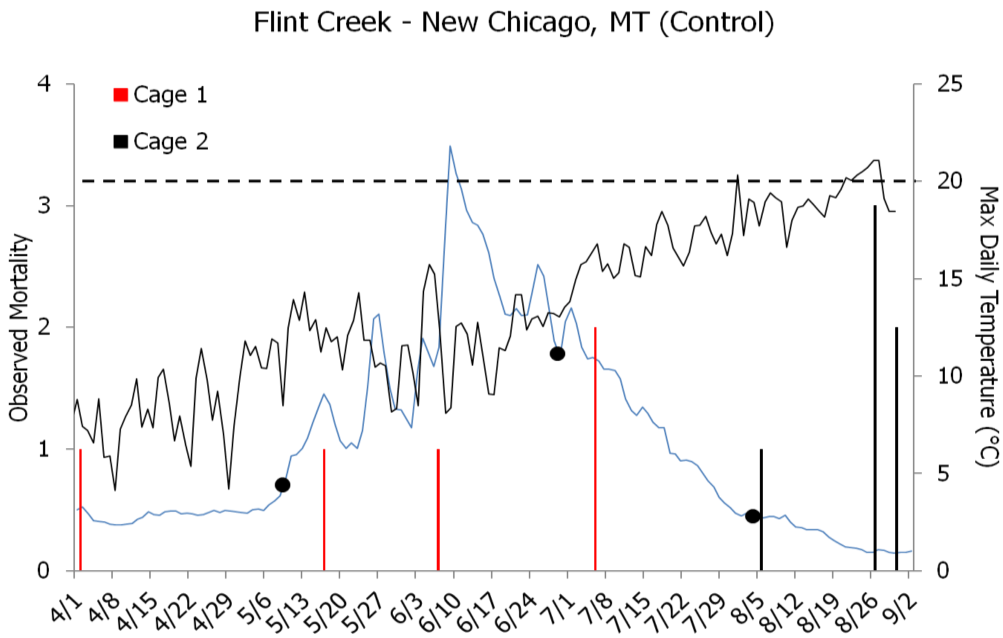

Figure 12. Mortalities, water temperatures (black) and discharges (blue) observed in Flint Creek.

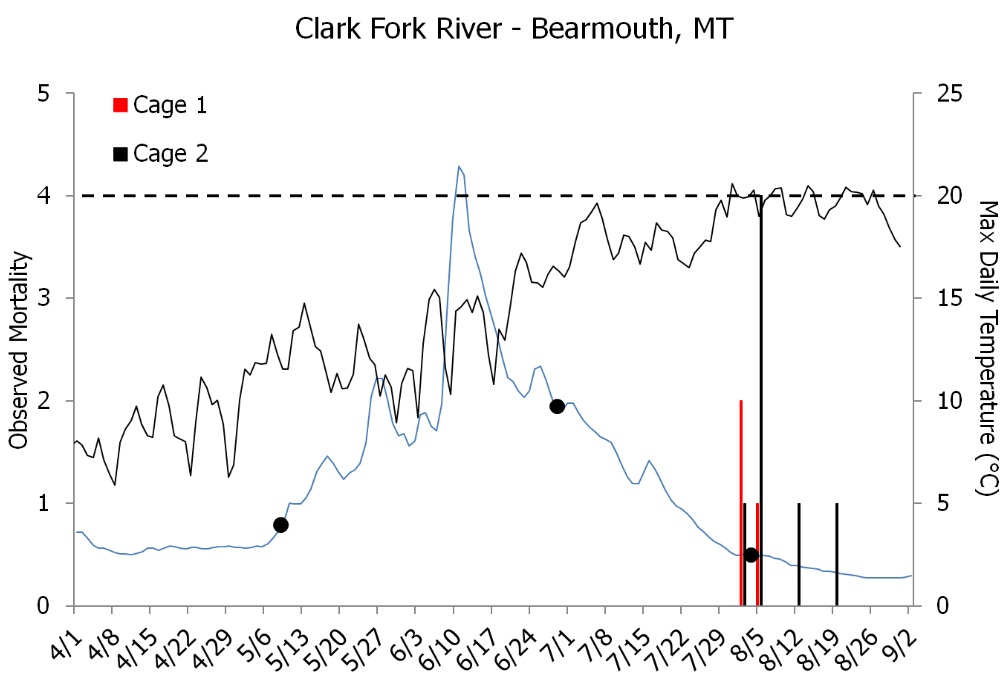

Figure 13. Mortalities, water temperatures (black) and discharges (blue) observed in the Clark Fork River near Bearmouth, MT.

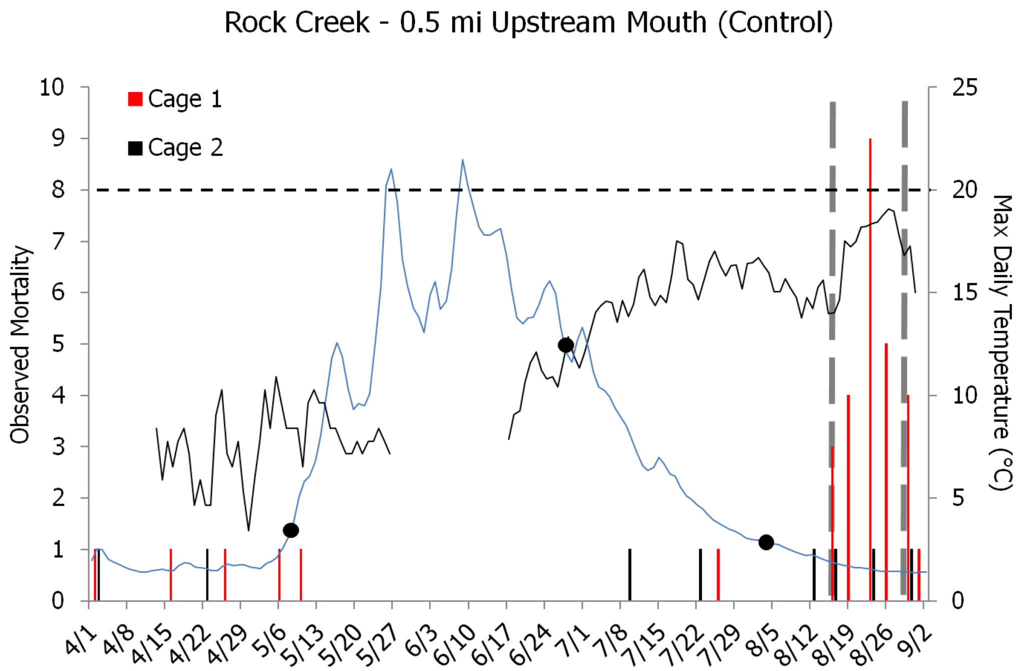

Figure 14. Mortalities, water temperatures (black) and discharges (blue) observed in Rock Creek. The vertical dashed lines on August 15 and 20 represent the dates when Cage 3 and Cage 2 were emptied, respectively. The missing temperature data were the result of equipment malfunctions. Temperature data before June 17 was obtained from the USGS gauging station.

