Upper Clark Fork River Caged Fish Study: The Distribution and Timing of Trout Mortality Final Report 2011-2012

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Cover Photograph: Brown trout in the replacement cage located at the handling control site near Clinton, Montana in April 2012.

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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 known that 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, Montana 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 (MTDEQ) 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, Montana), 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.

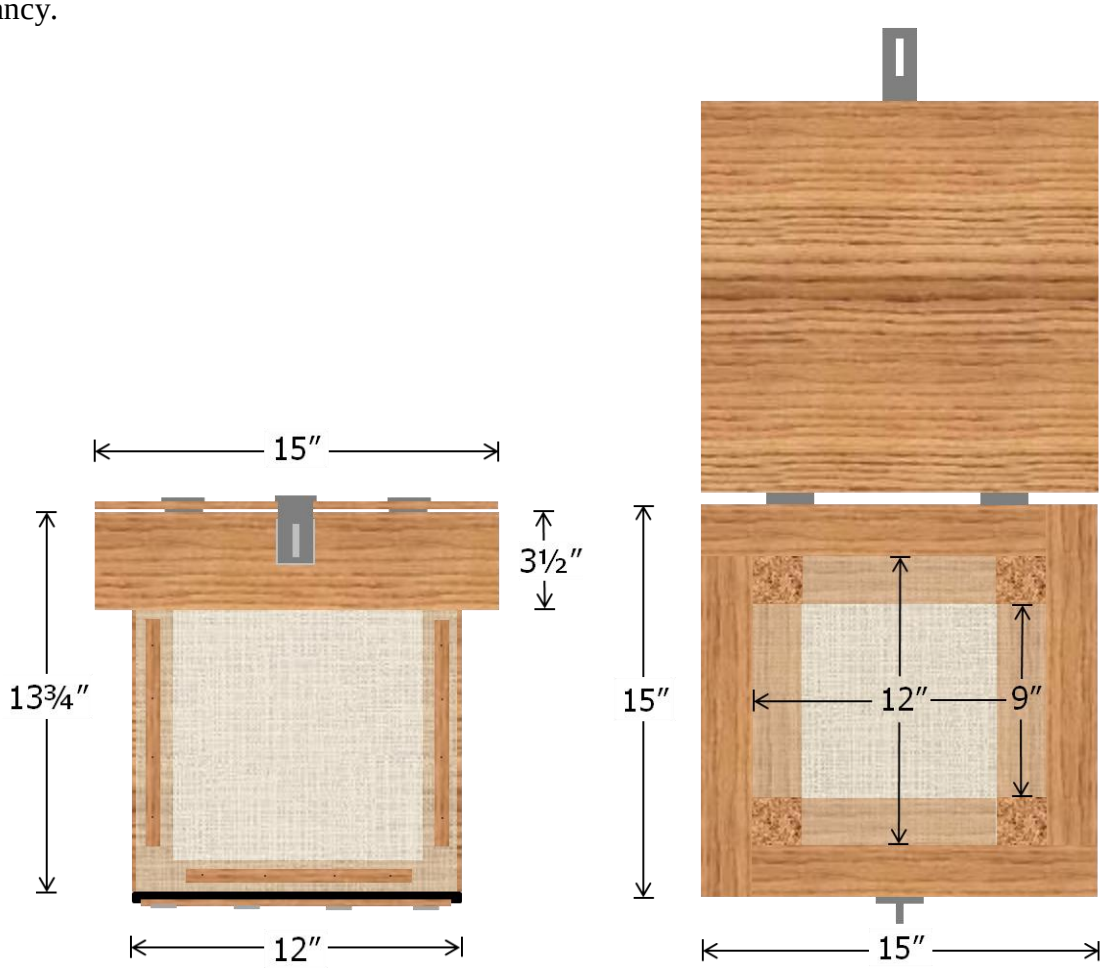

Figure 1. Dimensions of the cages constructed for the study.

Study Sites

Cages were deployed at eleven locations in the Upper Clark Fork River Drainage in late March 2011 and 2012 (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, Montana
3) Deer Lodge, Montana
4) Upstream of the Little Blackfoot River
5) Downstream of Gold Creek
6) Bearmouth, Montana
7) Turah, Montana

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, Montana

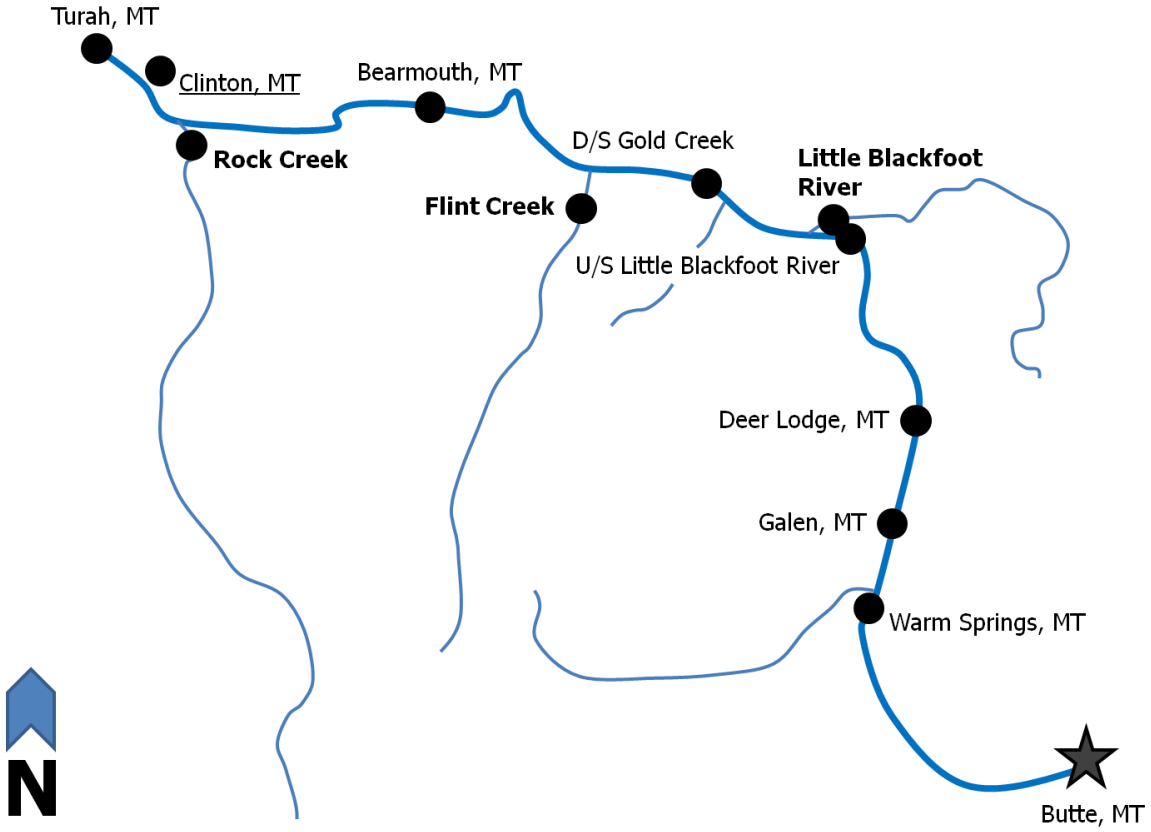

Figure 2. Distribution of the eleven study sites in the Upper Clark Fork River drainage. Control sites are shown in bold and the handling control is underlined.

All sites except the spring channel near Clinton, Montana (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.

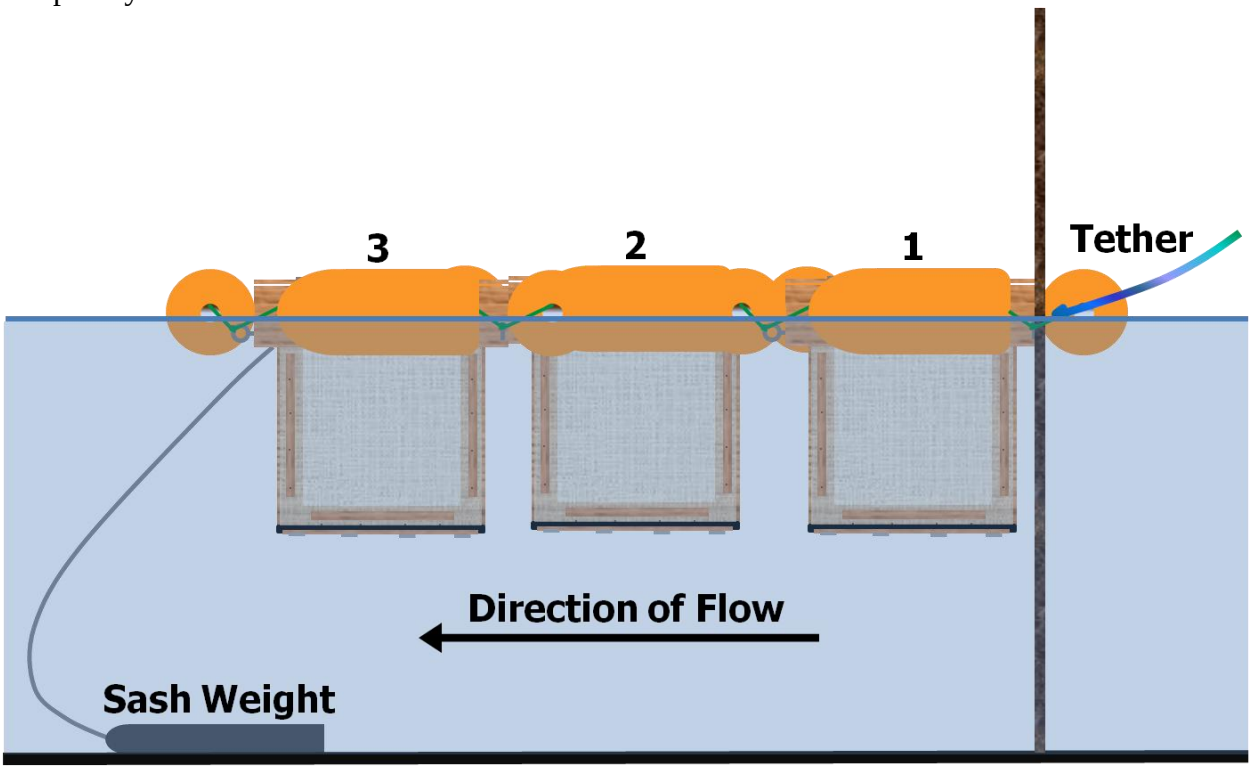

Figure 3. Representation of cage deployment (arrangement of cages differed by site, and cages often drifted together).

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 50-88 mm and were feed-trained on pellet feed upon delivery.

In late March of each year approximately 900 fingerling brown trout were obtained from Big Springs Hatchery in Lewistown, Montana. The trout were transported from the hatchery to Helena, Montana 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 water temperature at each site with the addition of onsite water. In 2011 at the first site stocked the hatchery water was 8.2 °C and water temperatures at the sites varied from 4.8 °C to 8.6 °C, and in 2012 at the first site stocked the hatchery water was 7.2 °C and water temperatures at the sites varied from 3.9 °C to 8.9 °C. Mean length of trout stocked in cages was 67.7 mm (SD = 6.6 mm) in 2011, and mean length of trout stocked in cages was 68.8 mm (SD = 6.2 mm) in 2012.

Mortality Monitoring

Beginning the first week of April each year, trout mortality was monitored twice per week. At each visit the trout in each cage were fed one tablespoon of pellet 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). Cages were repositioned to seams and eddies with reduced velocities as discharge varied at each site. Velocities around the cages were measured periodically to ensure velocities did not exceed 0.75 ft/s. The exterior of the cages were brushed clean as needed to provide for exchange of water between the cage and the site. At each visit mortalities were removed from the treatment cages (cages 1 and 2) and were replaced with individuals from the replacement cage (cage 3). All mortalities were measured to total length in millimeters and archived in a freezer at the Region 2 FWP headquarters.

Statistical analyses of trout survival at the seven treatment sites consisted of chi-square comparisons between observed and expected survival and mortality in 2011 and 2012 with $\alpha = 0.05$. Yates's correction for continuity was applied to all chi-square tests as the degrees of freedom for each test was one (Yates 1934). Expected mortality for each year was determined by using the mean mortality at the three control sites located in the Little Blackfoot River, Flint Creek, and Rock Creek (2011 mean mortality = 6.3; 2012 mean mortality = 6.0). Expected survival at each site was set to 50 as this was the number of live fish maintained in cages one and two combined. Mortalities during the first week of April each year were not included in the analyses to preclude any mortality due to acclimation. Mortalities after the end of July were not included in the analyses as all mortalities at the Clinton springs handling control occurred during August both years and any mortalities occurring at the treatment sites during this period may have been due to fish being held in cages.

Growth

All specimens placed in cages at the beginning of each field season were measured to the nearest millimeter and a one-way analysis of variance (ANOVA) was used to determine if initial lengths of fish placed in treatment cages differed among sites for each year. Initial lengths differed significantly among sites in 2011 (ANOVA: $F_{10, 539} = 7.19$, $P < 0.001$); thus, all surviving specimens were measured at the completion of each field season and growth was evaluated using change in mean total length by site for each year.

Histology

Cellular change can occur in various organs as a result of stressors and can serve as a biomarker of contaminant exposure (Bernet et al. 1999). 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. In 2012 specimens were collected at all sites in late August at the completion of the study season and individual fish that were near mortality but still alive at the time cages were checked were sacrificed for histology samples. This occurred three times during the 2012 field season, on May 17 downstream of Gold Creek, on July 30 in the Little Blackfoot River, and on August 23 at Warm Springs. In

2012 specimens were also collected March 27 immediately upon obtaining fish for the study from the Big Springs Hatchery for use as a control to establish baseline conditions. 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 ensure all organs were adequately preserved. After 72 hrs, specimens were transferred into alcohol. Specimens were submitted to the Bozeman Fish Health Center at the completion of each field season and were examined for cellular changes, physical irritants, bacteria levels and copper accumulations, as well as the general condition of the gills, kidney, liver and skin. Depending on size, fish were processed whole or dissected, then sectioned, mounted to slides, and stained with either hematoxylin and eosin or Giemsa stains. Slides were examined and observed cellular changes were scored on a scale of 1-5: 1 = minimal; 2 = mild; 3 = moderate; 4 = moderately severe; 5 = severe. In general, histological scores of 1 and 2 are considered normal or cellular changes of no significance; 3 is transitional or intermediary, indicating moderate cellular changes that may or may not be within the normal range depending on species, age, and sex of the fish; scores of 4 and 5 are indicative of pathological lesions.

Increased cellular turnover in kidney and liver tissue results in the proliferation of melanomacrophage aggregates indicative of chronic exposure to contaminants (Agius 1979; Herraez and Zapata 1986; Wolke 1992; Meinelt et al. 1997; Agius and Roberts 2003; Handy 2003; Schwindt et al. 2006; Rosseland et al. 2007). Melanomacrophage aggregates are considered reliable biomarkers of the effects of environmental exposure to pollutants and contaminants (Wolke et al. 1985; Goksoyr et al. 1996; Fournie et al. 2001). Melanomacrophage aggregates in the livers and kidneys of fish sampled were scored for degree of severity. Histology scores ≥ 4 for degree of melanomacrophage aggregation in juvenile brown trout kidneys and scores ≥ 1 in juvenile brown trout livers indicate chronic severe cell necrosis (B. MacConnell, Headwaters Fish Pathology LLC, personal communication).

Tissue Metals Burdens

Tissue metals burdens in fish can be used as a measure of exposure and can be correlated to histopathological effects (Hansen et al. 2004). Upon completion of the study, all mortalities from the replicate cages from May through August each year were submitted to the Montana Department of Health and Human Services Environmental Laboratory in Helena for analysis of tissue metals burdens. Mortalities from each site during each month of each year were combined and submitted as a single sample ($N = 55$) for analysis. In addition, in both years all fish surviving at the conclusion of the field season at the end of August at each site were also combined and submitted as a single sample ($N = 22$) for tissue analysis.

Samples were blended to a powder to ensure homogeneity, and then the samples were weighed, dried, and reweighed to determine moisture content. The dried samples were then crushed and dissolved with nitric acid, diluted with deionized water, and analyzed for barium, beryllium, chromium, copper, manganese, nickel, and zinc with inductively coupled plasma optical emission spectrometry (ICP-OES) using the U.S. Environmental Protection Agency (USEPA) Method 200.7 (USEPA 2001). The samples were also analyzed with inductively coupled plasma mass spectrometry (ICP-MS) for contaminants that have a lower detection limit including arsenic, cadmium, lead and selenium using USEPA Method 200.8 (USEPA 1999). All results were reported as µg/g dry weight.

Water Contaminants

In both years 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 using the techniques outlined by the MTDEQ Field Procedures Manual for Water Quality Assessment Monitoring (MTDEQ 2012a). Samples were collected on May 10, June 30, and August 1 in 2011 and on April 20, June 5, and August 9 in 2012. All samples were delivered to Energy Laboratories Inc. in Helena, Montana and were analyzed for dissolved and total recoverable metals including copper, arsenic, lead, cadmium, and zinc, as well as calcium, magnesium, and total ammonia nitrogen (NH_3-N). Atkins collected additional water data under a contract for MTDEQ during the quarterly monitoring of the Clark Fork River Operating Unit (CFROU) (Figure 4). This data is available for 2011 in a comprehensive report published by Atkins (Atkins 2012); the Atkins report detailing the 2012 data was not yet available at the time of preparation of this manuscript.

Performance standards have been identified for contaminants in the upper Clark Fork River (USEPA 2004; Atkins 2012) and are defined as the more stringent of the freshwater aquatic life standards (ALS) published by the MTDEQ (2012b). As the chronic aquatic life standard is the most stringent and this study focuses on chronic effects the chronic ALS was used to evaluate contaminant data. Freshwater ALS are a function of total water hardness and are evaluated on the basis of total recoverable metals concentrations (Atkins 2012; MTDEQ 2012b). Chronic freshwater ALS values were obtained from the table of standards for Montana waters or calculated using the hardness relationships described by MTDEQ (2012b). The chronic ALS values were calculated as:

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

where *mc* and *bc* = values listed by MTDEQ (2012b). Chronic ALS compliance ratios were calculated by dividing the measured contaminant values by the calculated chronic ALS values, and were plotted for each site and sampling period. Compliance ratio values <1 indicate contaminant levels below the chronic ALS, while values >1 indicate contaminant levels above the chronic ALS.

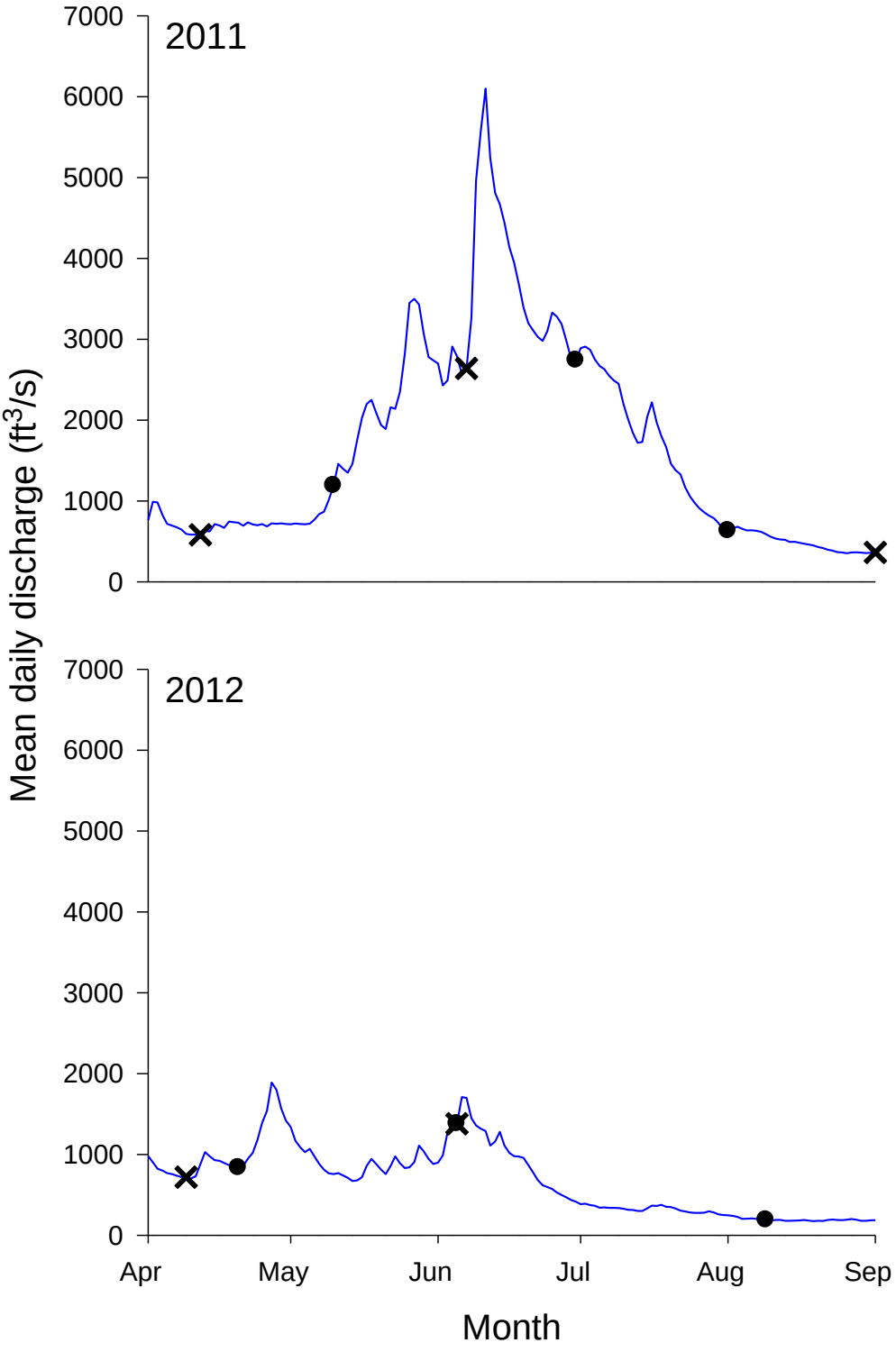

Figure 4. Clark Fork River hydrographs for 2011 and 2012 at the Gold Creek gauging station (roughly the midpoint of the study area). Dots represent FWP and crosses represent Atkins water collection dates.

Discharge and Water Temperature

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 2011 and on the Little Blackfoot River in June 2012 were the result of equipment malfunctions. No station exists at the site near Clinton, Montana.

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. Due to logger malfunctions temperature data may contain gaps at some sites; when available, this data was substituted with data from the appropriate USGS station.

Water Quality

Water quality parameters were recorded in the Clark Fork River at four sites in 2011 and at five sites in 2012 with continuously recording multiparameter water quality probes (Hydrolab ® MS5). Cross referencing of data collected with continuously recording multiparameter water quality probes was achieved by sampling intermittently at the seven treatment and four control sites using a handheld multiprobe (YSI ® 556 MPS). Hydrolab and YSI probes were calibrated at regular intervals during each field season. Probes were deployed at Galen, upstream of the Little Blackfoot River, upstream of Gold Creek, and at Bearmouth in 2011; in 2012 probes were once again deployed at these locations with the addition of a probe upstream of Warm Springs Creek. Water quality parameters recorded include temperature, pH, oxidation reduction potential (ORP), specific conductivity, and luminescent dissolved oxygen (LDO) at all sites, with the addition of total ammonia ($NH_4 + NH_3$) upstream of Warm Springs Creek and at Galen. Toxicity of total ammonia is dependent on other water parameters including water temperature and pH (Emerson et al. 1975; MTDEQ 2012b). The increased toxicity is due to the conversion of the generally inert form (NH_4) to the highly toxic form (NH_3) through the process of de-ionization (Barton 1996). Acute freshwater ALS for total ammonia based on hourly average measurements and chronic ALS based on a 30 day average were calculated based on equations published by MTDEQ (2012). The acute ALS values were calculated as:

$$\text{Acute} = (0.275/(1+10^{7.204-pH})) + (39.0/(1+10^{7.204-pH}))$$

and the chronic ALS were calculated as:

$$\text{Chronic} = ((0.0577/(1+10^{7.688-pH})) + (2.487/(1+10^{pH4-7.688}))) \times \text{MIN}(2.85, 1.45 \times 10^{0.028 \times (25-T)})$$

where T = temperature (°C). Thirty day averages for comparison to chronic ALS values were calculated around peaks in total ammonia measurements.

Results

Mortality, Discharge, and Water Temperature

Table 1 contains the results of chi-square comparisons between observed and expected survival and mortality, and Figures 5-15 depict total mortalities between cages one and two combined, maximum daily water temperatures, and mean daily discharges at cage sites in 2011 and 2012. The solid red horizontal line in each panel represents the upper critical temperature threshold for brown trout of 19.0 °C (Elliot 1994). At temperatures above this critical threshold, significant disturbances to normal brown trout behavior may occur, including cessation of feeding and growth and ultimately death (Elliot 1994). The dashed red horizontal line in each panel represents the upper incipient lethal temperature for brown trout of 24.7 °C, above which thermal stress is lethal with mortality a function of exposure time (Elliot 1994).

Generally, in both years the highest mortality occurred after the peak of the hydrograph as water temperatures approached or exceeded 19.0 °C (Figures 5-15). Sites deviating from this trend include Galen (2011 and 2012), Deer Lodge (2012), the Little Blackfoot River (2011), Gold Creek (2012), Flint Creek (2011 and 2012), Rock Creek (2011 and 2012), and Turah (2012) which all exhibited either consistent mortality throughout the study season or bimodal mortality with some mortality occurring early in the study season on the ascending limb of the hydrograph, and some mortality on the descending limb as water temperatures approached or exceeded 19 °C. Discharge was higher at all sites in 2011 than in 2012, resulting in lower maximum daily water temperatures in 2011 (Figures 5-15). Mean daily discharge, maximum daily water temperatures, and timing of mortalities at each site are outlined below in order from upstream to downstream.

Clark Fork River near Warm Springs

Peak mean daily discharge in the Clark Fork River at the Warm Springs site in 2011 (1,060 ft^3/s) was 271 % higher than in 2012 (286 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (24.8 °C) was 21 % higher than in 2011 (20.5 °C) (Figure 5). Maximum daily water temperature in 2011 exceeded 19.0 °C for 9 days and peaked at 20.5 °C on July 29, while maximum daily water temperature in 2012 exceeded 19.0 °C for 39 days and peaked at 24.8 °C on July 21, exceeding the upper incipient lethal temperature for brown trout of 24.7 °C for one day (Figure 5). In 2011 all mortalities at the Warm Springs site were on the descending limb of the hydrograph as water temperatures approached or exceeded 19.0 °C. Mortality at Warm Springs in 2012 was higher than in 2011, and was significantly higher than expected (Table 1). The majority of mortalities at Warm Springs in 2012 were on the descending limb of the hydrograph as water temperatures exceeded 19.0 °C (Figure 5).

Clark Fork River near Galen

Peak mean daily discharge in the Clark Fork River near Galen in 2011 (1,390 ft^3/s) was 151 % higher than in 2012 (554 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (22.9 °C) was 18 % higher than in 2011 (19.4 °C) (Figure 6). Maximum daily water temperature in 2011 exceeded 19.0 °C for 2 days and peaked at 19.4 °C on July 25, while maximum daily water temperature in 2012 exceeded 19.0 °C for 28 days and peaked at 22.9 °C

on July 21 (Figure 6). In 2011 mortality at the Galen site was significantly higher than expected (Table 1) and occurred throughout the field season with high mortality during April and early May. Mortality at Galen in 2012 was lower than in 2011 and also occurred throughout the field season. Half of the mortalities in 2012 occurred prior to maximum daily water temperatures exceeding 19.0 °C, while the remaining half occurred on the descending limb of the hydrograph as maximum daily water temperatures exceeded 19.0 °C (Figure 6).

Clark Fork River near Deer Lodge

Peak mean daily discharge in the Clark Fork River near Deer Lodge in 2011 (1,960 ft^3/s) was 151 % higher than in 2012 (781 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (24.0 °C) was 18 % higher than in 2011 (21.4 °C) (Figure 7). Maximum daily water temperature in 2011 exceeded 19.0 °C for 10 days and peaked at 21.4 °C on July 6, while maximum daily water temperature in 2012 exceeded 19.0 °C for 39 days and peaked at 24.0 °C on July 21 (Figure 7). In 2011, mortalities at the Deer Lodge site occurred on the descending limb of the hydrograph as maximum daily water temperatures approached or exceeded 19 °C (Figure 7). Mortality at Deer Lodge in 2012 was lower than in 2011 and occurred throughout the field season. The majority of the mortalities in 2012 occurred prior to maximum daily water temperatures exceeding 19.0 °C, while the remaining mortalities occurred on the descending limb of the hydrograph as maximum daily water temperatures exceeded 19.0 °C (Figure 7).

Clark Fork River upstream of the Little Blackfoot River

Peak mean daily discharge in the Clark Fork River upstream of the Little Blackfoot River in 2011 (2,560 ft^3/s) was 156 % higher than in 2012 (999 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (24.8 °C) was 22 % higher than in 2011 (20.3 °C) (Figure 8). Maximum daily water temperature in 2011 exceeded 19.0 °C for 13 days and peaked at 20.3 °C on July 10. Maximum daily water temperature in 2012 exceeded 19.0 °C for 41 days and peaked at 24.8 °C on July 8 and again on July 21, each time exceeding the upper incipient lethal temperature for brown trout of 24.7 °C for one day (Figure 8). In both years the majority of mortalities in the Clark Fork River upstream of the Little Blackfoot River occurred on the descending limb of the hydrograph as maximum daily water temperatures approached or exceeded 19.0 °C, with mortality higher in 2012 (Figure 8).

Little Blackfoot River (Control)

Peak mean daily discharge in the Little Blackfoot River in 2011 (2,540 ft^3/s) was 166 % higher than in 2012 (954 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (24.0 °C) was 19 % higher than in 2011 (20.1 °C) (Figure 9). Maximum daily water temperature in 2011 exceeded 19.0 °C for 8 days and peaked at 20.1 °C on July 18, while maximum daily water temperature in 2012 exceeded 19.0 °C for 32 days and peaked at 24.0 °C on July 29 (Figure 9). In 2011 mortality in the Little Blackfoot River occurred throughout the field season with the majority of mortalities on the ascending limb of the hydrograph. In 2012 mortality was lower than in 2011, with the majority of mortalities occurring on the descending limb of the hydrograph as maximum daily water temperatures approached or exceeded 19.0 °C (Figure 9).

Clark Fork River downstream of Gold Creek

Peak mean daily discharge in the Clark Fork River downstream of Gold Creek in 2011 (6,100 ft^3/s) was 223 % higher than in 2012 (1,890 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (23.6 °C) was 18 % higher than in 2011 (20.0 °C) (Figure 10). Maximum daily water temperature in 2011 exceeded 19.0 °C for 10 days and peaked at 20.0 °C on July 31, while maximum daily water temperature in 2012 exceeded 19.0 °C for 37 days and peaked at 23.6 °C on July 9 (Figure 10). In 2011 no mortality occurred in the Clark Fork River downstream of Gold Creek, and mortality was significantly less than expected (Table 1). In 2012, the Clark Fork River downstream of Gold Creek had a bimodal hydrograph, with half of the mortalities occurring on the ascending and descending limbs of the first peak and the remaining mortalities occurring on the descending limb of the second peak, as maximum daily water temperatures approached or exceeded 19.0 °C (Figure 10).

