

Upper Clark Fork River Fisheries Monitoring Study: 2014 Annual Report



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Cover Photograph: Fish cages at the Flint Creek tributary site.

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Introduction

Metal mining and milling operations began in Silver Bow Creek and the Upper Clark Fork River (UCFR) Basin as early as the 1860s. These operations expanded as the focus of mining shifted from gold to copper in the 1880s. Over the next century, an estimated 100 million tons of copper mine waste were deposited in the UCFR and the adjacent floodplain (Andrews 1987). Waste products from these mining operations contain high concentrations of metals that are known to be hazardous to fish (Wood 2012). These metals, especially copper, have been linked to increased mortality of adult and juvenile trout in the UCFR (Mayfield 2013, Richards et al. 2013, Schreck et al. 2011).

Metals such as copper and zinc have been shown to enter fish tissues through multiple pathways including diet and the uptake of water through the gills (Marr et al. 1995a, 1995b; Woodward et al. 1995a). Concentrations of these substances in fish tissues are a function of ambient metal concentration and duration of exposure to contaminated water (Marr et al. 1996; Gundogdu and Erdem 2008). Copper is transferred from the water into fish tissue through sodium (Na^+) and copper-specific uptake mechanisms (Wood 2012). Water-borne metals not only accumulate metals in fish tissue, but also can directly damage gill epithelium and inhibit olfaction (Wood 2012). Aquatic invertebrates are a large part of trout diets, and contaminants within these diet items are integrated into fish tissue when consumed. Several studies have demonstrated metal accumulation in fishes fed invertebrates from the UCFR (Farag et al. 1994; Woodward et al. 1995a; Louma et al. 2008). Aquatic invertebrates typically represent the largest source by which copper enters fish in the Clark Fork River. Regardless of the pathway into fish, metal exposure causes a variety of negative effects. Potential effects include cell damage (Farag et al. 1994; Woodward et al. 1995a), reduced growth (Marr et al. 1996), behavioral changes (Hansen et al. 1999; Woodward et al. 1995b), and mortality (Farag et al. 2003).

In addition to heavy metal contamination, high water temperatures are often cited as a factor that negatively affects fish populations in the UCFR Basin. Elevated water temperatures can cause stress and can worsen effects of other stressors and diseases (Wahli et al. 2002; Hari et al. 2006; Jonsson and Jonsson 2009). High water temperatures also increase susceptibility to metals exposure through increased respiration (Sorensen 1991). The upper thermal limit for Brown Trout is 19.0°C , above which growth rate approaches zero (Elliot 1994). During the summer months, temperatures routinely exceed 19°C in some reaches of the UCFR. For example, water temperatures in the Clark Fork River near Deer Lodge exceeded 20°C for 31-56 days annually between 2001 and 2004 (RESPEC 2014). These high water temperatures may make trout in the UCFR more likely to succumb to toxic effects of heavy metal contamination.

Effects on trout of various concentrations of water borne heavy metals have been well studied (e.g., Dixon and Sprague 1981; Marr 1995a; Hansen et al. 2002). However, metal concentrations and toxicities vary depending on flows and water chemistry, which makes getting an adequate representation of river contamination through water sampling difficult. Thus, using whole body metal tissue burdens have become an important tool in monitoring contamination and ongoing remediation in the UCFR. Other than a study conducted by Montana Fish, Wildlife and Parks (FWP) in 2013 (Leon et al. 2014), no studies have related fish survival directly to the concentration of heavy metals within fish tissue. More understanding of the relationship between tissue burdens and fish survival is needed.

In 2014, FWP received funding from Montana Department of Environmental Quality (MTDEQ) to complete a caged fish study similar to those conducted by Leon et al. (2014) and Richards et al. (2013) and Schreck et al. (2012) as well as to collect fish population information

on the mainstem Clark Fork River. The goals of this project are to document current levels of metals contamination in the Upper Clark Fork River, assess potential impacts these metals have on fishes, and collect baseline fish population monitoring data for future assessment of remediation efforts.

Objectives

1. Document status and trends of fish populations in the upper Clark Fork River.
2. Identify water quality factors affecting the growth, condition, and mortality of young trout.
3. Determine survival rates of age 0 Brown Trout in the upper Clark Fork River at nine sites (from Warm Springs Ponds to Bearmouth, Montana), two tributary streams, and one handling control site.
4. Draw comparisons between tissue burdens of: 1) tributary and mainstem sites, 2) sites upstream and downstream of the construction area in Warm Springs, Montana, and 3) fish collected in different months of the year.
5. Explore possible trends between data collected in previous years and the current year.
6. Provide information to remediation project managers that will aid in the planning and implementation of cleanup efforts.

Methods

Population Monitoring

Mark-recapture population estimates were calculated for the following sample reaches of the Upper Clark Fork River in 2014: Bearmouth, Flint Creek Mouth, Phosphate, Williams-Tavener, Below Sager Lane, and pH Shack. Field methods were conducted in the same manner as Lindstrom (2011). During the month of April, fish were collected with the use of a 14 ft long aluminum drift boat with a mounted electrofishing unit and two front boom anodes and one netter. The system was powered by a 5,000-watt generator and current was modified with a Coffelt VVP-15 or Smith-Root VVP-15B rectifying unit. Estimates were made using two mark passes and two recapture passes of which recapture passes were completed roughly one week later. All captured trout were identified to species, weighed (g) and measured (mm), and given a small fin clip unique to the sampling section and day. Resulting data were analyzed by sample reach and species and summarized by the population estimate (if available; standardized to number of fish per mile), 95% confidence interval with upper and lower bounds, capture efficiencies, number of fish handled, mean length, length range, and percent of species composition. Population estimates were generated using the Chapman modification (Chapman 1951) of the Petersen method provided in Montana Fish, Wildlife and Park's Fisheries Information System database. Estimates and capture efficiencies were calculated for trout species that had a minimum of 4 marked fish that were recaptured (B. Liermann, Montana, Fish, Wildlife, and Parks, personal communication, 2014). Due to low numbers and/or poor capture efficiency of smaller size classes, only estimates for fish greater than 175 mm (~7 in) in length were reported.

Estimates from previous years (2008-2013) included in this report are part of the long-term dataset required for this study. A Chapman modification of the Petersen method, as described above, was used to generate estimates in the Fisheries Information System for data from 2011-2014, two sample reaches from 2010 (Bearmouth and Flint Creek Mouth), and two sample reaches from 2009 (Bearmouth and Flint Creek Mouth). Estimates from 2008, remaining sample reaches in 2009 (pH Shack, Below Sager Lane, Williams-Tavener, and Phosphate), and remaining sample reaches in 2010 (pH Shack, Below Sager Lane, Williams-Tavener, and Phosphate) were generated using a Chapman estimator for the Peterson method provided in Montana Fish, Wildlife and Park's Fisheries Analysis Plus (FA+) software package, and are presented here as originally reported in Lindstrom 2011. Both programs produce identical population estimates, but confidence intervals around the estimates are calculated differently, with FA+ assuming sample data is normally distributed and the Fisheries Information System assuming sample data is binomially distributed (see Ogle 2010 for details).

When sampling for these population estimates, only trout and char (members of *Salmo*, *Oncorhynchus*, and *Salvelinus* genera) are netted. Thus, other species present in the Clark Fork River are not captured, enumerated, weighed, or measured during population estimate sampling events. Because remediation in the Upper Clark Fork River has the potential to affect all fish species present, two reaches were sampled in which all fish were netted, weighed, and measured. These reaches were one mile long and were located upstream of the town of Deer Lodge ("Above Deer Lodge") and upstream from the Jens Road Bridge ("Jens"). One electrofishing pass was conducted at each sampling reach using methods similar to those listed above. Resulting data were analyzed by sample reach and species and summarized by catch per unit

Fish Cage Construction

Technical drawings of the 'The Artist' wooden box. The left drawing is a front view showing a 15-inch wide lid with a central latch and a 12-inch by 13 3/4-inch base. The right drawing is a top-down view of the lid, showing a 15-inch width and a 15-inch height, with a central 12-inch by 9-inch panel.

Study Sites

- 1) Pond 2 Outlet at Warm Springs, Montana (Pond 2)
- 2) Silver Bow Creek at Warm Springs, Montana (Silver Bow)
- 3) Warm Springs Creek near the mouth (Warm Springs)
- 4) Clark Fork River at Perkins Lane Bridge (Perkins Lane)
- 5) Clark Fork River at Galen Road Bridge (Galen)
- 6) Clark Fork River upstream of Racetrack Creek confluence (Racetrack)
- 7) Clark Fork River at Deer Lodge, Montana (Deer Lodge)
- 8) Clark Fork River upstream of the Little Blackfoot River (U/S Lil Black)
- 11) Clark Fork River near the Bearmouth FAS (Bearmouth)

Two control sites were located on tributaries:

- 9) Lower Little Blackfoot River (Lil Black)
- 10) Flint Creek (Flint)

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

- 12) Clinton, Montana (Spring)

The Clinton Spring handling control served as a reference to establish baseline mortality rates. The Clinton site was used to determine if handling during cage checks (e.g., cleaning and relocating) or stress from initial fish delivery to the cages negatively impacted survival, independent of water quality. All sites except Pond 2, Galen, Racetrack, and Spring were located near U.S. Geological Survey (USGS) gauging stations equipped to measure discharge four times per hour.

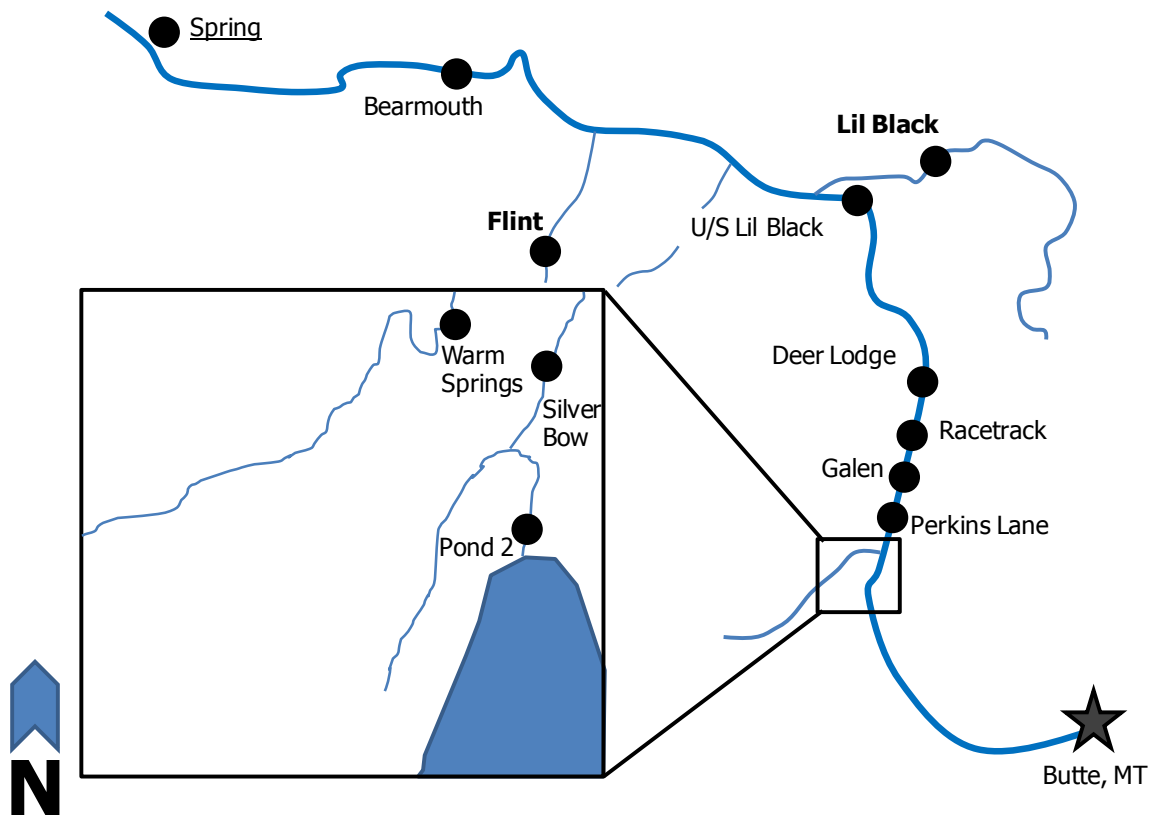


Figure 2. Distribution of the twelve study sites in the Upper Clark Fork River drainage. Tributary control sites are shown in bold and the handling control is underlined.

Cage Deployment

Within each site 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. 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. Temperature loggers (HOBO ® U22 Pro v2) were attached to the rebar securing the cages in the channel and the units were most often set 6-12 inches above the substrate. The loggers were programmed to take a measurement once every half hour.

Two cages served as treatment cages (i.e., one replicate) and the third held fish for replacement of individuals in the treatment cages and live fish collection. 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. However, high fish mortality during 2014 led to the third cage at most sites becoming empty of fish before the field season was completed. This required that fish from the treatment cages (cages one and two) be used for live fish collections and resulted in fewer than 25 fish in most treatment cages at most sites.