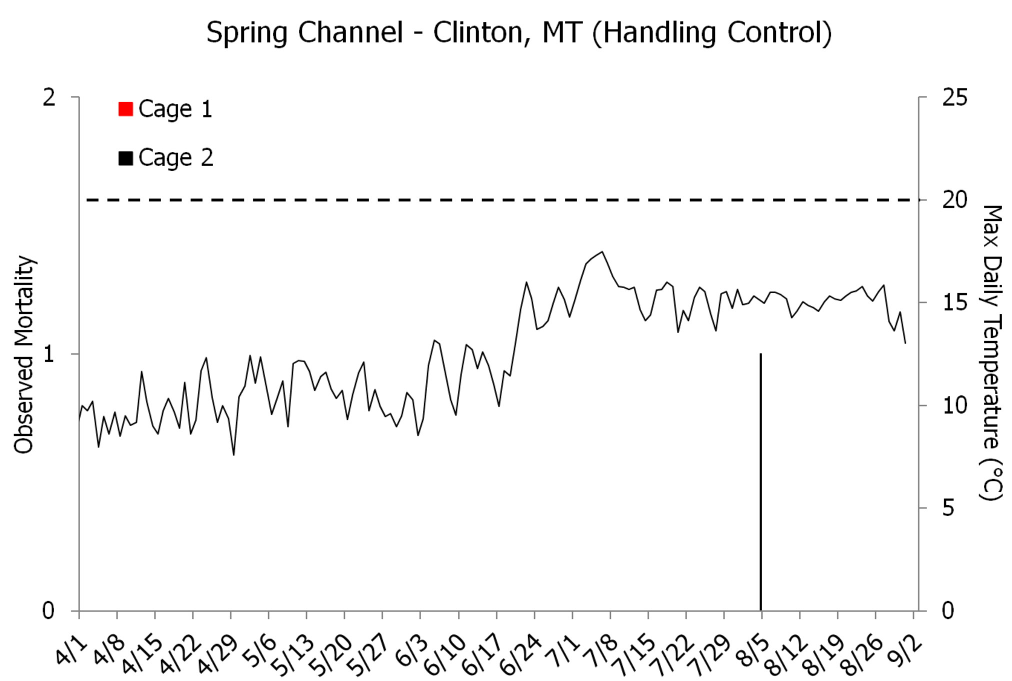

Figure 15. Mortalities, water temperatures (black) and discharges (blue) observed in the spring channel near Clinton, MT.

Growth

Initial lengths were measured at all the sites on March 31. Individuals were also measured during the second stocking of the Little Blackfoot cages on April 4. Final lengths were measured on either August 31 or September 1. A one-way Analysis of Variance (ANOVA) indicated initial lengths differed among sites (mean range: 64-70mm). Thus, growth was evaluated by mean change in total length (Figure 16). Growth varied by site and appeared to be related to location on the mainstem. Growth at the mainstem sites followed a decreasing trend from upstream to down, and growth in the tributary and handling control sites was slightly faster than at adjacent mainstem sites.

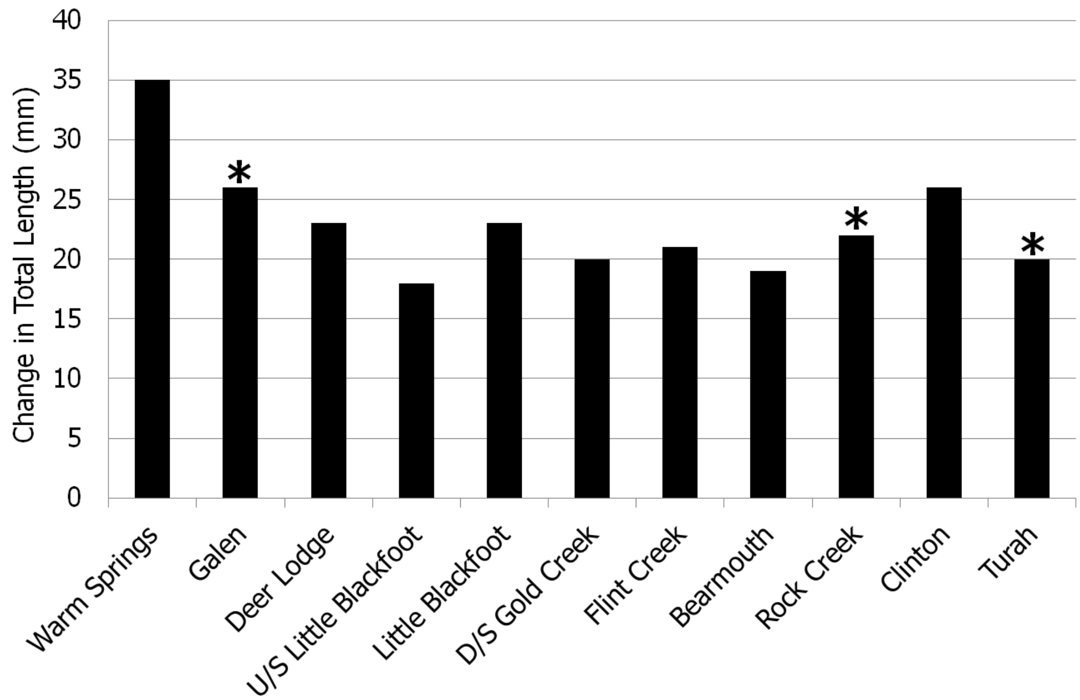

Figure 16. Mean change in total length observed in 2011, arranged from upstream to downstream. Asterisks denote estimates derived from means of one cage at the completion of the study. This occurred when fewer than 25 fish remained in the replacement cage (the original replicate cage).