Flint Creek (Control)

Peak mean daily discharge in Flint Creek in 2011 (1,390 ft^3/s) was 179 % higher than in 2012 (499 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (23.5 °C) was 27 % higher than in 2011 (18.5 °C) (Figure 11). Maximum daily water temperature in 2011 did not exceed 19.0 °C and peaked at 18.5 °C on July 18, while maximum daily water temperature in 2012 exceeded 19.0 °C for 38 days and peaked at 23.5 °C on July 29 (Figure 11). In 2011 mortality in Flint Creek occurred throughout the field season with one mortality on the ascending limb, one mortality near the peak, and one mortality on the descending limb of the hydrograph as maximum daily water temperatures approached or exceeded 19.0 °C. In 2012 Flint Creek had a bimodal hydrograph, with the majority of the mortality occurring on the ascending limb of the first peak, one mortality on the ascending limb of the second peak, and the remaining mortalities on the descending limb of the second peak as maximum daily water temperatures approached or exceeded 19.0 °C (Figure 11).

Clark Fork River near Bearmouth

Peak mean daily discharge in the Clark Fork River near Bearmouth in 2011 (7,740 ft^3/s) was 255 % higher than in 2012 (2,180 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (23.7 °C) was 15 % higher than in 2011 (20.6 °C) (Figure 12). Maximum daily water temperature in 2011 exceeded 19.0 °C for 5 days and peaked at 20.6 °C on July 31, while maximum daily water temperature in 2012 exceeded 19.0 °C for 39 days and peaked at 23.7 °C on July 29 (Figure 12). In 2011, no mortality occurred in the Clark Fork River near Bearmouth, and mortality was significantly less than expected (Table 1). In 2012, the Clark Fork River near Bearmouth had a bimodal hydrograph with all mortalities occurring on the descending limb of the second peak as maximum daily water temperatures exceeded 19.0 °C (Figure 12).

Rock Creek (Control)

Peak mean daily discharge in Rock Creek in 2011 (5,150 ft^3/s) was 52 % higher than in 2012 (3,380 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (20.9 °C) was

19 % higher than in 2011 (17.5 °C) (Figure 13). Maximum daily water temperature in 2011 did not exceed 19.0 °C and peaked at 17.5 °C on July 18, while maximum daily water temperature in 2012 exceeded 19.0 °C for 9 days and peaked at 20.9 °C on July 21 (Figure 13). In 2011 in Rock Creek the majority of mortalities were on the ascending limb and the remaining mortalities on the descending limb of the hydrograph. In 2012, Rock Creek had a bimodal hydrograph, with three mortalities occurring on the ascending limb of the first peak, one mortality on the ascending limb of the second peak, and the remaining mortalities on the descending limb of the second peak, as maximum daily water temperatures approached or exceeded 19.0 °C (Figure 13).

Spring channel near Clinton (Handling control)

Peak maximum daily water temperature in 2011 at the handling control in the spring channel near Clinton did not exceed 19.0 °C and peaked at 15.9 °C on July 18. Peak maximum daily water temperature in 2012 did not exceed 19.0 °C and peaked at 17.5 °C on July 6. Peak maximum daily water temperature in 2012 was 10 % higher than in 2011. There were no mortalities at the handling control in the spring channel near Clinton during the analysis period in either year (Figure 14).

Clark Fork River near Turah

Peak mean daily discharge in the Clark Fork River near Turah in 2011 (12,700 ft^3/s) was 70 % higher than in 2012 (7,490 ft^3/s). Conversely, peak maximum daily water temperature in 2012 (22.1 °C) was 9 % higher than in 2011 (20.3 °C) (Figure 15). Maximum daily water temperature in 2011 exceeded 19.0 °C for 6 days and peaked at 20.3 °C on July 31, while maximum daily water temperature in 2012 exceeded 19.0 °C for 30 days and peaked at 22.1 °C on July 18 and again on July 22 (Figure 15). In 2011, observed mortality was significantly higher than expected in the Clark Fork River near Turah (Table 1), with the majority of mortalities occurring on the descending limb of the hydrograph as maximum daily water temperatures approached or exceeded 19.0 °C (Figure 15). In 2012, the Clark Fork River near Turah had a bimodal hydrograph, with two mortalities on the ascending limb and two mortalities on the descending limb of the first peak. One mortality also occurred on the descending limb of the second peak as maximum daily water temperatures exceeded 19.0 °C (Figure 15).

Table 1. Results of χ^2 tests between expected and observed survival and mortality for 2011 and 2012, with Yates's correction for continuity applied; df = 1 for all tests. Red asterisks denote significantly higher than expected mortality at $\alpha = 0.05$; black asterisks denote significantly lower than expected mortality.

	Year	
Site	2011	2012
Warm Springs	$P = 0.14$	$P = 0.007$*
Galen	$P < 0.001$*	$P = 0.20$
Deer Lodge	$P = 0.93$	$P = 0.86$
Upstream of Little Blackfoot R.	$P = 0.93$	$P = 0.20$
Downstream of Gold Creek	$P = 0.02$*	$P = 0.83$
Bearmouth	$P = 0.02$*	$P = 0.87$
Turah	$P < 0.001$*	$P = 0.83$

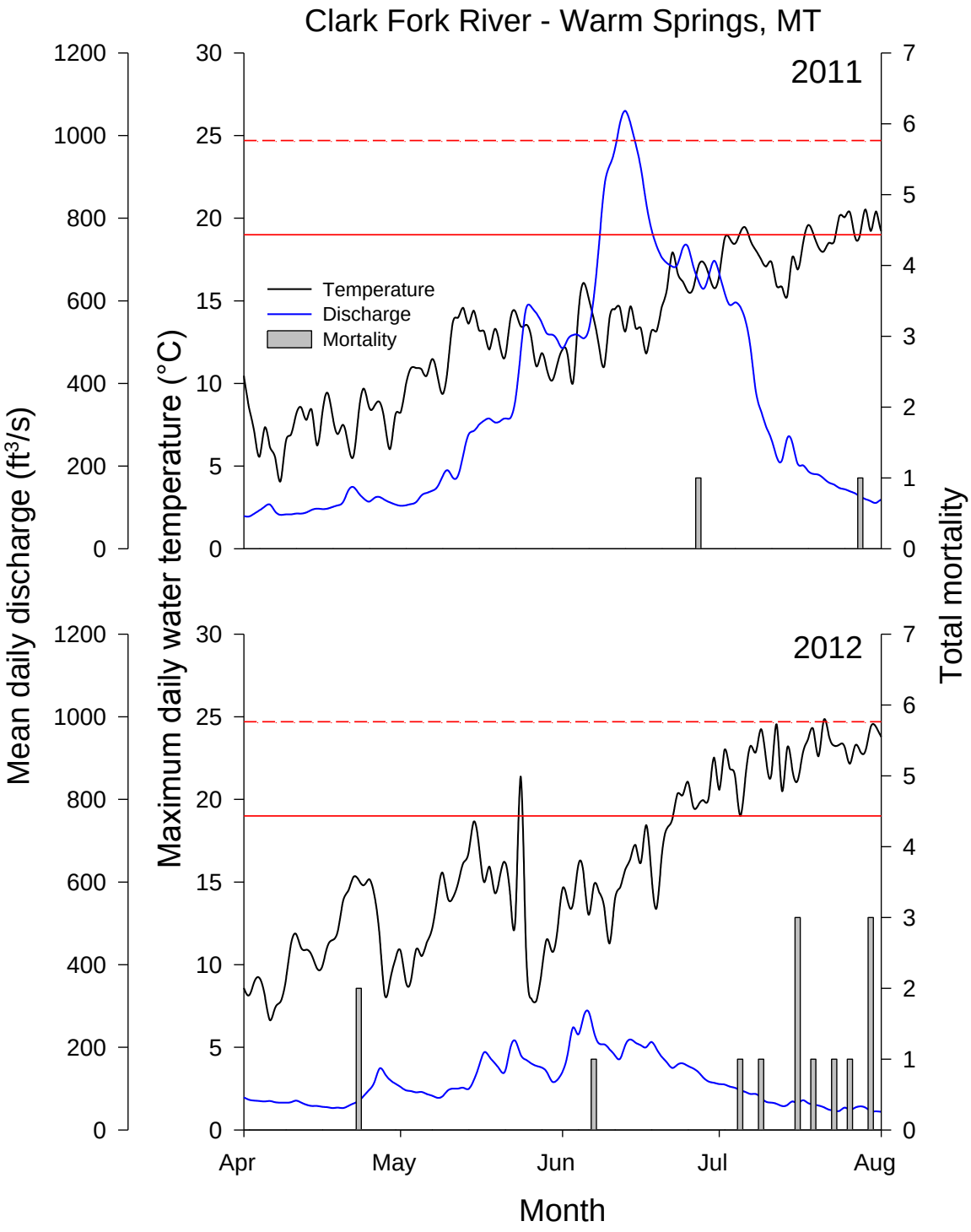

Figure 5. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River at the Warm Springs site. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

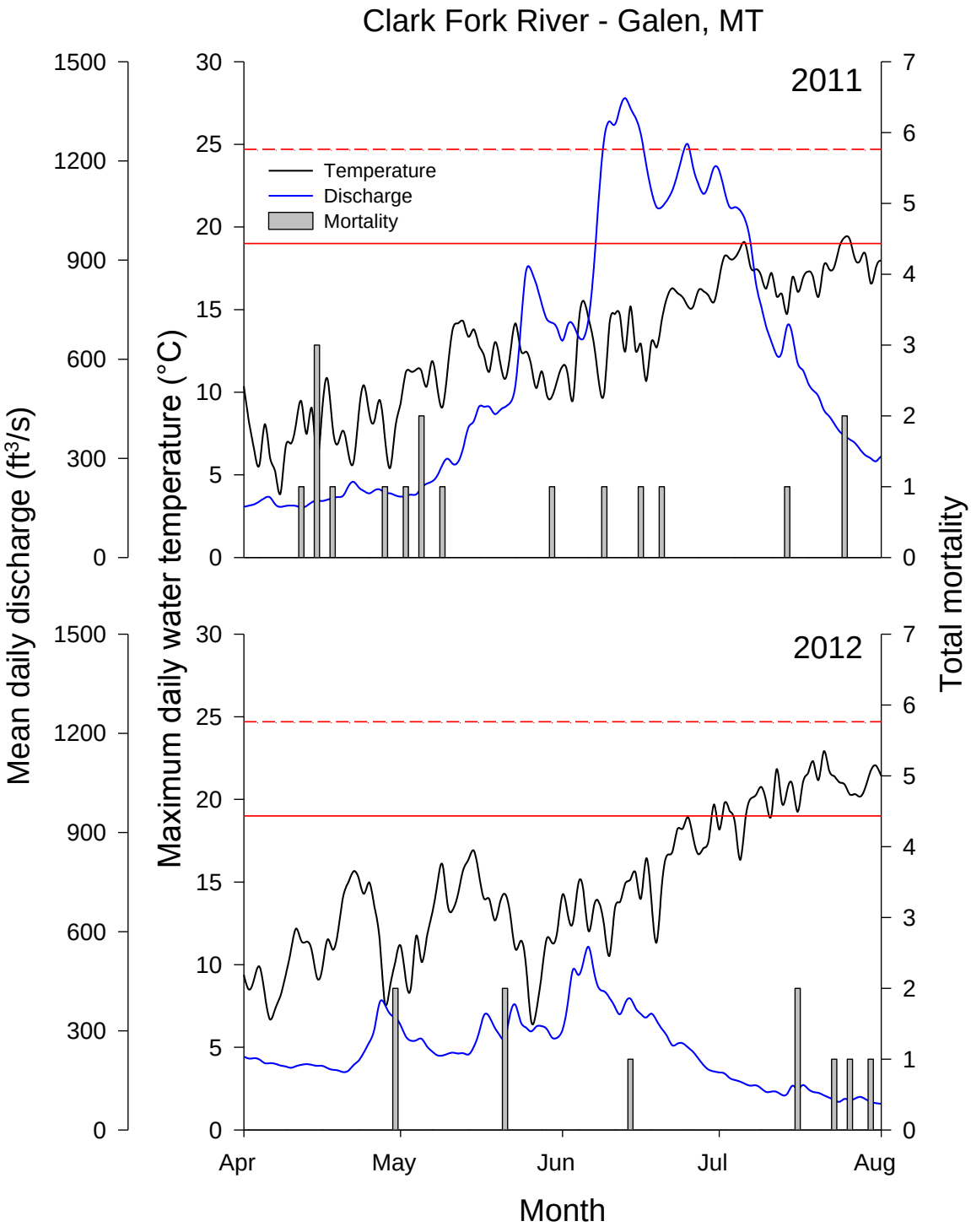

Figure 6. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River at the Galen site. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

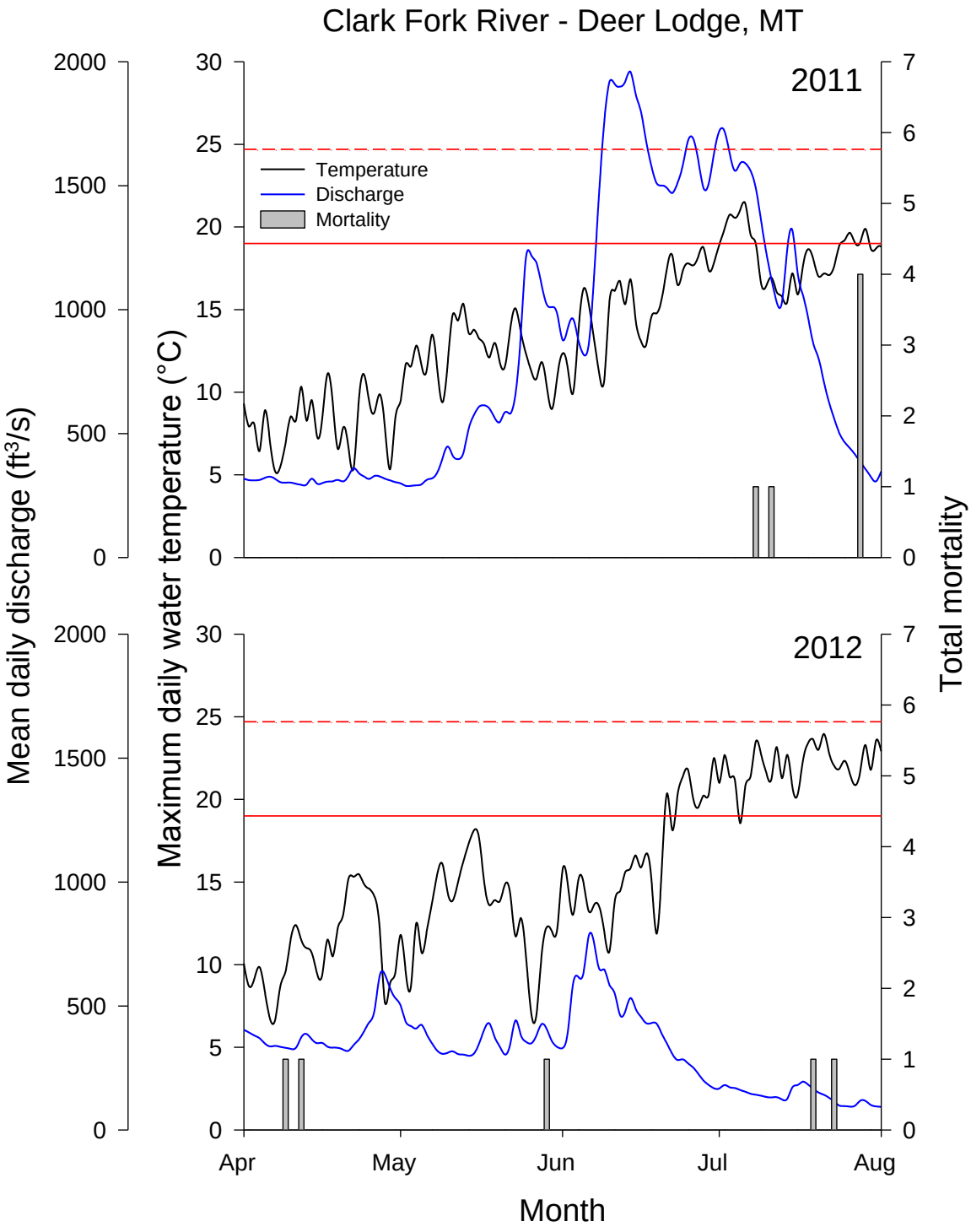

Figure 7. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River at the Deer Lodge site. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

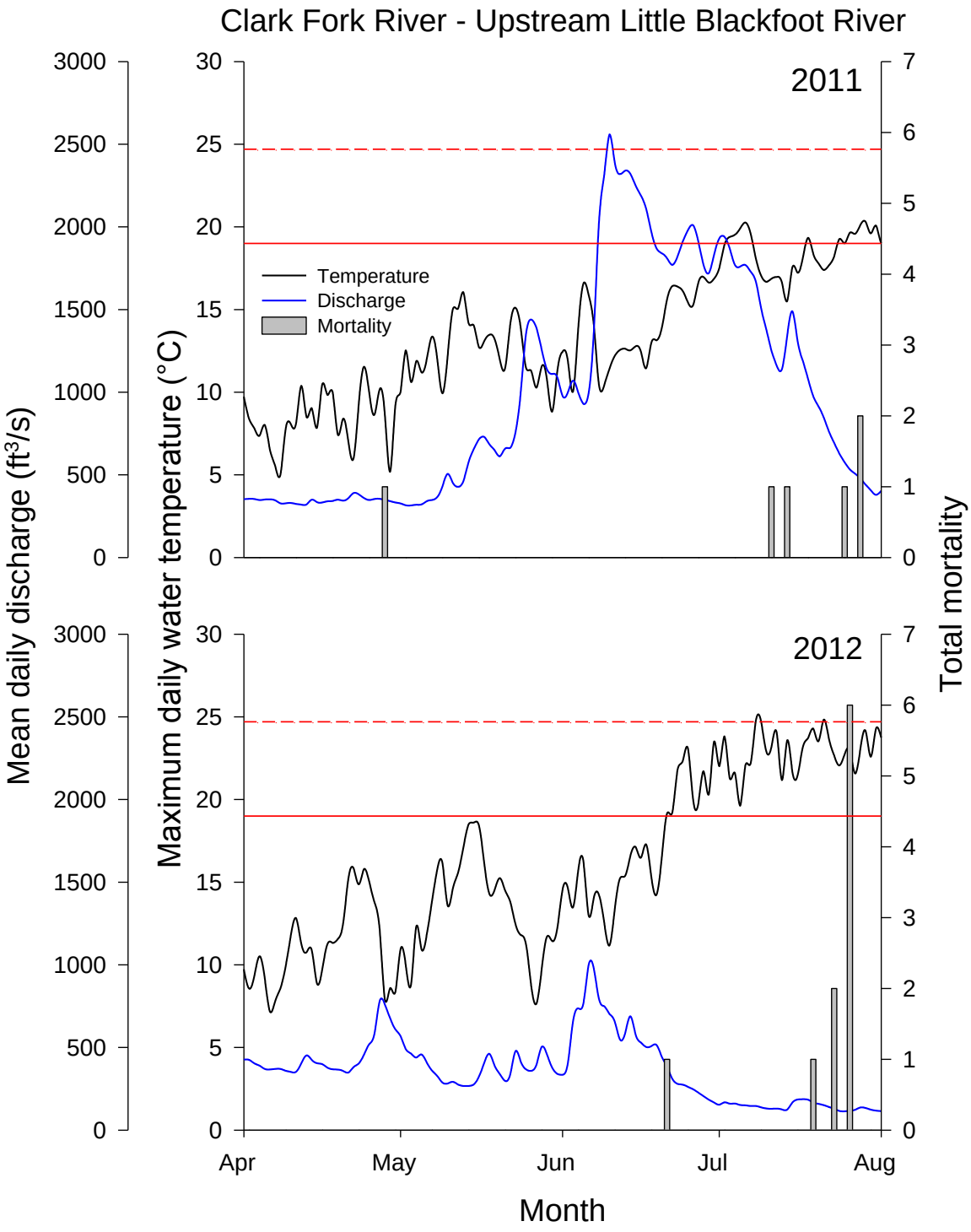

Figure 8. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River at the site upstream of the Little Blackfoot River. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

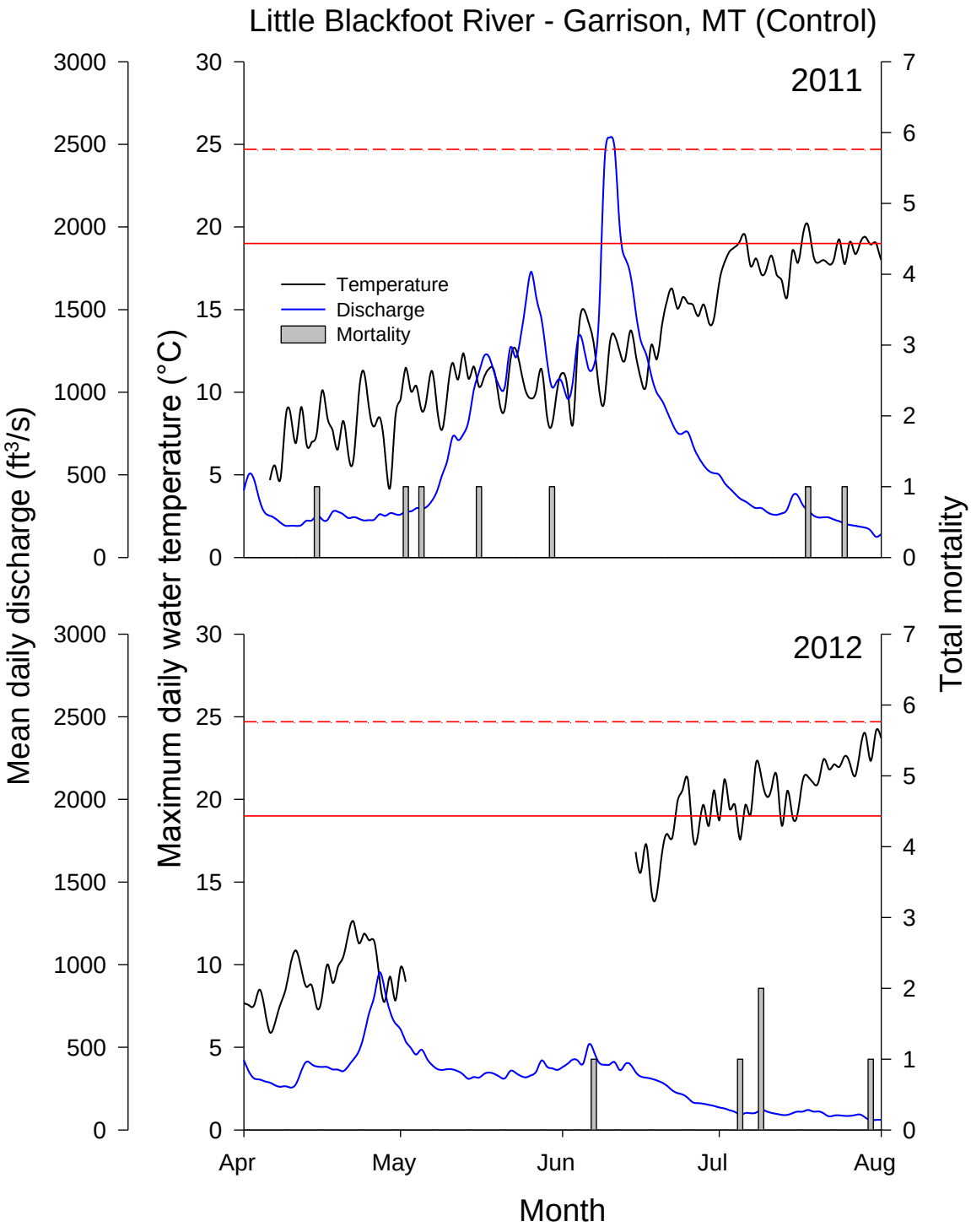

Figure 9. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 at the control site in the Little Blackfoot River. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

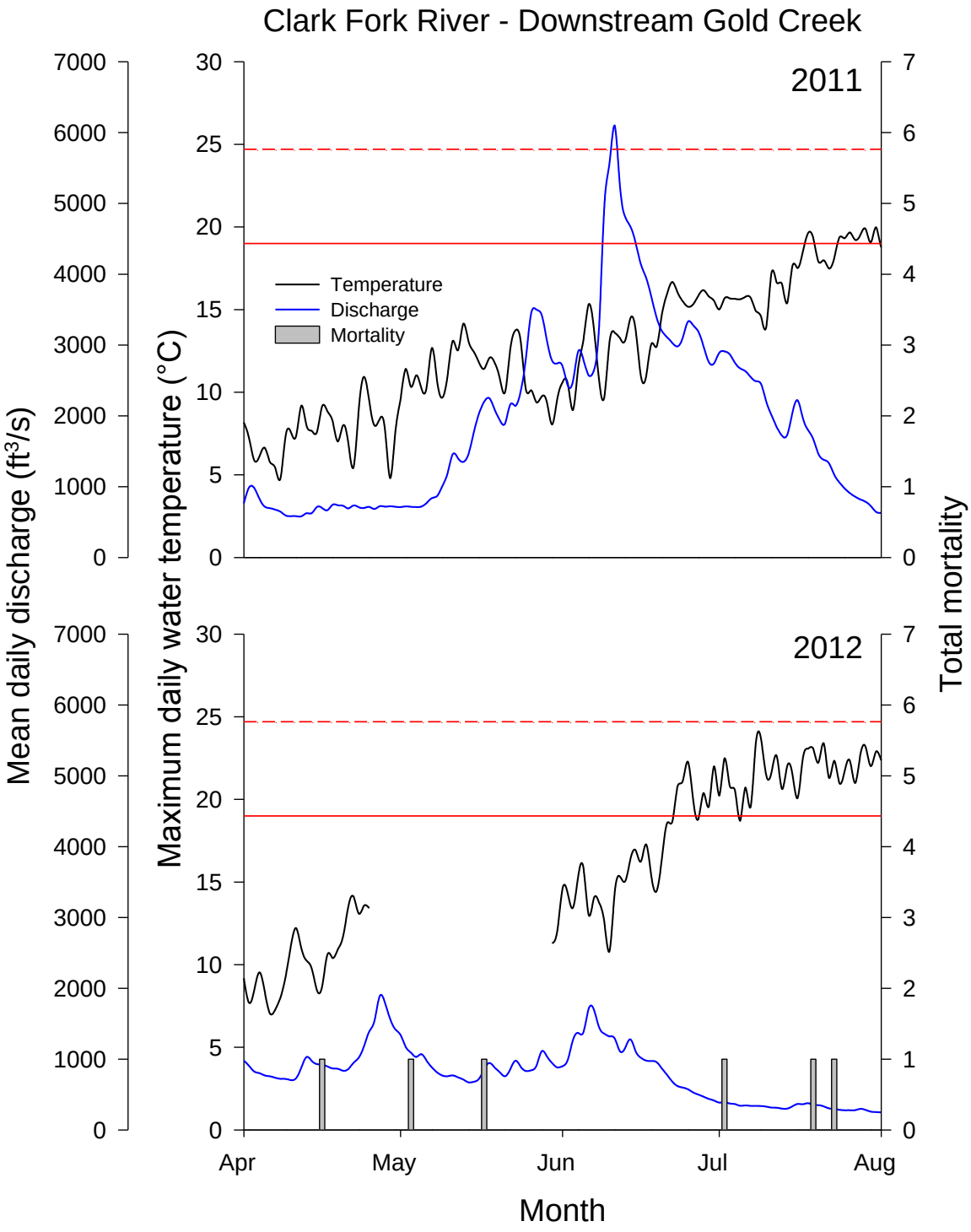

Figure 10. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River downstream of Gold Creek. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

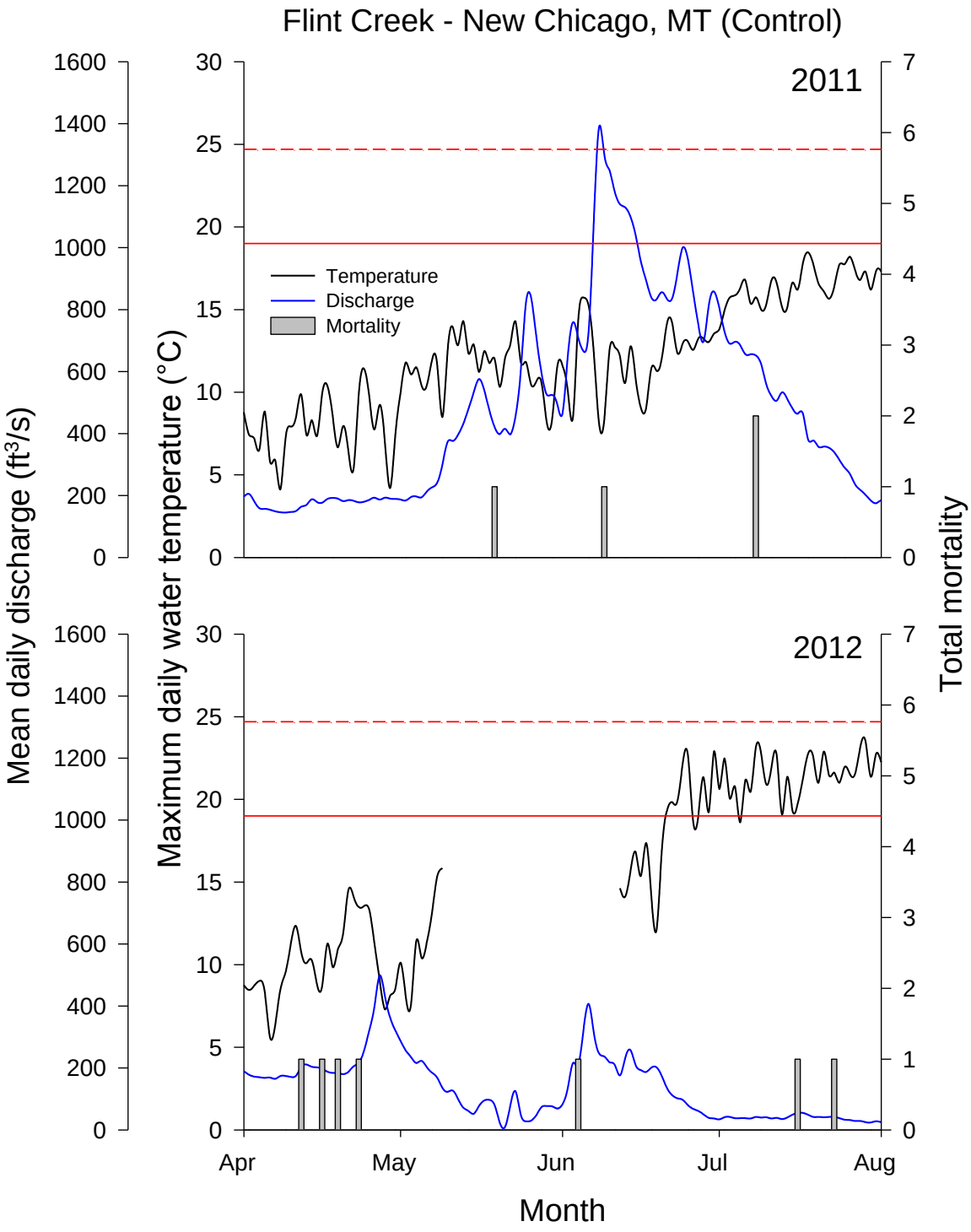

Figure 11. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 at the control site in Flint Creek. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