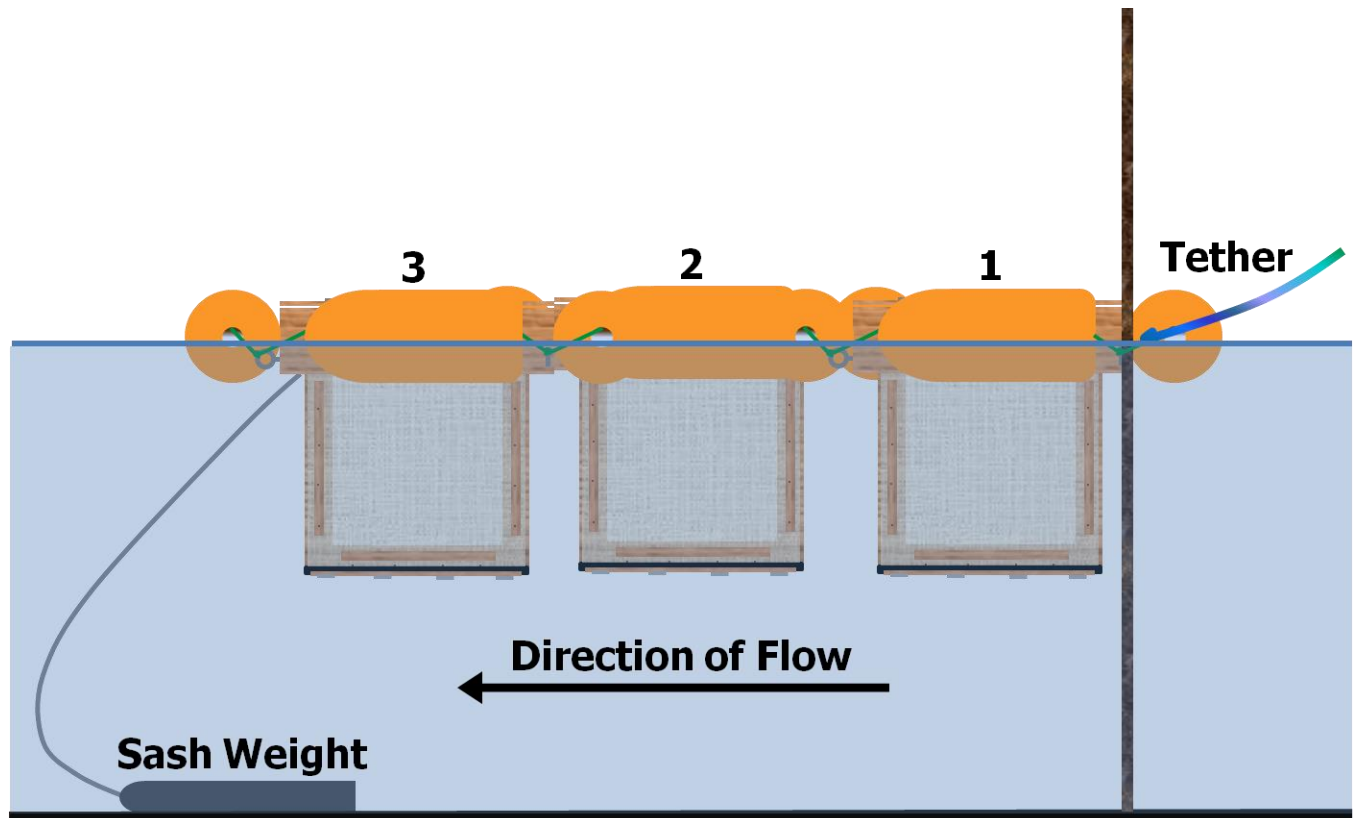


Figure 3. Representation of cage deployment (arrangement of cages differed by site, and cages often drifted together).

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, fingerling study specimens were obtained from a state hatchery. In late March approximately 900 fingerling Brown Trout were obtained from Big Springs Hatchery in Lewistown, Montana. The trout were transported from the hatchery via an aerated cooler.

At each site trout were anesthetized with clove oil, measured for total length (mm), weighed to the nearest 0.1 g and divided into one of the three cages. Lengths of fingerlings ranged from 56-95 mm (mean = 75 mm) and weights ranged from 1.9-9.8 g (mean = 4.1 g). Fingerlings were feed-trained on pellet feed prior to leaving the hatchery. Prior to being anesthetized, fish were acclimated to the water temperature at each site with the addition of onsite water. Water temperature in the coolers was 6.7 °C before stocking. Water temperatures at the first six sites stocked ranged from 5.0 °C to 5.6 °C.

Mortality Monitoring

Beginning the last week of March, trout mortality was monitored twice per week. At each visit the trout in each cage were fed one tablespoon of Bio Oregon BioClark's Starter #1 pellet feed (pellet size 0.6 mm). It should be noted that both the size and brand of feed was different in 2014 than previous years. For example, in the first three months of the 2013 study, trout were fed 1.0 mm sinking feed (Silver Cup Extruded Salmon). During the remaining months of 2013, trout were fed slightly larger No. 3 sinking feed (Silver Cup Crumbled Salmon/Trout).

Cages were repositioned to seams and eddies as needed to maintain water velocities near 0.75 ft/s around the cages. Velocities around the cages were measured periodically to ensure they were near to 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 cages and weighed and measured. In previous years, mortalities removed from the treatment cages (cages 1 and 2) were replaced with live individuals from the replacement cage (cage 3). However, the rapid depletion of fish caused by high mortality and live fish sampling meant that most sites ran out of replacement fish at some point during the 2014 study. As a result, most treatment cages could not be maintained at 25 fish. All mortalities were held in a freezer at the Region 2 FWP headquarters after collection.

As in previous years of the caged fish study, the only time period considered for survival analysis was after an acclimation period and before August. The acclimation period included mortalities that were thought to be due to moving fish from a controlled hatchery environment to cages in more variable stream environments. In previous years the acclimation period was considered the first week of the study. In 2014 the acclimation period was extended to two weeks (ending April 10) because mortality tended to be high at most sites up to this date. August mortalities are typically excluded because of significant mortality at the Clinton Spring control site during this month. Survival within a cage was expressed as the number of fish remaining in the cage on July 31 divided by the net number of fish placed in the cage up to that time. Survival can be expressed as:

$$\text{Survival} = (\text{Fish remaining}) / (\text{net number of fish added})$$

or

$$\text{Survival} = (\text{Fish remaining}) / (\text{Initial 50 fish} + \text{replacements} - \text{removals})$$

Numbers of fish remaining and added were combined for cages one and two at each site to yield an overall survival estimate for that site. Survival at each of the nine mainstem treatment sites were compared to survival at the tributary sites (Lil Black and Flint) with chi-square tests incorporating Yate's correction for continuity (Yates 1934). This test is identical to a test of two proportions where fish remaining are "hits" or "successes" and total fish added are "events". Numbers of fish remaining and fish added at Lil Black and Flint were averaged for analysis and these averages were used as the control to which survival at each treatment site was compared. Alpha was set as 0.05 for statistical analyses.

Growth and Condition

Lengths and weights of half (450) of the total number (900) of specimens placed in cages were taken prior to stocking the fish cages. Initial lengths did not differ significantly among sites in 2014 ($F_{5,444} = 1.1230$, $P = 0.3473$), so mean of all measured fish was used as the initial length to compare growth over the field season. At the completion of the field season a subsample of 30 fish (10 surviving fish randomly selected from cages 1, 2, and 3) were measured and weighed. If there were less than 30 surviving fish at the end of the field season all surviving fish at a site were sampled. Growth was calculated as the mean change in length at each site. Relative weight (Wr) was used as an index of conditions. Relative weight was calculated using the standard weight equation of Milewski and Brown (1994). Although Milewski and Brown (1994) developed their standard weight equation for Brown Trout > 140 mm, and fish in this caged fish study were all < 140 mm, Wr still provides a meaningful way to compare body condition between live and dead fish, between sites, and over time. Mean Wr for live and dead fish each month at each site were depicted graphically. Only fish from cages one and two were used for growth and condition calculations.

Because most sites were depleted of replacement (cage 3) fish by the end of the field season, cages one and two contained different numbers of fish by the end of the season at all sites except Deer Lodge. There was some concern that growth and condition would be dependent on the density of fish in the cages. All cages received the same amount of food, so it is possible that competition would result in less food available for each individual in the cages with more fish. To test for density dependent growth and condition, two general linear models were performed. Mean increase in length and mean Wr for each cage (cages one and two at each site), were considered response variables in separate models. For each of these models, fish remaining in the cages (an index of fish density) was the continuous predictor variable and site was used as a categorical predictor variable. The site variable was necessary to account for significantly different growth and condition between sites (see results).

Rates of feeding, digestion, absorption, excretion, and metabolism for fish are heavily dependent on water temperature (Elliot 1994; Ojanguren et al. 2001). As a result water temperature is a primary determinant of growth. Elliot et al. (1995) developed a model to quantify the effects of varying water temperatures on growth in weight of Brown Trout in a

controlled laboratory setting. This model predicts increased growth at water temperatures near the optimum temperature of 13.1 °C and slower growth as temperatures approach the lower (3.6 °C) and upper (19.5 °C) thermal limits for Brown Trout growth. Specifically, the Elliot et al. (1995) model predicts the final weight of a fish of a given initial weight after a given length of time at a given temperature. Mean weight of the 450 Brown Trout weighed prior to cage stocking (4.2 g) was used for the initial weight in the model. Mean daily temperatures recorded by temperature loggers mounted to the fish cages at each site were input into the model to predict daily growth. These daily growth increments were summed for the entire time fish were in the cages (March 27 to the time the fish was sampled), resulting in a predicted final weight of individual fish at each site. The observed mean weight of surviving live fish at each site was plotted against weights predicted by the temperature based model. Differences in observed weights from those predicted by the temperature model could be evidence of influences of factors other than temperature (i.e., food availability, heavy metal toxicity) on growth.

Tissue Metals Burdens

Three live fish were collected from each site the last week of the month April-July for tissue burden analysis. Three fish from each site were also collected upon the completion of the field season on September 2, 2014. Five fish from the hatchery were sacrificed prior to stocking fish cages in order to determine baseline tissue metals burdens. In addition to live fish, a subsample of fish that died during the 2014 season were collected for tissue burden analysis. However, preliminary analyses indicated that tissue burdens of the dead fish were abnormally, perhaps artificially high. A previous study conducted on an estuarine species (Mummichog, *Fundulus heteroclitus*) suggested that fish corpse may gain copper and zinc after death, thus limiting the research value of whole body metal concentrations from dead fish (Eisler and Gardener 1973). Due to these concerns, only tissue burden data from fish collected alive will be discussed in the remainder of this report.

Fish samples were submitted to the Montana Department of Health and Human Services Environmental Laboratory in Helena for determination of whole-fish metal concentrations. Fish 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 copper and zinc with inductively coupled plasma optical emission spectrometry (ICP-OES) using the U.S. Environmental Protection Agency (USEPA) Method 200.7 (USEPA 2001). All results were reported as µg/g dry weight.

Graphical comparisons were made between tissue metals burdens (copper and zinc) and each of the following variables: site, month, and site location (hatchery controls vs. tributary sites vs. mainstem sites, upstream construction vs. downstream construction.) For the purposes of these comparisons between tributary and mainstem sites, Clinton Spring was not included because it does not experience significant temperature and flow fluctuations typical of the flowing water sites. For each comparison, 95% confidence intervals were displayed and tissue burden values were considered statistically different if their confidence intervals did not overlap. Statistical differences in tissue burdens between sites were also assessed using an analysis of variance (ANOVA). Pairwise *T* tests (with Bonferroni-adjusted *P* values to account for multiple

comparisons) were then conducted to identify pairs of sites with statistically different tissue burdens.

To evaluate possible temporal trends in copper and zinc tissue burdens, annual mean tissue burdens at each site were compared. Mean tissue burdens from caged fish studies conducted 2011-2014 (Schreck et al. 2012, Richards et al. 2013, Leon et al. 2014, this study) were compared graphically by site. Tissue samples from individual fish were combined into composite samples in 2011 and 2012 to reduce costs, which did not allow for measures of variation such as confidence intervals or ANOVA. Tissue burdens in 2013 and 2014 were analyzed for individual fish, so confidence intervals could be generated for these years. Average annual survival at each site used in caged fish studies 2011-2014 were also compared to evaluate potential temporal trends in fish survival. Annual survival comparisons could also reveal sites that have consistently low fish survival due to high metal tissue burdens, high water temperatures, or some combination of these factors.

Water Contaminants

Montana FWP collected water samples at each of the twelve sites on 4/21/14 and 7/28/14. An additional collection was done on 8/14/14 at the eight sites upstream of confluence of the Little Blackfoot River. One sample was collected at the U/S Lil Black site on 7/21/14 which was four days after a large mortality event at that site. Samples were collected using the techniques outlined by the MTDEQ Field Procedures Manual for Water Quality Assessment Monitoring (MTDEQ 2012a). 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 (NH₃-N). RESPEC Consulting collected additional water data under a contract for MTDEQ during the quarterly monitoring of the Clark Fork River Operating Unit (CFROU).

Performance standards have been identified for contaminants in the upper Clark Fork River (USEPA 2004) and are defined as the more stringent of the freshwater aquatic life standards (ALS) published by the MTDEQ (2012b). Because the chronic ALS is the most stringent and since 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 (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:

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

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. Compliance ratio values <1 indicate contaminant levels below the chronic ALS, while values >1 indicate contaminant levels above the chronic ALS.

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. It is important to note that not all estimates presented in this report have been reviewed and approved for publication. No station existed at the Pond 2, Galen, Racetrack, and Spring sites. Maximum daily water temperatures were obtained for each site with water temperature data loggers mounted to fish cages described above.