Water Quality

Water samples collected during 2011 indicate concentrations of toxic metals were elevated or exceeded Freshwater Aquatic Life Standards (ALS) during the study. The ALS were calculated using the hardness relationships described by DEQ or obtained from the table of standards for Montana waters (DEQ 2010). Aquatic Life Standards were calculated using the mean hardness values from across all sites at each sampling event. These values are represented by dashed horizontal lines or listed in the upper right in the following graphs. No water quality standards were listed for magnesium.

Arsenic concentrations exceeded neither the Acute nor the Chronic ALS, but often exceeded the human health and drinking water (HH/DW) standard of 0.010 mg/L (Figures 17). Calcium concentrations varied across sites, but remained relatively low at the Rock Creek control site (Figure 18). Cadmium concentrations never reached the Acute ALS, but total recoverable cadmium exceeded the chronic standard at five of the mainstem sites and approached the chronic standard at two additional sites in early May (Figures 19). Dissolved and total recoverable cadmium concentrations at Deer Lodge exceeded the Chronic ALS in late June, as did total recoverable cadmium in the Little Blackfoot River and at Gold Creek. Dissolved copper concentrations exceeded the Chronic ALS at Deer Lodge and upstream of the Little Blackfoot River and total recoverable copper exceeded both standards at six mainstem sites in early May (Figure 20). All mainstem sites exceeded the Acute ALS for dissolved copper concentrations except for Turah in late June, and all mainstem sites, as well as the Clinton handling control, exceeded the Acute ALS for total recoverable copper (Figure 20). Dissolved copper concentrations at the site upstream of the Little Blackfoot River exceeded the Chronic ALS, and total recoverable copper exceeded the Acute ALS in early August. Total recoverable copper concentrations at the Galen and Deer Lodge sites also exceeded the Acute ALS. Dissolved lead concentrations were low across all sites and seasons, but total recoverable lead exceeded the Chronic ALS at all but three sites in early May and seven sites total in late June (Figure 21). Similar to calcium, concentrations of magnesium varied across sites, but remained relatively low at the Rock Creek control site (Figure 22). The Acute and Chronic ALS of zinc were calculated as the same value based on the constants presented by DEQ (2010). Total recoverable zinc concentrations exceeded the Acute/Chronic ALS once in early May at the Bearmouth site (Figure 23). Both dissolved and total recoverable zinc concentrations at Deer Lodge exceeded the combined ALS in late June. All dissolved and total recoverable zinc levels were below standards in early August. Ammonia (NH_3-N) was only detected at the Deer Lodge site on May 10 at a concentration of 0.06 mg/L.

At low gradient sites on the mainstem, small changes in discharge often yielded relatively large changes in elevation. In a few instances this left temperature loggers near the surface. Thus temperatures recorded between checks (once every 3-4 days) at these sites may not represent the exact temperatures experienced within the cages. Loggers were noted near the surface once at Warm Springs in mid-July, twice at Deer Lodge in mid and late-July, thrice at the site upstream of the Little Blackfoot River and once at Bearmouth in late-July. With these caveats in mind, maximum daily water temperatures recorded exceeded 20°C at eight of the eleven sites (Figures 6-15). On the mainstem, water temperatures exceeded 20°C on 27 days at Warm Springs, 11 days at Deer Lodge, 17 days upstream of the Little Blackfoot, 9 days at Gold Creek, 12 days at Bearmouth and 5 days at Turah. In control tributaries, water temperatures exceeded 20°C on 10 days in the Little Blackfoot, and 8 days in Flint Creek.