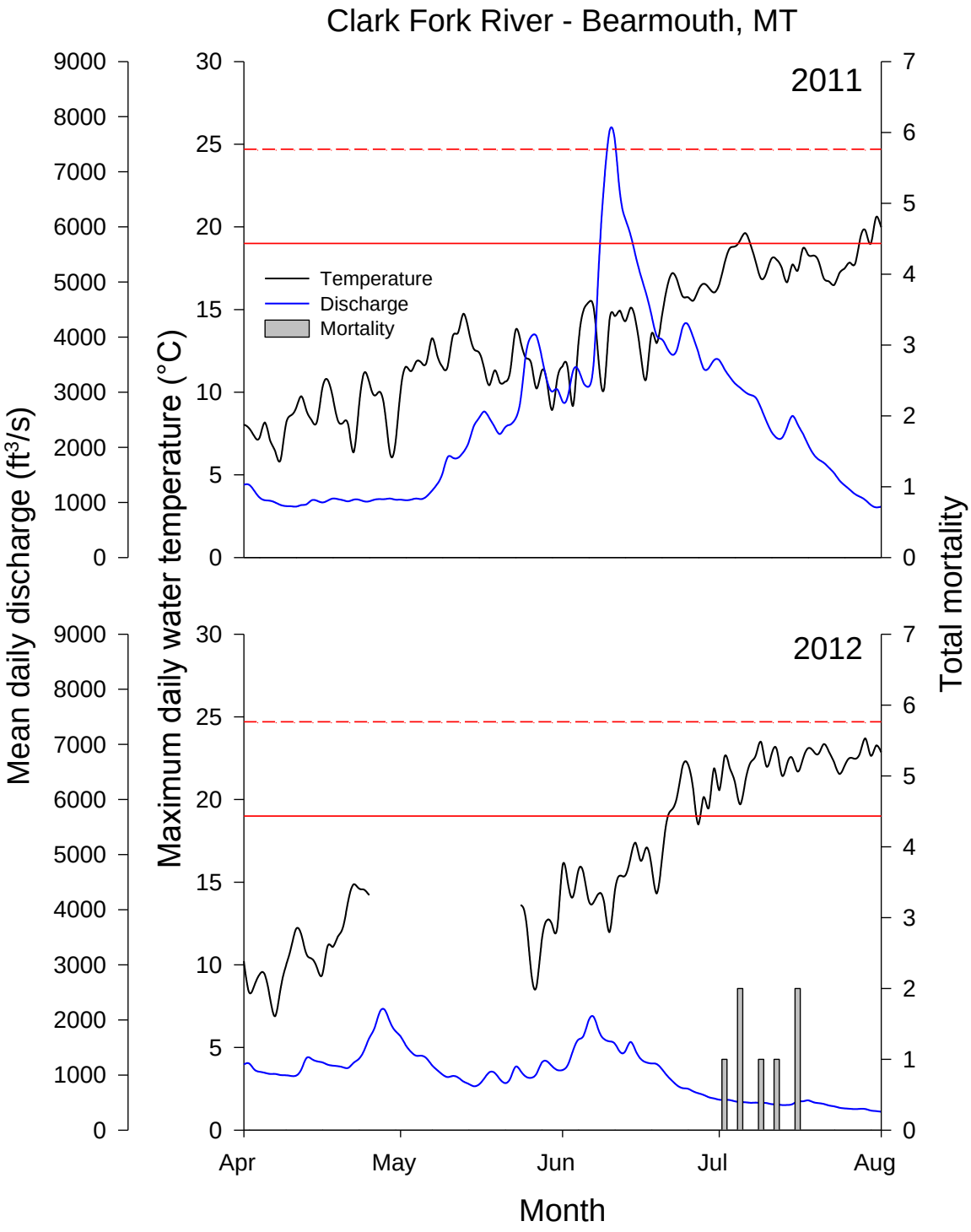

Figure 12. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River near Bearmouth, Montana. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

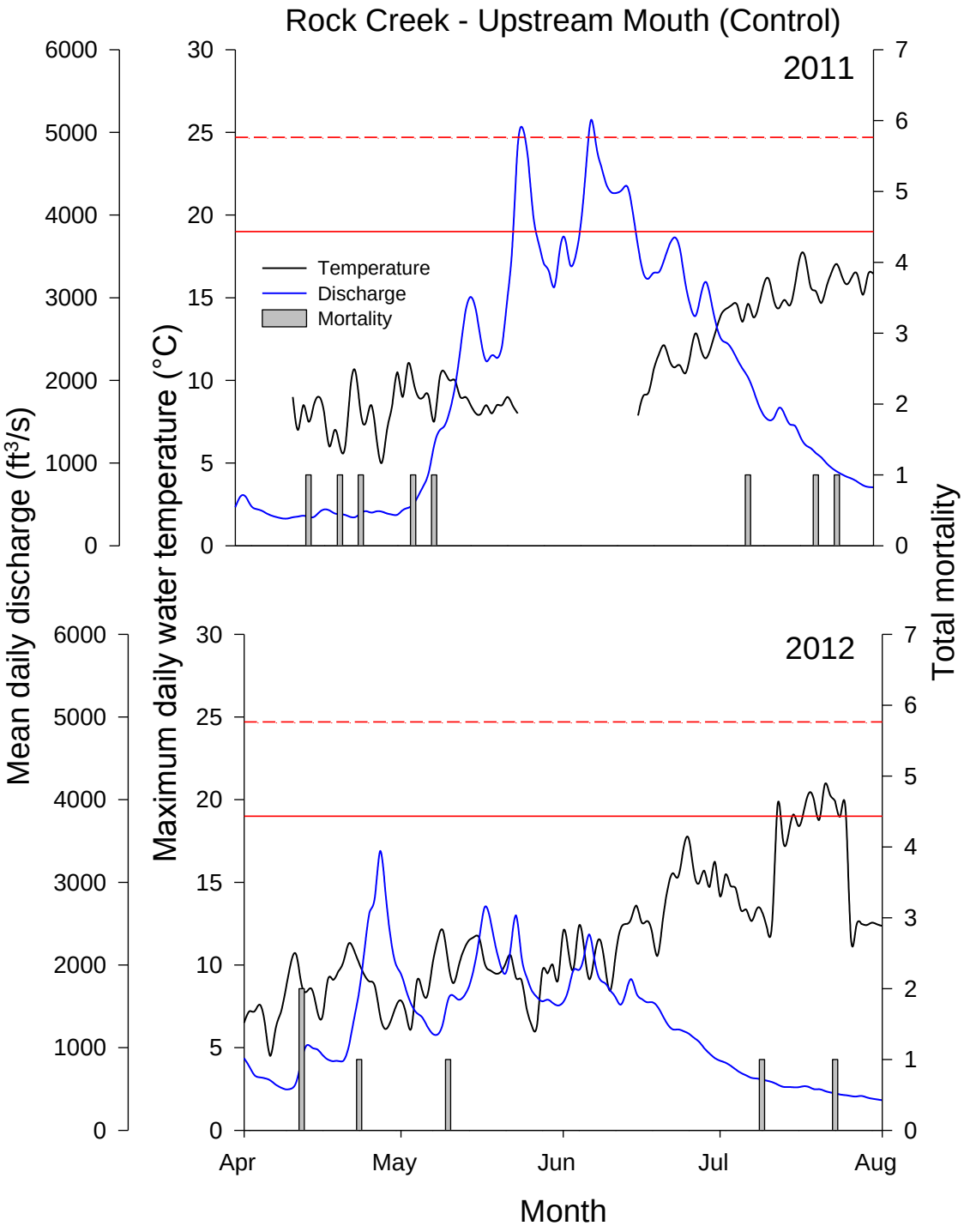

Figure 13. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 at the control site in Rock Creek. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

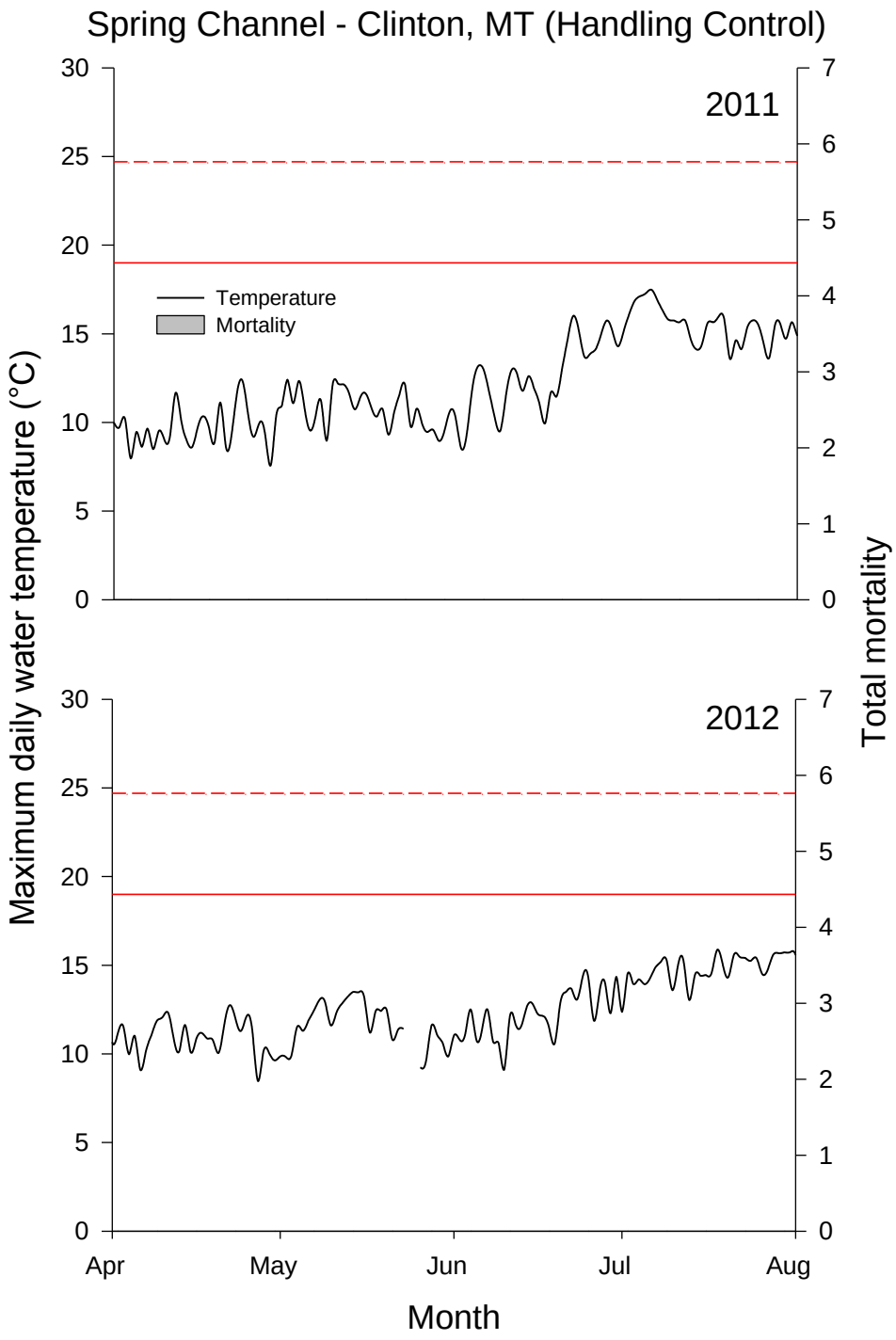

Figure 14. Total mortalities between cages one and two combined and maximum daily water temperature for 2011 and 2012 at the control site in the spring channel near Clinton, Montana. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

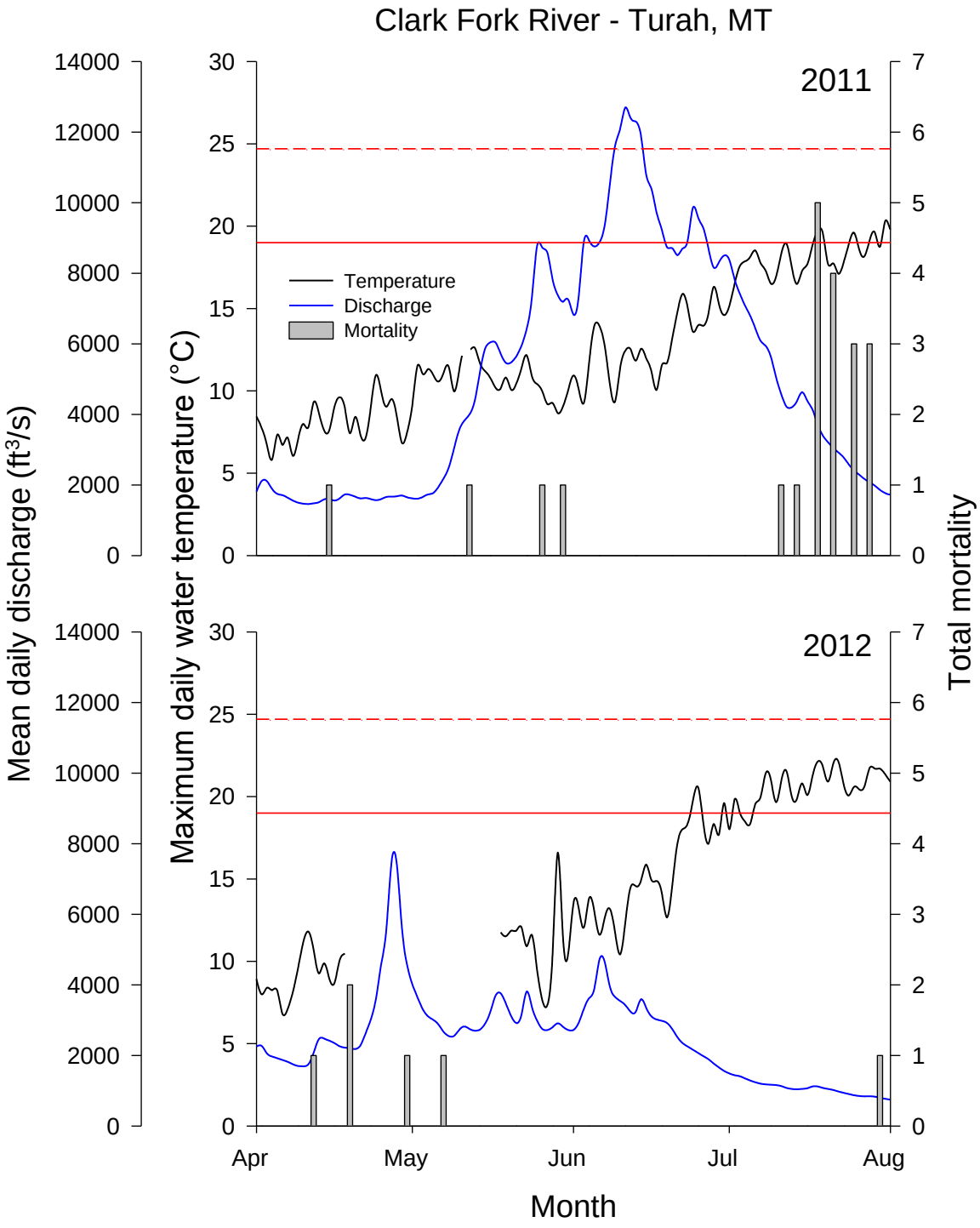

Figure 15. Total mortalities between cages one and two combined, maximum daily water temperature, and mean daily discharge for 2011 and 2012 in the Clark Fork River near Turah, Montana. In each panel the solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for brown trout.

Spatial Distribution of Brown Trout Survival

Cumulative survival (%) at each site was calculated by dividing the number of live fish at the end of the analysis period in cages one and two combined by the total number of fish placed in both cages over the entire season. Cumulative survival in 2011 (April 8 to July 31) from the Warm Springs site downstream was as follows; Warm Springs 96 %, Galen 73 %, Deer Lodge 89 %, upstream of the Little Blackfoot River 89 %, within the Little Blackfoot River 88 %, downstream of Gold Creek 100 %, Flint Creek 93 %, Bearmouth 100 %, Rock Creek 86 %, Clinton 100 % and Turah 69 % (Figure 16). Cumulative survival in 2012 (April 8 to July 31) from the Warm Springs site downstream was as follows; Warm Springs 78 %, Galen 83 %, Deer Lodge 91 %, upstream of the Little Blackfoot River 83 %, within the Little Blackfoot River 91 %, downstream of Gold Creek 89 %, Flint Creek 88 %, Bearmouth 88 %, Rock Creek 89 %, Clinton 100 % and Turah 89 % (Figure 16).

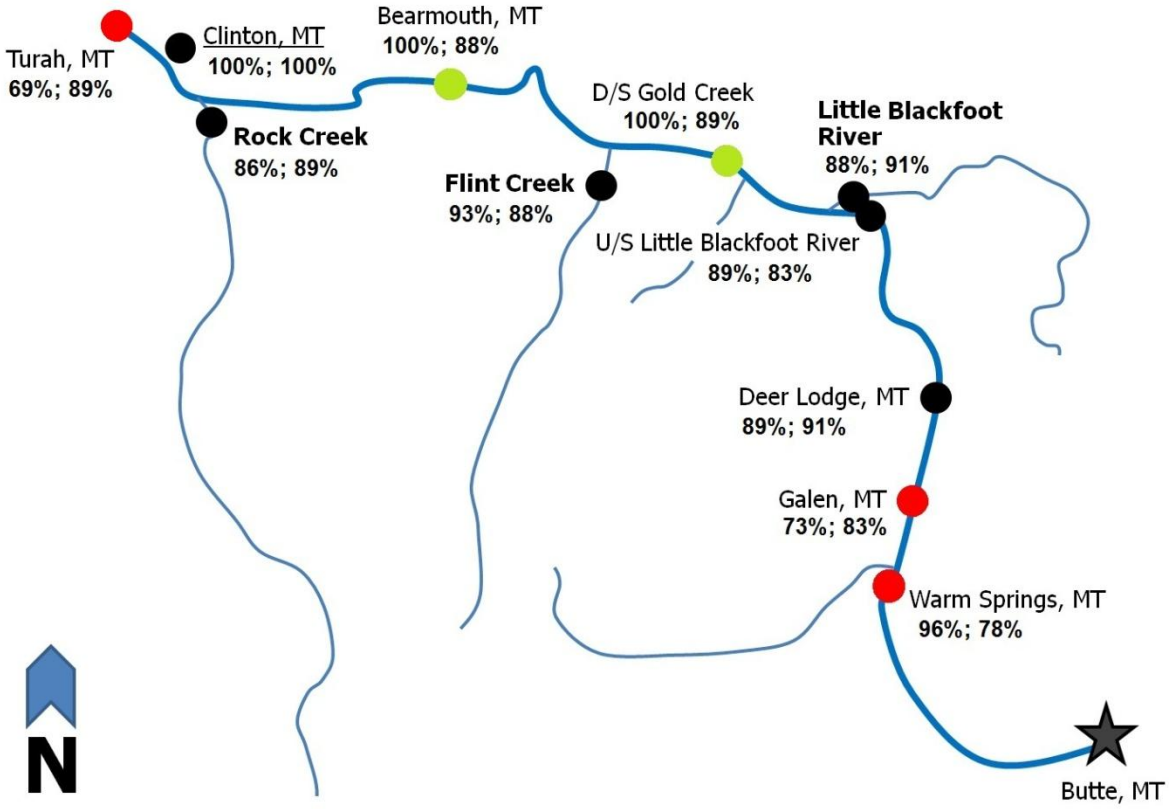

Figure 16. Cumulative brown trout survival calculated from April 8th to July 31st across sites for 2011 and 2012 respectively. Control sites are shown in bold and the handling control is underlined. Red dots denote sites with significantly lower than expected survival in at least one year; green dots denote sites with significantly higher than expected survival in at least one year.

Survival in the control tributaries was high, between years averaging 90 % in the Little Blackfoot River, 91 % in Flint Creek, and 88 % in Rock Creek. Survival at the handling control in the spring channel near Clinton was 100 % in both years indicating that mortalities observed at the experimental mainstem sites were not due to conditions inside the cages. Survival in the Clark Fork River near Warm Springs was significantly less than expected in 2012 and survival in

the Clark Fork River near Galen was significantly less than expected in 2011. Survival then increases at downstream mainstem sites and was significantly higher than expected downstream of Gold Creek and near Bearmouth in 2011 (Table 1; Figure 16). This may indicate a point source of contaminants contributing to mortality that is diluted by input from numerous tributaries entering the Clark Fork River below the two upper sites. In 2011 survival in the Clark Fork River near Turah was less than expected at 69 %; however, survival in 2012 was relatively high at 89 % which is within the range seen in the control tributaries. Why survival was so low at Turah in 2011 but not in 2012 is unknown; however, the low survival seen in 2011 may have been due to environmental conditions or other factors in the immediate area of the cages that were unobserved and out of the control of the researchers. Additional monitoring at this site may identify such conditions.

Growth

Growth varied by site and by year and was overall higher in 2012 (Figure 17). In 2011 growth appeared to be related to location on the mainstem and generally followed a decreasing trend from upstream to down, with growth in the tributary and handling control sites slightly higher than at adjacent mainstem sites (Figure 17). In 2012, growth was higher than in 2011 at all sites excluding the Clark Fork River near Warm Springs. As in 2011, growth was higher at the handling control at Clinton and in Rock Creek than at adjacent mainstem sites; however, growth at the other two control sites, (Little Blackfoot River and Flint Creek) was lower than at the adjacent mainstem sites (Figure 17). In 2012 growth was higher at the sites with the lowest water temperatures (Rock Creek and Clinton) indicating that high temperature in 2012 had an effect on growth at the remaining sites. The high growth rates at Warm Springs and Galen are expected as there is a "tail water" effect of the upstream ponds resulting in increased nutrients and additional food sources including freshwater shrimp and isopods.

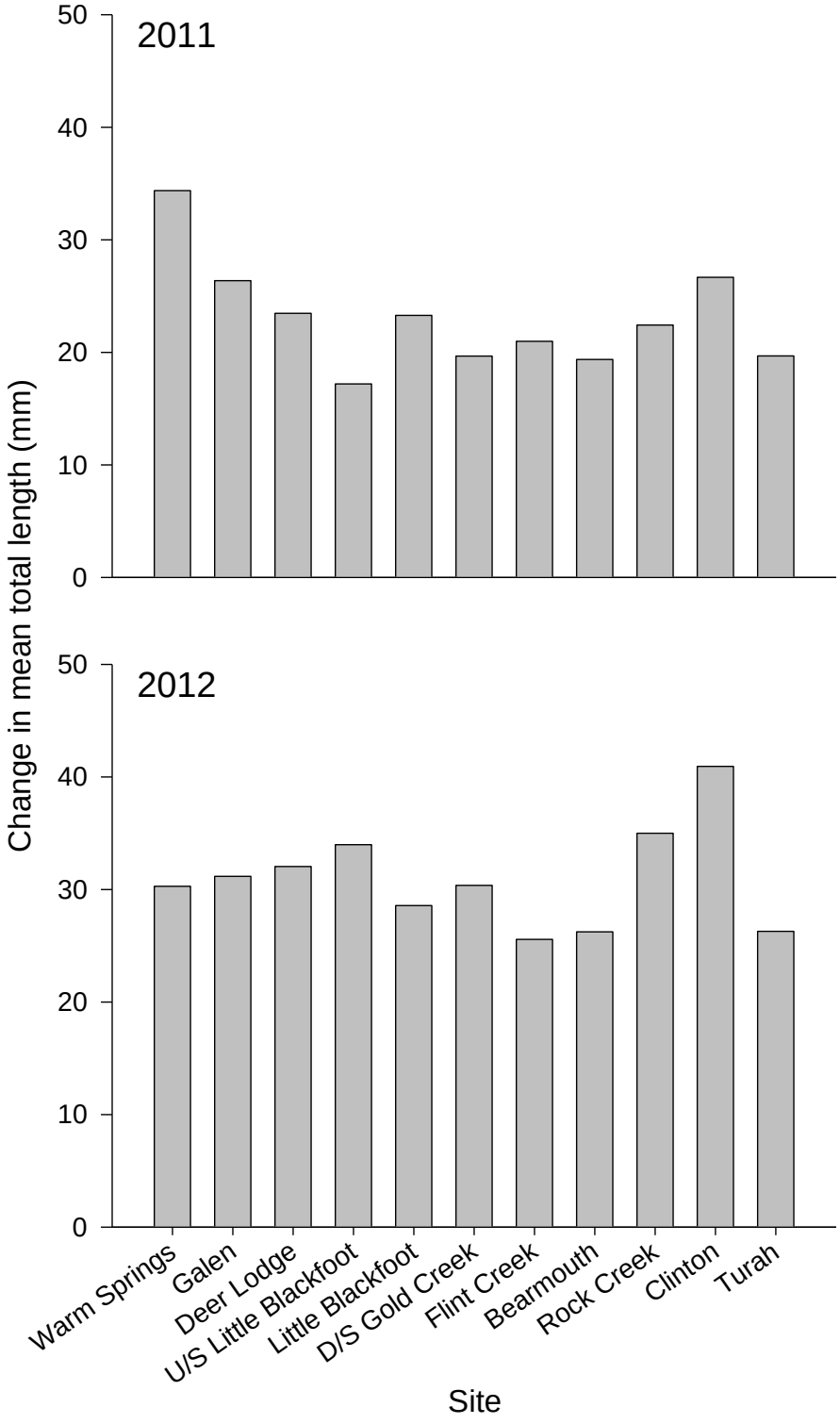

Figure 17. Change in mean total length by site for juvenile brown trout held in cages by site in 2011 and 2012, arranged from upstream to downstream.

Histology

The liver is the primary cite of toxic action for fish (Lage et al. 2006). Results from histological analyses show varying degrees of cellular changes within brown trout livers at all sites that only occur with exposure to contaminants. These changes include excessive glycogen vacuolation (histology score ≥ 4) and nuclear vacuolation (histology score ≥ 2) of hepatocytes, as well as pleomorphic nuclei within hepatocytes. Degeneration or necrosis of hepatocytes is indicative of exposure to contaminants which may include heavy metals. The relative effect of contaminant exposure on brown trout at each site for both years was evaluated using histology scores for hepatocytic degeneration and necrosis from fish sampled at the end of each field season as well as a hatchery control collected in late March 2012 when fish for the study were obtained (Table 2).

Table 2. Scores representing degree of hepatocytic necrosis of histology samples collected at the end of each season by site and year. Scores are on a scale of 1-5: 1 = minimal; 2 = mild; 3 = moderate; 4 = moderately severe; 5 = severe.

Site	2011	2012
Hatchery	NA	2
Warm Springs	2	4
Galen	2	3-4
Deer Lodge	3	4
U/S Little Blackfoot	NA	3-4
Little Blackfoot	NA	4
D/S Gold Creek	3	3-4
Flint Creek	NA	4
Bearmouth	3	4
Rock Creek	2-3	3-4
Clinton Spring	3-4	2-4
Turah	NA	2-4

Histology scores for degree of melanomacrophage aggregation in kidneys and livers from samples collected at the end of each field season by site are presented in Table 3. Histology scores for melanomacrophage aggregations indicate contaminant exposure was chronic across the study area in both years. In 2011 the highest score for melanomacrophage aggregation in kidneys was at Warm Springs, with scores varying from 4-5. However all remaining sites excluding Turah had scores of 4 for kidney melanomacrophage aggregation indicating exposure to contaminants was chronic. In 2011 the highest scores for melanomacrophage aggregations in livers were seen downstream of Gold Creek, with scores varying from 2-3; all remaining sites with an available score had scores of 2.

Overall scores for degree of melanomacrophage aggregation in both kidneys and livers were higher in 2012. Fish from the hatchery control had a score of 3 for melanomacrophage aggregation in kidney tissue indicating moderate cellular changes that may or may not be within the normal range. However, the score for melanomacrophage aggregations in kidneys of

hatchery fish were lower than any of the treatment or control sites, with scores at all sites ≥ 4. Scores for melanomacrophage aggregation in livers of hatchery fish varied from 2-3 indicating that fish from the hatchery were potentially subjected to chronic exposure to contaminants prior to placement in cages; similar values were seen at all treatment and control sites with the lowest score (1-2) seen at Rock Creek.

Table 3. Scores representing degree of melanomacrophage aggregation in kidneys and livers of histology samples collected at the end of each season by site and year. Scores are on a scale of 1-5: 1 = minimal; 2 = mild; 3 = moderate; 4 = moderately severe; 5 = severe.

	2011		2012	
Site	Kidney	Liver	Kidney	Liver
Hatchery	NA	NA	3	2-3
Warm Springs	4-5	2	4-5	NA
Galen	4	2	3-4	NA
Deer Lodge	4	2	3-5	NA
U/S Little Blackfoot	4	2	3-5	2
Little Blackfoot	4	2	4-5	2-3
D/S Gold Creek	3-4	2-3	3-4	2-3
Flint Creek	4	2	4	3
Bearmouth	4	2	4-5	2-3
Rock Creek	3-4	NA	4-5	1-2
Clinton Spring	4	NA	4	2-3
Turah	3	NA	4	2-3

Although fish were exposed to contaminants to some degree at all sites, histological analysis showed gill tissue to be very healthy across all sites and both years indicating that exposure to contaminants was either via ingestion or was non-toxic to gill epithelium. Severity of cellular changes in gills, livers, and kidneys of fish collected at the end of the 2011 field season was similar across all sites, both treatment and control, with the exception of fish collected at Turah which appeared healthier with less severe cellular changes than the other sites. Overall, fish collected at the end of the 2012 field season showed similar cellular changes and severity scores among sites. In addition to cellular changes related to exposure to contaminants, fish from the study area were found to have evidence of infection with parasites including *Myxobolus cerebralis*, as well as bacterial infections, and steatitis, an inflammation of the adipose tissue that is usually associated with a dietary deficiency in trout (Smith 1979; Herman and Kircheis 1985). Results of histological analysis for the hatchery controls collected in March 2012, fish remaining at the end of each field season at each site, and fish collected prior to the end of each field season are discussed below.

Hatchery Control

Histological analysis of brown trout from the hatchery control indicated that fish were overall in good health prior to the study. Prior to being placed in cages fish had normal gills,

good glycogen vacuolation indicating stored energy, mild hepatocytic necrosis indicating normal cells or cellular changes of no consequence (Table 2), and mild to moderate numbers of melanomacrophages in the kidneys and liver (Table 3). Fish from the hatchery also exhibited moderate steatitis, indicating a possible dietary deficiency. These same cellular changes were observed in fish from the treatment and control sites with increased severity, especially in the kidneys.

Clark Fork River near Warm Springs

Fish collected from cages at Warm Springs at the completion of the field season in 2011 had mild to moderate (histology scores = 2-3) swelling of gill epithelium, indicating cellular changes ranging from normal or changes of no consequence to those that may or may not be within the normal range. Degeneration and necrosis of hepatocytes was mild (Table 2) indicating normal tissue or tissue with cellular changes of no consequence. Scores for melanomacrophage aggregation in kidney tissue varied from moderately severe to severe, and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. Fish from Warm Springs had moderately severe glycogen vacuolation within hepatocytes (histology score = 4) indicating exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2011 field season at Warm Springs showed evidence indicative of a bacterial skin lesion.

In 2012 fish collected from cages at Warm Springs at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Scores for melanomacrophage aggregations in kidney tissue varied from moderately severe to severe indicating that exposure to contaminants was chronic (Table 3).

Clark Fork River near Galen

Fish collected from cages at Galen at the completion of the field season in 2011 had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was mild (Table 2) indicating normal tissue or tissue with cellular changes of no consequence. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, all fish collected at the end of the 2011 field season at Galen had steatitis indicating a potential dietary deficiency.