Water Quality

Water quality parameters were recorded in the Clark Fork River at five sites in 2014 with continuously recording multiparameter water quality probes (Hydrolab ® MS5). Cross referencing of Hydrolab data was achieved by sampling intermittently at the nine mainstem and three control sites using a handheld multiprobe (YSI ® 556 MPS). Hydrolab and YSI probes were calibrated periodically during the field season. Probes were deployed at Pond 2, Silver Bow, Galen, Racetrack, and U/S Lil Black in 2014. Water quality parameters recorded include temperature, pH, specific conductivity, and luminescent dissolved oxygen (LDO) at all sites, with the addition of total ammonia ($\text{NH}_4 + \text{NH}_3$) at Pond 2 and Silver Bow. 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 ammonia ALS values were calculated as:

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

and the chronic ALS were calculated as:

$$\text{Chronic} = [0.0577/(1+10^{7.688-\text{pH}}) + 2.487/(1+10^{\text{pH}4-7.688})] \times \text{MIN}(2.85, 1.45 \times 10^{0.028 \times (25-T)})$$

where T = temperature (°C). Ammonia and ALS value were then plotted graphically to determine if and when exceedance events occurred.

Results

Trout Population Estimates

Figure 4 displays all Brown Trout population estimates by sample reach from 2008-2014, including population estimates reported in Lindstrom (2011). Population estimates from 2008-2010 for the Below Sager Lane, Williams-Tavener, and Phosphate electrofishing sections from Lindstrom (2011) are included in an appendix to this report (Appendix I). The pH shack Section had the highest Brown Trout population estimate in 2014 with 1,177 fish/mile. Conversely, the

Bearmouth Section had the lowest Brown Trout population estimate, with 57 fish/mile in 2014. Flint Creek Mouth, Below Sager Lane, Williams-Tavener, and Phosphate sections had 2014 Brown Trout population estimates of 199, 594, 618, and 596 fish/mile respectively.

Across all years that Brown Trout population estimates were available, Bearmouth consistently had the lowest numbers, while pH Shack had the highest numbers (Figure 5). Estimates at Flint Creek Mouth tended to be relatively low while Phosphate, Williams-Tavener, and Below Sager Lane tended to have intermediate Brown Trout numbers. At most sections, Rainbow or Cutthroat trout recaptures were too low to generate population estimates. Generally speaking, the Bearmouth section tends to have higher numbers of Cutthroat and Rainbow trout than other reaches (Tables 1-6).

At the two sampling sections where all fish species were netted, a total of eight species were captured including Brown Trout, Longnose Dace (*Rhinichthys cataractae*), Longnose Sucker (*Catostomus catostomus*), Largescale Sucker (*Catostomus macrocheilus*), Mountain Whitefish (*Prosopium williamsoni*), Redside Shiner (*Richardsonius balteatus*), Slimy Sculpin (*Cottus cognatus*), and Westslope Cutthroat Trout (Tables 7-8). Mountain Whitefish were the most commonly captured species at both sections. Brown Trout were the second most common species found at the Jens section whereas Largescale Sucker were the second most common species captured at the Above Deer Lodge section.

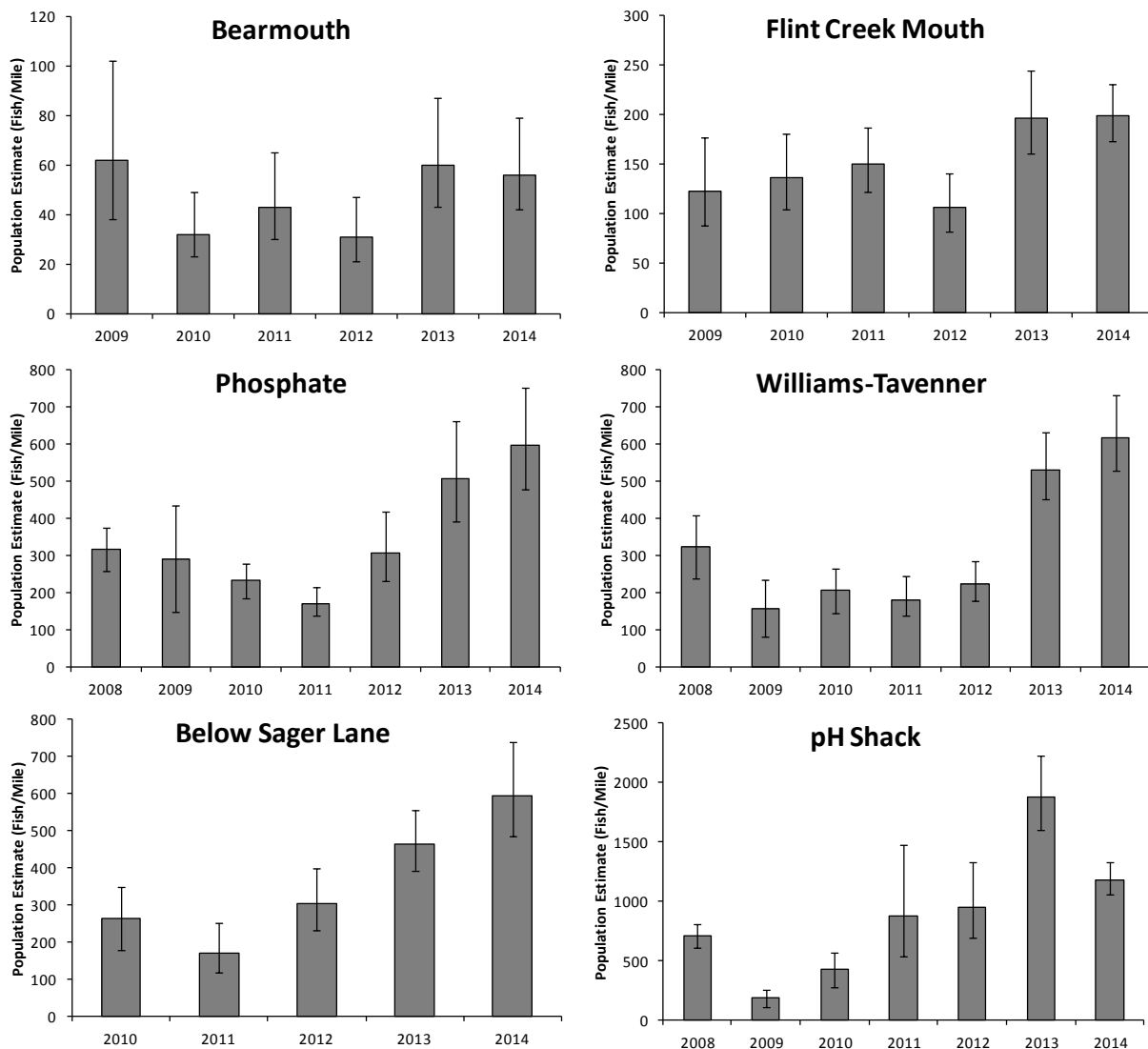


Figure 4. Clark Fork River Brown Trout population estimates from 2008-2014 by sample reach. Sample reaches are displayed downstream to upstream, left to right then top to bottom. Please note that x-axis and y-axis values are not the same for every sample reach.

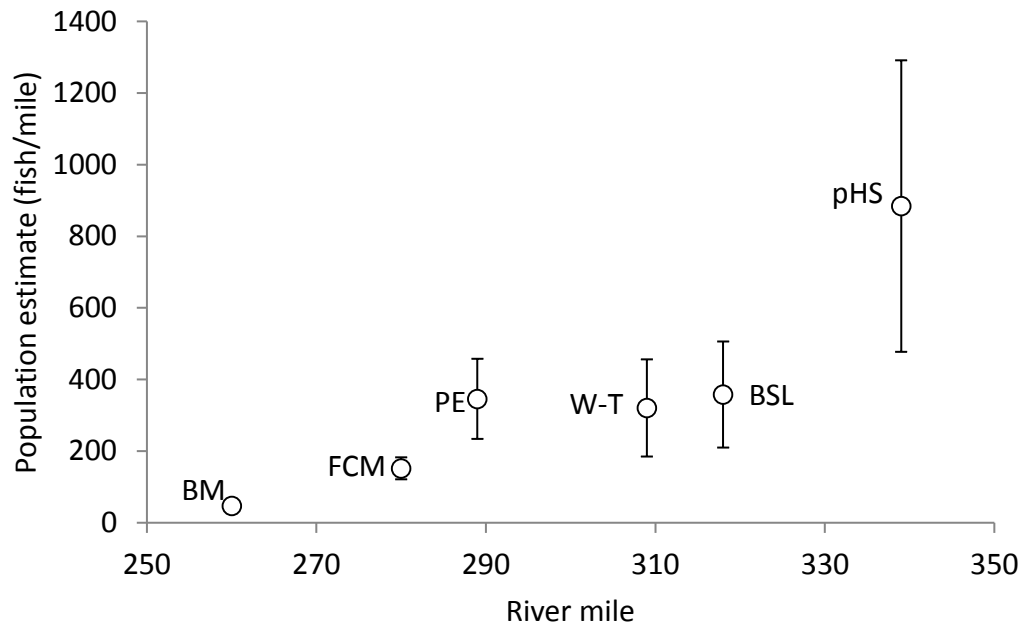


Figure 5. Average Brown Trout population estimates and 95% confidence intervals for the six monitoring sections in the upper Clark Fork River by river mile. All years of available estimates were averaged for each section. Number of years with estimates varied among (see Figure 4 for years averaged for each). Station abbreviations are Bearmouth (BM), Flint Creek Mouth (FCM), Phosphate (PE), Williams-Tavener (W-T), Below Sager Lane (BSL), pH Shack (pHS).

Table 1. Electrofishing data collected on the Upper Clark Fork River at the pH Shack Section from 2011-2014. Population estimates and capture efficiencies are for trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval. Cutt x Rbow represents a phenotypic hybrid between a Cutthroat and Rainbow trout.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2011	Brown	878 (531-1476)	13	265	311	89-498	98
	Rainbow	-	-	2	531	472-590	1
	Cutthroat	-	-	3	350	292-424	1
	Cutt x Rbow	-	-	1	423	-	< 1
2012	Brown	943 (686-1322)	17	403	293	105-473	98
	Rainbow	-	-	7	369	256-540	2
	Cutthroat	-	-	2	306	292-319	< 1
	Cutt x Rbow	-	-	1	323	-	< 1
2013	Brown	1,878 (1,595-2,223)	19	1,056	296	156-630	98
	Rainbow	-	-	13	447	314-610	1
	Cutthroat	-	-	6	327	271-352	1
	Cutt x Rbow	-	-	1	282	-	< 1
2014	Brown	1,177 (1054-1322)	38	1,018	323	160-518	99
	Rainbow	-	-	12	367	240-541	1

Table 2. Electrofishing data collected on the Upper Clark Fork River at the Below Sager Lane Section from 2011-2014. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2011	Brown	170 (119-251)	20	205	313	103-495	98
	Cutthroat	-	-	4	335	280-392	2
	Brook	-	-	1	202	-	< 1
2012	Brown	302 (232-397)	17	533	240	90-595	96
	Cutthroat	-	-	6	314	277-347	1
	Brook	-	-	15	216	134-273	3
2013	Brown	462 (390-553)	25	655	308	139-497	99
	Rainbow	-	-	1	324	-	< 1
	Cutthroat	-	-	2	323	308-337	< 1
	Brook	-	-	6	245	194-275	1
2014	Brown	594 (484-737)	19	666	350	122-532	99
	Rainbow	-	-	1	197	-	< 1
	Cutthroat	-	-	2	321	300-342	< 1
	Brook	-	-	2	297	245-350	< 1

Table 3. Electrofishing data collected on the Upper Clark Fork River at the Williams-Tavener Section from 2011-2014. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2011	Brown	182 (140-244)	26	247	311	108-514	90
	Cutthroat	15 (9-28)	29	24	275	213-328	9
	Brook	-	-	2	203	196-209	1
2012	Brown	224 (180-285)	29	351	266	109-497	88
	Cutthroat	23 (18-34)	46	48	301	170-373	12
	Brook	-	-	1	221	-	< 1
2013	Brown	532 (453-632)	26	636	317	129-507	93
	Cutthroat	33 (22-56)	32	47	295	193-383	7
	Brook	-	-	1	320	-	< 1
2014	Brown	618 (528-731)	25	712	368	138-535	95
	Cutthroat	-	-	34	351	260-443	4
	Brook	-	-	2	292	272-312	< 1

Table 4. Electrofishing data collected on the Upper Clark Fork River at the Phosphate Section from 2011-2014. Population estimates and capture efficiencies are for trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval. Cutt x Rbow represents a phenotypic hybrid between a Cutthroat and Rainbow trout.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2011	Brown	171 (140-215)	41	239	300	104-474	97
	Cutthroat	-	-	7	294	207-378	3
	Cutt x Rbow	-	-	1	367	-	< 1
2012	Brown	308 (231-419)	21	282	270	111-464	92
	Rainbow	-	-	2	423	215-630	1
	Cutthroat	-	-	23	267	187-364	7
	Brook	-	-	1	305	-	< 1
2013	Brown	506 (393-664)	22	387	301	120-461	96
	Cutthroat	-	-	14	305	255-357	3
	Cutt x Rbow	-	-	1	389	-	< 1
2014	Brown	596 (479-751)	22	490	328	124-452	98
	Cutthroat	-	-	10	354	289-416	2
	Cutt x Rbow	-	-	1	415	-	< 1

Table 5. Electrofishing data collected on the Upper Clark Fork River at the Flint Creek Mouth Section from 2009-2014. Population estimates and capture efficiencies are for trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval. Cutt x Rbow represents a phenotypic hybrid between a Cutthroat and Rainbow trout. Brook x Bull represents a phenotypic hybrid between an eastern Brook and Bull trout.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2009*	Brown	123 (88-177)	18	273	369	97-550	95
2010	Brown	136 (105-181)	20	377	345	115-535	94
	Rainbow	-	-	4	389	326-421	1
	Cutthroat	-	-	16	284	227-355	4
	Cutt x Rbow	-	-	4	332	305-352	1
2011	Brown	150 (122-187)	25	481	311	110-509	89
	Rainbow	-	-	3	441	425-468	1
	Cutthroat	14 (8-24)	20	54	275	195-390	10
	Brook	-	-	1	287	-	< 1
	Brook x Bull	-	-	1	393	-	< 1
2012	Brown	107 (82-141)	19	334	293	124-515	87
	Rainbow	-	-	6	352	232-468	2
	Cutthroat	-	-	42	289	186-445	11
	Bull	-	-	2	374	373-375	1
2013	Brown	197 (161-245)	20	572	315	195-502	96
	Cutthroat	6 (3-11)	21	25	326	220-378	4
	Bull	-	-	1	273	-	< 1
2014	Brown	199 (173-231)	26	778	357	185-519	96
	Rainbow	-	-	2	294	250-374	< 1
	Cutthroat	4 (2-7)	36	25	351	202-451	3
	Bull	-	-	2	270	252-288	< 1

* In 2009 entire Upper Clark Fork River was sampled and as a result the Flint Creek Mouth Section is roughly half a mile longer than in other years.