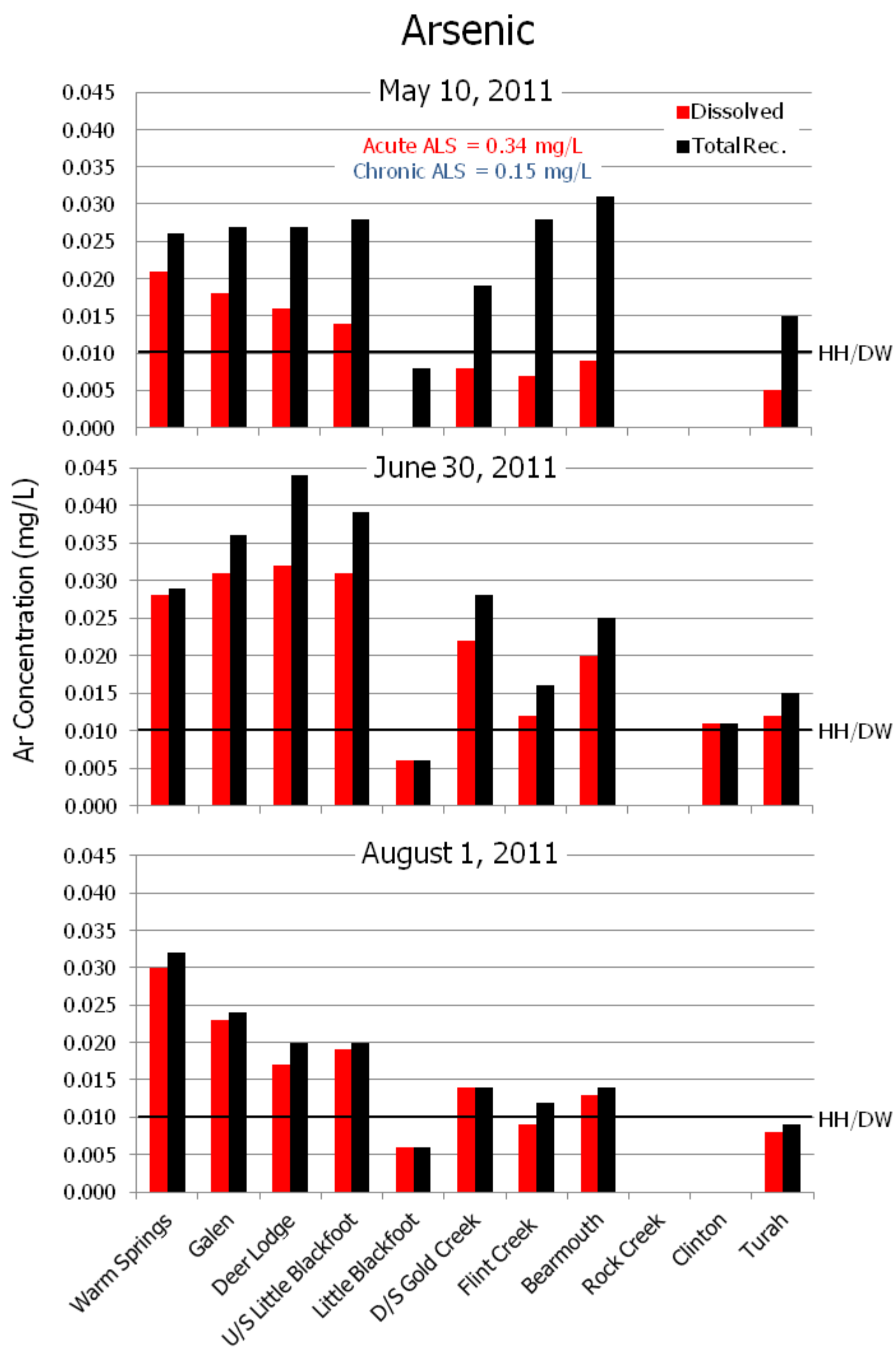

Figure 17. Arsenic concentrations at the 11 cage sites arranged from upstream to downstream. The calculated Acute ALS and Chronic ALS were 0.34 mg/L and 0.15 mg/L, respectively.

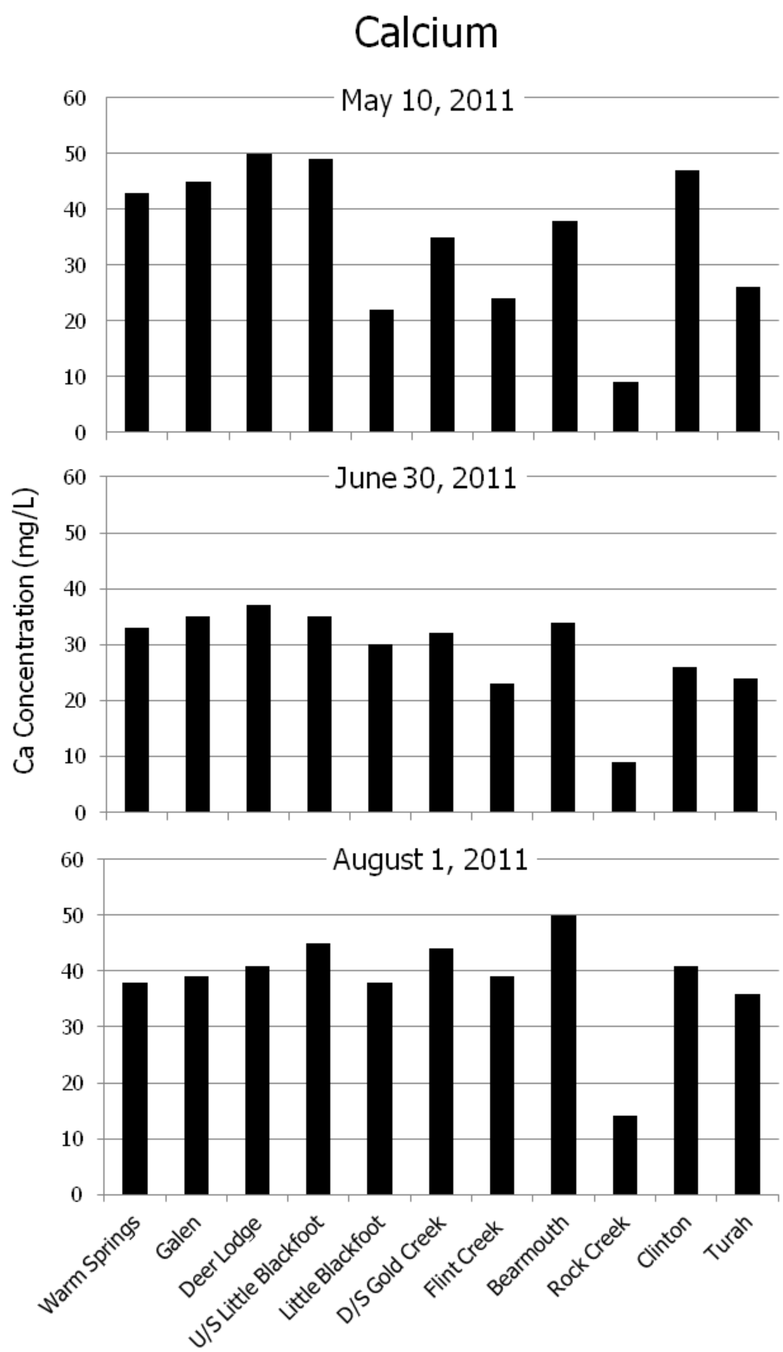

Figure 18. Calcium concentrations at the 11 cage sites arranged from upstream to downstream.