In 2012 six fish collected from cages at Galen at the completion of the field season had mostly normal gill tissue, while four fish showed signs of gill inflammation. Degeneration and necrosis of hepatocytes was moderate to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Fish from Galen in 2012 also exhibited nuclear vacuolation within hepatocytes which indicates exposure to contaminants. Scores for melanomacrophage aggregations in kidney tissue varied from moderate to moderately severe indicating that exposure to contaminants was chronic (Table 3).

Clark Fork river near Deer Lodge

Fish collected from cages at Deer Lodge at the completion of the field season in 2011 had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderate (Table 2) indicating cellular changes that may or may not be within the normal range. Pleomorphic nuclei within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe, and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. The parasite *Myxobolus cerebralis* was observed in two fish at Deer Lodge in 2011.

In 2012 fish collected from cages at Deer Lodge at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Pleomorphic nuclei within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregations in kidney tissue varied from moderate to severe indicating that exposure to contaminants was chronic (Table 3). In addition to cellular changes associated with exposure to contaminants, two fish collected at the end of the 2012 field near Deer Lodge had eosinophilic granular cells in cranial nerves which are commonly seen in brown trout exposed to *M. cerebralis* (Hedrick et al. 1999).

Clark Fork River upstream of the Little Blackfoot River

Fish collected from the cages upstream of the Little Blackfoot River at the completion of the field season in 2011 had moderate (histology score = 3) cellular changes to gill tissue that may or may not be within the normal range. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2011 field season upstream of the Little Blackfoot River showed evidence of an eye fluke as well as an oral cavity wound. Many of the fish from the cages upstream of the Little Blackfoot River in 2011 had eosinophilic granular cells in cranial nerves which are commonly seen in brown trout exposed to *M. cerebralis*.

In 2012 fish collected from the cages upstream of the Little Blackfoot River at the completion of the field season had mostly normal gill tissue with mild to moderate (histology score = 2-3) cellular changes that varied from normal or changes of no consequence to those that may or may not be within the normal range. Degeneration and necrosis of hepatocytes was moderate to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Pleomorphic nuclei and nuclear vacuolation within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue varied from moderate to severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2012 field season upstream of the Little Blackfoot River was infected with parasitic *Trichodina* spp. Presence of *Trichodina* spp. may indicate habitats experiencing eutrophication or poor water quality (Schmidt et al. 2003; Ogut and Palm 2005).

Little Blackfoot River (Control)

Fish collected from the Little Blackfoot River at the completion of the field season in 2011 had moderate (histology score = 3) cellular changes to gill tissue that may or may not be within the normal range. Fish from the Little Blackfoot River had moderately severe glycogen vacuolation within hepatocytes (histology score = 4) indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, fish collected at the end of the 2011 field season from the Little Blackfoot River had steatitis indicating a dietary deficiency.

In 2012 fish collected from the Little Blackfoot River at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and pleomorphic nuclei within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe to severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Clark Fork River downstream of Gold Creek

Fish collected from the cages downstream of Gold Creek at the completion of the field season in 2011 had moderate (histology score = 3) swelling of gill tissue. Degeneration and necrosis of hepatocytes was moderate (Table 2) indicating cellular changes that may or may not be within the normal range. Scores for melanomacrophage aggregation in kidney tissue were moderate to moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

In 2012 fish collected from the cages downstream of Gold Creek at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderate to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Excessive nuclear vacuolation (histology score = 3) and pleomorphic nuclei within hepatocytes were observed indicating contaminant exposure. Scores for melanomacrophage aggregation in kidney tissue were moderate to moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Flint Creek (Control)

Fish collected from Flint Creek at the completion of the field season in 2011 had moderate to moderately severe (histology score = 3-4) swelling of gill tissue. Moderately severe glycogen vacuolation (histology score = 4) of hepatocytes was observed indicating contaminant exposure. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2011 field season in Flint Creek was infected

with parasitic *Trichodina* spp., the presence of which could indicate habitat experiencing eutrophication or poor water quality.

In 2012 fish collected from Flint Creek at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and pleomorphic nuclei within hepatocytes were observed indicating contaminant exposure. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Clark Fork River near Bearmouth

Fish collected near Bearmouth at the completion of the field season in 2011 had moderate to moderately severe (histology score = 3-4) swelling of gill tissue. Degeneration and necrosis of hepatocytes was moderate (Table 2) indicating cellular changes that may or may not be within the normal range; however excessive glycogen vacuolation (histology score = 3-4) and nuclear vacuolation (histology score = 2) within hepatocytes indicates exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2011 field season near Bearmouth was infected with parasitic *Trichodina* spp., the presence of which could indicate habitat experiencing eutrophication or poor water quality.

In 2012 fish collected near Bearmouth at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and pleomorphic nuclei were observed within hepatocytes indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe to severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Rock Creek (Control)

Fish collected from Rock Creek at the completion of the field season in 2011 had moderate to moderately severe (histology score = 3-4) swelling of gill tissue. Degeneration and necrosis of hepatocytes was moderate to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and moderate to moderately severe glycogen vacuolation (histology score = 3-4) and pleomorphic nuclei within hepatocytes were observed indicating contaminant exposure. Scores for melanomacrophage aggregation in kidney tissue were moderate to moderately severe (Table 3) indicating chronic exposure to contaminants. In addition to cellular changes associated with exposure to contaminants, one fish collected at the end of the 2011 field season from Rock Creek had a severe bacterial infection of the heart. Many of the fish from Rock Creek in 2011 also had eosinophilic granular cells in cranial nerves, a condition commonly seen in brown trout exposed to *M. cerebralis*.

In 2012 fish collected from Rock Creek at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was mild to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Mild to

moderately severe nuclear vacuolation (histology score = 2-4) and pleomorphic nuclei within hepatocytes were also observed indicating contaminant exposure. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Spring channel near Clinton (Handling control)

Fish collected from the handling control near Clinton at the completion of the field season in 2011 had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was moderate to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and moderately severe (histology score = 4) glycogen vacuolation within hepatocytes was observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe (Table 3), indicating chronic exposure to contaminants. In 2011, one fish from the handling control had eosinophilic granular cells in cranial nerves, a condition commonly seen in brown trout exposed to *M. cerebralis*.

In 2012 fish collected from the handling control near Clinton at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was mild to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2), and pleomorphic nuclei within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Clark Fork River near Turah

Fish collected near Turah at the completion of the field season in 2011 had mostly normal gill tissue. Moderately severe (histology score = 4) glycogen vacuolation within hepatocytes was observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderate and were below the threshold indicating chronic exposure to contaminants.

In 2012 fish collected near Turah at the completion of the field season had mostly normal gill tissue. Degeneration and necrosis of hepatocytes was mild to moderately severe indicating pathological lesions associated with contaminant exposure (Table 2). Excessive nuclear vacuolation (histology score = 2) and pleomorphic nuclei within hepatocytes were observed indicating exposure to contaminants. Scores for melanomacrophage aggregation in kidney tissue were moderately severe and melanomacrophage aggregations were observed in livers (Table 3), indicating chronic exposure to contaminants.

Mid-Season Histology Samples

In addition to samples collected at the conclusion of each field season, histology samples were collected at various points during the summer. On August 4, 2011 following an increase in mortalities in mid- to late-July, samples were collected for histology from all sites excluding the Little Blackfoot River. In 2012 specimens were collected when an individual fish that was near mortality but still alive at the time cages were checked was found. This occurred three times during the 2012 field season, on May 17 downstream of Gold Creek, on July 30 in the Little

Blackfoot River, and on August 23 at Warm Springs. Fish collected prior to the end of each field season showed more severe cellular changes than fish collected at the end of the season. Results for histological analysis of fish collected prior to the end of each field season are summarized below.

Overall, fish collected in early August 2011 showed the same cellular changes as fish collected at the end of the 2011 field season, only the changes were more severe. Moderately severe (histology score = 4) swelling of gill tissue was present in 63 % of histology samples submitted in early August compared to 18 % of those collected at the end of the field season. When observed, degeneration and necrosis of hepatocytes was moderately severe (histology score = 4) indicating pathological lesions in 83 % of histology samples submitted in early August compared to 33 % of those collected at the end of the field season. Overall, fish from Galen had the most severe lesions while those at Warm Springs had the least severe lesions among samples collected in early August.

In addition to cellular changes associated with exposure to contaminants, the moribund fish collected near Galen in May of 2012 had fungal meningitis of the spine, a very uncommon and serious condition. Histological analysis of the fish collected in July 2012 from the Little Blackfoot River revealed proliferation and fusion of gill lamellae and moderate (histology score = 3) degeneration of hepatocytes with no sign of bacterial or parasitic infection, indicating that the fish was moribund due to exposure to contaminants. The moribund fish collected at Warm Springs in August 2012 had normal gills and moderately severe (histology score = 4) cellular changes within the liver indicative of toxicant exposure. In addition to cellular changes associated with exposure to contaminants, this fish also showed evidence of a cranial deformity.

Tissue Metals Burdens

Tissue metals burdens from fish held at cage sites were compared to reported values from previous studies assessing growth or mortality effects using whole body burdens in salmonids. Of the ten metals detected, studies reporting effects on salmonids from whole body burdens of arsenic, cadmium, copper, lead, selenium, and zinc were found (Table 4). Beryllium was the only metal not detected in any of the tissue samples analyzed; the remaining metals were detected in samples from one or more sites.

Table 4. Summary of studies relating whole body metals burdens to growth or mortality effects in salmonids. All values reported were the minimum concentrations causing an effect.

Species	Metal	Metal concentration (µg/g dry weight)	Reference
Rainbow trout	Arsenic	6.80	McGeachy and Dixon 1990
Rainbow trout	Cadmium	4.64	Hollis et al. 2001
Rainbow trout	Copper	8.57	Marr et al. 1996
Eastern brook trout	Lead	20.10	Holcombe et al. 1976
Rainbow trout	Selenium	4.00	Hilton and Hodson 1983
Rainbow trout	Zinc	105.09	Gundogdu and Erdem 2008

Copper and zinc consistently exceeded these thresholds, therefore tissue metals burdens for copper and zinc were depicted through each field season graphically (Figures 18-39) while

the remaining metals detected were summarized as mean values weighted by sample size (Tables 5 and 6). Arsenic was the only metal besides copper and zinc to exceed the identified threshold at any site. Arsenic exceeded the threshold only 3 times throughout the study; in Flint Creek in May (13.70 µg/g, N=1) and June (9.57 µg/g, N=1) of 2011, and once in the Clark Fork River downstream of Gold Creek in May 2012 (8.45 µg/g, N=2); however, when weighted by sample size, average arsenic tissue burdens did not exceed the identified threshold at any site (Tables 5 and 6). Generally, tissues metals burdens were lower at control sites than at mainstem sites; however, Flint Creek was an exception to this as some of the highest metals burdens of any site occurred there (Figures 18-39; Tables 5 and 6). Copper and zinc burdens typically peaked mid-season at most sites; however some sites had burdens that were highest at the beginning of the season and decreased through time (e.g. copper at Galen in 2011, zinc and copper at Rock Creek in 2012) (Figures 18-39).

Table 5. Average tissue metals burdens weighted by sample size for mortalities and fish remaining alive at the end of the field season in 2011. All measurements are µg/g, dry weight; ND indicates all samples below detectable limit.

Site	Ar	Ba	Cd	Cr	Mn	Ni	Pb	Se
Warm Springs	1.22	4.11	0.27	ND	10.98	ND	0.70	1.75
Galen	2.00	7.95	0.40	ND	23.02	5.12	2.54	1.77
Deer Lodge	2.52	11.47	0.30	ND	24.98	ND	3.89	1.53
U/S Little Blackfoot R.	1.78	2.81	0.34	ND	22.66	7.69	0.75	1.35
Little Blackfoot R.	1.71	3.30	ND	ND	16.05	ND	0.92	1.71
Gold Creek	1.76	2.17	ND	ND	15.32	ND	0.44	1.65
Flint Creek	2.60	10.72	ND	2.04	49.21	4.21	1.50	1.54
Bearmouth	2.04	4.17	0.25	ND	29.16	ND	0.96	1.56
Rock Creek	2.52	14.19	ND	ND	9.92	ND	0.45	1.44
Clinton Spring	1.50	2.05	ND	ND	7.41	ND	0.33	1.55
Turah	2.01	10.01	0.26	ND	36.60	ND	1.33	1.47

Table 6. Average tissue metals burdens weighted by sample size for mortalities and fish remaining alive at the end of the field season in 2012. All measurements are µg/g, dry weight; ND indicates all samples below detectable limit.

Site	Ar	Ba	Cd	Cr	Mn	Ni	Pb	Se
Warm Springs	1.51	7.76	ND	ND	21.08	5.07	0.41	1.15
Galen	1.94	2.51	0.36	ND	23.15	49.40	0.94	1.13
Deer Lodge	1.97	3.58	ND	ND	36.68	41.40	1.03	1.11
U/S Little Blackfoot R.	1.36	6.68	0.27	ND	11.76	ND	1.12	0.85
Little Blackfoot R.	1.37	6.53	ND	4.30	15.75	ND	0.33	0.83
Gold Creek	1.55	16.70	0.67	4.04	11.35	2.71	3.53	1.04
Flint Creek	2.05	5.28	ND	ND	40.56	ND	1.27	1.15
Bearmouth	1.34	7.42	0.26	ND	17.10	ND	0.31	1.14
Rock Creek	1.05	3.41	0.40	ND	3.47	4.22	0.27	1.08
Clinton Spring	0.95	1.65	ND	ND	10.57	ND	0.29	1.11
Turah	1.31	3.78	0.72	ND	17.06	2.59	0.47	1.16

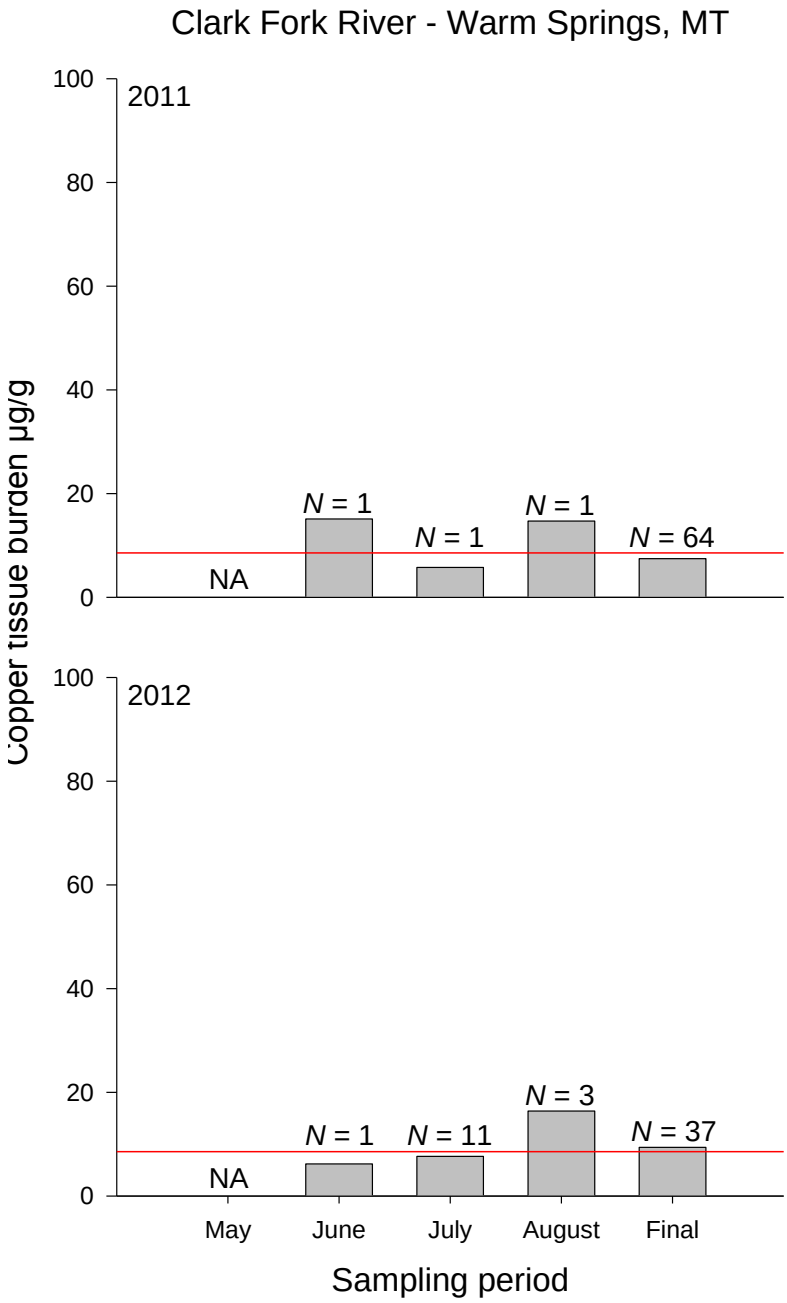

Figure 18. Copper tissue burdens in the Clark Fork River near Warm Springs, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

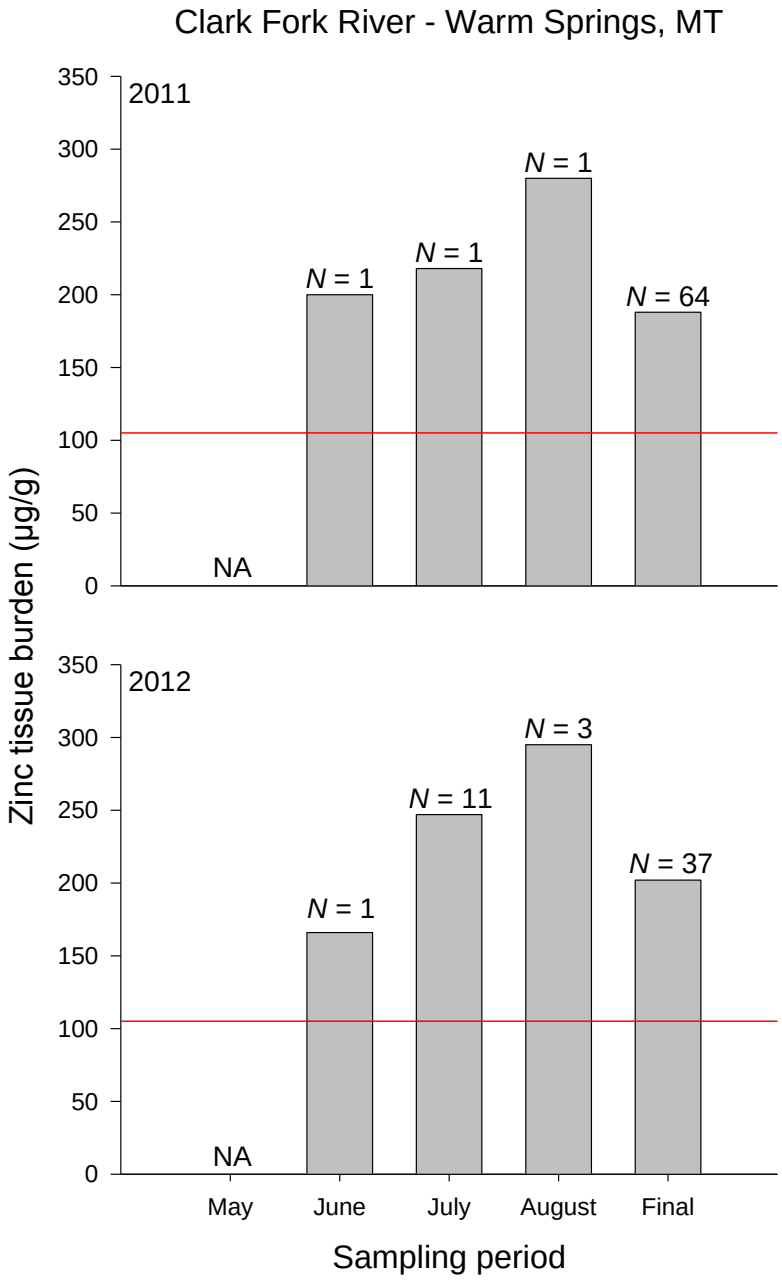

Figure 19. Zinc tissue burdens in the Clark Fork River near Warm Springs, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

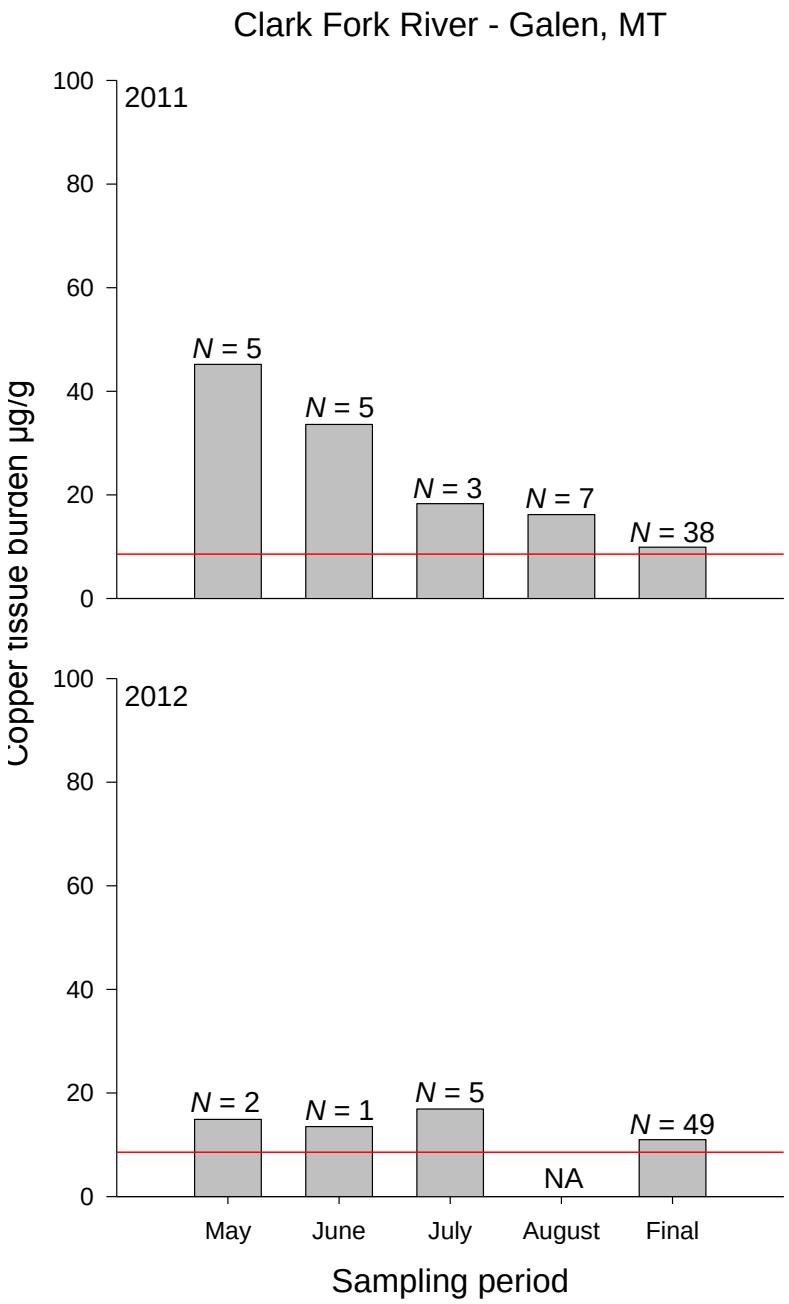

Figure 20. Copper tissue burdens in the Clark Fork River near Galen, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

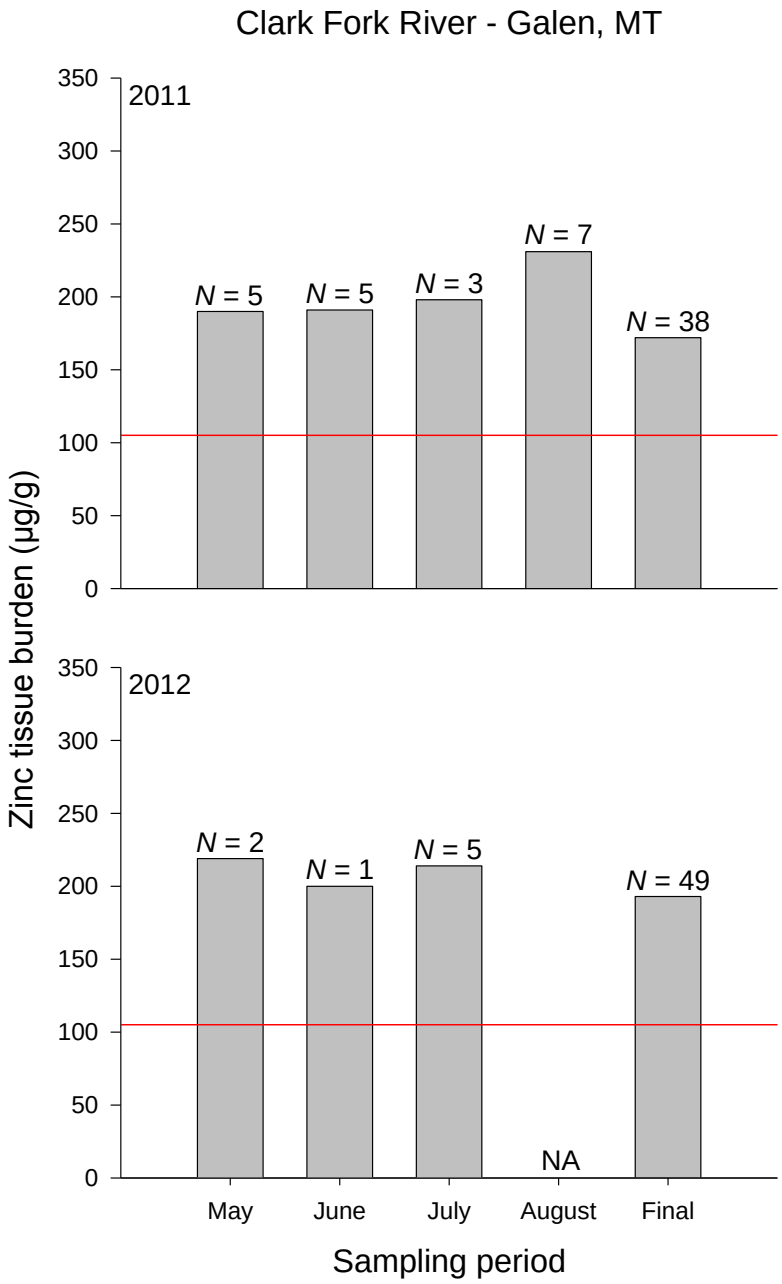

Figure 21. Zinc tissue burdens in the Clark Fork River near Galen, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

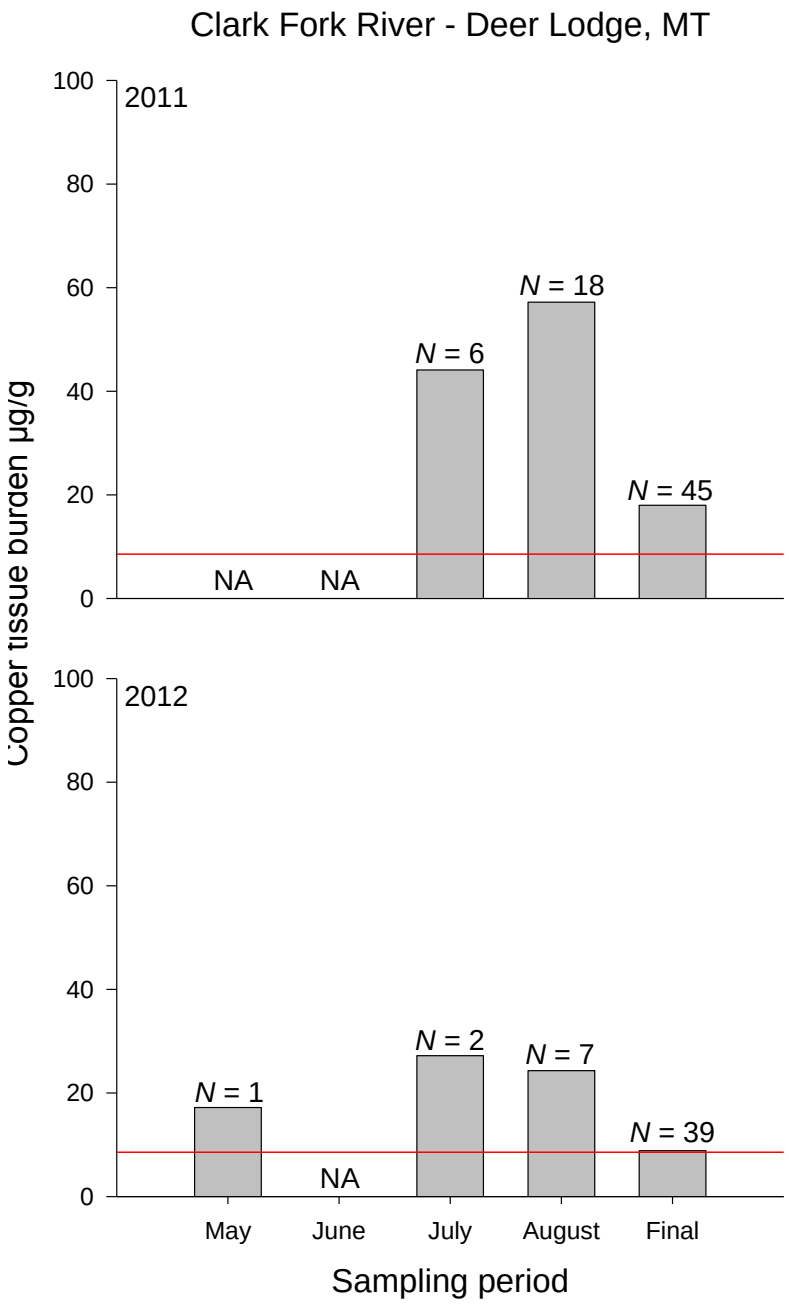

Figure 22. Copper tissue burdens in the Clark Fork River near Deer Lodge, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