Table 6. Electrofishing data collected on the Upper Clark Fork River at the Bearmouth Section from 2009-2014. Population estimates and capture efficiencies are for trout greater than 175 mm (~7") in total length. Numbers following the population estimate (in parentheses) represent the 95 % confidence interval. Cutt x Rbow represents a phenotypic hybrid between a Cutthroat and Rainbow trout.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2009*	Brown	62 (38-102)	13	134	358	119-528	84
	Cutthroat	7 (4-14)	27	26	314	152-410	16
2010	Brown	32 (23-49)	35	106	362	157-525	68
	Rainbow	-	-	13	345	242-442	8
	Cutthroat	6 (4-11)	42	27	308	100-400	17
	Bull	-	-	2	321	297-345	1
	Cutt x Rbow	-	-	8	371	320-458	5
2011	Brown	43 (30-65)	27	123	342	152-523	59
	Rainbow	7 (4-13)	38	28	342	152-479	14
	Cutthroat	13 (9-20)	38	54	309	182-414	26
	Bull	-	-	2	424	362-486	1
2012	Brown	31 (21-47)	29	95	326	177-502	32
	Rainbow	21 (14-34)	31	69	285	178-467	23
	Cutthroat	41 (30-59)	27	134	290	168-434	45
	Bull	-	-	2	266	260-272	< 1
2013	Brown	60 (43-87)	21	169	339	191-476	48
	Rainbow	19 (11-35)	24	49	344	230-455	14
	Cutthroat	45 (32-66)	27	134	321	175-426	38
	Bull	-	-	3	379	337-400	< 1
2014	Brown	56 (42-79)	24	173	367	183-534	55
	Rainbow	28 (16-49)	21	68	331	188-493	21
	Cutthroat	19 (14-28)	36	74	355	180-452	25

* In 2009, entire Upper Clark Fork River was sampled and as a result the Bearmouth Section is roughly a tenth of a mile longer than in other years.

Table 7. Electrofishing data collected on the Upper Clark Fork River at the Jens CPUE section.

Year	Species	CPUE (fish/mile)	CPUE (fish/min)	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2014	Brown Trout	58	1.98	343	165-460	29
	Cutthroat Trout	1	0.03	405	-	< 1
	Mountain Whitefish	129	4.41	338	228-445	64
	Largescale Sucker	10	0.34	507	440-578	5
	Sculpin	1	0.03	74	-	< 1
	Redside Shiner	1	0.03	87	-	< 1
	Longnose Dace	1	0.03	97	-	< 1

Table 8. Electrofishing data collected on the Upper Clark Fork River at the Above Deer Lodge CPUE section.

Year	Species	CPUE (fish/mile)	CPUE (fish/min)	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2014	Brown Trout	36	1.40	349	261-440	14
	Mountain Whitefish	181	7.03	323	142-463	70
	Largescale Sucker	39	1.52	505	-	15
	Longnose Sucker	1	0.04	116	-	< 1

Caged Fish Survival, Mortality, Discharge, and Water Temperature

Figures 6-17 depict total mortalities in cages one and two combined, maximum daily water temperatures, and mean daily discharges at cage sites in 2014. The solid red horizontal line in each figure 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 figure represents the upper incipient lethal temperature for Brown Trout of 24.7 °C, above which thermal stress is lethal with mortality being a function of exposure time (Elliot 1994).

In 2014, most cage sites displayed 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 and/or exceeded 19 °C. Early season mortality was generally high until early- to mid- April, although sites such as Pond 2, Silver Bow, Warm Springs, Little Blackfoot, and Flint had significant early season mortality that continued until May. Mortality at most sites was relatively low during May and early June then increased as flows decreased and temperatures increased during the summer. Site specific descriptions of discharge, water temperatures, and timing of mortalities at each site are outlined below in order from upstream to downstream.

Of the mainstem sites, U/S Lil Black had the lowest survival at 44% and the Deer Lodge site had the highest survival at 90% (Table 9). Survival at the Flint Creek tributary site was 72% and 79% at the Lil Black tributary site. The average survival estimate at the two tributary sites (0.76) was compared to each mainstem site with chi-square tests. Results of these tests revealed that U/S Lil Black, Silver Bow, and Pond 2 had significantly lower survival than the tributary sites (Table 9). No sites had survival that was significantly higher than tributaries in 2014. From a spatial perspective, survival was $\geq 85\%$ at mainstem sites from Perkins Lane to Deer Lodge (Figure 18). The three most upstream treatment sites (Pond 2, Silver Bow, and Warm Springs) had survival $\leq 60\%$.

Pond 2

There are no discharge data available for Pond 2 in 2014 because there is not a USGS station present at this site. Peak maximum daily water temperature at Pond 2 in 2014 was 24.1 °C on July 14 (Figure 7). Maximum daily water temperature in 2014 exceeded 19.0 °C for 63 days and never exceeded the upper incipient lethal temperature for Brown Trout of 24.7 °C (Figure 6). Pond 2 experienced lower survival than tributary sites (Table 9), with most mortality occurring in April. Another peak in mortality occurred at this site in early July after temperatures exceeded 19.0 °C (Figure 7).

Silver Bow

Peak mean daily discharge at Silver Bow in 2014 was 331 ft³/s on May 26. In 2014 peak maximum daily water temperature at Silver Bow was 23.6 °C on August 11 (Figure 8). Maximum daily water temperature in 2014 exceeded 19.0 °C for 52 days and never exceeded the upper incipient lethal temperature for Brown Trout of 24.7 °C (Figure 8). Silver Bow

experienced significantly lower survival than tributary sites (Table 9), with most mortality occurring early and late in the study season.

Warm Springs

Peak mean daily discharge at Warm Springs in 2014 was 244 ft³/s on May 29. In 2014 peak maximum daily water temperature at Warm Springs was 19.1 °C on August 11 (Figure 9). August 11 was the only day maximum daily water temperature exceeded 19.0 °C. The upper incipient lethal temperature for Brown Trout of 24.7 °C was never exceeded (Figure 9). Warm Springs experienced significantly lower survival than the tributary sites (Table 9), with most mortality occurring early in the study season before runoff, as well as on the descending limb of the hydrograph water temperatures approached 19.0 °C (Figure 9).

Perkins Lane

Peak mean daily discharge at Perkins Lane in 2014 was 526 ft³/s on May 27. In 2014 peak maximum daily water temperature at Perkins Lane was 21.9 °C on August 1 (Figure 10). Maximum daily water temperature in 2014 exceeded 19.0 °C for 49 days and the upper incipient lethal temperature for Brown Trout of 24.7 °C was never exceeded (Figure 10). Survival rate of fish at Perkins Lane was not significantly different from tributaries (Table 9). Most mortalities at this site occurred on the ascending and descending limbs of the hydrographs (Figure 10).

Galen

There are no discharge data available for Galen in 2014 because there is not a USGS station present at this site. In 2014 peak maximum daily water temperature at Galen Right was 21.9 °C on August 1 (Figure 11). Maximum daily water temperature in 2014 exceeded 19.0 °C for 45 days and the upper incipient lethal temperature for Brown Trout of 24.7 °C was never exceeded (Figure 11). Survival rate of fish at Galen was not significantly different from tributaries (Table 9). Most mortalities at this site occurred during the time period when water temperatures were above 19.0 °C, although four mortalities did occur earlier in the season (Figure 11).

Racetrack

There are no discharge data available for Racetrack in 2014 because there is not a USGS station present at this site. In 2014 peak maximum daily water temperature at Racetrack was 22.7 °C on August 1 (Figure 12). Maximum daily water temperature in 2014 exceeded 19.0 °C for 44 days and never exceeded 24.7 °C (Figure 12). Survival rate of fish at Racetrack was not significantly different from tributaries (Table 9). Nine mortalities (69%) at this site occurred during the time period when water temperatures were above 19.0 °C, although four mortalities also occurred in April and May (Figure 12).

Deer Lodge

Peak mean daily discharge at Deer Lodge in 2014 was 748 ft³/s on June 28. In 2014 peak maximum daily water temperature at Deer Lodge was 24.3 °C on July 13 (Figure 13). Maximum daily water temperature in 2014 exceeded 19.0 °C for 50 days and never exceeded 24.7 °C (Figure 13). Survival rate of fish at Deer Lodge was not significantly different from tributaries (Table 9). Mortality at this site exhibited a bimodal pattern, occurring in the first few weeks of study season on, as well as on the descending limb of the hydrograph as water temperatures began to exceed 19.0 °C (Figure 13).

U/S Lil Black

Peak mean daily discharge at U/S Lil Black in 2014 was 978 ft³/s on June 28. In 2014 peak maximum daily water temperature at U/S Lil Black was 25.1 °C on July 13 (Figure 14). Maximum daily water temperature in 2014 exceeded 19.0 °C for 52 days and exceeded the upper incipient lethal temperature for Brown Trout of 24.7 °C for one day (Figure 14). Fish at the U/S Lil Black site experienced significantly lower survival than the tributary sites (Table 9), with 28 (93%) of the mortalities occurring when water temperatures were above 19.0 °C (Figure 14).

Lil Black (Tributary)

Peak mean daily discharge at Lil Black in 2014 was 1010 ft³/s on June 5. In 2014 peak maximum daily water temperature at Lil Black was 21.4 °C on July 12 (Figure 15). Maximum daily water temperature in 2014 exceeded 19.0 °C for 36 days and never exceeded 24.7 °C (Figure 15). Nineteen (90%) of the 21 mortalities occurred during the month of April, with the other two mortalities occurring when water temperatures were above 19.0 °C (Figure 15).

Flint (Tributary)

Peak mean daily discharge at Flint in 2014 was 282 ft³/s on April 9. In 2014 peak maximum daily water temperature at Flint was 19.6 °C on July 13 and August 14 (Figure 16). Maximum daily water temperature in 2014 exceeded 19.0 °C for 8 days and never exceeded 24.7 °C (Figure 16). Twenty-six (90%) of the mortalities at this site occurred between the beginning of the study and May 12 (Figure 16).

Bearmouth

Peak mean daily discharge at Bearmouth was 1,080 ft³/s on May 31. In 2014 peak maximum water temperature was 23.8 °C on July 13. Maximum daily water temperature in 2014 exceeded 19.0 °C for 52 days and never exceeded 24.7 °C (Figure 17). Survival rate of fish at Bearmouth was not significantly different from tributaries (Table 9). The number of mortalities at this site generally increased after flows went down and water temperatures exceeded 19 °C (Figure 17).

Clinton Spring (Handling Control)

There are no discharge data available for Clinton Spring because there is not a USGS station present at this site. In 2014 peak maximum daily water temperature at Clinton Spring was 15.9 °C on August 18 (Figure 18). Maximum daily water temperature never exceeded 19.0 °C or 24.7 °C in 2014 (Figure 18). A relatively large mortality event occurred at this site between August 14-18, when nine fish died. Other mortalities occurred near the beginning of the study and one mortality occurred in June.