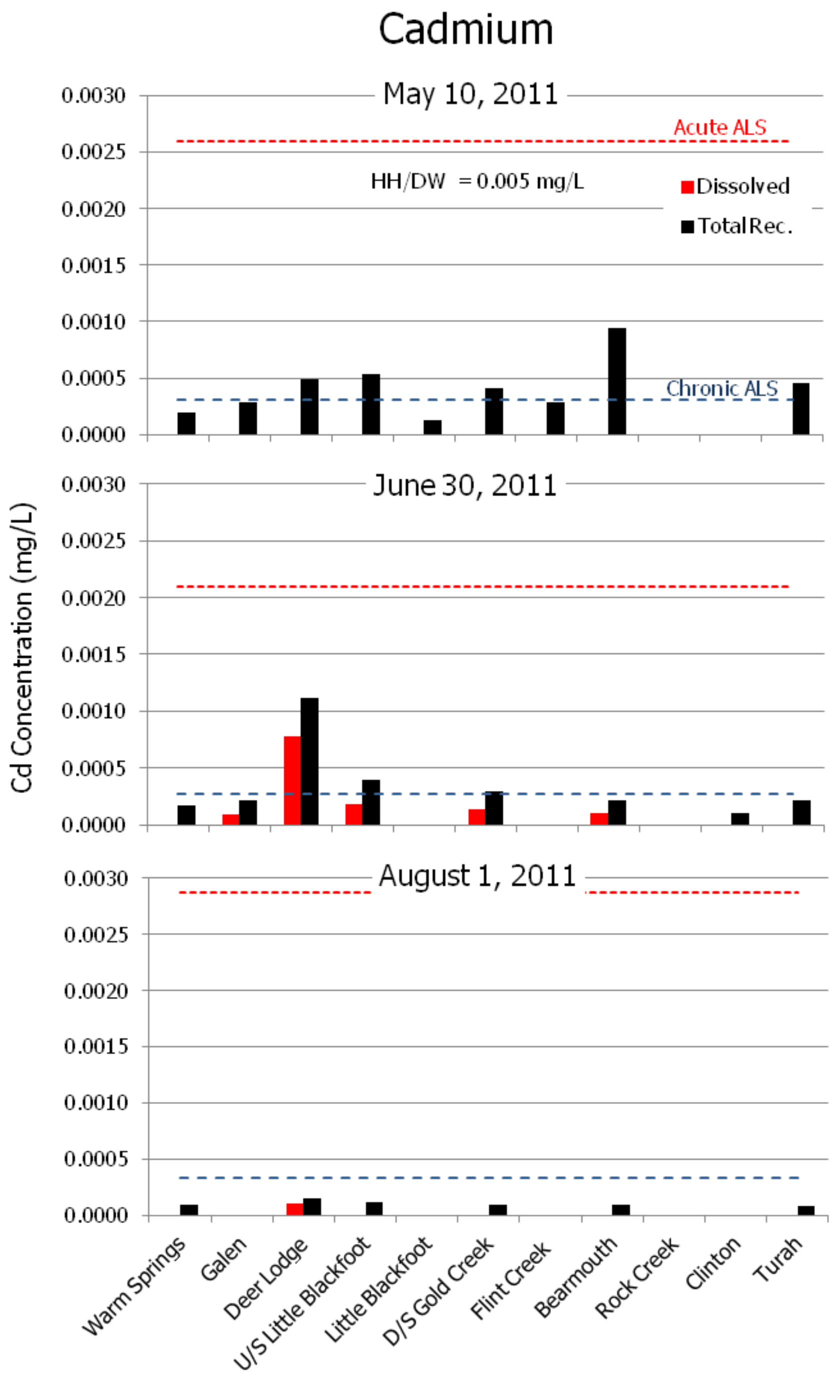

Figure 19. Cadmium concentrations at the 11 cage sites arranged from upstream to downstream.

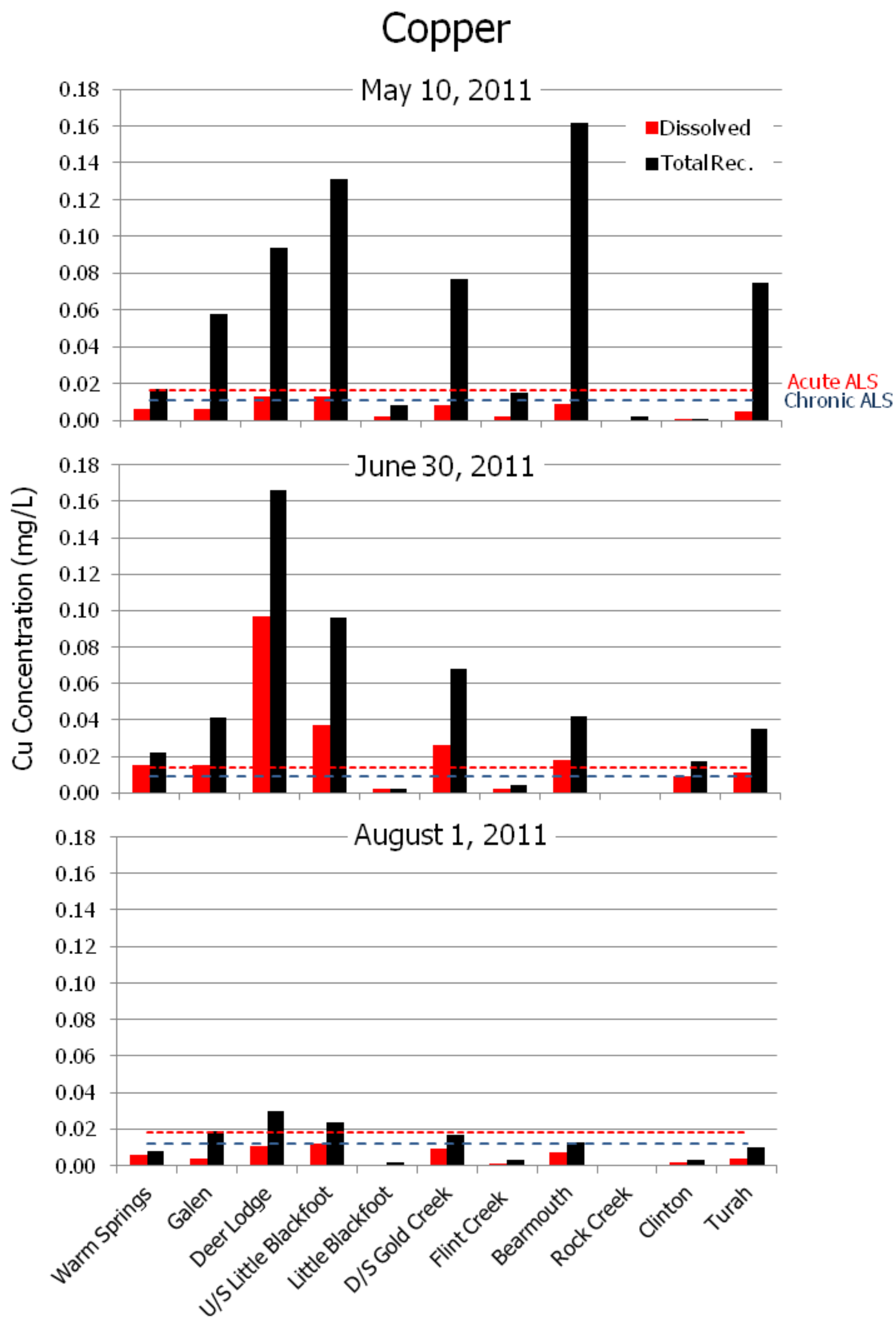

Figure 20. Copper concentrations at the 11 cage sites arranged from upstream to downstream.