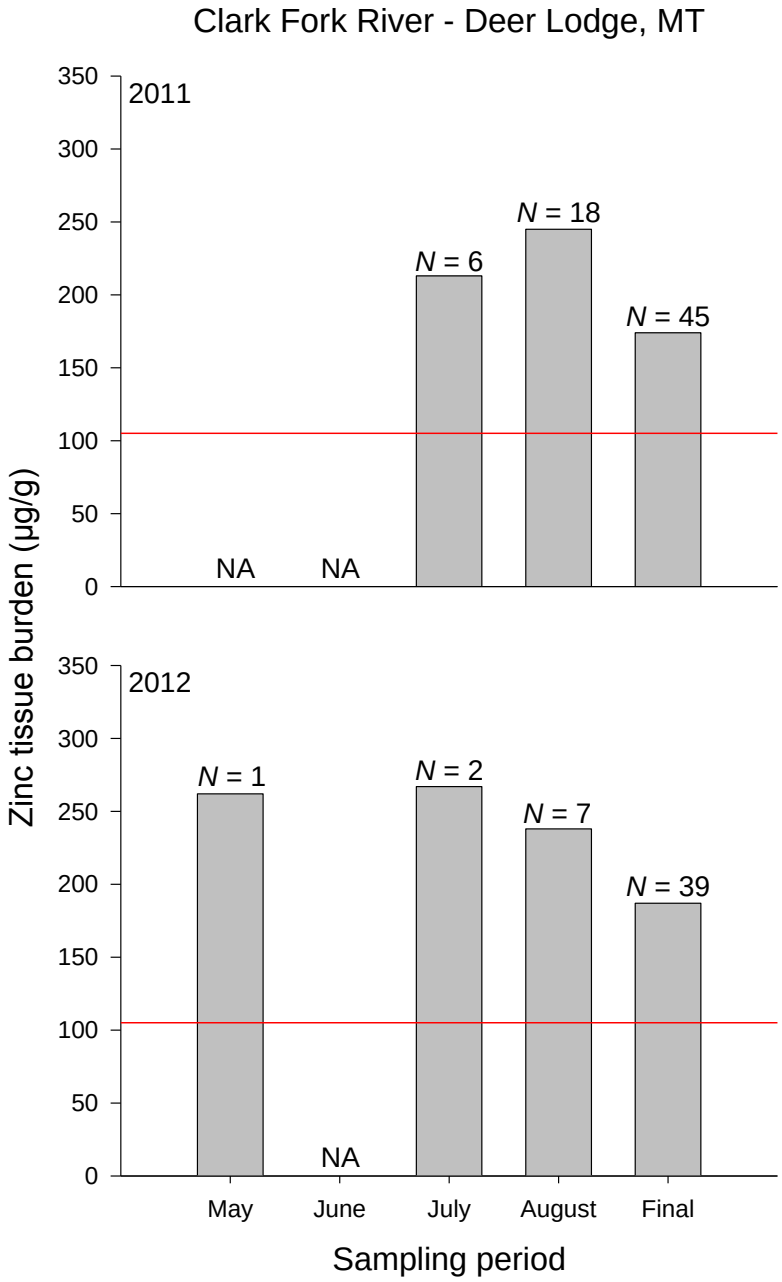

Figure 23. Zinc tissue burdens in the Clark Fork River near Deer Lodge, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

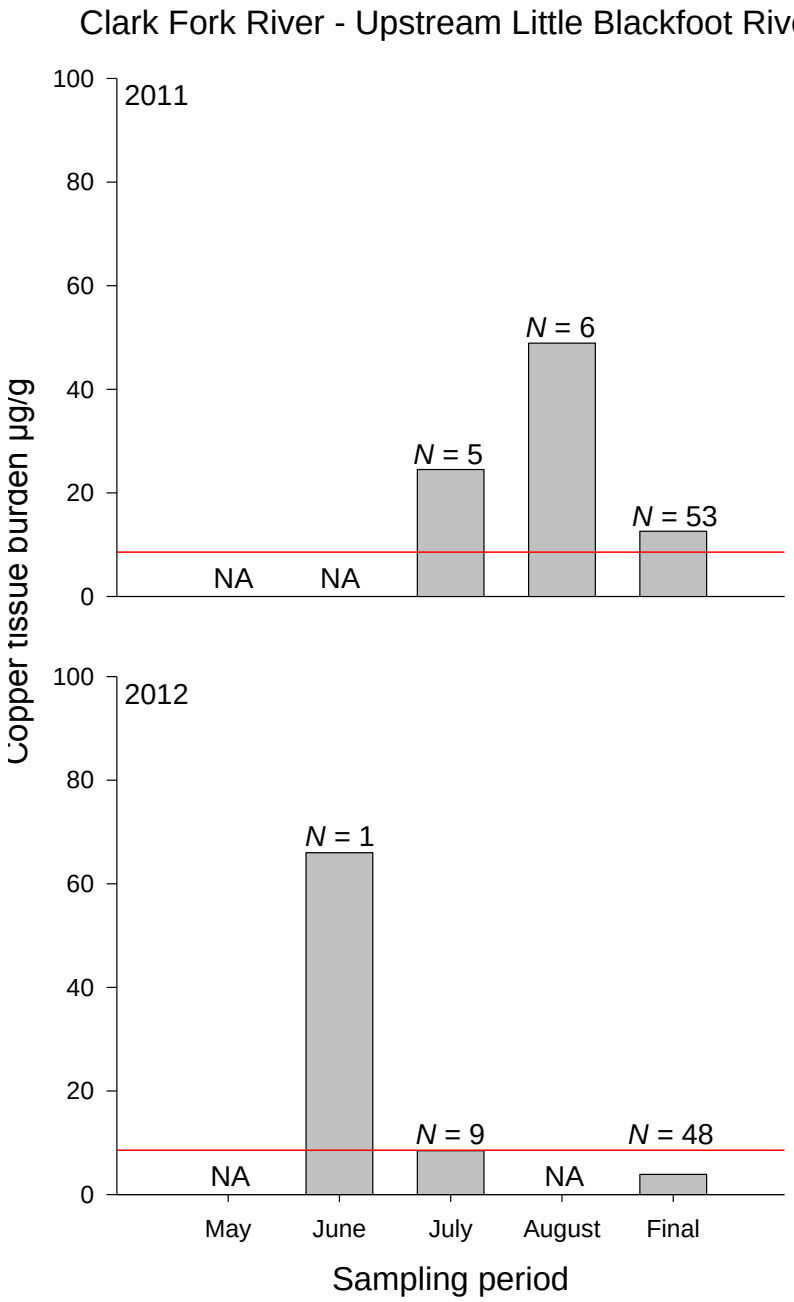

Figure 24. Copper tissue burdens in the Clark Fork River upstream of the Little Blackfoot River. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

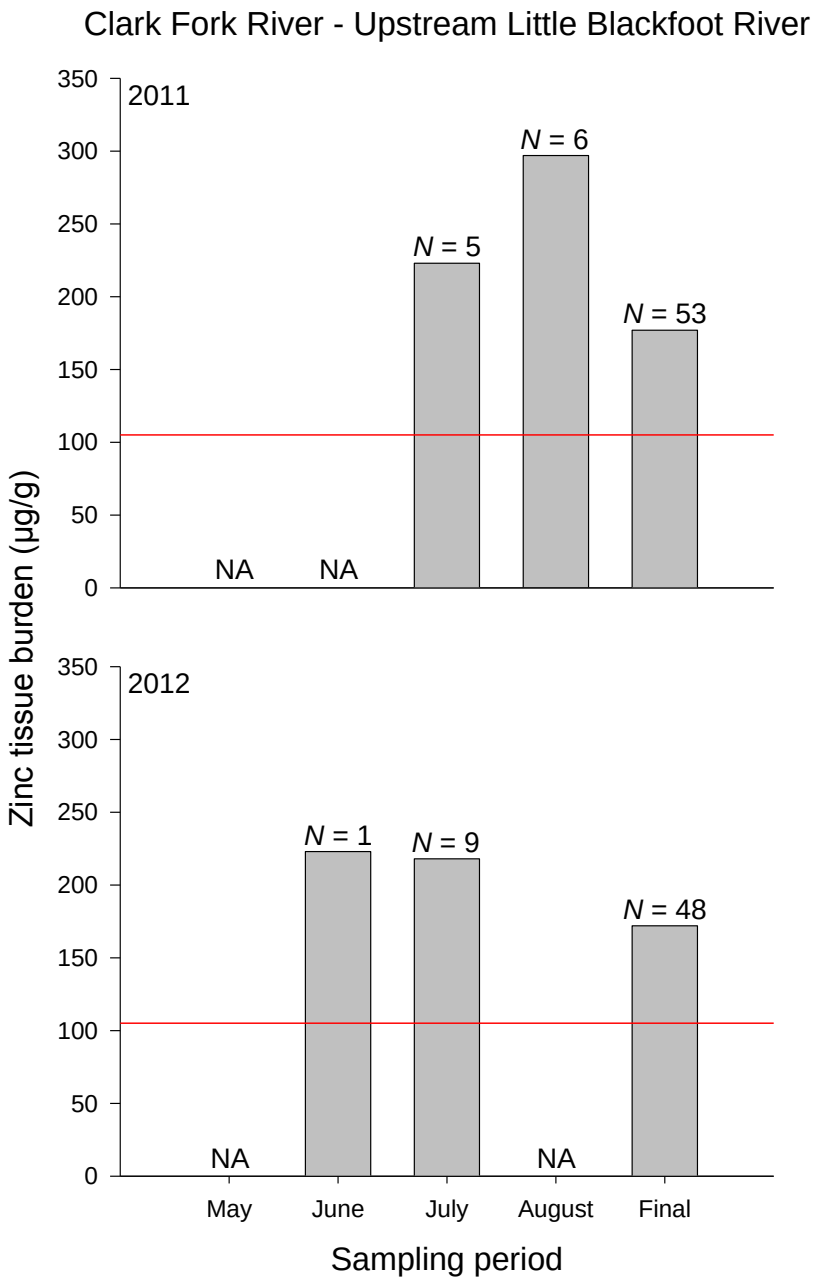

Figure 25. Zinc tissue burdens in the Clark Fork River upstream of the Little Blackfoot River. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

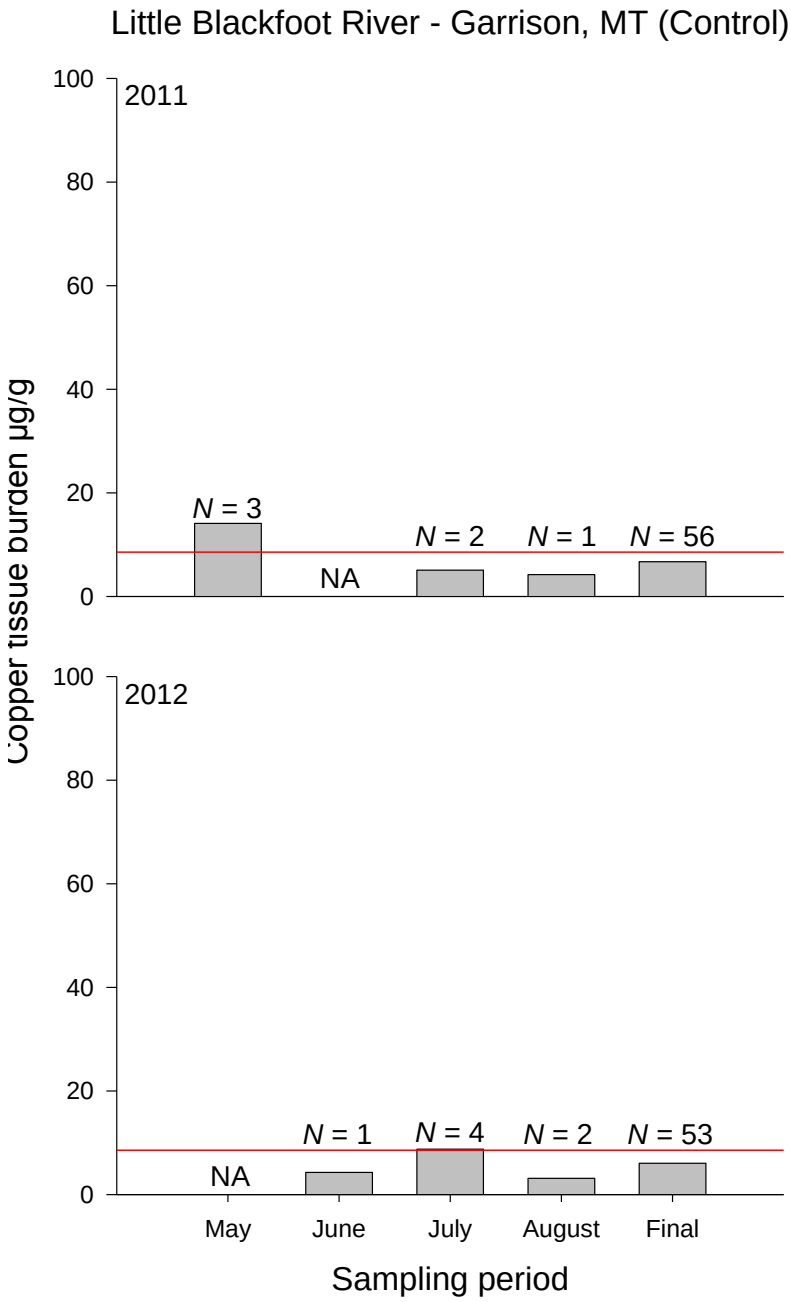

Figure 26. Copper tissue burdens in the Little Blackfoot River near Garrison, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

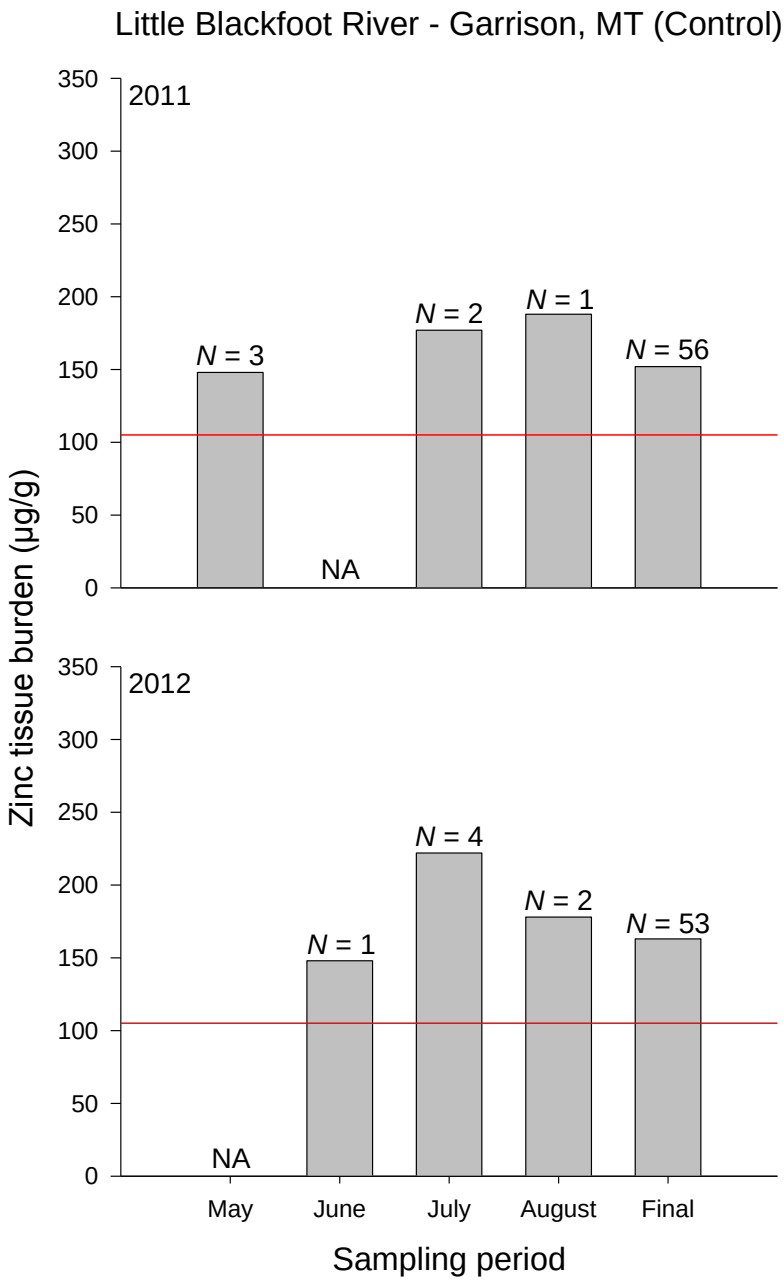

Figure 27. Zinc tissue burdens in the Little Blackfoot River near Garrison, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

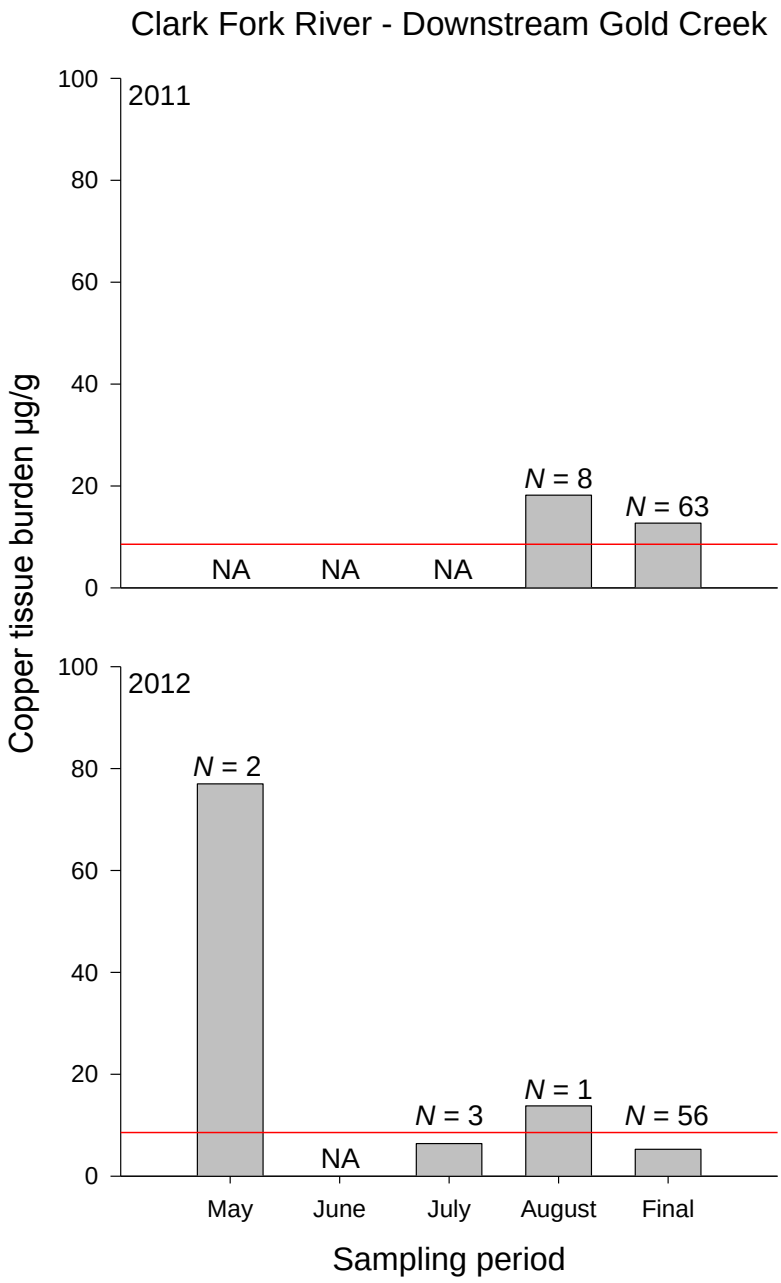

Figure 28. Copper tissue burdens in the Clark Fork River downstream of Gold Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

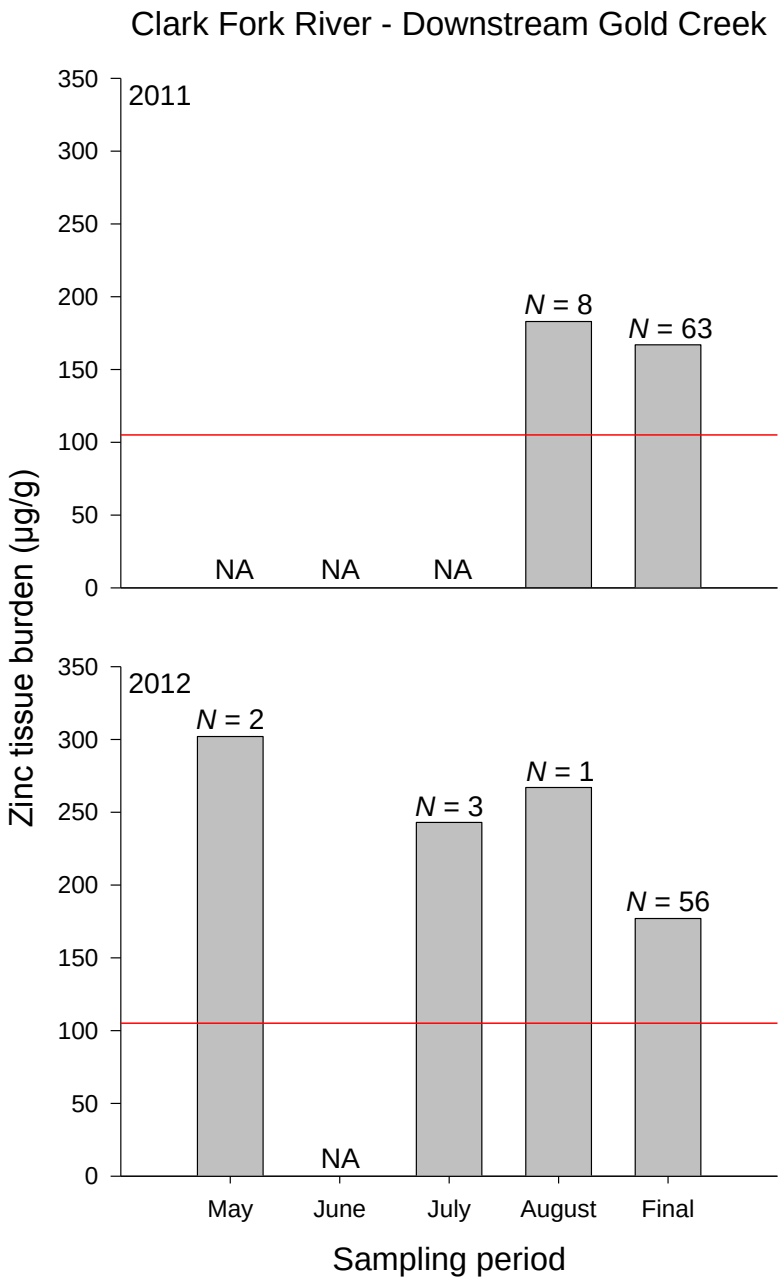

Figure 29. Zinc tissue burdens in the Clark Fork River downstream of Gold Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

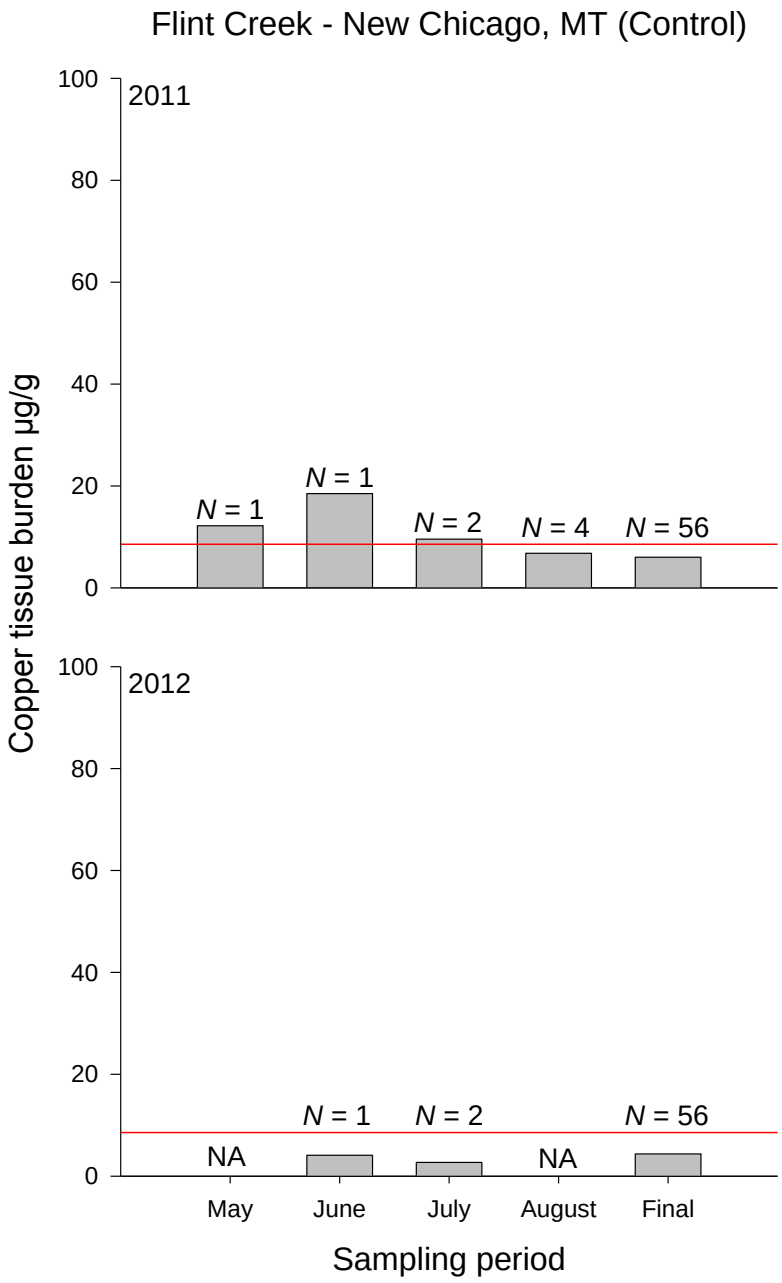

Figure 30. Copper tissue burdens in Flint Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

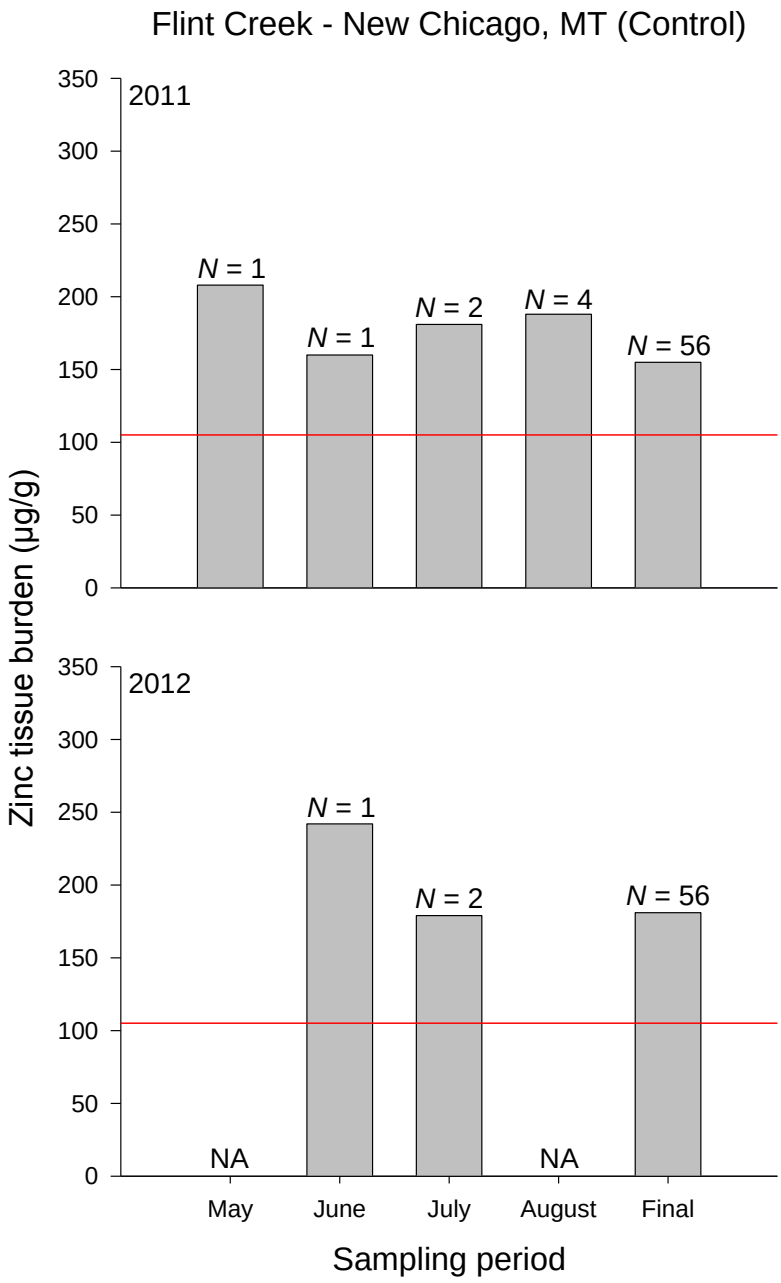

Figure 31. Zinc tissue burdens in Flint Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

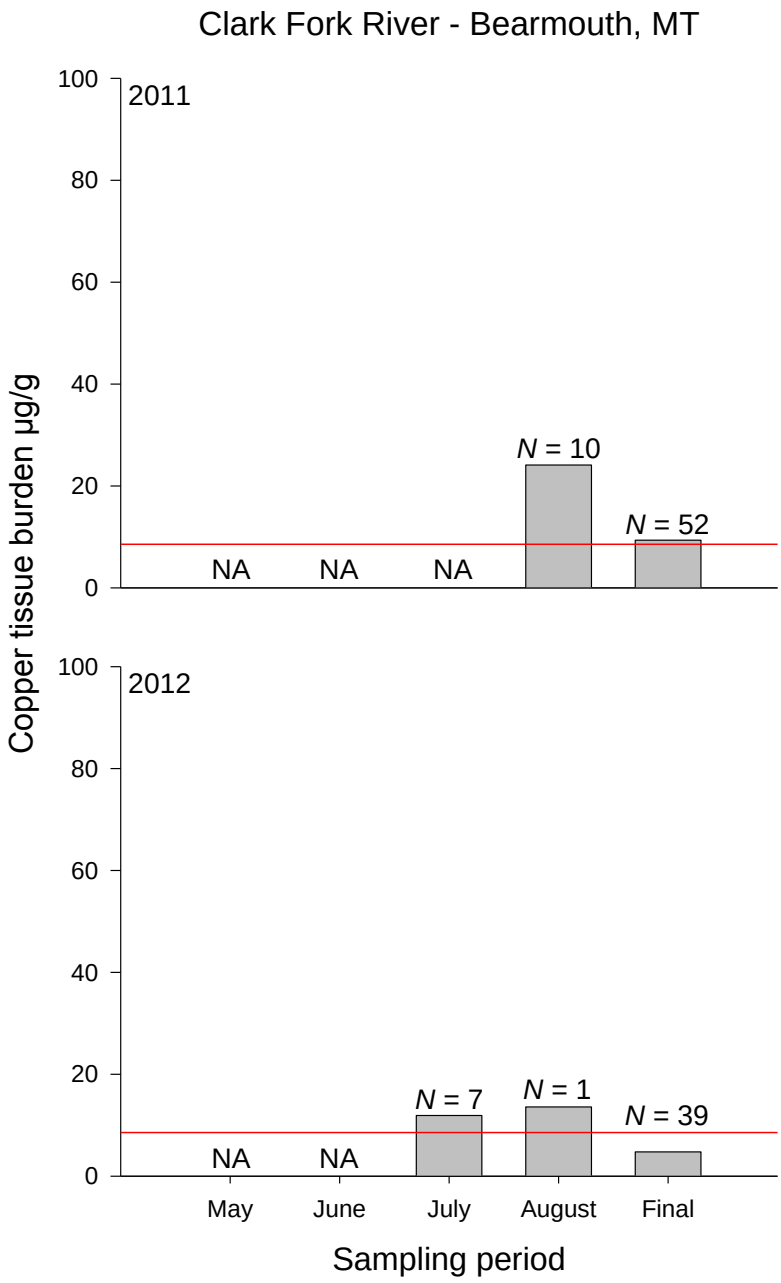

Figure 32. Copper tissue burdens in the Clark Fork River near Bearmouth, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