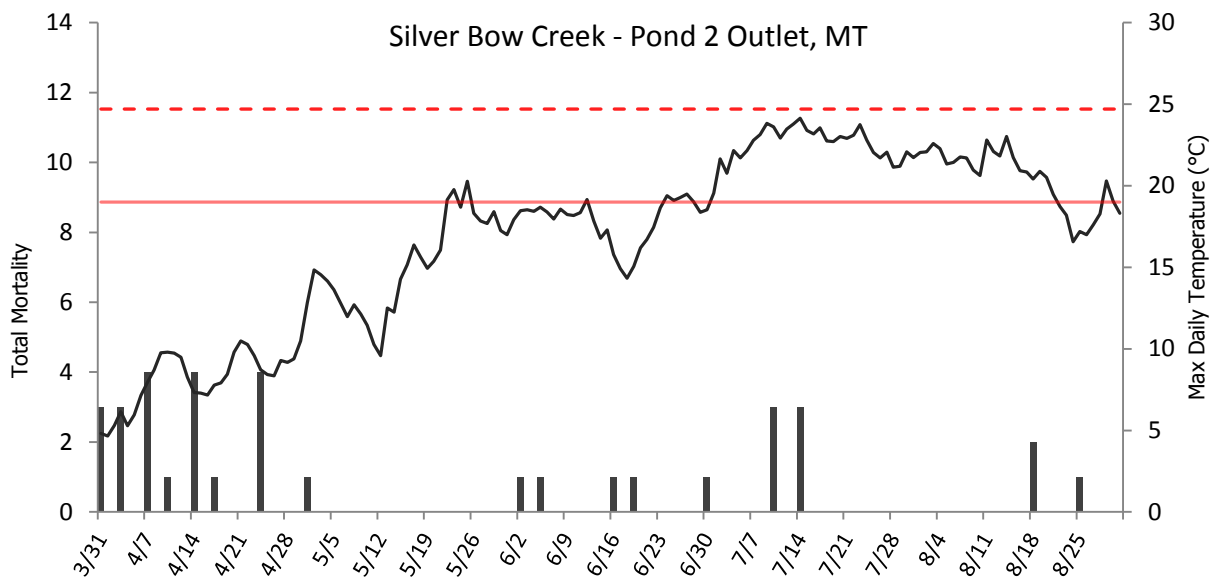


Figure 6. Total mortalities between cages one and two combined (gray bars) and maximum daily water temperature (black line) for 2014 in Silver Bow Creek at the Pond 2 outlet site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

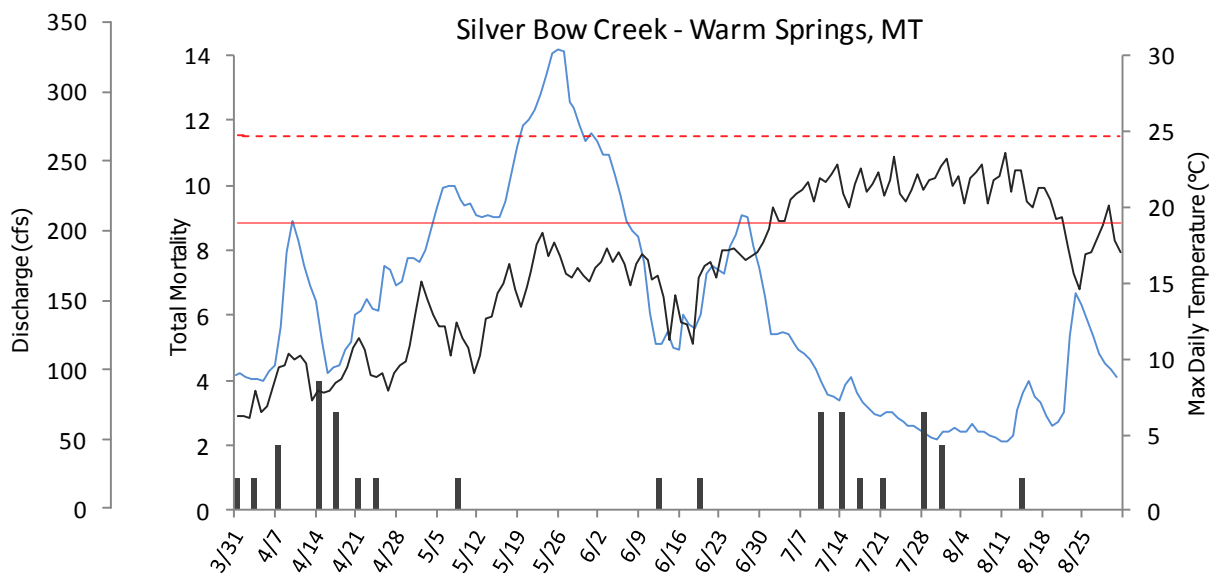


Figure 7. Total mortalities between cages one and two combined (gray bars) and maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 in Silver Bow Creek, Warm Springs, MT. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

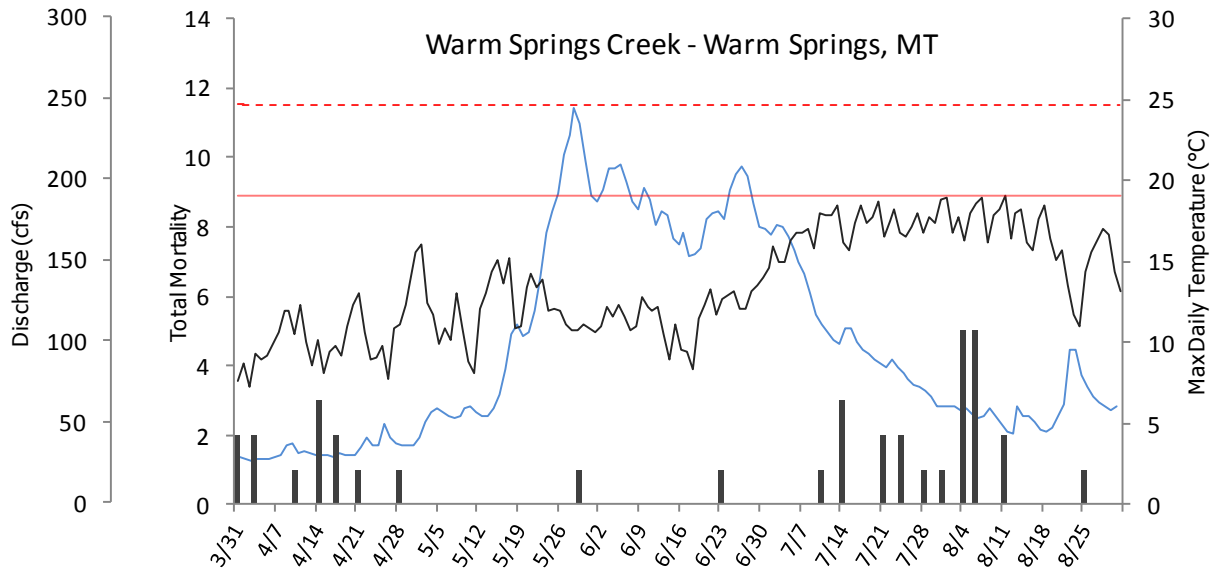


Figure 8. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 in Warm Springs Creek at Warm Springs, MT. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

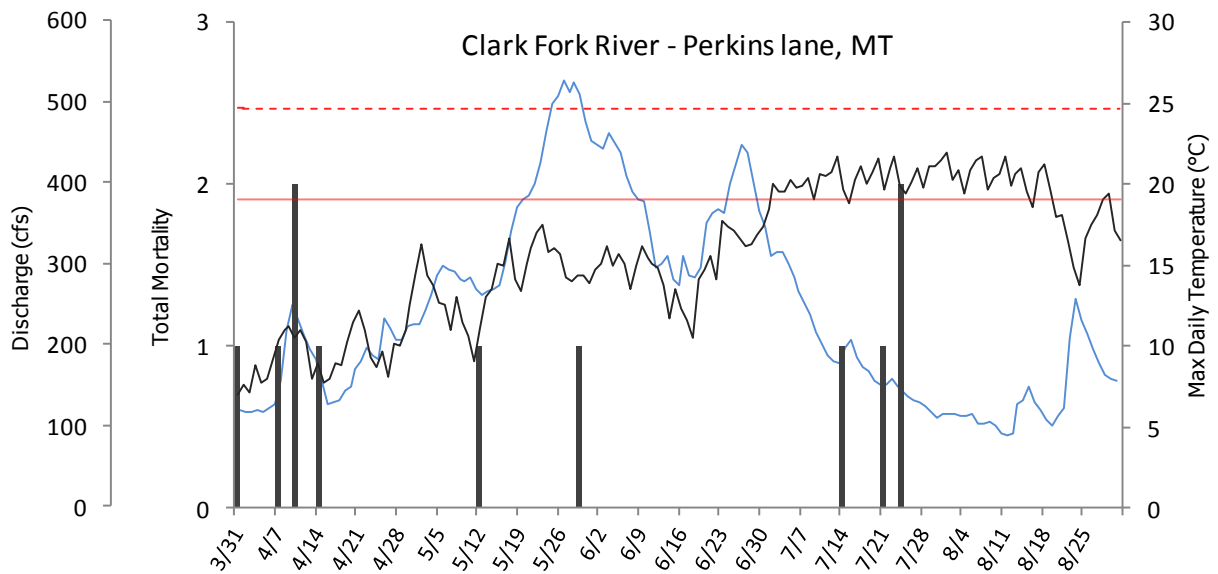


Figure 9. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 in the Clark Fork River at the Perkins Lane site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

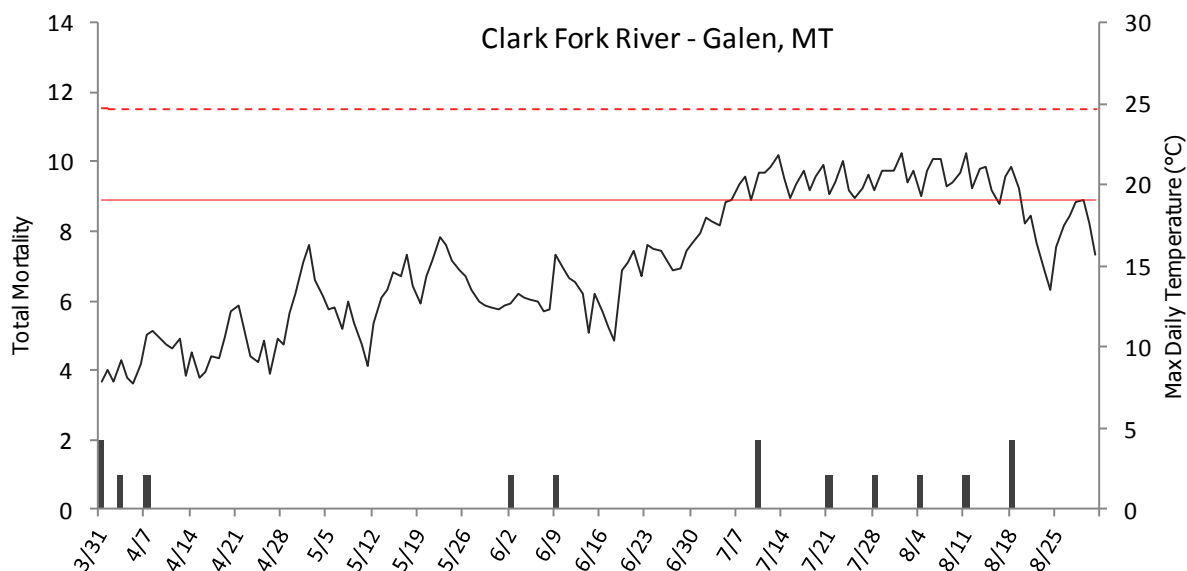


Figure 10. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line) in the Clark Fork River at the Galen site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

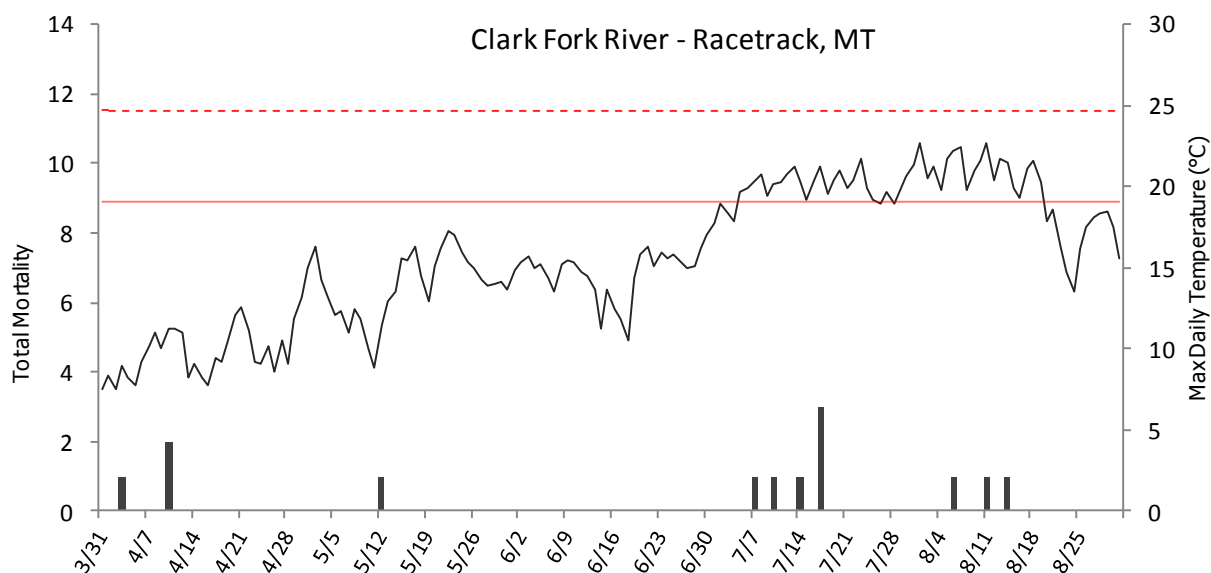


Figure 11. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line) in the Clark Fork River at the Racetrack site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