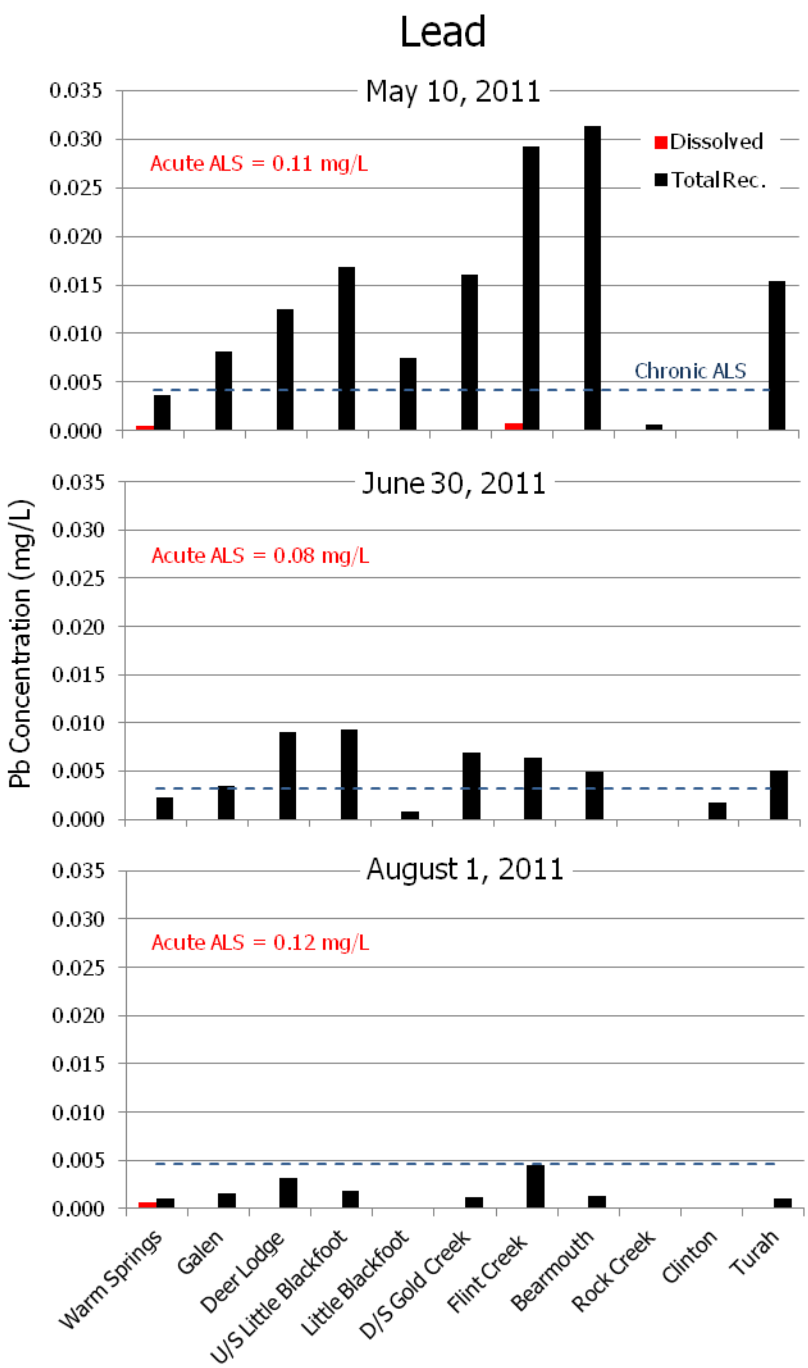

Figure 21. Lead concentrations at the 11 cage sites arranged from upstream to downstream.

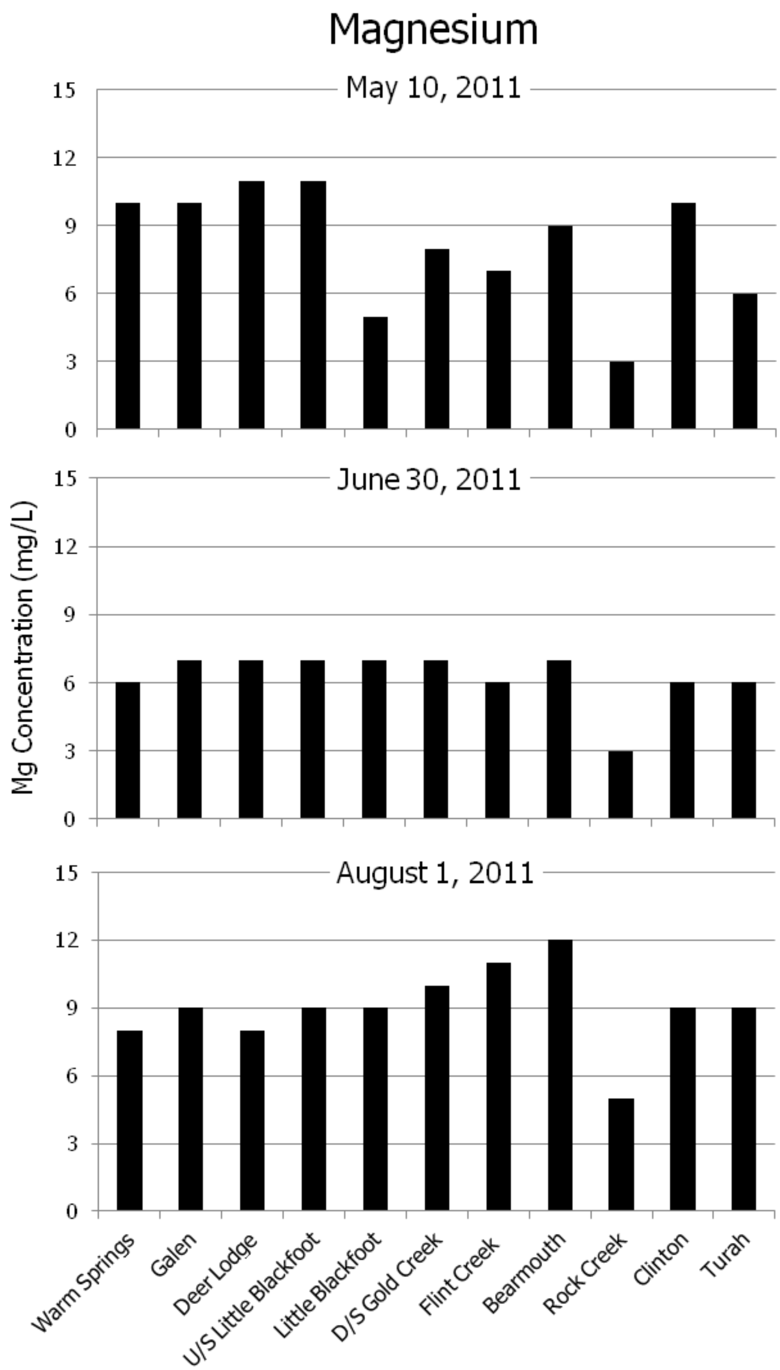

Figure 22. Magnesium concentrations at the 11 cage sites arranged from upstream to downstream.