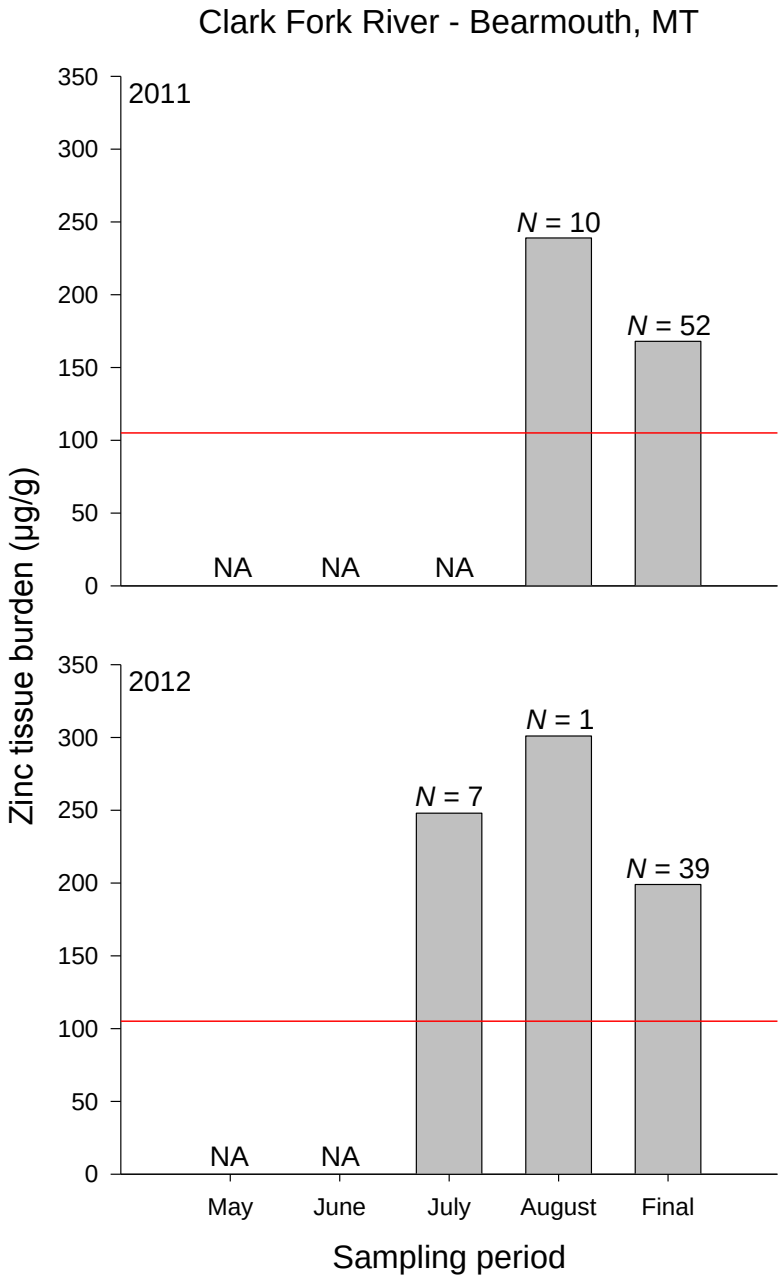

Figure 33. Zinc tissue burdens in the Clark Fork River near Bearmouth, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

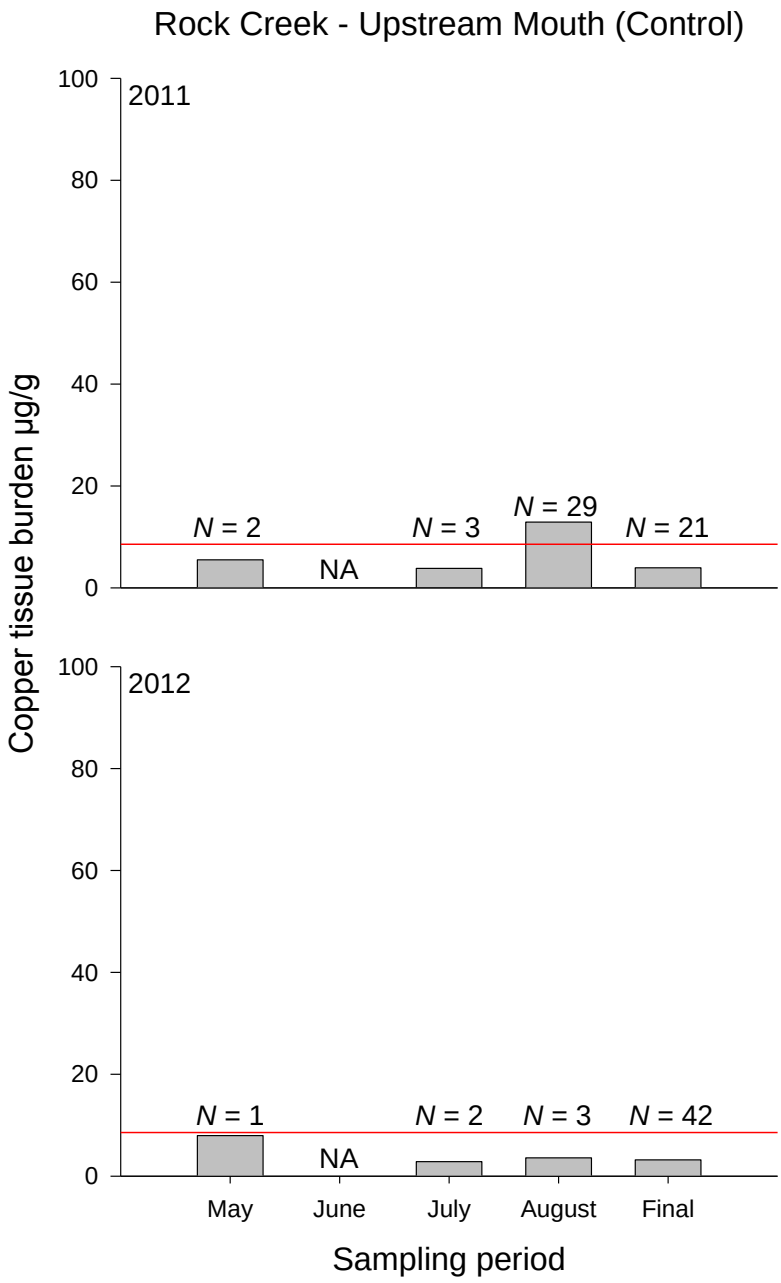

Figure 34. Copper tissue burdens in Rock Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

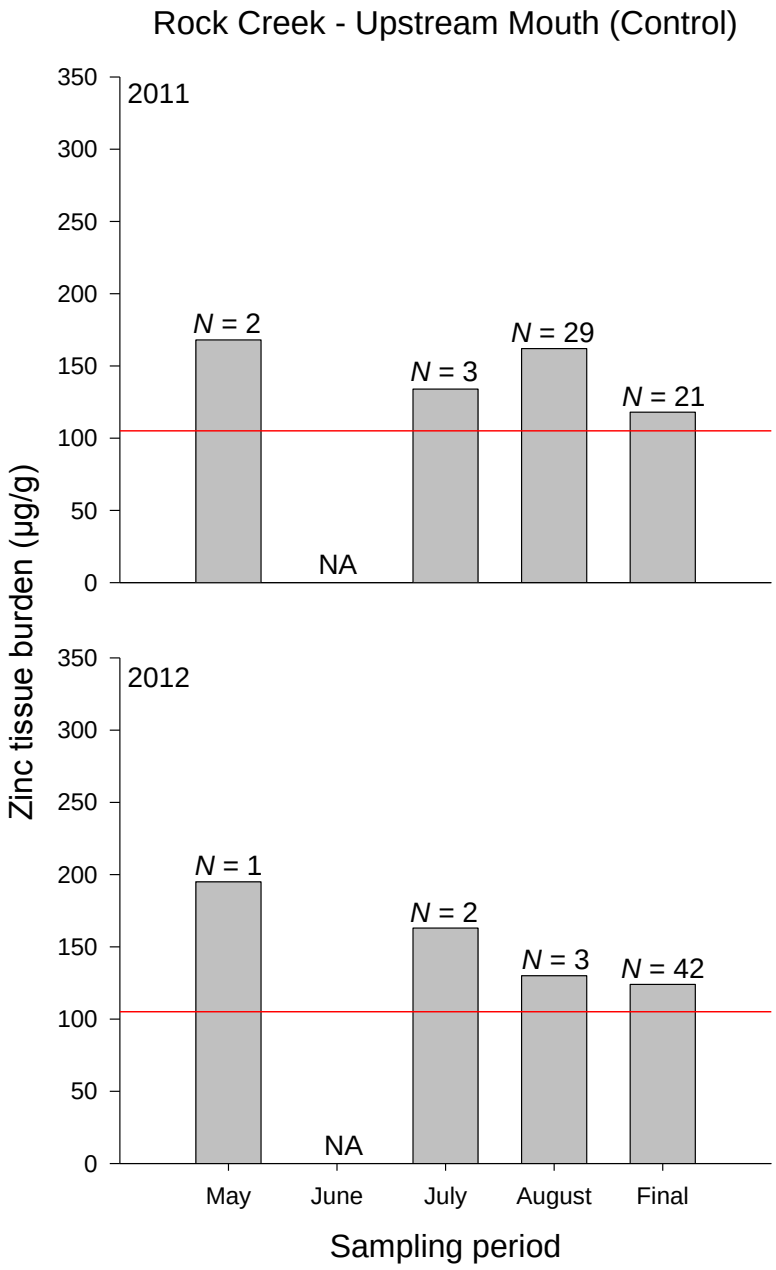

Figure 35. Zinc tissue burdens in Rock Creek. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

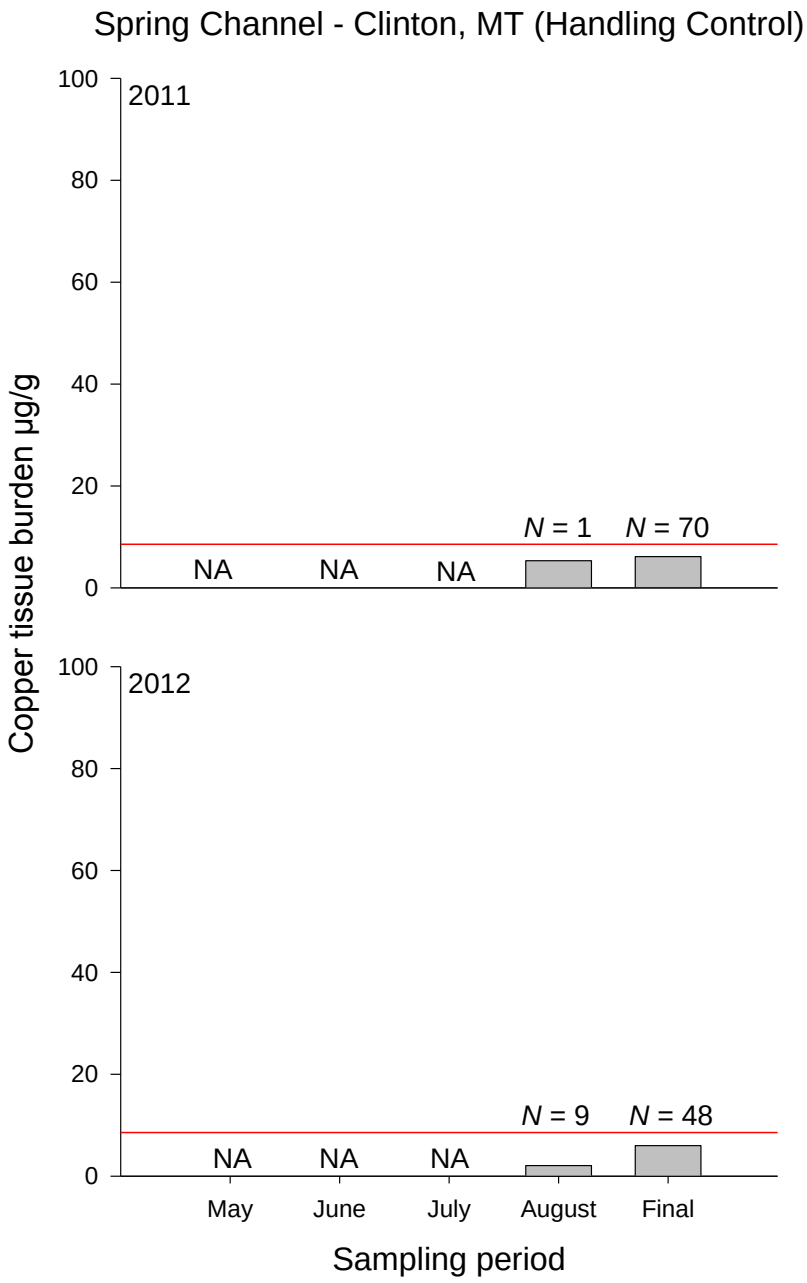

Figure 36. Copper tissue burdens in the spring channel near Clinton, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

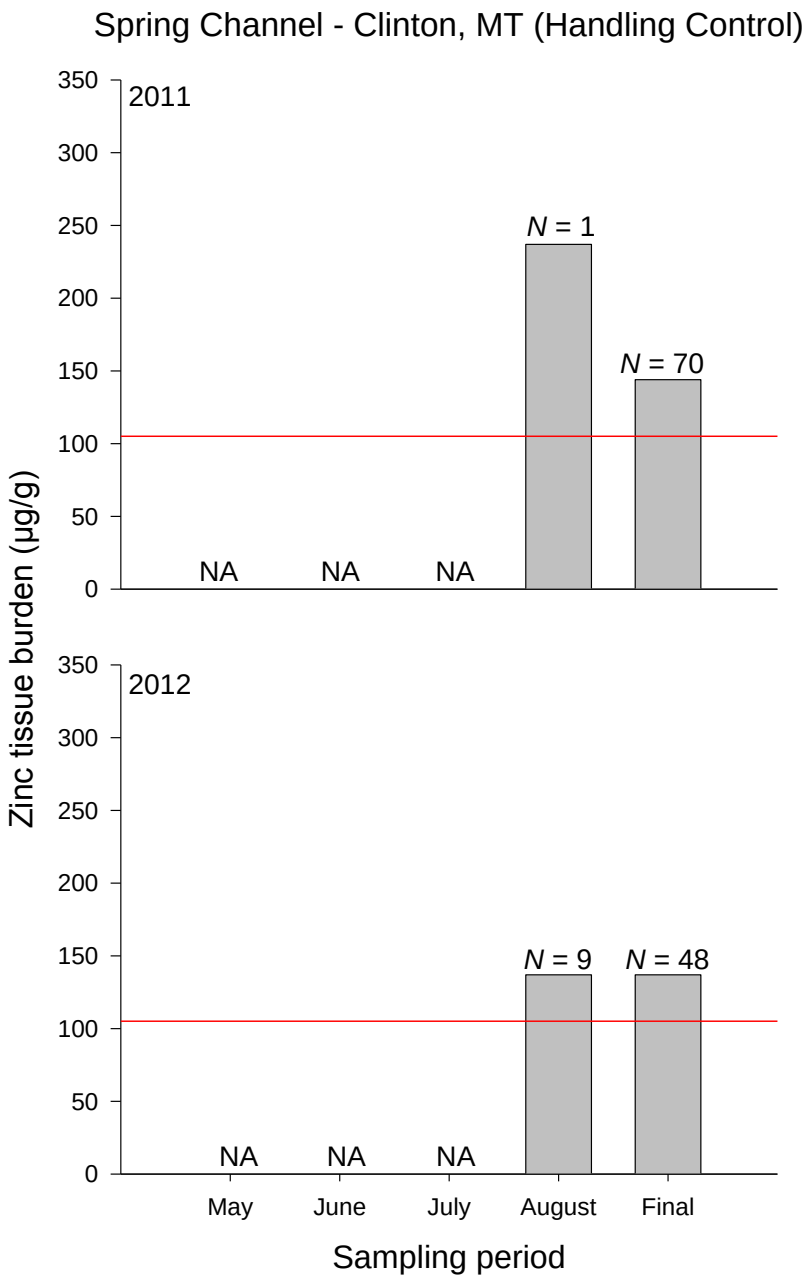

Figure 37. Zinc tissue burdens in spring channel near Clinton, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

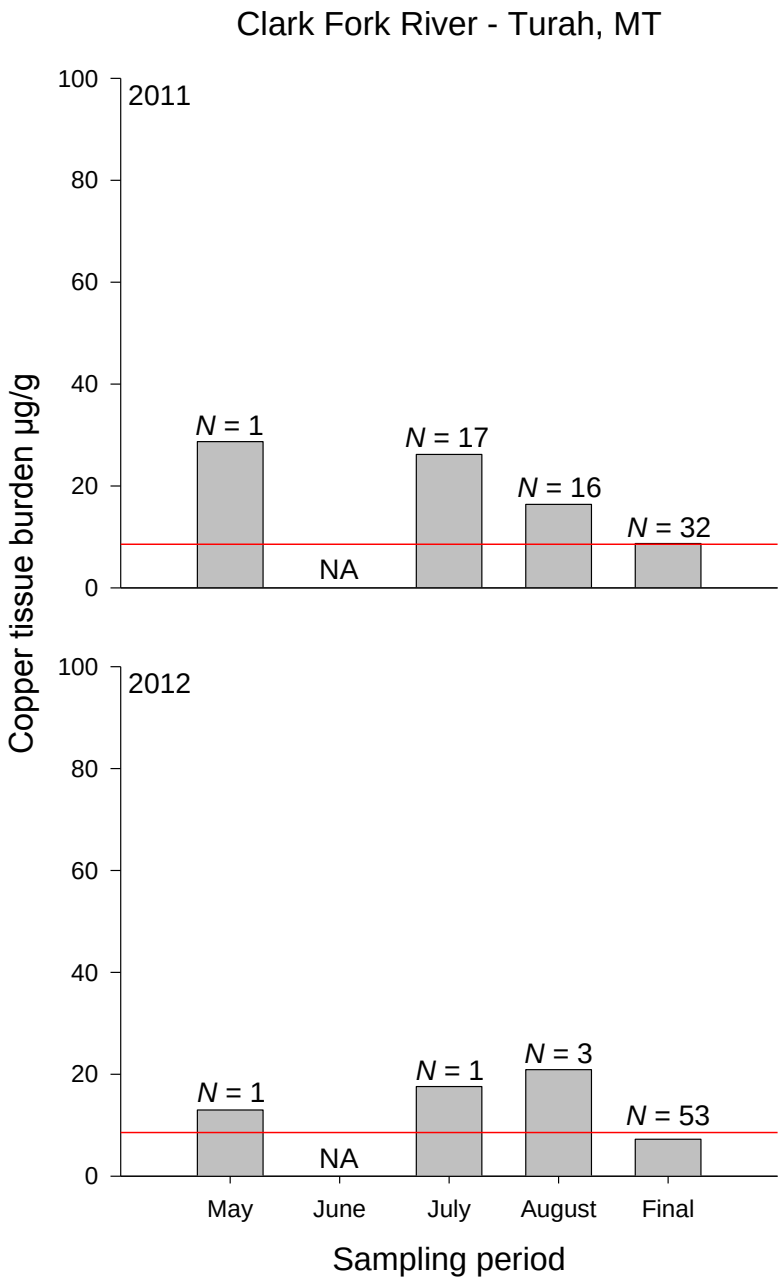

Figure 38. Copper tissue burdens in the Clark Fork River near Turah, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

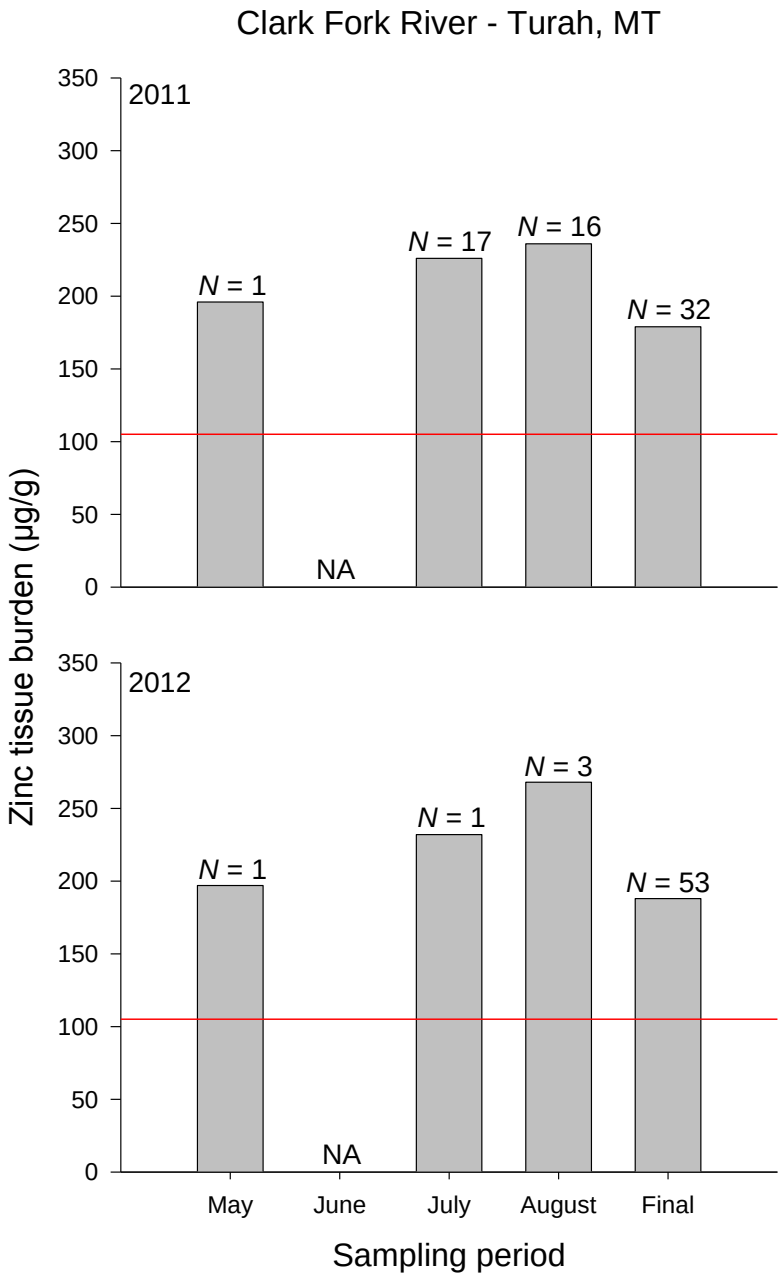

Figure 39. Zinc tissue burdens in Clark Fork River near Turah, Montana. The red line in each panel indicates the minimum effect threshold identified for salmonids. The sample sizes for May through August represent the number of mortalities in each sample (NA = 0), while the final sample size represents the number of fish remaining alive in each sample at the end of each field season.

Water Contaminants

Chronic freshwater ALS values for metals in surface water are evaluated based upon the analysis of samples following a total recoverable method (Atkins 2012; MTDEQ 2012); therefore discussion of water sampling results will focus on total recoverable levels. Dissolved metals concentrations generally followed the same trends as total recoverable concentrations. Ammonia nitrogen (NH_3-N) was only detected once at Deer Lodge on May 10, 2011 at a concentration of 0.06 mg/L.

In 2011, total recoverable arsenic concentration was lower at control sites (mean = 0.012 mg/L; SD = 0.008) than at mainstem treatment sites (mean = 0.025 mg/L; SD = 0.009). In 2012, total recoverable arsenic concentration was also lower at control sites (mean = 0.012 mg/L; SD = 0.008) than at mainstem treatment sites (mean = 0.017 mg/L; SD = 0.007). Total recoverable arsenic did not exceed the chronic ALS values in either year (Figures 40 and 41). Overall, total recoverable arsenic concentrations at the mainstem sites were highest at the four upper sites and decreased downstream in both years. The exception is the sample collected on May 10, 2011 where elevated arsenic concentrations remained similar at the six upper sites.

In 2011, total recoverable cadmium concentration was lower at control sites (mean = 0.00017 mg/L; SD = 0.00010) than at mainstem treatment sites (mean = 0.00033 mg/L; SD = 0.00028). Total recoverable cadmium concentration in 2012 was also lower at control sites (mean = 0.00018 mg/L; SD = 0.00014) than at mainstem treatment sites (mean = 0.00021 mg/L; SD = 0.00009). Total recoverable cadmium concentrations in 2011 exceeded chronic ALS values at least once at one control site (Flint Creek), and all mainstem treatment sites excluding the two furthest upstream sites (Warm Springs and Galen) (Figure 42). In 2012, total recoverable cadmium concentrations did not exceed chronic ALS values (Figure 43). Overall, total recoverable cadmium concentration at the mainstem sites in 2011 was highest at Deer Lodge and decreased downstream, with the exception of the samples collected on May 10 where the highest cadmium concentration was seen at Bearmouth. In 2012, overall total recoverable cadmium concentration at the mainstem sites was highest upstream of the Little Blackfoot River and decreased at sites both up- and downstream of there.

In 2011, total recoverable copper concentration was lower at control sites (mean = 0.006 mg/L; SD = 0.006) than at mainstem treatment sites (mean = 0.057 mg/L; SD = 0.049). Total recoverable copper was also lower at control sites (mean = 0.003 mg/L; SD = 0.003) than at mainstem treatment sites (mean = 0.032 mg/L; SD = 0.029) in 2012. Total recoverable copper exceeded the chronic ALS at least once at all seven mainstem treatment sites as well as two control sites (Flint Creek and the Clinton spring) in 2011 (Figure 44). In 2012, total recoverable copper exceeded the chronic ALS at all mainstem treatment sites (Figure 45). In 2011, overall total recoverable copper at the mainstem sites was highest at Deer Lodge and decreased at sites both up- and downstream of there. However, on May 10, 2011 the highest total recoverable copper was seen at Bearmouth followed by the site upstream of the Little Blackfoot River. In 2012, overall total recoverable copper at the mainstem sites was highest upstream of the Little Blackfoot River and decreased at sites both up- and downstream of this site.

In 2011 total recoverable lead concentration was similar at control sites (mean = 0.0072 mg/L; SD = 0.0101) and mainstem treatment sites (mean = 0.0074 mg/L; SD = 0.008). Total recoverable lead concentration in 2012 was higher at control sites (mean = 0.0071 mg/L; SD = 0.0118) than at mainstem treatment sites (mean = 0.0047 mg/L; SD = 0.0039). Total recoverable lead concentrations in 2011exceeded the chronic ALS value at all mainstem treatment sites and

two tributary control sites (Little Blackfoot River and Flint Creek) (Figure 46). Total recoverable lead concentrations in 2012 exceeded the chronic ALS values at one control site (Flint Creek) and all mainstem treatment sites excluding the two furthest upstream sites (Warm Springs and Galen) (Figure 47). Overall, total recoverable lead at the mainstem sites in both years was highest upstream of the Little Blackfoot River and decreased at sites up- and downstream of this site; however, on May 10, 2011 the highest total recoverable lead concentration was seen at Bearmouth and decreased at sites up- and downstream of this site.

In 2011, total recoverable zinc concentration was lower at control sites (mean = 0.03 mg/L; SD = 0.03) than at mainstem treatment sites (mean = 0.07 mg/L; SD = 0.07). Total recoverable zinc concentration was also lower at control sites (mean = 0.03 mg/L; SD = 0.03) than at mainstem treatment sites (mean = 0.04 mg/L; SD = 0.02) in 2012. Total recoverable zinc concentration exceeded the chronic ALS value at three mainstem treatment sites in 2011 (Deer Lodge, Bearmouth, and Turah) (Figure 48). Total recoverable zinc concentration in 2012 did not exceed the chronic ALS value at any of the treatment or control sites (Figure 49). Overall, total recoverable zinc concentration at the mainstem sites in 2011 was highest at Deer Lodge and decreased at sites up- and downstream of there, with the exception of the samples collected on May 10, where the highest zinc concentration was seen at Bearmouth. In 2012, overall total recoverable zinc concentration at the mainstem sites in 2012 was highest upstream of the Little Blackfoot River and at Bearmouth and decreased at sites up- and downstream of those sites.