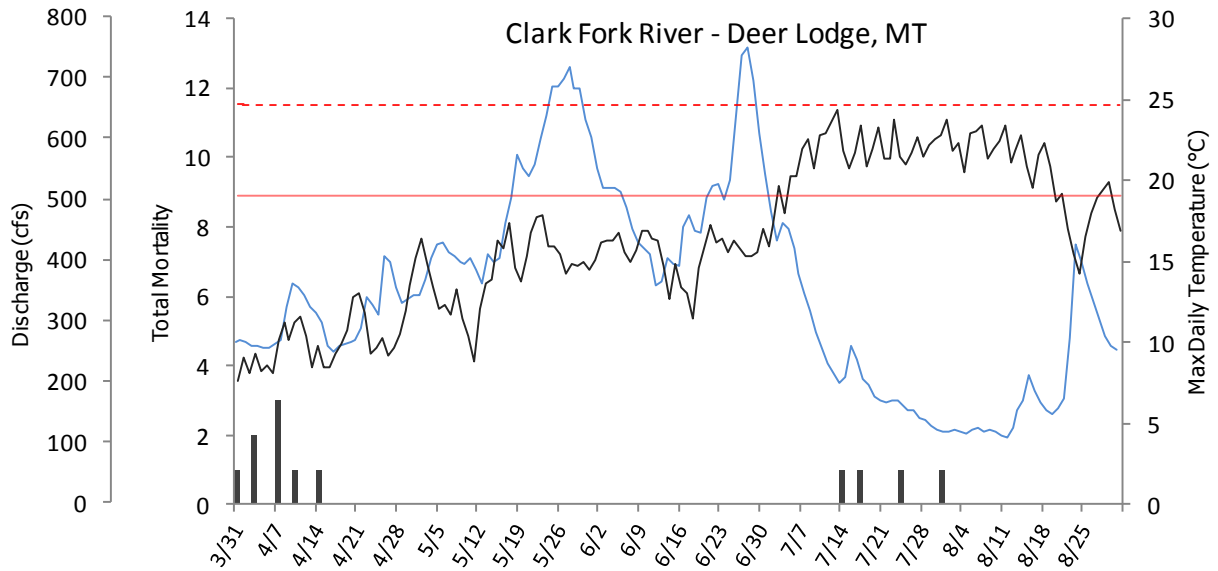


Figure 12. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 in the Clark Fork River at the Deer Lodge site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

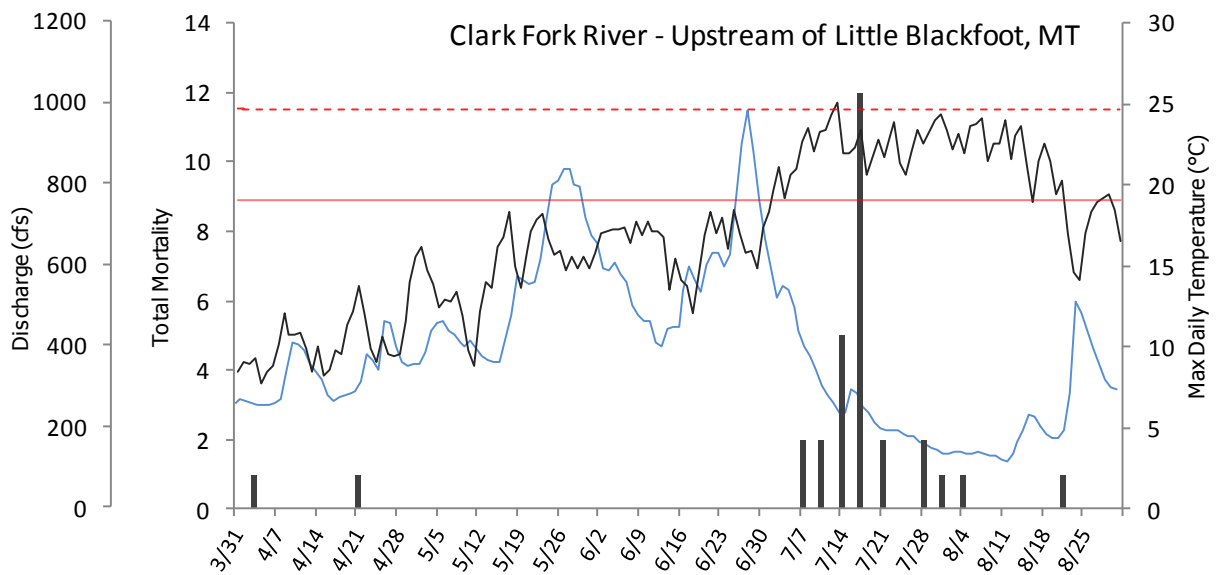


Figure 13. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 in the Clark Fork River at the site upstream of the Little Blackfoot River. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

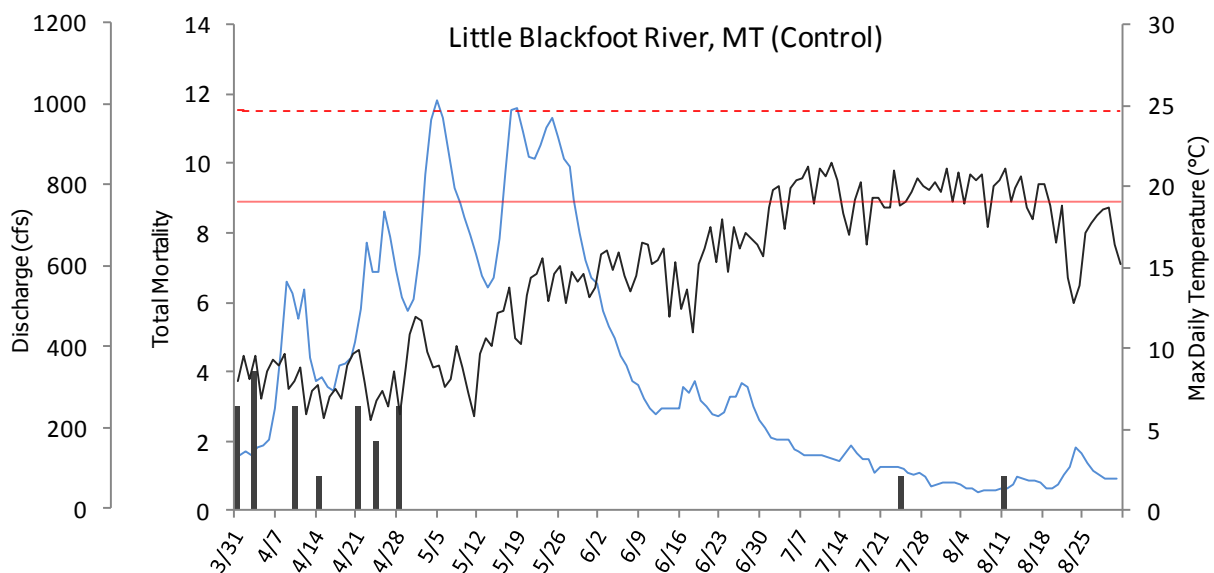


Figure 14. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 at the tributary site in Little Blackfoot River. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

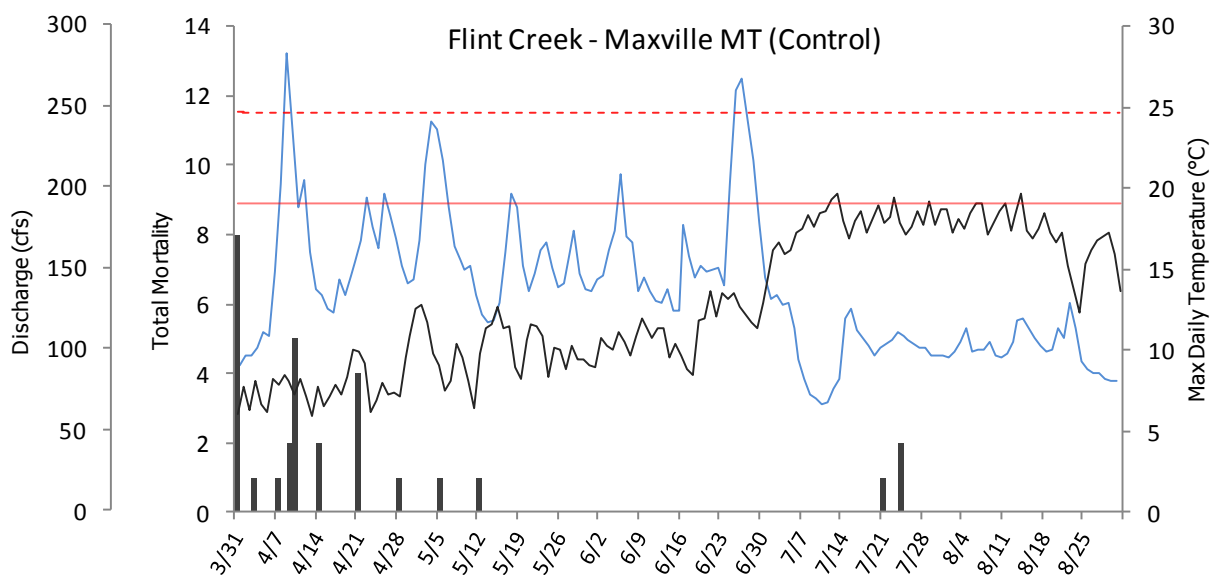


Figure 15. Total mortalities between cages one and two combined (gray bars), maximum daily water temperature (black line), and mean daily discharge (blue line) for 2014 at the tributary site in Flint Creek. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

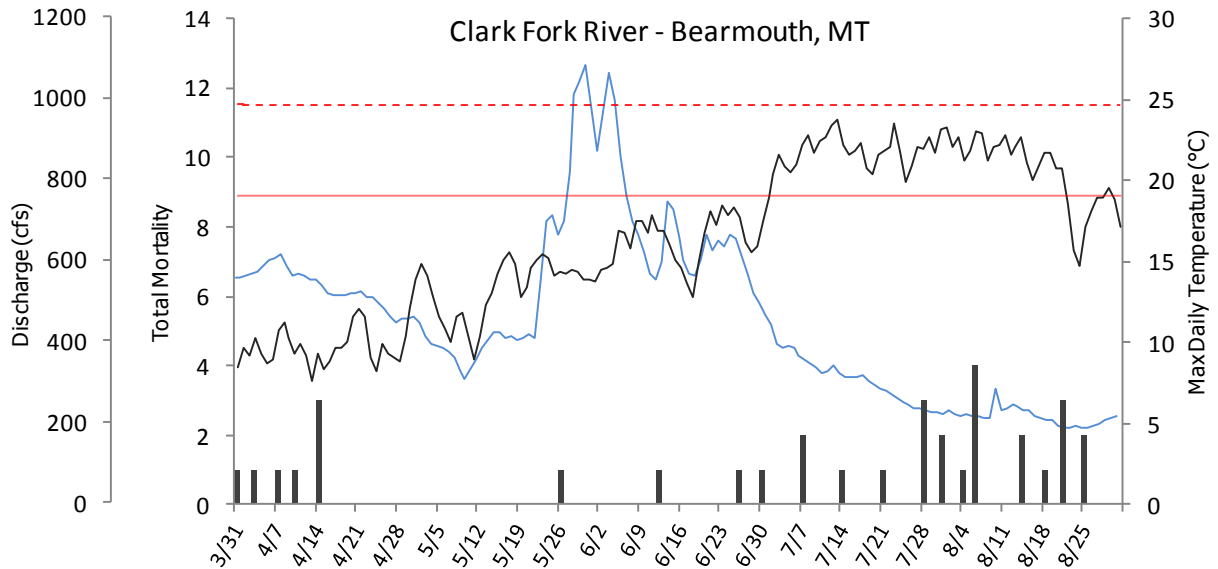


Figure 16. Total mortalities between cages one and two combined (gray bars) and maximum daily water temperature (black line) and mean daily discharge (blue line) for 2014 in the Clark Fork River at the Bearmouth site. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

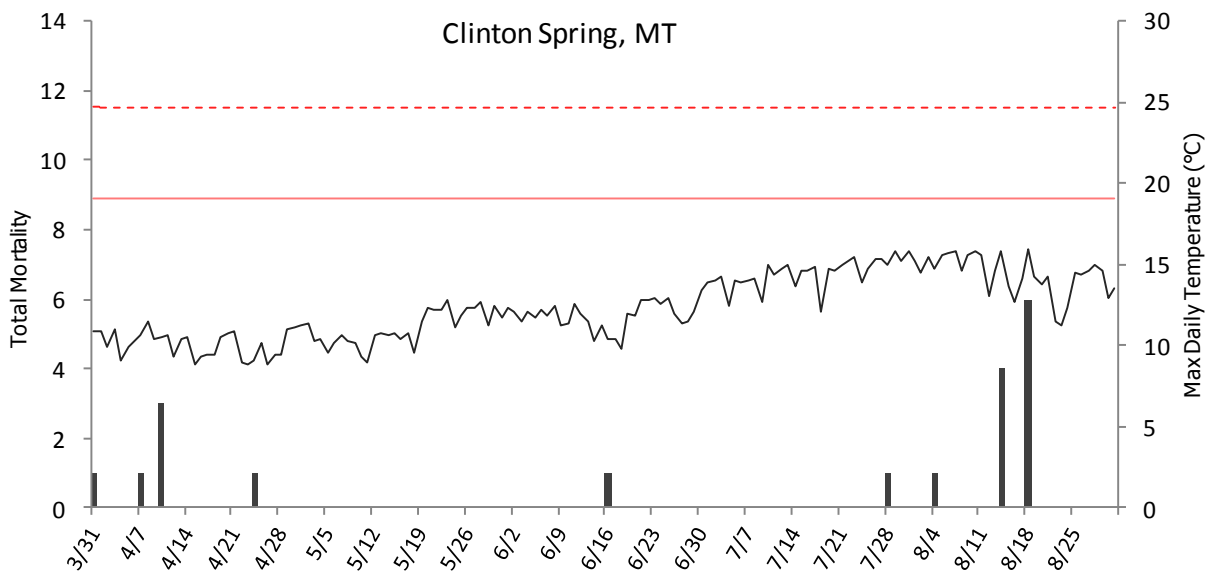


Figure 17. Total mortalities between cages one and two combined (gray bars) and maximum daily water temperature (black line) for 2014 at the control site in the spring channel near Clinton, Montana. The solid red line indicates the upper critical temperature threshold and the dashed red line represents the upper incipient lethal temperature for Brown Trout.