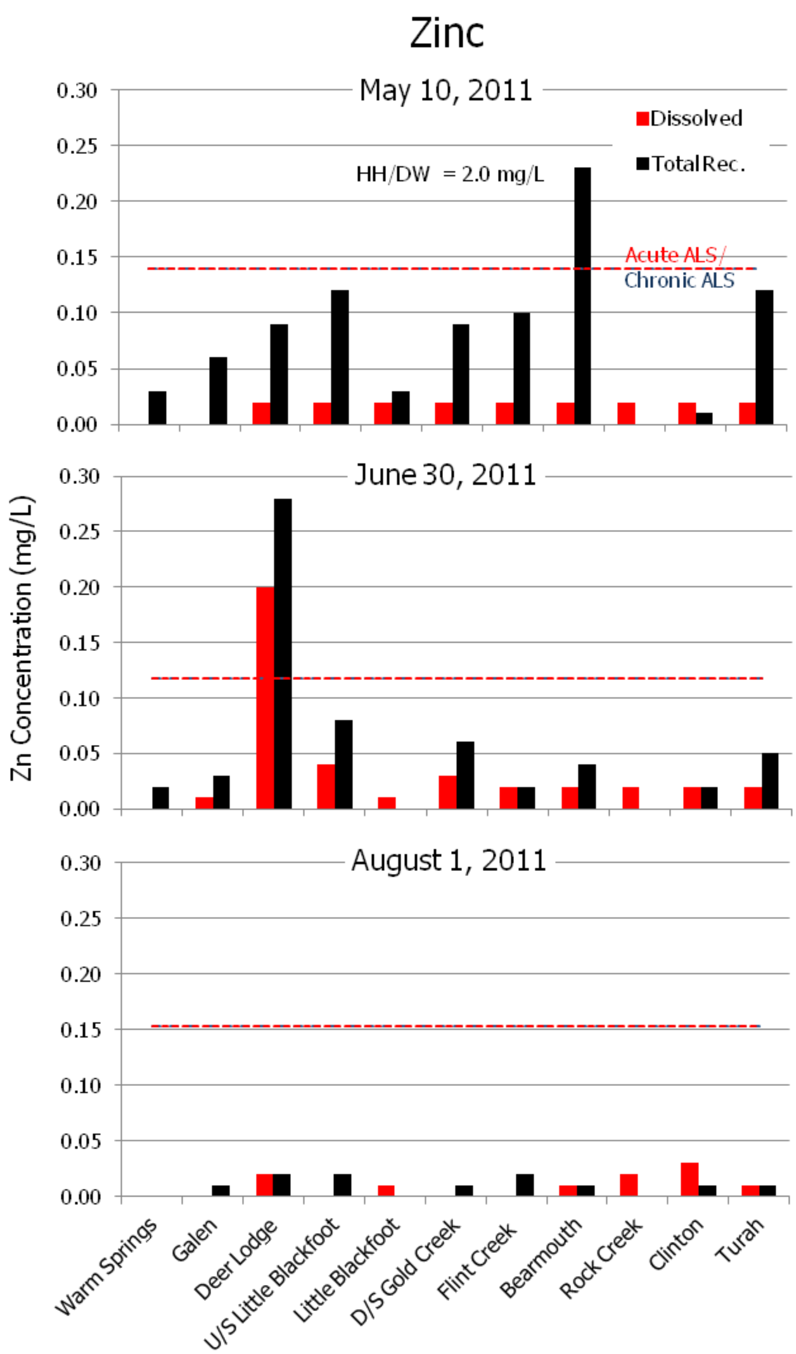

Figure 23. Zinc concentrations at the 11 cage sites arranged from upstream to downstream.

Histology

Histology analyses were performed by a consultant located in Bozeman, MT. Specimens from seven of the eleven sites were submitted to evaluate physiological condition. The specimens were collected on August 4, 2011 following substantial increases in mortality. At this point the trout had been held in the cages approximately four months. The seven sites submitted included; Warms Springs, Galen, Gold Creek and Turah and the three controls (Little Blackfoot River, Flint Creek, and Rock Creek). Histology results indicated liver and kidney conditions varied across the seven sites. The least severe changes in liver and kidney tissues were noted at the Warm Springs site, while the most severe changes in kidney tissue were noted at the Galen site. In general, most of the downstream cages were not as affected as at Galen, and specimens from the Warm Springs site were the only fish showing signs of recovery from tissue injury. No bacteria or parasites were found, nor was there evidence of infectious disease.

Kidney lesions were observed in the specimens (both hematopoietic and nephron elements), indicating the occurrence of a blood borne toxicant that resulted in a hemolytic crisis. Proliferation of interrenal cells (produce corticosteroids) in head kidney tissue was also noted in several fish, indicating a stress response that has been reported in fish exposed to heavy metals or other stressors. Cellular changes observed in liver hepatocytes also suggest exposure to toxicants.

Discussion

Trout are exposed to hazardous substances (i.e., metals) in the Clark Fork River through the surface water pathway and the food chain pathway as suggested by Lipton et al. (1995). This study investigated the affect of direct contact with metals (i.e., the surface water pathway). Caged juvenile trout were fed an uncontaminated diet and thus exposure to metals via diet was minimized (i.e., the food chain pathway). As with most field research, *in situ* toxicity studies must consider variability in discharge, climate, and water temperature, but additionally specimen diet, physiology and origin (e.g., resident stream or hatchery origin). This study occurred during a high-water year and used age-0 hatchery brown trout fed a commercial diet.