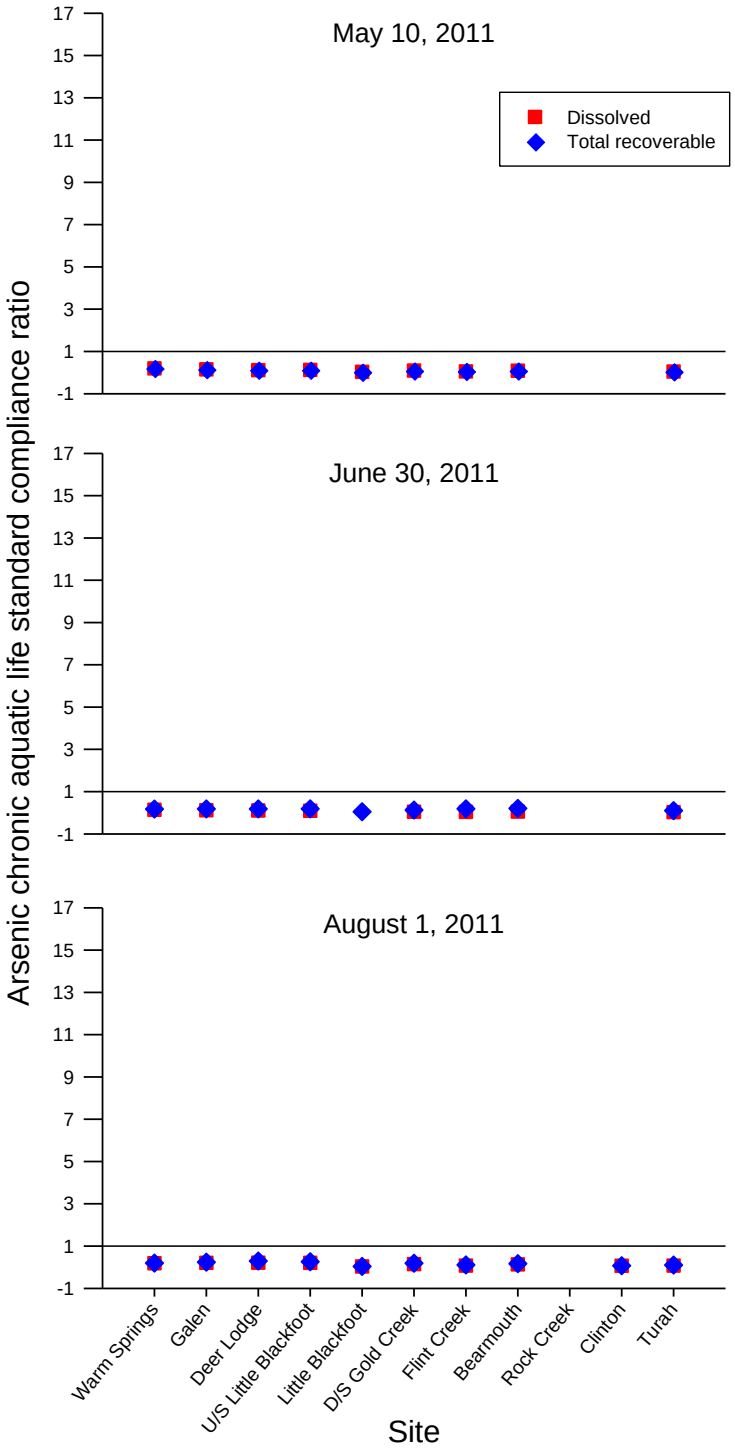

Figure 40. Arsenic compliance ratios at the cage sites in 2011 arranged from upstream to downstream. Compliance ratios were calculated by dividing arsenic concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate arsenic levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

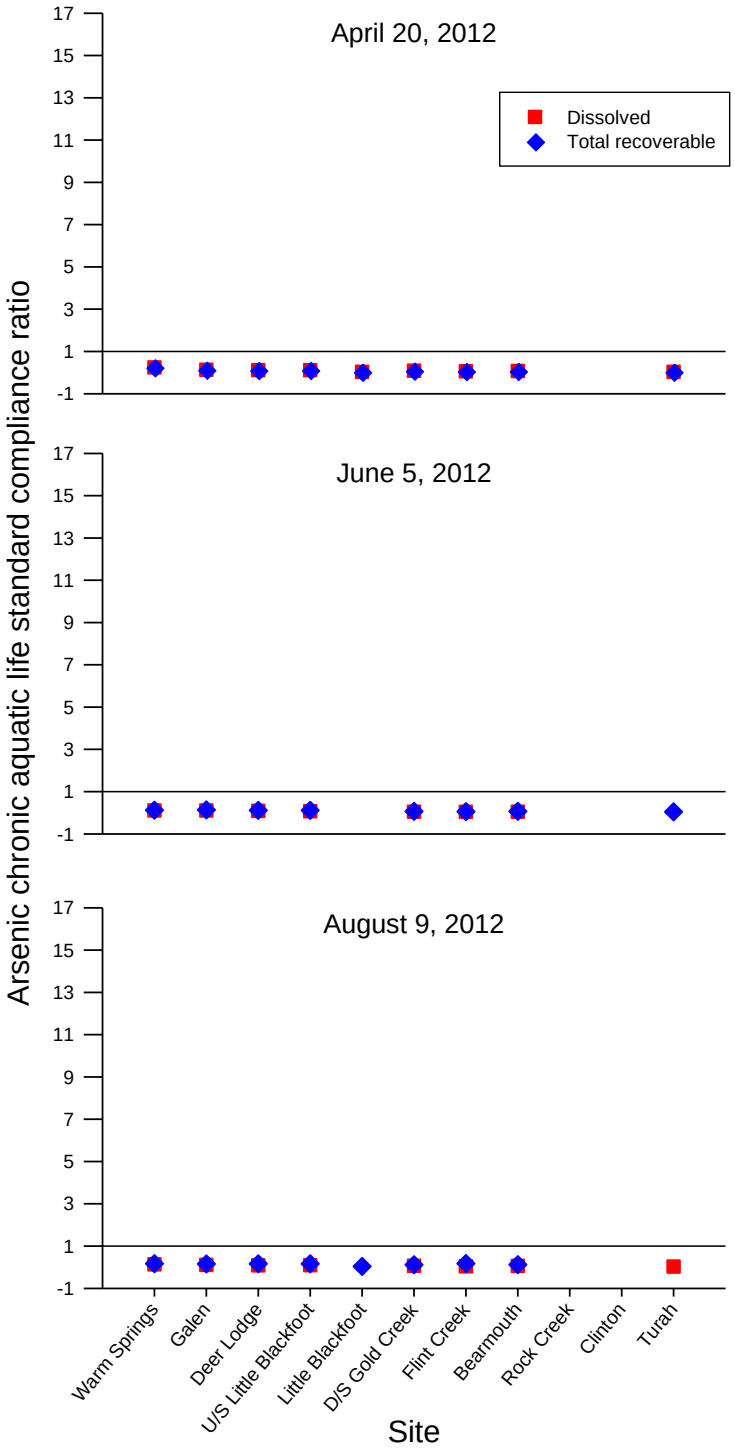

Figure 41. Arsenic compliance ratios at the cage sites in 2012 arranged from upstream to downstream. Compliance ratios were calculated by dividing arsenic concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate arsenic levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

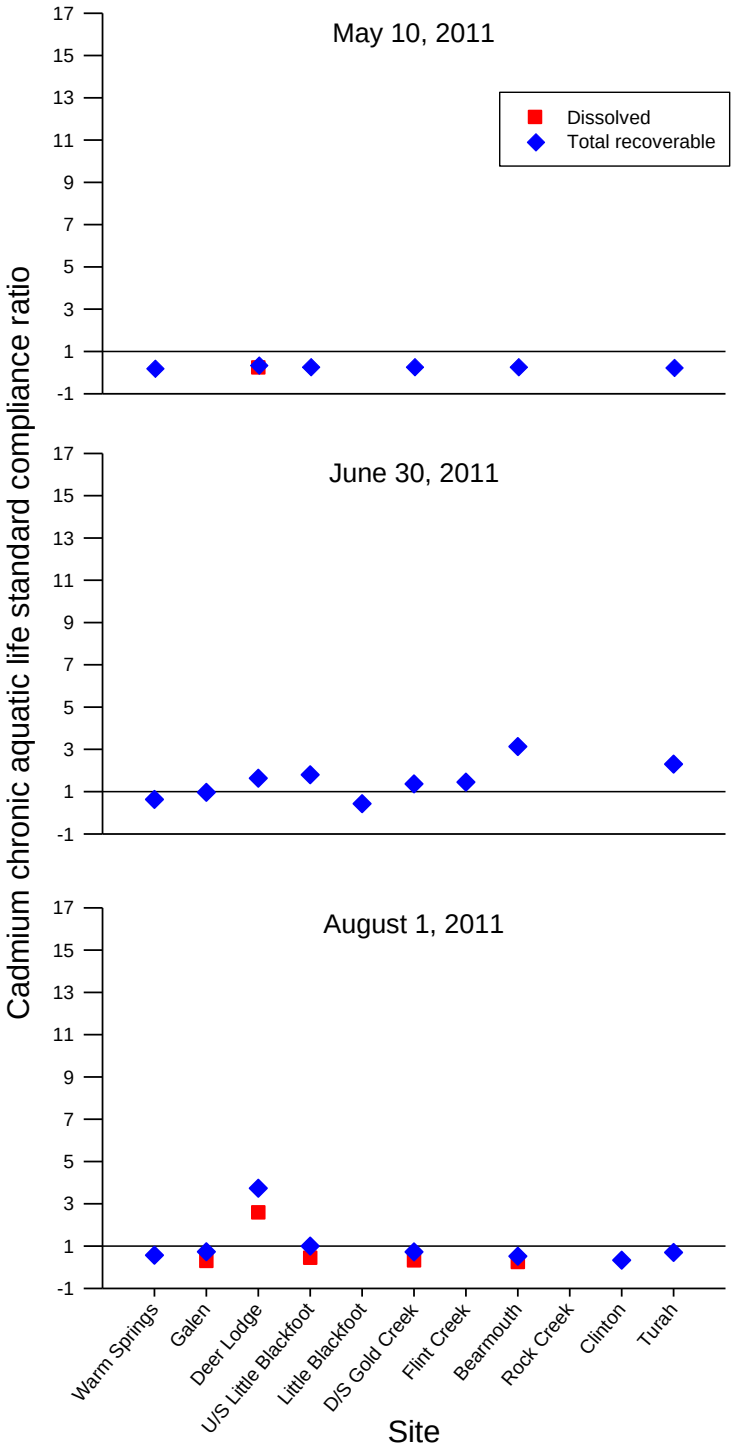

Figure 42. Cadmium compliance ratios at the cage sites in 2011 arranged from upstream to downstream. Compliance ratios were calculated by dividing cadmium concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate cadmium levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

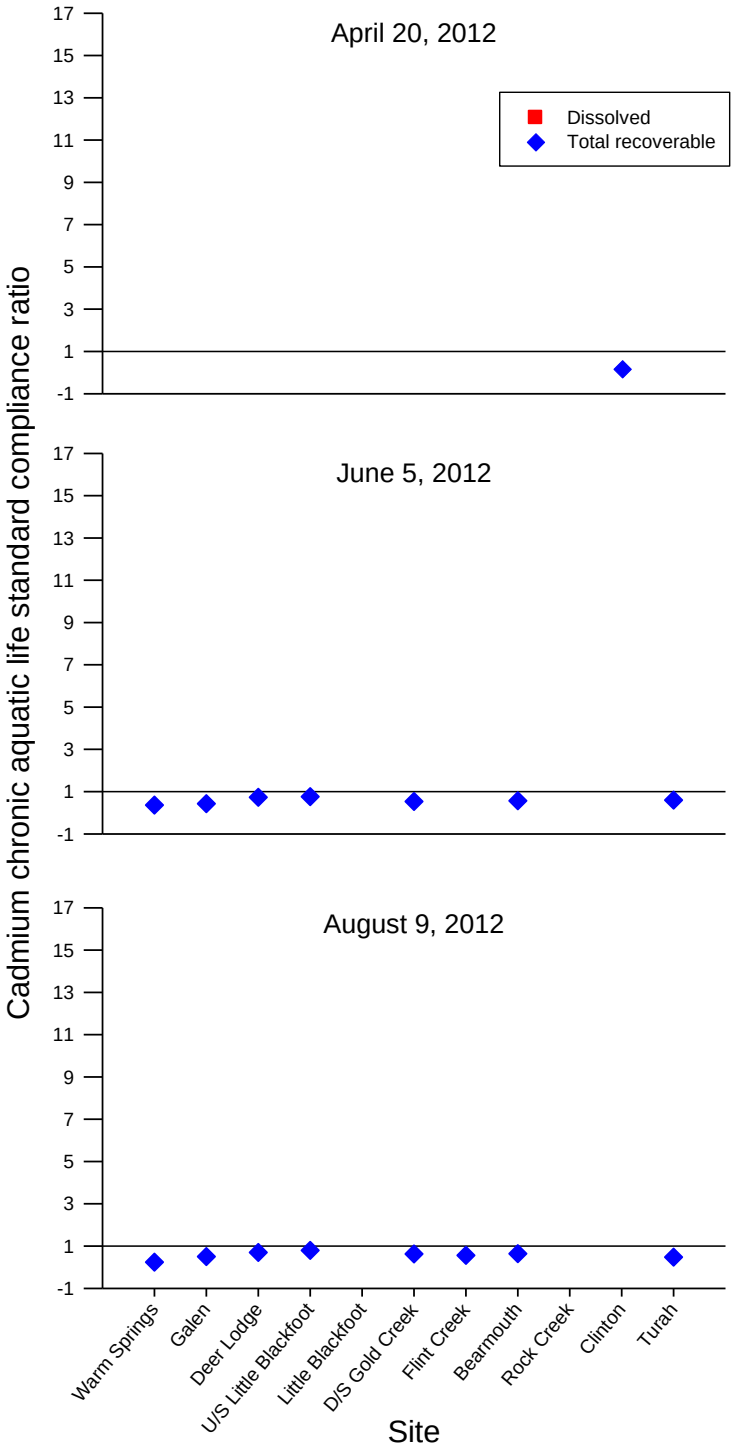

Figure 43. Cadmium compliance ratios at the cage sites in 2012 arranged from upstream to downstream. Compliance ratios were calculated by dividing cadmium concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate arsenic levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

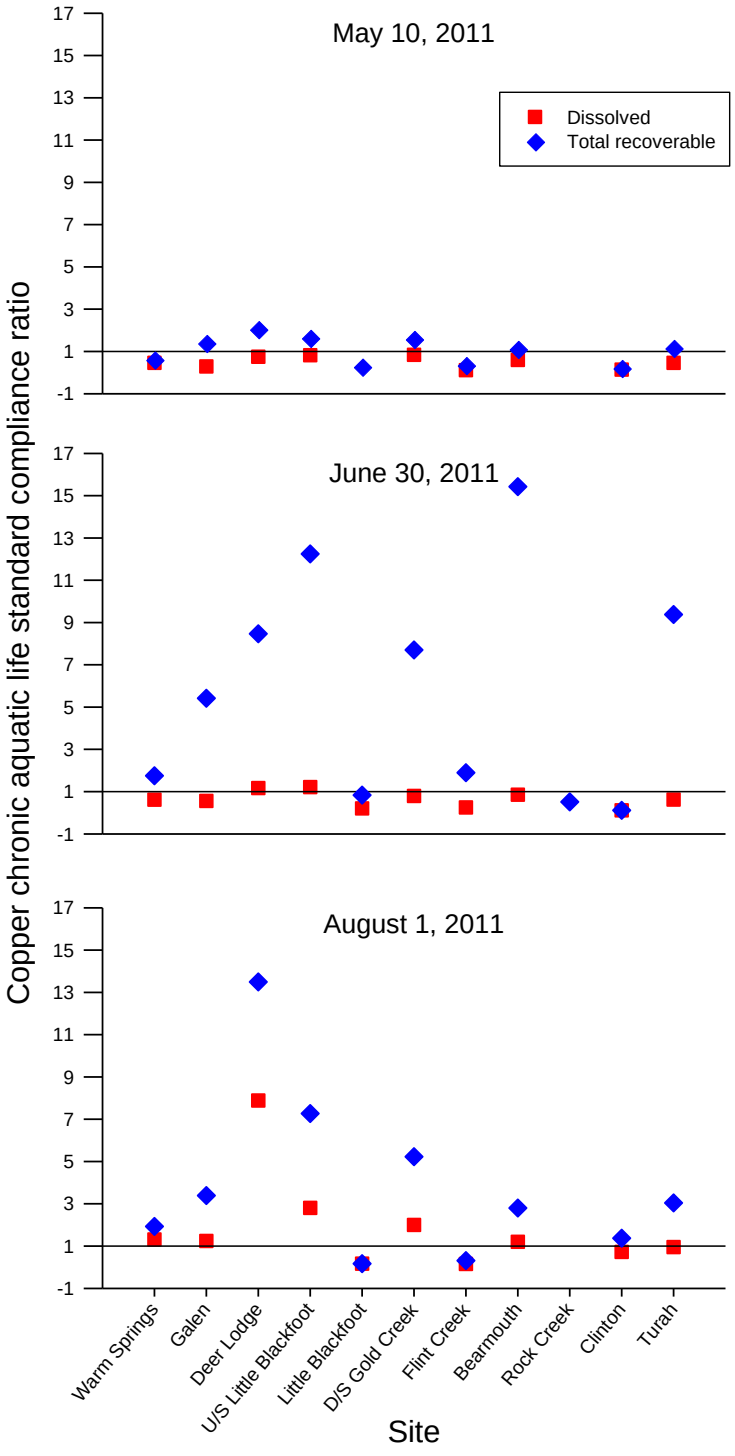

Figure 44. Copper compliance ratios at the cage sites in 2011 arranged from upstream to downstream. Compliance ratios were calculated by dividing arsenic concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate copper levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

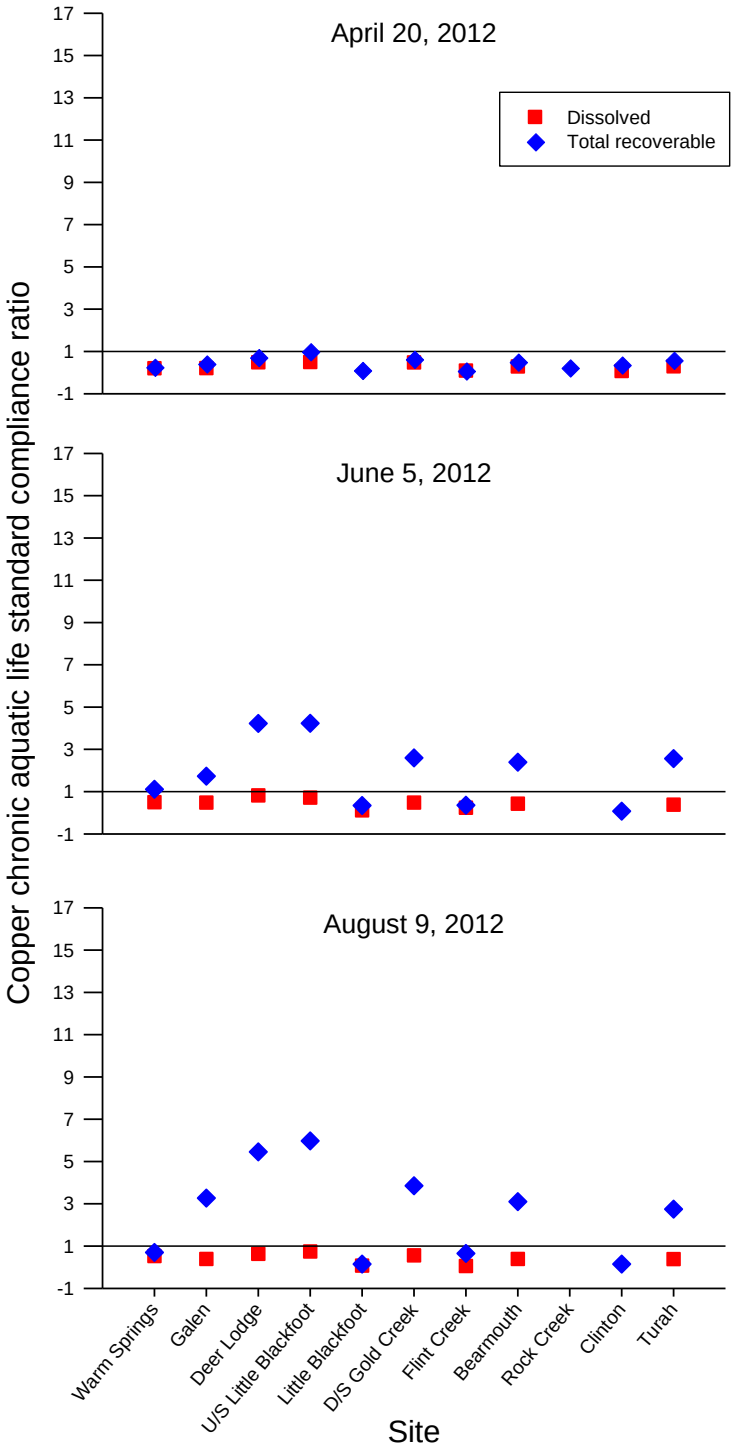

Figure 45. Copper compliance ratios at the cage sites in 2012 arranged from upstream to downstream. Compliance ratios were calculated by dividing copper concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate copper levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

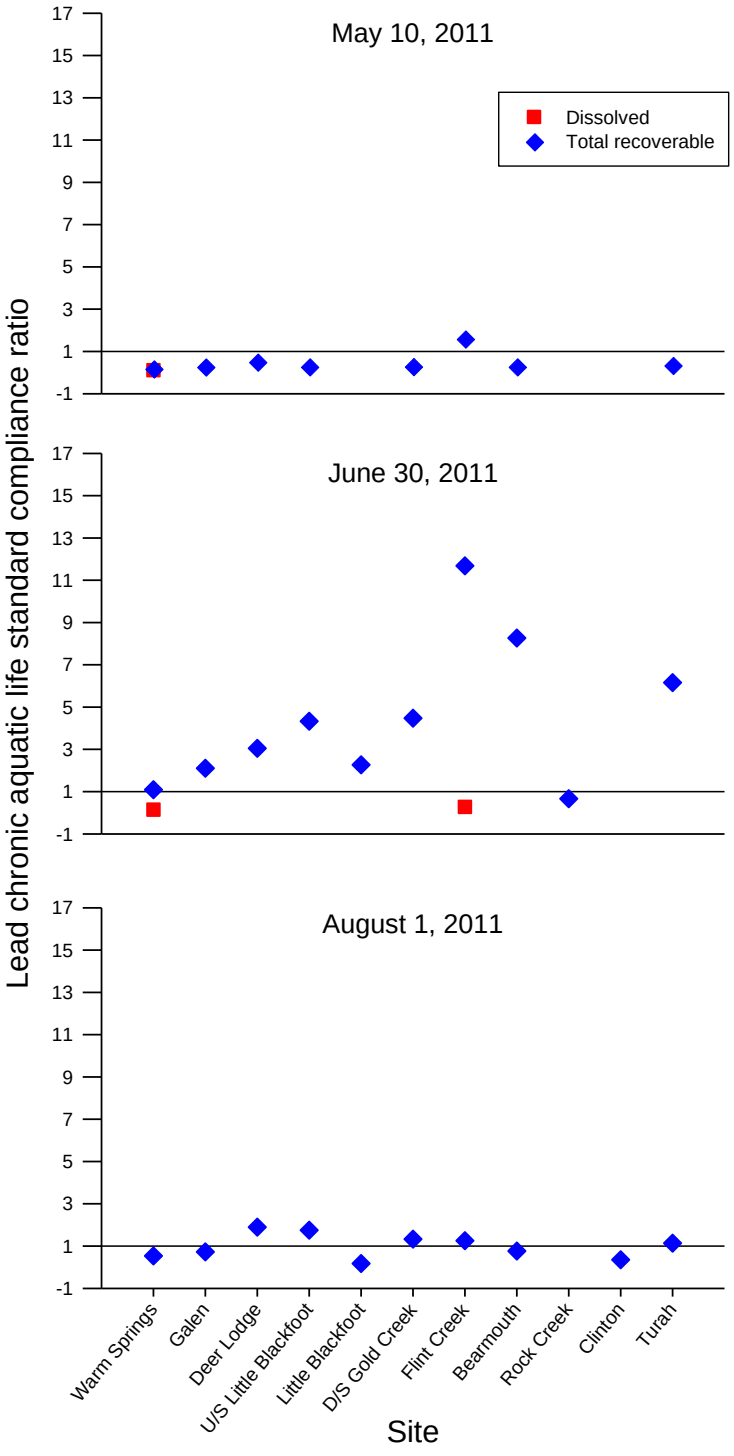

Figure 46. Lead compliance ratios at the cage sites in 2011 arranged from upstream to downstream. Compliance ratios were calculated by dividing lead concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate lead levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

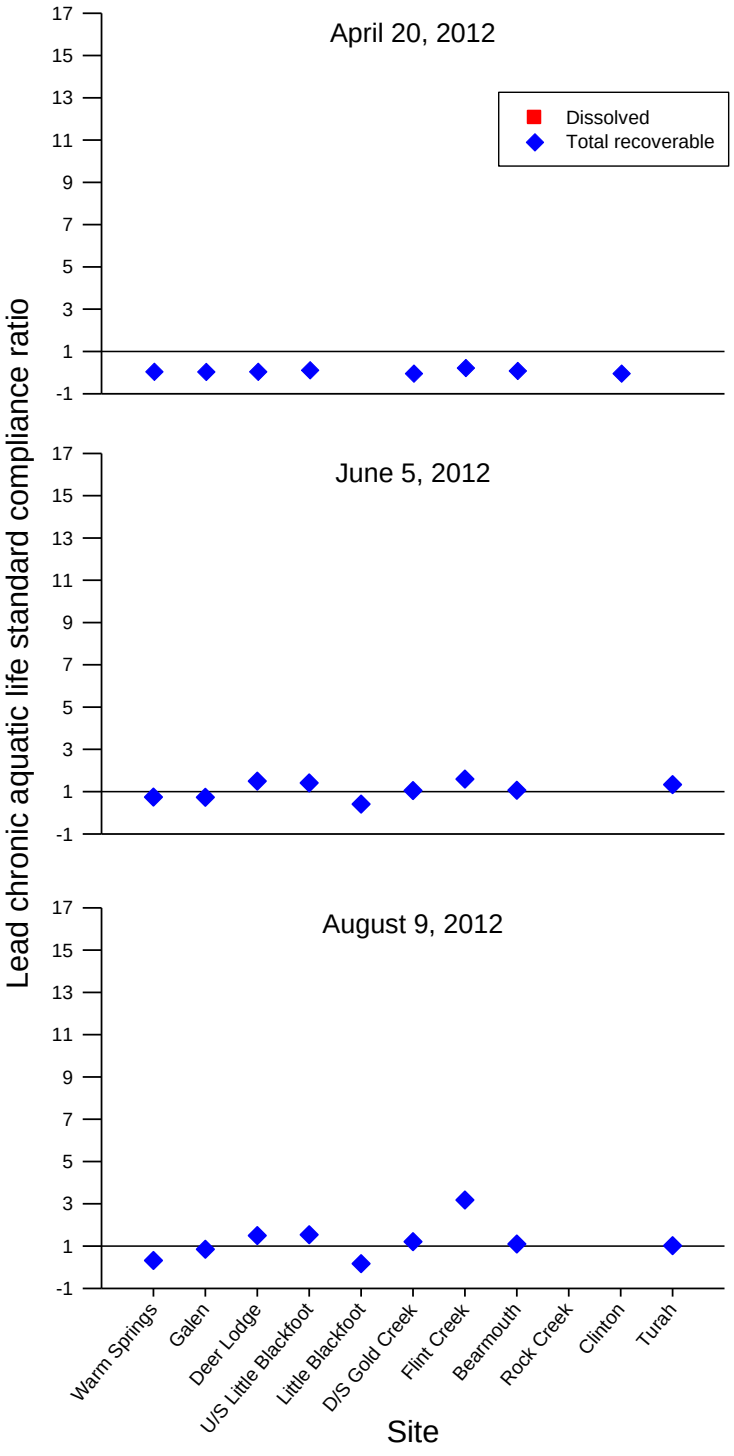

Figure 47. Lead compliance ratios at the cage sites in 2012 arranged from upstream to downstream. Compliance ratios were calculated by dividing lead concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate lead levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

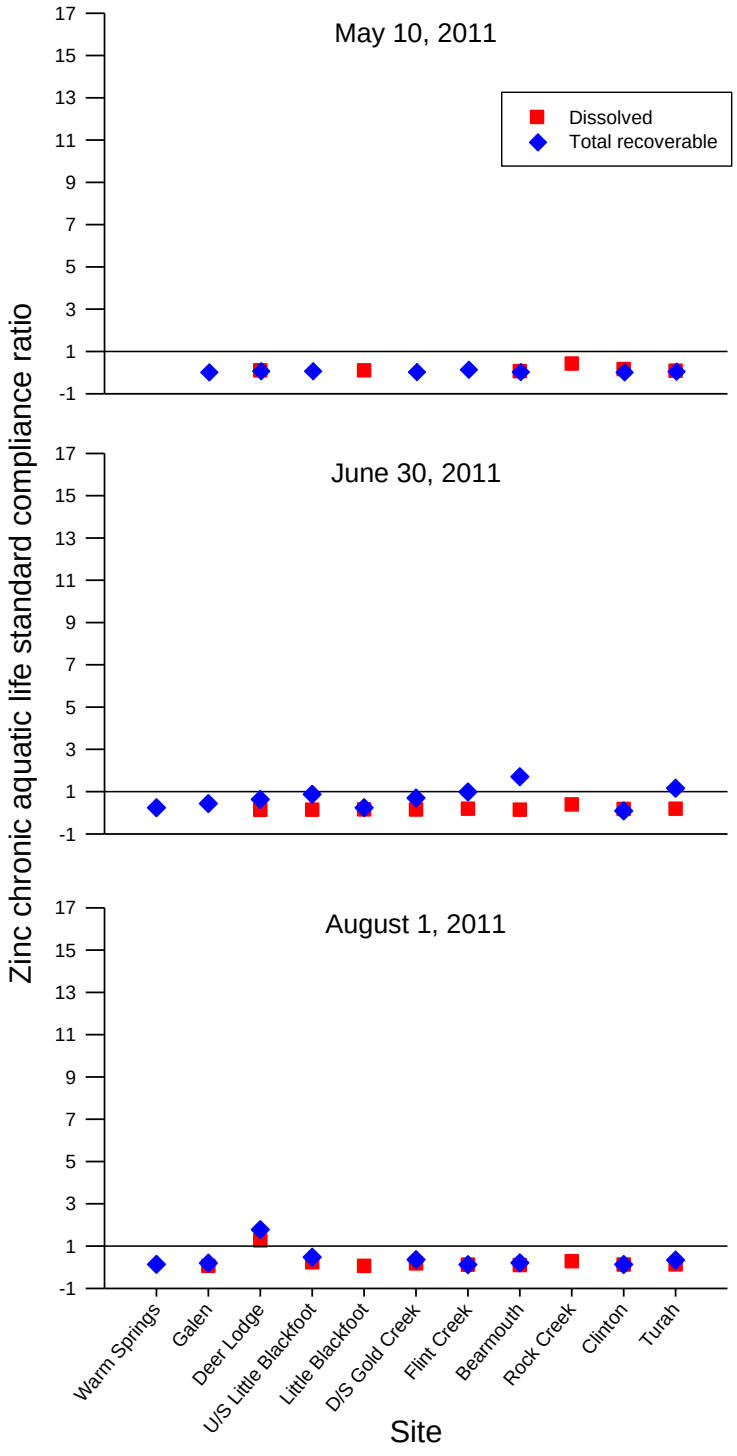

Figure 48. Zinc compliance ratios at the cage sites in 2011 arranged from upstream to downstream. Compliance ratios were calculated by dividing zinc concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate zinc levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

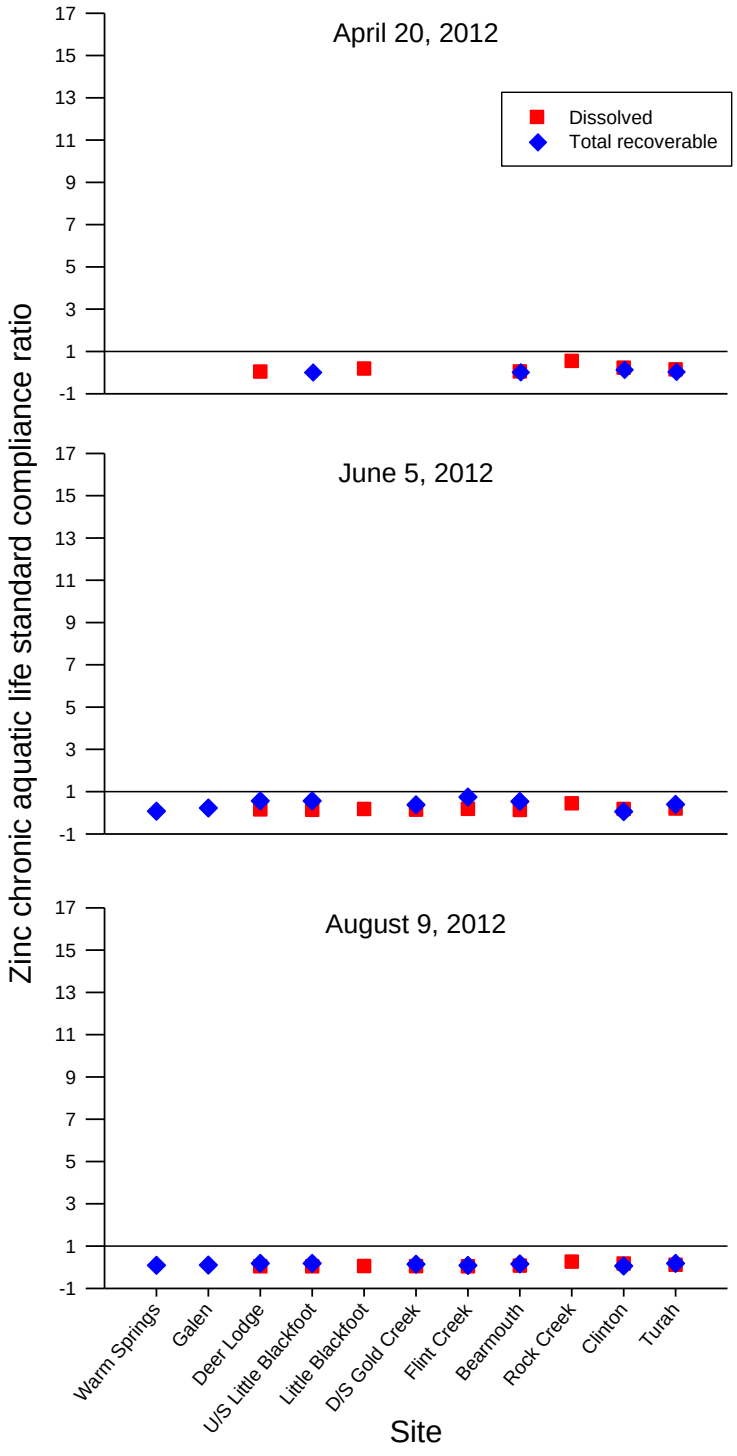

Figure 49. Zinc compliance ratios at the cage sites in 2012 arranged from upstream to downstream. Compliance ratios were calculated by dividing zinc concentrations by the calculated chronic aquatic life standards. Compliance ratio values <1 indicate zinc levels below the aquatic life standard (compliance) while values >1 indicate levels above the standard (non-compliance).