Table 9. Survival, net number of fish added during the survival study period (April 14 – July 31) and fish remaining in cages one and two on July 31. Results of χ^2 tests (df = 1 for all tests) between survival at mainstem treatment sites and mean survival at two tributary control sites are also presented. Statistically significant *P* values are in bold.

Site type	Site	Fish remaining	Net fish added	Survival	χ^2	<i>P</i>
Mainstem	Bearmouth	39	56	0.70	0.22	0.6386
	U/S Lil Black	21	48	0.44	8.68	0.0032
	Deer Lodge	46	51	0.90	2.63	0.1051
	Racetrack	49	56	0.88	1.61	0.2040
	Galen	38	44	0.86	1.00	0.3167
	Perkins Lane	40	47	0.85	0.75	0.3872
	Warm Springs	29	48	0.60	1.89	0.1696
	Silver Bow	25	50	0.50	5.71	0.0169
	Pond 2	22	43	0.51	4.81	0.0284
	Mainstem Average	34.3	49.2	0.70	0.02	0.6631
Tributary	Flint	31	43	0.72		
	Lil Black	38	48	0.79		
	Tributary average	34.5	45.5	0.76		

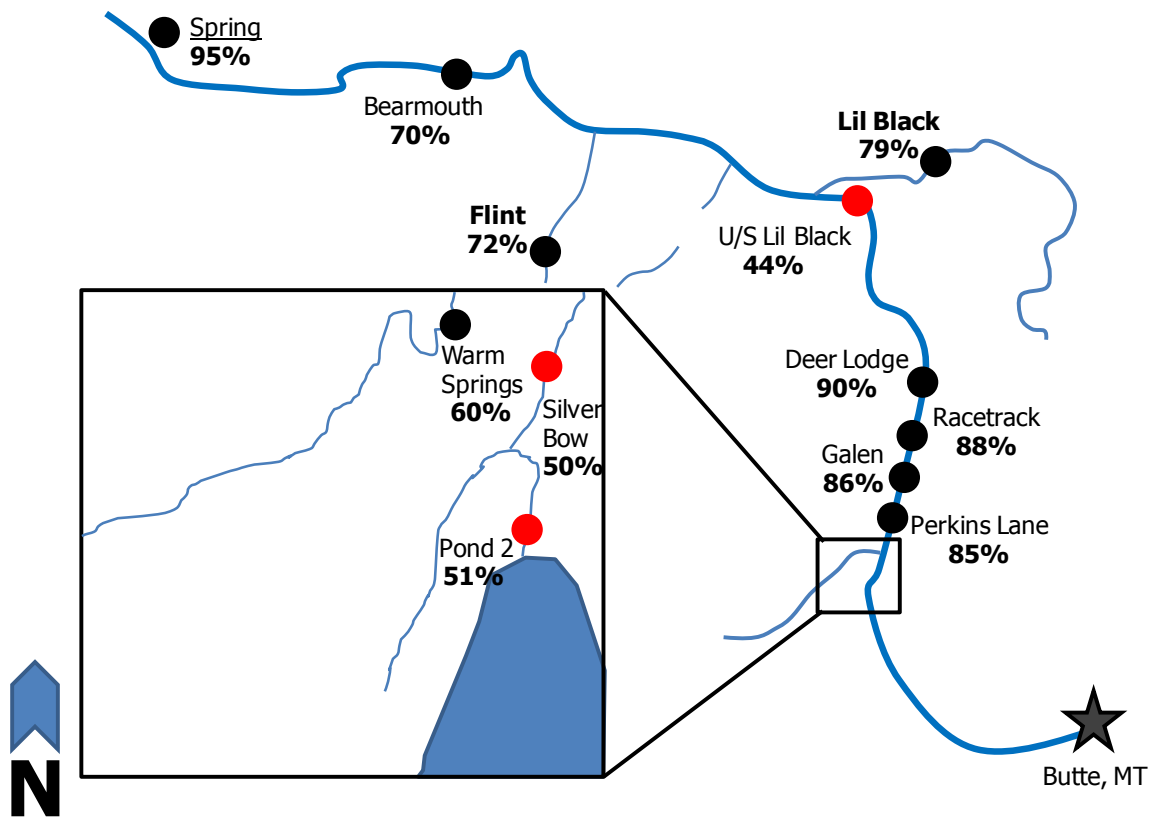


Figure 18. Cumulative brown trout survival from April 14th to July 31st, 2014. Tributary sites are shown in bold and the handling control is underlined. Red dots denote sites with survival that was significantly lower than the average of the two tributary control sites. No sites had significantly higher survival than control sites in 2014.

Growth and Condition

Fish at the Deer Lodge site had the lowest increase in length of all sites, growing an average of 17.6 mm over the course of the study (Figure 19a). Fish in the Spring control site grew 34.1 mm on average, the most of any site. Bearmouth fish had the lowest Wr for fish surviving to the end of the field season (mean = 71; Figure 19b) whereas the Warm Springs site had fish in the best condition at the end of the study (mean Wr = 95). Dead fish tended to have higher Wr than live fish at all sites and during most months (Figure 20). Mean Wr of all dead fish measured and weighed in 2014 was 99.5 ($n = 202$, $SD = 24.2$) compared to a mean Wr of 83.3 ($n = 417$, $SD = 8.7$) for all live fish. The Wr data of dead fish should be interpreted with caution because many of this fish had saprolegnia coating their bodies, which may have absorbed water and increased the weight of these specimens. Also, fish in freshwater tend to gain water when osmoregulation is disrupted by stress or death, which would also increase *post-mortem* weight (Mazeaud et al. 1977; Bronstein et al. 1985). There were not statistically significant relationships between the number of fish remaining in the cages and the increase in mean length ($P = 0.879$) or Wr ($P = 0.778$) within cages. Thus, there was no evidence of density dependent growth or condition.

Growth (increase in weight) at all but one site was lower than the Elliot et al. (1995) temperature based model predicted (Figure 21). Fish at the Pond 2 site was predicted to have the lowest increase in weight of any site, but growth at this site was actually greater than at any other site. High growth and productivity at this site has been attributed to a tail water effect in previous caged fish studies (Richards et al. 2013, Leon et al. 2014). After removing the Pond 2 site from analysis, a linear regression of observed weights versus predicted weights indicated a significant relationship ($P = 0.003$, $R^2 = 0.776$), suggesting a strong influence of temperature on Brown Trout growth in this study

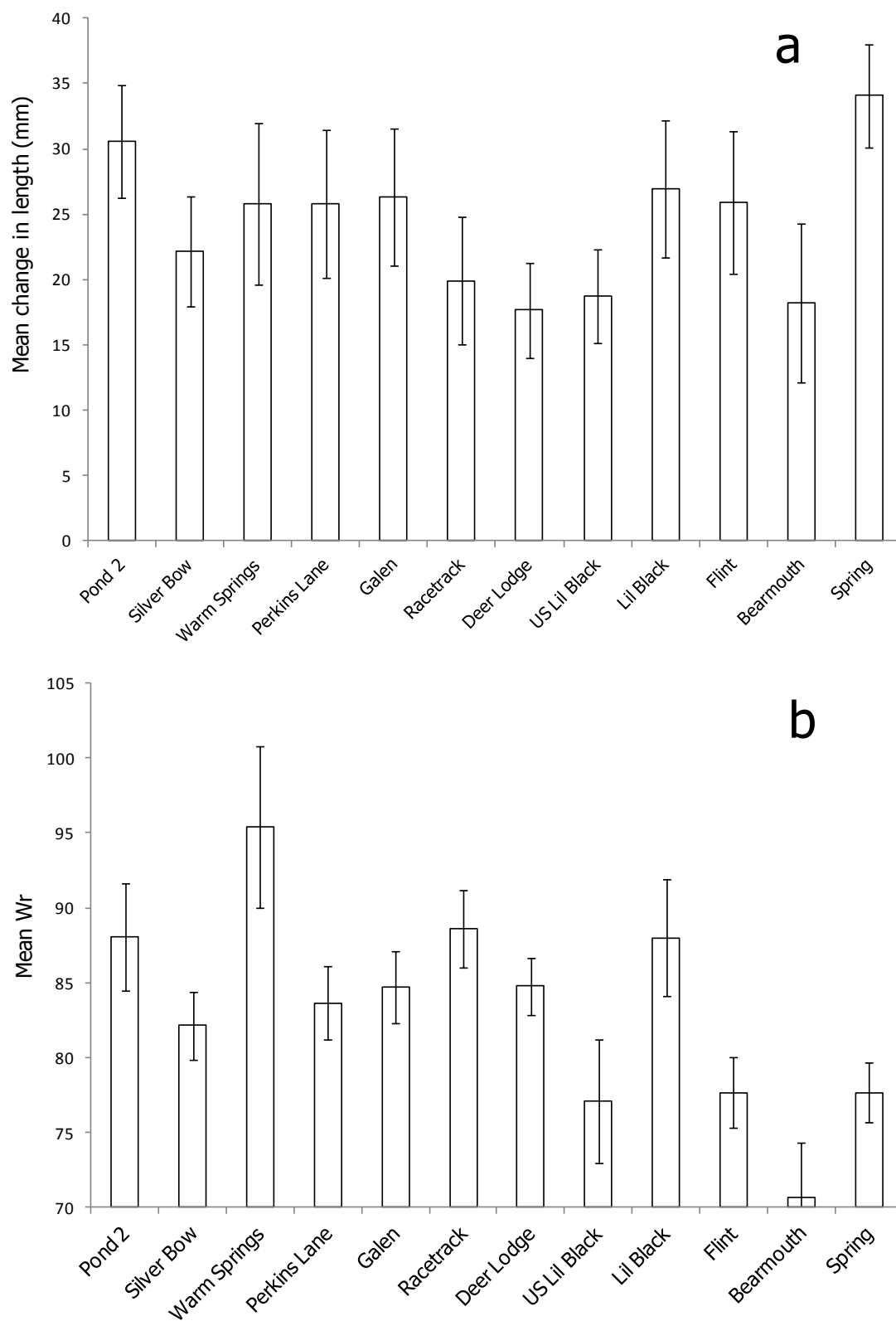


Figure 19. Mean change in length (a) and mean relative weight (b) by site for live fish at the end of the 2014 caged fish study. Error bars are 95% confidence intervals.

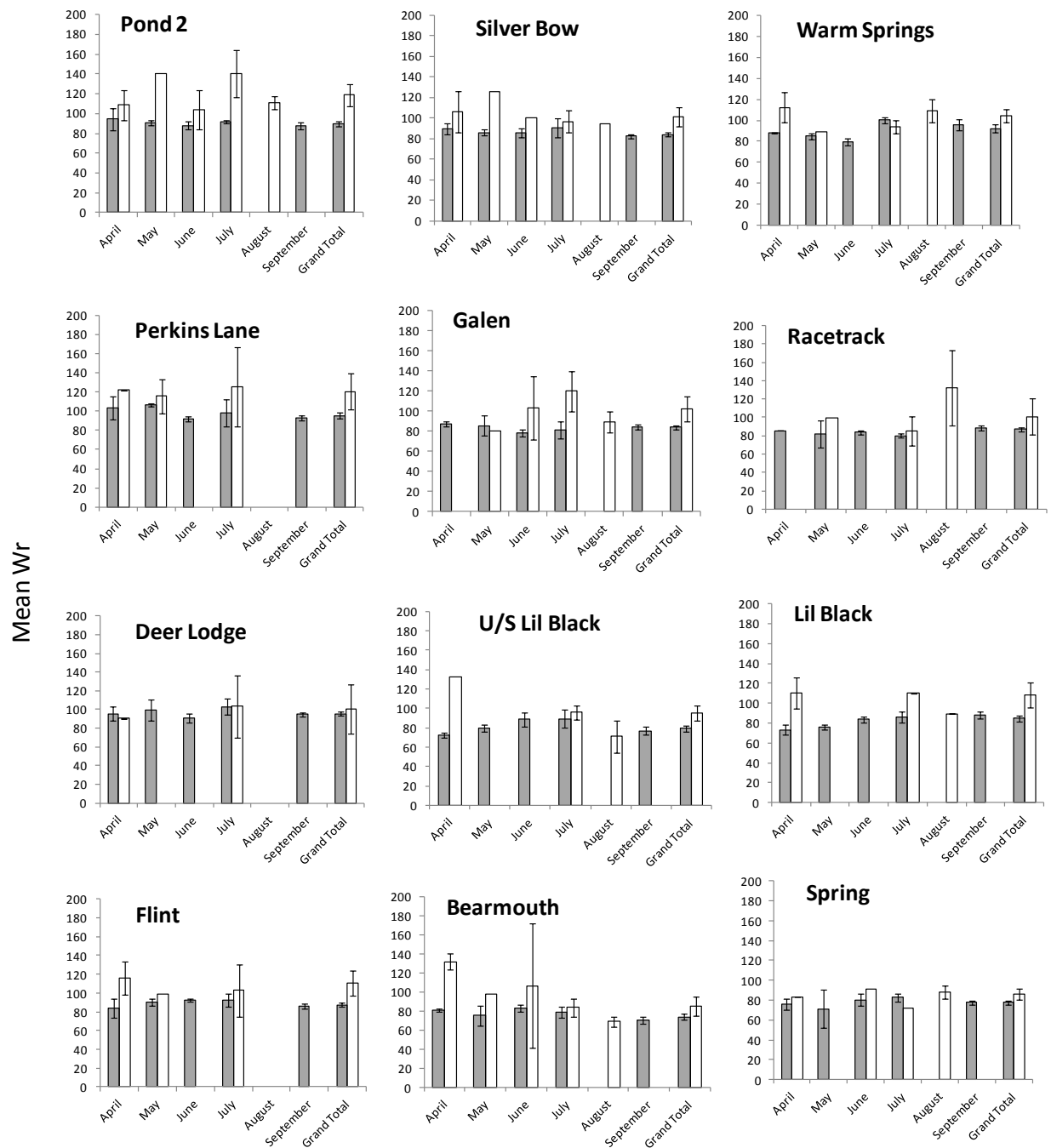


Figure 20. Mean relative weight (Wr) for live (white bars) and dead (grey bars) fish by site and month for the 2014 caged fish study. Error bars are 95% confidence intervals.