Brown trout mortality varied on spatial and temporal scales. In the early seasons (April through June) mortality was highest at the Galen site and at the Rock Creek site. Similar trends continued as the field season progressed, but mortalities became more widespread. Mortality was generally reduced from late May to mid-July, and increased from late July to August (Figures 6-15). Reduced mortality coincided with elevated discharges, as well as the highest concentrations of arsenic, cadmium, copper and zinc documented by the three water collections (Figures 17-23). The increased volume of water in the system did not appear to dilute metals concentrations. This trend was also documented by Atkins (2012), and has been noted historically on the Upper Clark Fork River (e.g., Hornberger et al. 2009). During this period water temperatures fell within suitable ranges (<20°C). Increased mortality coincided with reduced discharges and generally the lowest concentrations of cadmium, copper and zinc. Water temperatures rose during this period and temperatures at or above 20°C were recorded (higher values tentative at four sites). Although water temperatures may have been a contributing factor to late season mortality, brown trout are known to be more tolerant of elevated water temperatures, and it was unlikely the primary stressor. Unexpectedly high mortality at the Rock Creek control site raises a few questions. Although water temperatures and metals concentrations remained low at Rock Creek (Figures 17-37), considerable mortality was observed in August (Figure 14). The potential causes of these mortalities and the effects on Turah downstream will be further investigated in 2012.

Chi-Square analyses supported our preliminary interpretations that survival at the Galen site through June 20 differed from expected, given the observations at the Warm Springs site upstream. There was no significant difference between observed and expected survival in the lower sites (Little Blackfoot River and below). However, observations of mortality in the lower section following peak runoff suggest contaminated sediments were redistributed downstream, as documented in mine-impacted rivers such as the Clark Fork River (Hornberger et al. 2009). The intermittency of water data made it difficult to associate specific mortality events with changes in metals concentrations. Philips and Spoon (1990) also declared periodic water sampling may not be sufficient for assessing toxicity potential during their *in situ* studies in the late-1980s. In 2008 however, a caged fish study conducted by FWP on Silverbow Creek was able to document the effects of a rain event on instream metals concentrations and fish survival (Selch 2009). During the one month study, water samples were collected daily for the first week and every second or third day following. Despite what was considered a relatively small event; a rainstorm spiked concentrations of arsenic, copper, lead and zinc, and caused acute mortality of westslope cutthroat trout. The 2008 study showed westslope cutthroat trout, of hatchery origin, survive degraded water quality even during August when water temperatures are generally highest. Thus, it was proposed trout could survive in the study section during other times of the year when water quality conditions are more favorable. However, as with this study, the hatchery westslope cutthroat trout used in Silverbow Creek may have been more tolerant of metals due to their naivety to the Creek (i.e., shorter exposure to toxicants relative to resident fishes; Lipton et al. 1995).

Most of the fish kills documented on the Upper Clark Fork River have been attributed to thunderstorms (Averett 1961; Johnson and Schmidt 1988; Philips and Spoon 1990; Phillips 1992; Phillips and Lipton 1995). Intense events have released pulses of metals into the mainstem which have resulted in fish kills dating back to the 1950's (Phillips 1992; Lipton et al. 1995). During 2011, no thunderstorms were observed on the mainstem, but localized events may have occurred. However, during the peak of 2011 runoff, over-land flow was noted on slickens just downstream of the Warm Springs Ponds and in other locations along the Upper Clark Fork River.

Although metals concentrations were not elevated at the Galen site (Figures 17-23), mortality and histology observations indicate stressors are present in the vicinity. This aligns well with the reduced fish densities documented in the mainstem 1-2 miles downstream of the Warm Springs Ponds (Louma et al. 2008; Lindstrom 2011), and increased mortality of adult radio-tagged trout (Mayfield and McMahon 2010). The continuation of the caged fish study in 2012 will provide additional information and may reinforce or contradict this trend. The floodplain below the Warm Springs Ponds is scheduled for remediation (i.e., removal of slickens) within coming years and this effort will likely lessen the impacts of metals downstream (Hornberger et al. 2009).

Growth varied during the study, but growth on the mainstem followed a decreasing trend moving downstream (Figure 16). Greater growth at the Warm Springs site may have been a result of increased productivity and invertebrate availability below the Warm Springs Ponds (Louma et al. 2008, Lindstrom 2011). Although trout fed well on pelleted feed throughout the study, a variety of aquatic insects invertebrates were also available from April to early June. Trout were observed feeding on these insects numerous occasions and other invertebrates were likely consumed as well. The greatest abundances were noted below the Warm Springs Ponds, and other studies support these anecdotal observations (e.g., Louma et al. 2008). Although diet can be a significant pathway for bioaccumulation of metals (Lipton et al. 1995; Louma et al. 2008), the consumption of invertebrates at the Warm Springs site did not appear to affect growth or survival.

Trends in this study compare favorably to recent studies on the Clark Fork River. Mortality of caged rainbow trout on the Middle Clark Fork River also coincided with low elevation run-off and the descending limb of the hydrograph when water temperatures rose (D. Schmetterling, Montana Fish, Wildlife and Parks [FWP], personal communication). A recent radio-telemetry study on the Upper Clark Fork River documented < 50% survival of adult trout tagged in the spring through fall (Mayfield and McMahon 2010, 2011). Most mortalities during the telemetry study occurred during high spring discharges. These patterns have also been noted on the Middle Clark Fork River, where radio-tagged adults expire prior to caged juveniles (D. Schmetterling, Montana Fish, Wildlife and Parks [FWP], personal communication). Thus, since greater size is generally accompanied by greater resistance to metals (Lipton et al. 1995; Louma et al. 2008), these studies suggest resident fish, even as adults have lower tolerances of metals concentrations than young hatchery origin trout.

The results of this study suggest acute mortalities of caged fish may be more related to over-land flow following thunderstorms, as has been documented historically (e.g., Phillips 1992), than due to bank erosion and limited over-land flow during flood events. Although mortality and discharge were generally inverse in 2011, additional field seasons will be necessary to better identify the distribution and timing of caged trout mortality in the Upper Clark Fork River.

Future Study Plans

During 2011, mortalities often occurred more consistently in one cage than others at a site. Cages were brushed clean to insure water exchange, but water quality may have been lower when carcasses were present. This will be closely monitored in 2012, and mortalities may be removed more frequently.

The locations of the cages will remain the same at all sites except downstream of Gold Creek and in Rock Creek. Debris washed downstream during peak runoff 2011 changed the hydrodynamics of the Gold Creek site and the cages will be deployed a few hundred meters upstream in 2012. The new location will remain downstream of the Gold Creek confluence. The cages at the Rock Creek site may also be relocated, dependent on 2012 discharges.

The 2011 study season provides trout survival data during a high-water year. These observations will be used as the expected values in 2012 to identify longitudinal trends in brown trout survival within the Upper Clark Fork River, as well as the variability between years.

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