Water Quality

Water quality parameters recorded on continuously recording water quality probes upstream of Warm Springs Creek, at Galen, upstream of the Little Blackfoot River, upstream of Gold Creek, and at Bearmouth are outlined in the following sections.

pH

Elevated pH was observed at Warm Springs and Galen (Figure 50). Extended exposure to pH > 9 may be harmful to trout (Colt 1979). Mean daily values for pH exceeded 9 in late May and early June 2011 at Galen, and at Warm Springs in every month during the analysis period in 2012; pH at Galen in 2012 was consistently > 8.5. In contrast, mean daily pH at the remaining mainstem sites with probes deployed rarely exceeded 8.5 and generally varied from 7.5 to 8.5 (Figure 50) which is considered within the ranges suitable for trout (Colt 1979). For comparison, mean pH as measured with a handheld probe varied from 6.5 to 7.5 at control sites.

Oxidation Reduction Potential

Oxidation reduction potential (ORP) is a general indicator of water quality with positive measurements indicating aerobic or oxidative conditions and negative measurements indicating anaerobic or reductive conditions (Pearson and Black 2001; Apps 2012). Typically surface waters have an ORP from 100 to 200 mV, with more pristine, oxygenated water having an ORP of up to 400 mV; ORP < 200 mV indicates reduced levels of dissolved oxygen and/or oxidative potential (Apps 2012). Mean daily oxidation reduction potential was low throughout the study area in 2012, with most sites having ORP values centered on zero with many negative values (Figure 51). Two sites show deviation from this trend including upstream of the Little Blackfoot River which has a negative trend in June and achieves values < -500 mV, and Bearmouth which has a positive trend in June and achieves values > 900 mV (Figure 51). Neither of these deviations coincided with increased mortality.

Specific Conductivity

Specific conductivity is a measure of the ability of water to conduct electricity and can be used as a relative measure of water quality. Specific conductivity typically varies from 10 to 1000 µS/cm, but may exceed 1000 µS/cm in polluted waters or waters receiving large quantities of land runoff (Chapman 1996). Mean daily specific conductivity at all sites in both years was within normal ranges (Figure 52). However, specific conductivity increased sharply upstream of the Little Blackfoot River in June 2012. While this may reflect an actual increase in specific conductivity it is likely this is the result of a probe failure as none of the other sites exhibit the same increase (Figure 52).

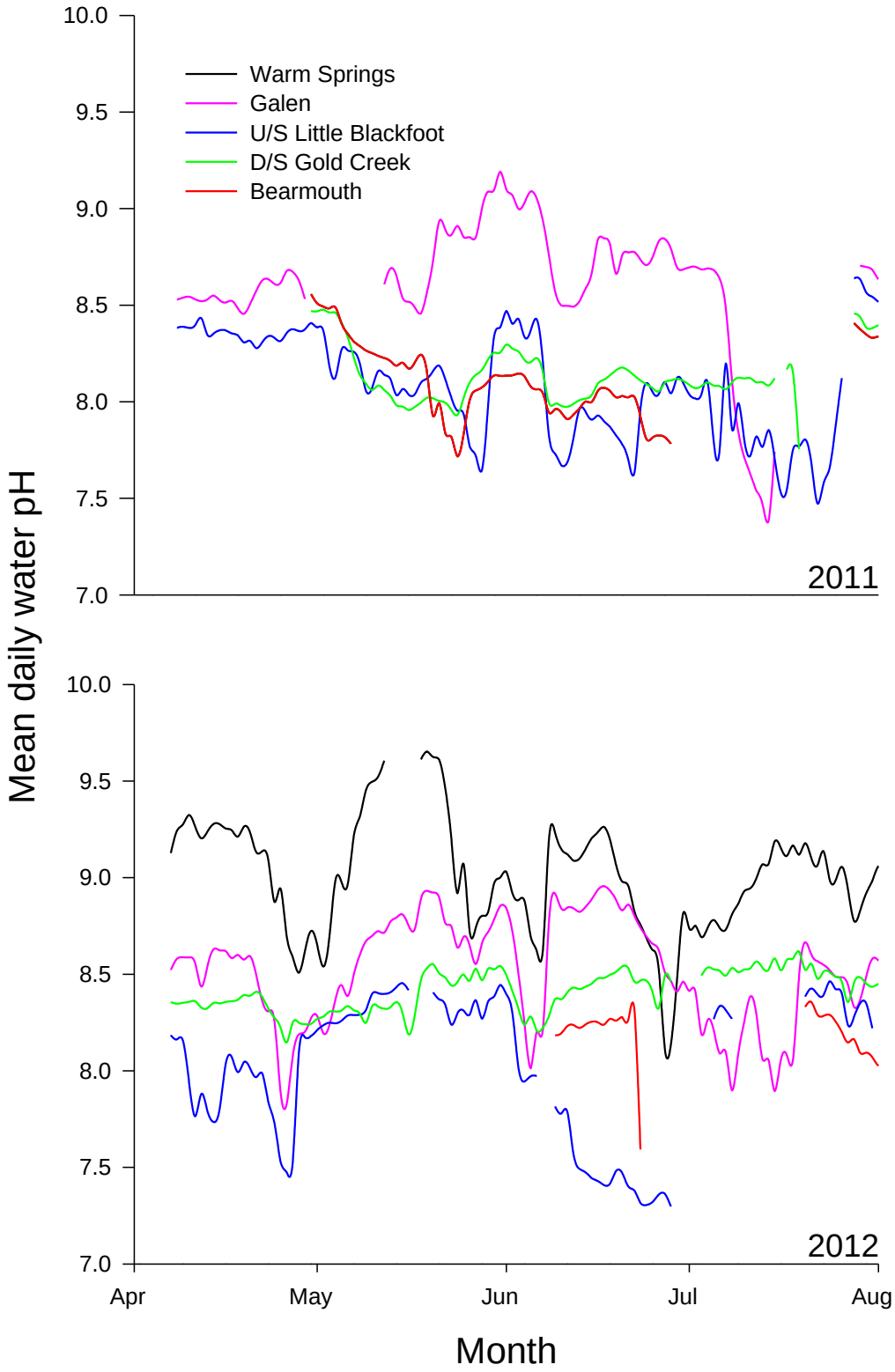

Figure 50. Mean daily water pH at sites with probes deployed in 2011 and 2012.

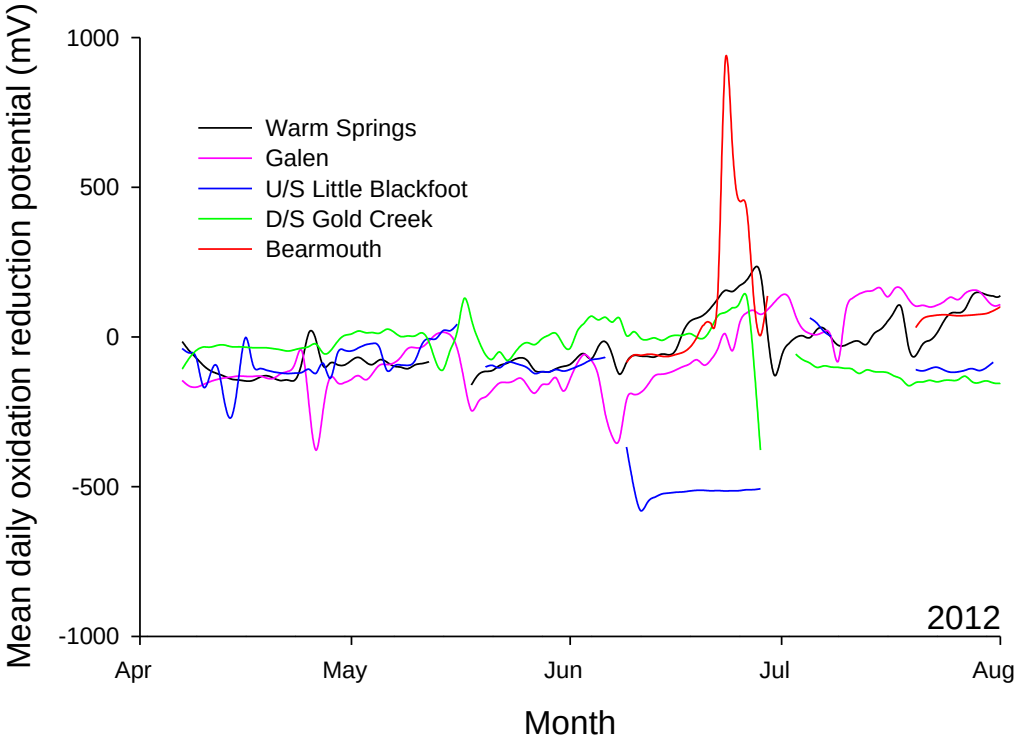

Figure 51. Mean daily oxidation reduction potential at sites with probes deployed in 2012.

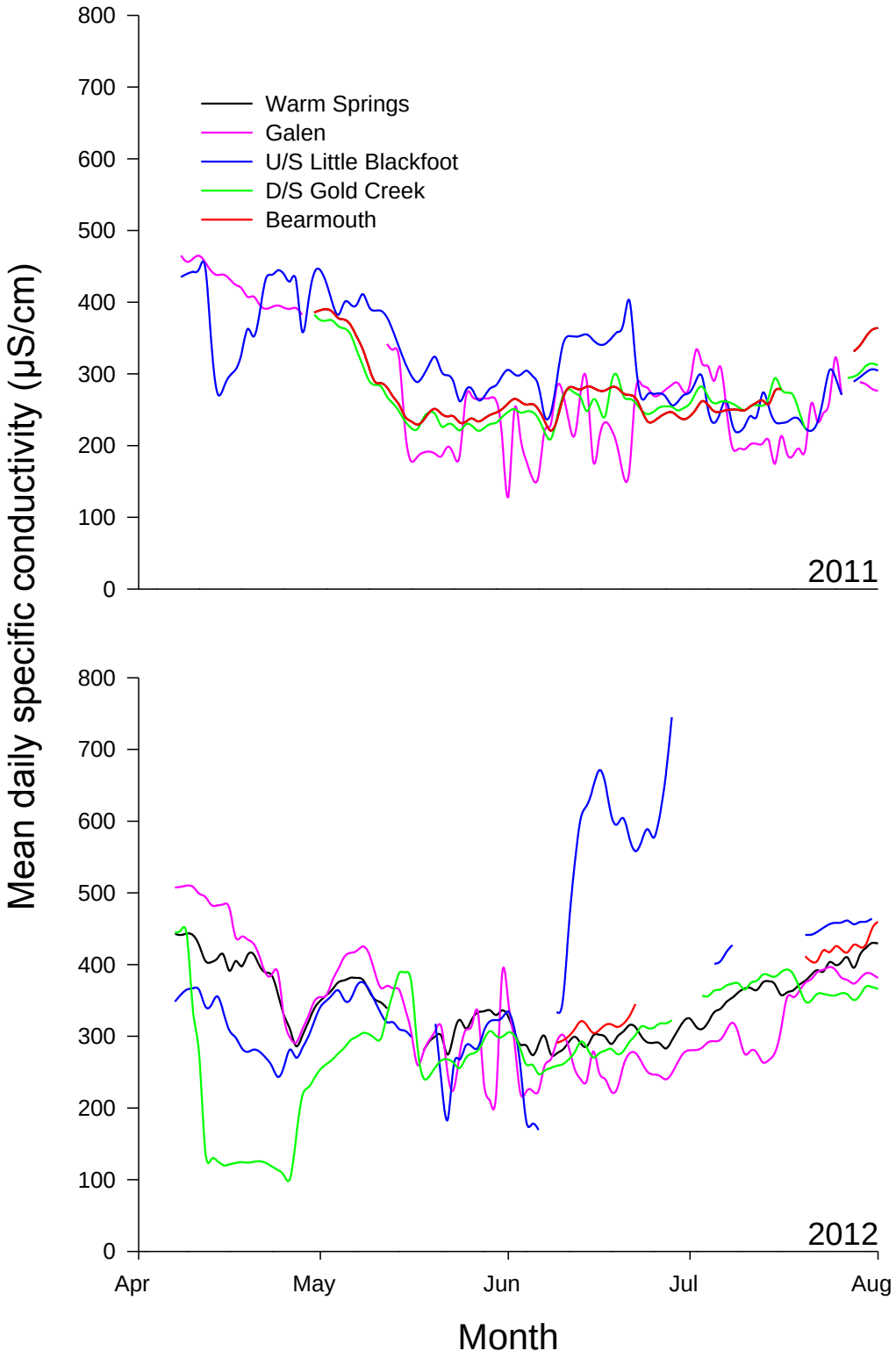

Figure 52. Mean daily specific conductivity at sites with probes deployed in 2011 and 2012.

Luminescent Dissolved Oxygen

The freshwater ALS one day minimum for dissolved oxygen for fish > 30 days post-hatch in the Clark Fork River is 4.0 mg/L (MTDEQ 2012b). Mean daily dissolved oxygen levels were below this threshold in early June 2011 at Galen, and twice in April 2012 upstream of the Little Blackfoot River. Mean daily dissolved oxygen levels also approached this threshold in early June 2012 at Warm Springs (Figure 53). Reduced dissolved oxygen levels at Galen in 2011 and upstream of the Little Blackfoot River in 2012 likely represent actual oxygen depression at those sites rather than probe failures as the oxygen levels rebound after dropping whereas probe failure results in dropping oxygen levels that do not rebound. The drop in oxygen at Warm Springs in 2012 rebounded after probe calibration (Figure 53) making it difficult to ascertain if this was an actual depression or the result of probe failure. None of the depressed oxygen levels coincided with increased mortality in either year. The overall trend in mean daily dissolved oxygen levels was values > 10.0 mg/L at all sites at the beginning of each field season that gradually decreased to values of approximately 7 to 9 mg/L by the end of July each year (Figure 53).

Total Ammonia

Total ammonia ($NH_4 + NH_3$) was measured at Galen in 2011 and at Galen and Warm Springs in 2012. Average daily total ammonia concentrations were below 0.5 mg/L (Figure 54). The calculated acute or chronic ALS values for total ammonia were never exceeded in 2011 or 2012 during the periods for which complete data existed. Toxicity of total ammonia is dependent on other water parameters including water temperature and pH (Emerson et al. 1975; MTDEQ 2012b). The increased toxicity is due to the conversion of the generally inert form (NH_4) to the highly toxic form (NH_3) through the process of de-ionization which is intensified at pH > 8 (Barton 1996; Stickney 1991). Emerson et al. (1975) reported an increase in the percent of toxic NH_3 in the total ammonia makeup from <1% at 0 °C and pH of 7.0 to 72 % at 30 °C and a pH of 9.5. The exposure of trout to ammonia can be sublethal or acutely toxic (Molony 2001). Both high water temperature and pH > 9 were recorded at Galen and Warm Springs (see previous sections) likely indicating a synergistic effect of multiple water quality parameters on brown trout survival at those sites.

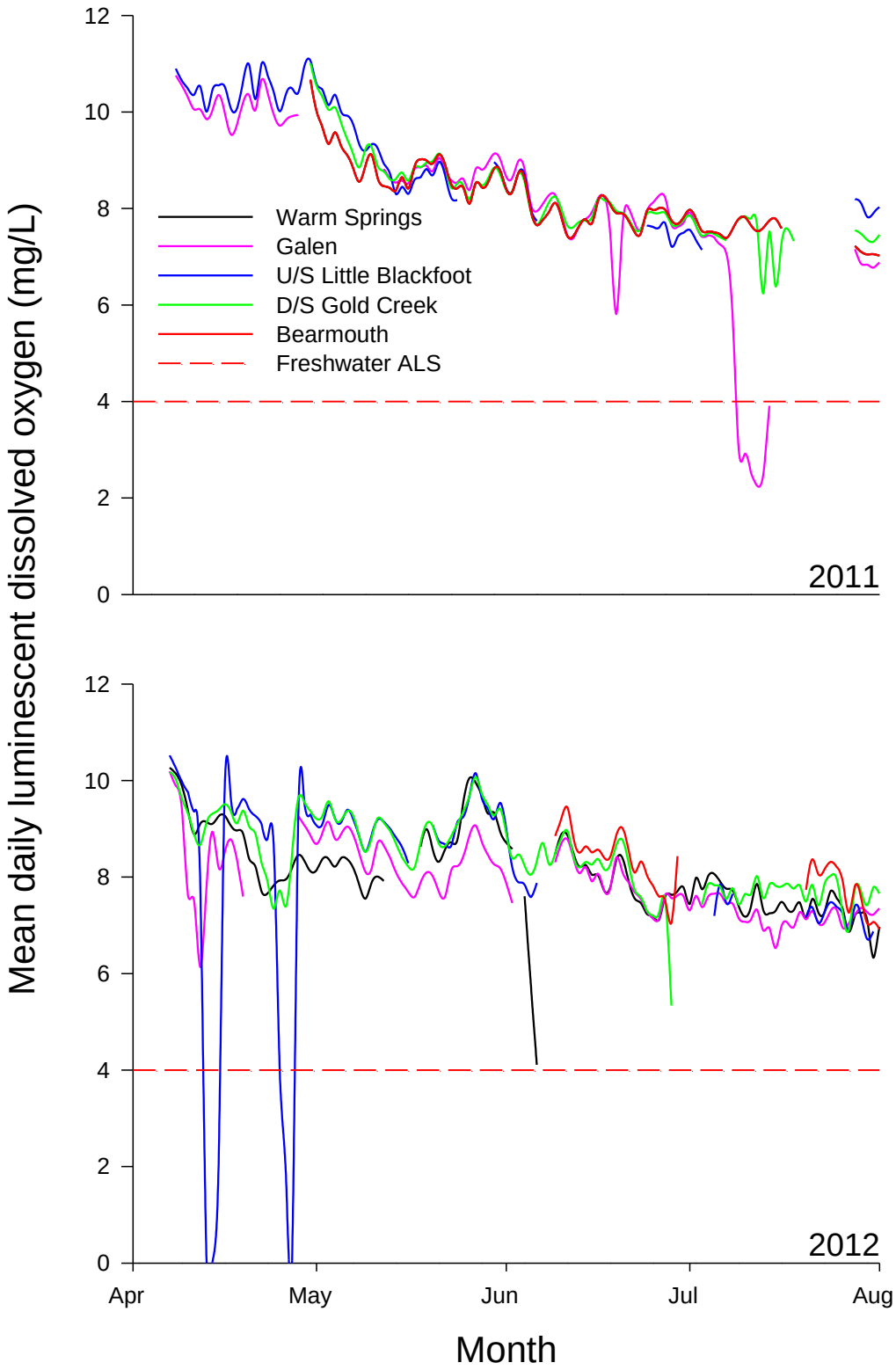

Figure 53. Mean daily luminescent dissolved oxygen at sites with probes deployed in 2011 and 2012. The red dashed horizontal lines denote the freshwater ALS one day minimum.

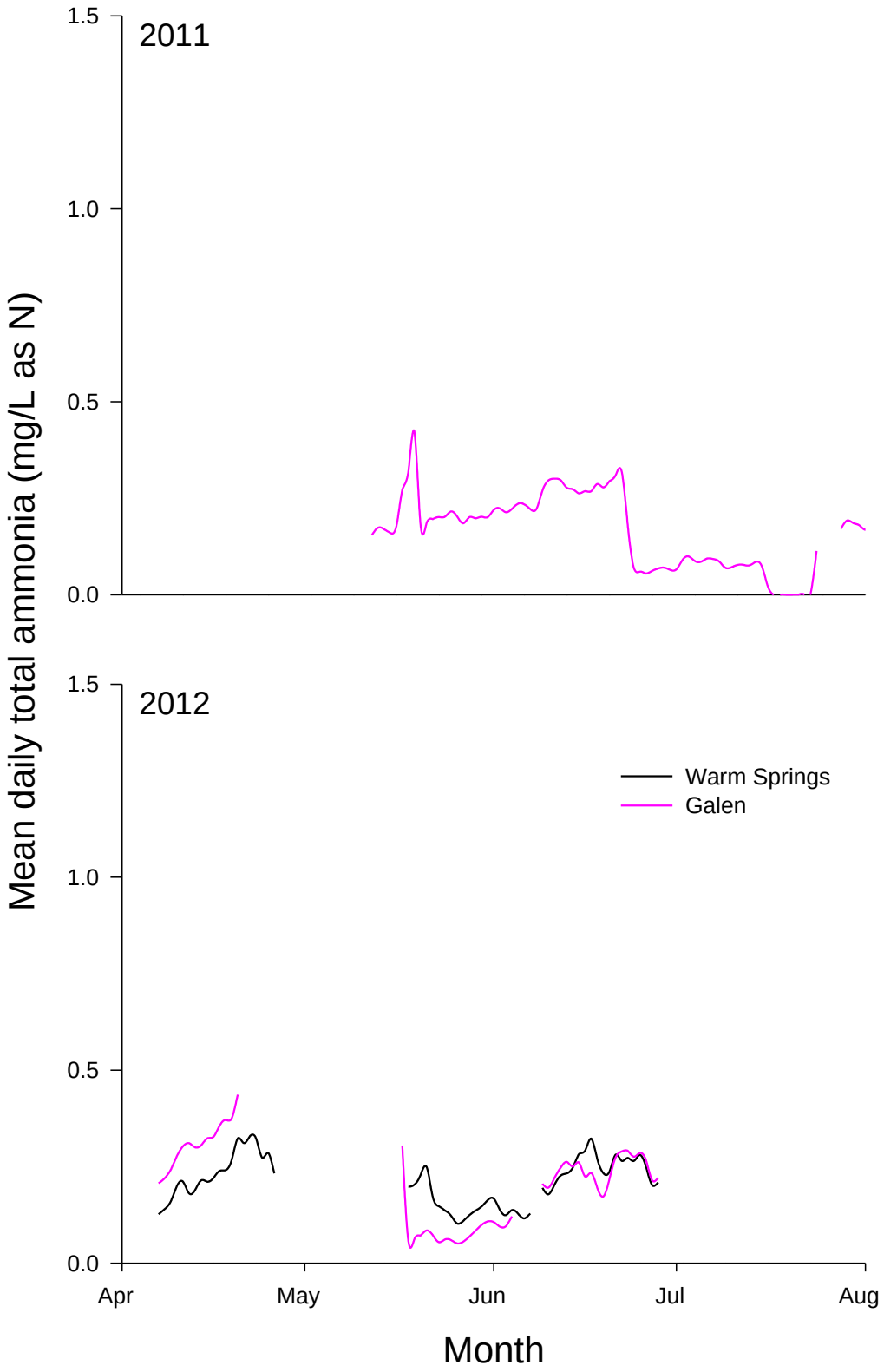

Figure 54. Mean daily total ammonia at sites with probes deployed in 2011 and 2012.

Discussion

The synergistic effect of elevated concentrations of metals, high water temperatures, and high pH induced high mortality of brown trout at some monitoring sites. The disparities seen in water quality from upstream to downstream could explain the spatial distribution of brown trout mortality, and alludes to the importance of site specific field studies such as this to determine the effect of mining contamination on trout populations in the upper Clark Fork River. This study further documented impairment of trout habitat in the upper Clark Fork River. Many of the detrimental environmental conditions observed are more likely to result in sub-lethal effects than in direct mortality (Blazer et al. 1987; Molony 2001) and these conditions often interact synergistically or cumulatively to influence fish growth, survival, and ultimately populations (Driedzic and Hochachka 1978; Hellawell 1986; Boyd and Tucker 1998; Molony 2001; Kiser et al. 2010).

The majority of mortality observed during the study was on the descending limb of the hydrograph as water temperatures approached or exceeded the upper critical temperature threshold for brown trout of 19.0 °C (Elliot 1994). However, when high water temperatures were observed without elevated metals concentrations or high pH, mortality was not elevated. For example, water temperatures were elevated in the control tributaries but overall metals concentrations were lower than mainstem sites and pH was within normal ranges. We generally observed minimal mortality in these streams. Water temperatures in the Clark Fork River exceeded the upper incipient lethal temperature for brown trout of 24.7 °C (Elliot 1994) at only two sites, Warm Springs and upstream of the Little Blackfoot River in 2012; however water temperatures were elevated in late summer and approached upper incipient lethal values at numerous sites. In addition to direct mortality, elevated water temperatures can influence feeding and growth of brown trout which can result in decreases in condition (Elliot 1994; Elliot and Hurley 2001) and increased susceptibility to other environmental stressors and diseases which can ultimately affect populations (Wahli et al. 2002; Hari et al. 2006; Jonsson and Jonsson 2009). High water temperature can also make fish more susceptible to metals exposure. As water temperature increases so does respiration, resulting in increased metals uptake by fish (Sorensen 1991).

Brown trout from cages in the Clark Fork River drainage showed evidence of cellular changes that occur due to chronic exposure to mining contaminants. The effects of chronic exposure to mining contaminants on the cellular structure of trout have been well documented in previous studies. These same cellular changes, including moderately severe necrosis of hepatocytes, increased numbers of macrophage aggregates, and swelling of gill epithelium were seen in cutthroat trout exposed to metals at elevated concentrations (Farag et al. 1999). These types of cellular changes attributed to elevated metals exposure would likely reduce growth and survival of fish in the wild and have an effect at the population level (Farag et al. 1999). Woodward et al. (1995) observed degeneration of hepatocytes in rainbow trout fed a diet contaminated with metals. Similar results were seen in histologies of the livers of bull trout subjected to mining contaminants in Idaho (Kiser et al. 2010). In that study, waterborne metals concentrations were below acute and chronic thresholds while those in invertebrates and sediments were elevated. Histologies of bull trout livers revealed degeneration and necrosis of hepatocytes indicating contaminant exposure (Kiser et al. 2010). These cellular changes were also seen in the livers of brown trout from cages in the Clark Fork River during this study. Kiser et al. (2010) concluded that the stress associated with dynamic natural environments likely made

trout more susceptible to exposure to mining contaminants. Additionally, it was concluded that exposure to metals likely decreased the ability of young salmonids to compete for food, cover, and elude predators, as well as compromised the ability to cope with environmental stressors such as thermal, oxygen, and water chemistry regimes which could lead to effects on populations (Kiser et al. 2010).

Tissue metals burdens have been shown to compromise trout health (Woodward et al. 1995a; Farag et al. 1999, 2003), and tissue metals burdens may be correlated to histopathological effects (Hansen et al. 2004). Results of tissue metals burden analyses indicate effects from copper and zinc in the Clark Fork River; the average tissue metals burdens for the remaining metals tested for were below previously identified effect thresholds. Average whole body arsenic concentrations in brown trout held in cages in the Clark Fork River (0.95-2.60 µg/g) were lower than the level previously reported to cause decreased growth in juvenile rainbow trout, 6.8 µg/g (McGeachy and Dixon 1990). A previous study has shown no effect on growth in juvenile rainbow trout at tissue cadmium burdens of 4.64 µg/g (Hollis et al. 2001). Cadmium burdens were detected at all mainstem sites in the Clark Fork River as well as Rock Creek and were well below the threshold identified above with average burdens varying from 0.26-0.72 µg/g. Average whole body lead burdens at cage sites varied from 0.27-3.89 µg/g, well below the threshold of 20.1 µg/g identified as causing an effect on growth in Eastern brook trout (Holcombe et al. 1976). One study that identified whole body selenium burden thresholds for salmonids utilized rainbow trout (Hilton and Hodson 1983). That study identified an effect level of 4.00 µg/g for whole body tissue selenium burdens, well above the average values seen in brown trout held in cages in the Clark Fork River drainage which were 0.83-1.77 µg/g. Whole body copper burdens were found to have an effect on rainbow trout at 8.57 µg/g after exposure for 60 days (Marr et al. 1996). In 2011, whole body copper burdens exceeded this value at all mainstem sites and all control sites excluding the spring channel at Clinton; this value was exceeded at all mainstem sites as well as the Little Blackfoot River in 2012. Difficulty in respiration, decreased feeding, and decreased growth were observed in rainbow trout with average whole body zinc burdens varying from 105.09 to 178.66 µg/g after experimental exposure (Gundogdu and Erdem 2008). The lower value in the range for whole body zinc burden from that study was surpassed at least once at all sites in both years while the upper value in that range was surpassed at all mainstem sites and control sites excluding Rock Creek in 2011, and at all mainstem sites and control sites excluding the spring channel at Clinton in 2012.

The highest mortality rates did not occur at sites with the highest water temperatures, waterborne metals concentrations, or tissue metals burdens, but rather at sites exhibiting a combination of these factors or others such as increased pH. This is likely due to a cumulative effect of environmental stressors (Kiser et al. 2010). For example, chronic freshwater ALS values for cadmium, copper, lead, and zinc were exceeded in water samples collected during the study, indicating that waterborne metals influenced mortality in fish cages. However, metals concentrations were highest on the ascending limb and peak of the hydrograph and the majority of mortalities were on the descending limb as discharges achieved or approached base flow. This likely indicates a chronic effect of waterborne metals that was exacerbated by environmental conditions such as elevated water temperature and pH, the cumulative effect of which resulted in increased mortalities. It should be kept in mind that fish placed in these cages came from a hatchery environment with no past exposure to metals. Thus, these fish likely required longer exposure to metals in the Clark Fork River to cause mortality than wild fish that are rearing or inhabiting the Clark Fork River. Mayfield (2013) found significant mortality in

adult brown trout implanted with radio transmitters in the Clark Fork River. The high mortality observed was believed to be due to long term chronic exposure to metals endured by these wild adult fish.

Values for pH at Warm Springs and Galen often exceeded those tolerable to trout (Colt 1979). However, in addition to direct adverse effects, pH may influence toxicity of metals (Couture and Pyle 2012). Studies have shown that cadmium, copper, and zinc are more toxic to steelhead trout (*Salmo gairdneri*) at high pH values (Cusimano et al. 1986). In addition to influencing the toxicity of metals, high pH results in a higher concentration of toxic NH_3 in the total ammonia makeup (Emerson 1975). Average daily pH was high at Warm Springs and Galen and generally decreased at sites downstream of those. This is due to the addition of lime to the settling ponds upstream of these sites that is an attempt to decrease water acidity and reduce metals contamination leaving Warm Springs Ponds. An unintended consequence to the addition of lime to the settling ponds is likely an increase in metals toxicity, that when combined with other stressors such as high water temperature, results in increased mortality at the upstream sites. Additionally, low dissolved oxygen levels were observed at Galen and potentially at Warm Springs. This could indicate a synergistic effect of pH, metals, high water temperature and periods of low dissolved oxygen, and explains why mortality was higher at Warm Springs and Galen than directly downstream at Deer Lodge which had similar tissue metals burdens.

Our results indicate that mortality was statistically significantly higher than expected at Warm Springs, Galen, and Turah. Mortality at Turah in 2011 was likely the result of the cumulative effect of many environmental stressors including increased zinc concentrations and high water temperatures, although further work is necessary to better understand the conditions causing the mortality observed at Turah. The cumulative effect of environmental stressors such as unsuitable pH, dissolved oxygen, ammonia, and water temperature in addition to metals exposure likely explain the high mortality seen at Warm Springs and Galen and could potentially indicate a point source of poor water quality. Water quality appears to improve downstream of these sites, potentially due to inflow of clean water from tributaries, with lower than expected mortality observed downstream of Gold Creek and at Bearmouth.

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