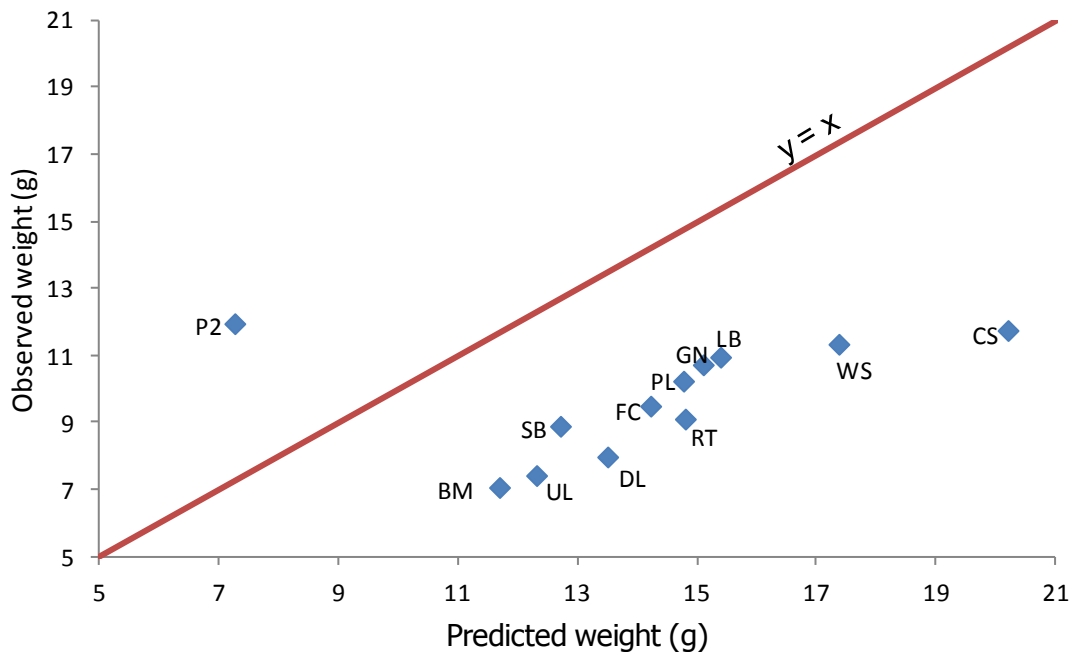


Figure 21. Observed mean final weight of live fish versus weights predicted by the temperature based model of Elliot et al. (1995) for twelve caged fish sites in the Upper Clark Fork River drainage, 2014. Site abbreviations are Pond 2 (P2), Silver Bow (SB), Warm Springs (WS), Perkins Lane (PL), Galen (GN), Racetrack (RT), Deer Lodge (DL), Upstream of the Little Blackfoot (UL), Little Blackfoot (LB), Flint Creek (FC), Bearmouth (BM), and Clinton Spring (CS). The red line represents the 1:1 line.

Tissue Metals Burdens

Mean (\pm 95% CI) whole body metal concentrations in the five hatchery control Brown Trout were $4.31 (\pm 1.26) \mu\text{g/g}$ for copper and $136.8 (\pm 9.45) \mu\text{g/g}$ for zinc. Therefore, concentrations above these values for fish held in cages represent accumulation of copper or zinc while in the cages. U/S Lil Black had the highest average copper tissue burden ($11.4 \mu\text{g/g}$; SD = 2.9; Figure 22), followed by Deer Lodge ($9.3 \mu\text{g/g}$; SD = 3.5), and Perkins Lane ($8.67 \mu\text{g/g}$; SD = 4.5). Copper tissue burdens at U/S Lil Black were significantly higher than every site except Deer Lodge and Perkins Lane (Table 10). Copper tissue burdens at Deer Lodge were significantly higher than Pond 2, Silver Bow, and the tributary and control sites. Perkins Lane had higher tissue burdens than the Lil Blackfoot site. Of the mainstem sites, Silver Bow had the lowest copper tissue burdens ($5.7 \mu\text{g/g}$; SD = 1.7), followed by Pond 2 ($6.0 \mu\text{g/g}$; SD = 1.5), and Galen ($6.83 \mu\text{g/g}$; SD = 1.3). The tributary sites and Clinton Spring the lowest copper tissue burdens of all the sites. Copper tissue burdens generally increased upstream to downstream from the Pond 2 to the U/S Lil Black sites.

The Pond 2 site had the highest zinc tissue burdens ($216.8 \mu\text{g/g}$; SD = 65.1), followed by Silver Bow ($198.7 \mu\text{g/g}$; SD = 34.5), and U/S Lil Black (178.7 ; 27.0). Zinc tissue burdens at Pond 2 were significantly higher than all sites except Silver Bow (Table 10). Silver Bow had zinc tissue burdens significantly higher than Warm Springs, Galen, Racetrack, Lil Black, and

Spring. Racetrack had the lowest zinc tissue burdens ($156.9 \mu\text{g/g}$; $\text{SD} = 156.9$) of the mainstem sites, followed by Galen ($162.3 \mu\text{g/g}$; $\text{SD} = 16.8$), and Perkins Lane ($167.2 \mu\text{g/g}$; $\text{SD} = 25.1$).

Copper Tissue Burdens reached the highest levels of the season in July and or September at Pond 2, Silver Bow, Warm Springs, Galen, Deer Lodge, Lil Black, and Bearmouth (Figures 23a – 23d). Other sites had less distinct patterns in tissue burdens over the season. Zinc Tissue Burdens were highest in July and/or September at Pond 2, Silver Bow, and Bearmouth (Figures 23a – 23d).

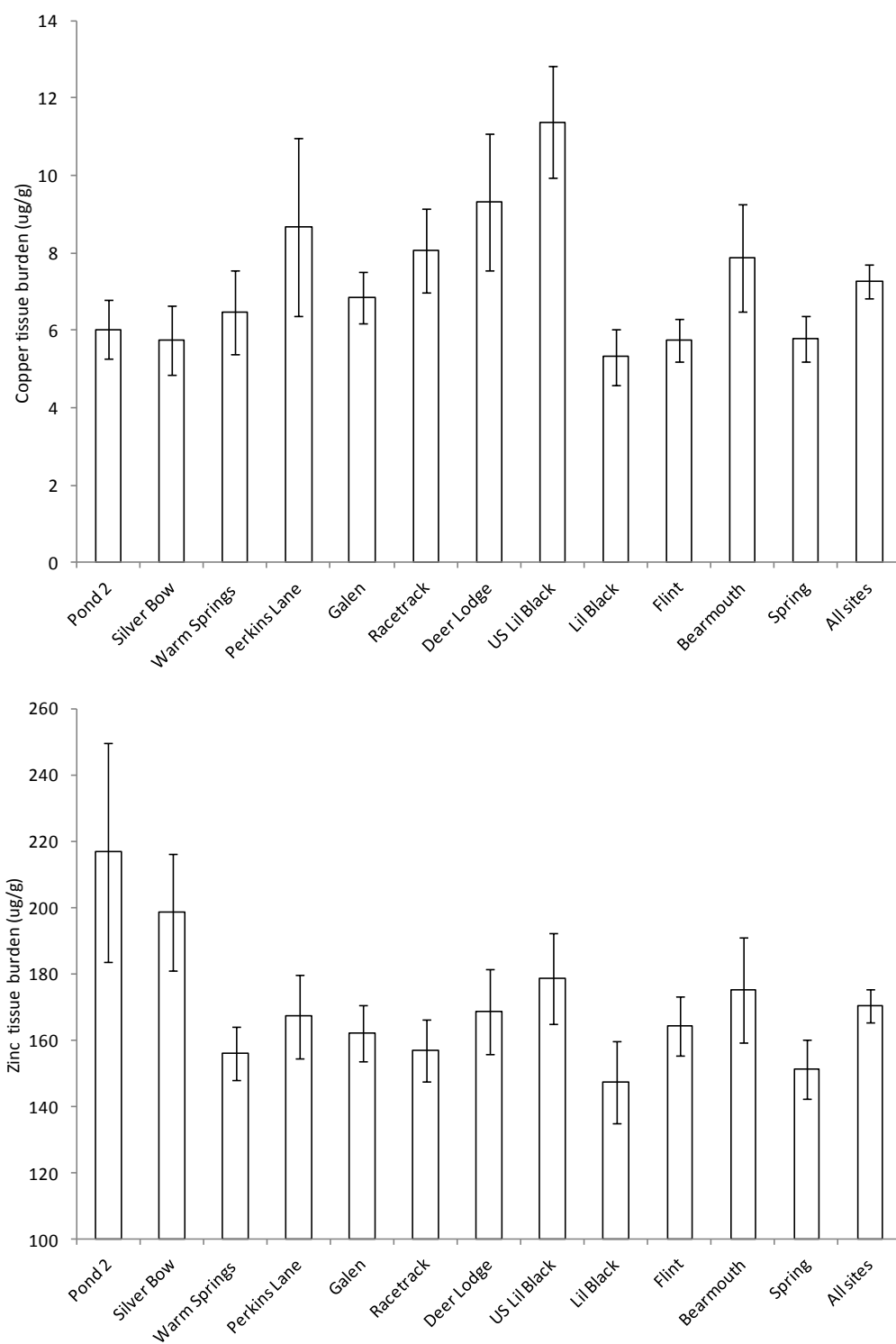


Figure 22. Mean whole body concentrations of copper (a) and zinc (b) at twelve study sites in the 2014 Upper Clark Fork River Drainage caged fish study. Error bars are 95% confidence intervals.

Table 10. Bonferroni-corrected P values from pairwise T tests of whole body copper tissue burdens between 12 sites in the Upper Clark Fork River Drainage. Values < 0.05 are in bold.

	Pond 2	Silver Bow	Warm Springs	Perkins Lane	Galen	Racetrack	Deer Lodge	US Lil Black	Lil Black	Flint	Bearmouth
Silver Bow	1.0000	-	-	-	-	-	-	-	-	-	-
Warm Springs	1.0000	1.0000	-	-	-	-	-	-	-	-	-
Perkins Lane	0.1912	0.0685	0.8570	-	-	-	-	-	-	-	-
Galen	1.0000	1.0000	1.0000	1.0000	-	-	-	-	-	-	-
Racetrack	1.0000	0.5868	1.0000	1.0000	1.0000	-	-	-	-	-	-
Deer Lodge	0.0146	0.0044	0.0879	1.0000	0.2620	1.0000	-	-	-	-	-
US Lil Black	0.0000	0.0000	0.0000	0.1552	0.0000	0.0144	1.0000	-	-	-	-
Lil Black	1.0000	1.0000	1.0000	0.0124	1.0000	0.1364	0.0006	0.0000	-	-	-
Flint	1.0000	1.0000	1.0000	0.0708	1.0000	0.6035	0.0046	0.0000	1.0000	-	-
Bearmouth	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0065	0.2645	1.0000	-
Spring	1.0000	1.0000	1.0000	0.0790	1.0000	0.6617	0.0052	0.0000	1.0000	1.0000	1.0000

Table 11. Bonferroni-corrected *P* values from pairwise *T* tests of whole body zinc tissue burdens between 12 sites in the Upper Clark Fork River Drainage. Values < 0.05 are in bold.

	Pond 2	Silver Bow	Warm Springs	Perkins Lane	Galen	Racetrack	Deer Lodge	US Lil Black	Lil Black	Flint	Bearmouth
Silver Bow	1.0000	-	-	-	-	-	-	-	-	-	-
Warm Springs	0.0000	0.0080	-	-	-	-	-	-	-	-	-
Perkins Lane	0.0006	0.2673	1.0000	-	-	-	-	-	-	-	-
Galen	0.0000	0.0393	1.0000	1.0000	-	-	-	-	-	-	-
Racetrack	0.0000	0.0101	1.0000	1.0000	1.0000	-	-	-	-	-	-
Deer Lodge	0.0010	0.3945	1.0000	1.0000	1.0000	1.0000	-	-	-	-	-
US Lil Black	0.0350	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	-	-	-
Lil Black	0.0000	0.0003	1.0000	1.0000	1.0000	1.0000	1.0000	0.2755	-	-	-
Flint	0.0002	0.1204	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	-
Bearmouth	0.0111	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6911	1.0000	-
Spring	0.0000	0.0014	1.0000	1.0000	1.0000	1.0000	1.0000	0.8118	1.0000	1.0000	1.0000

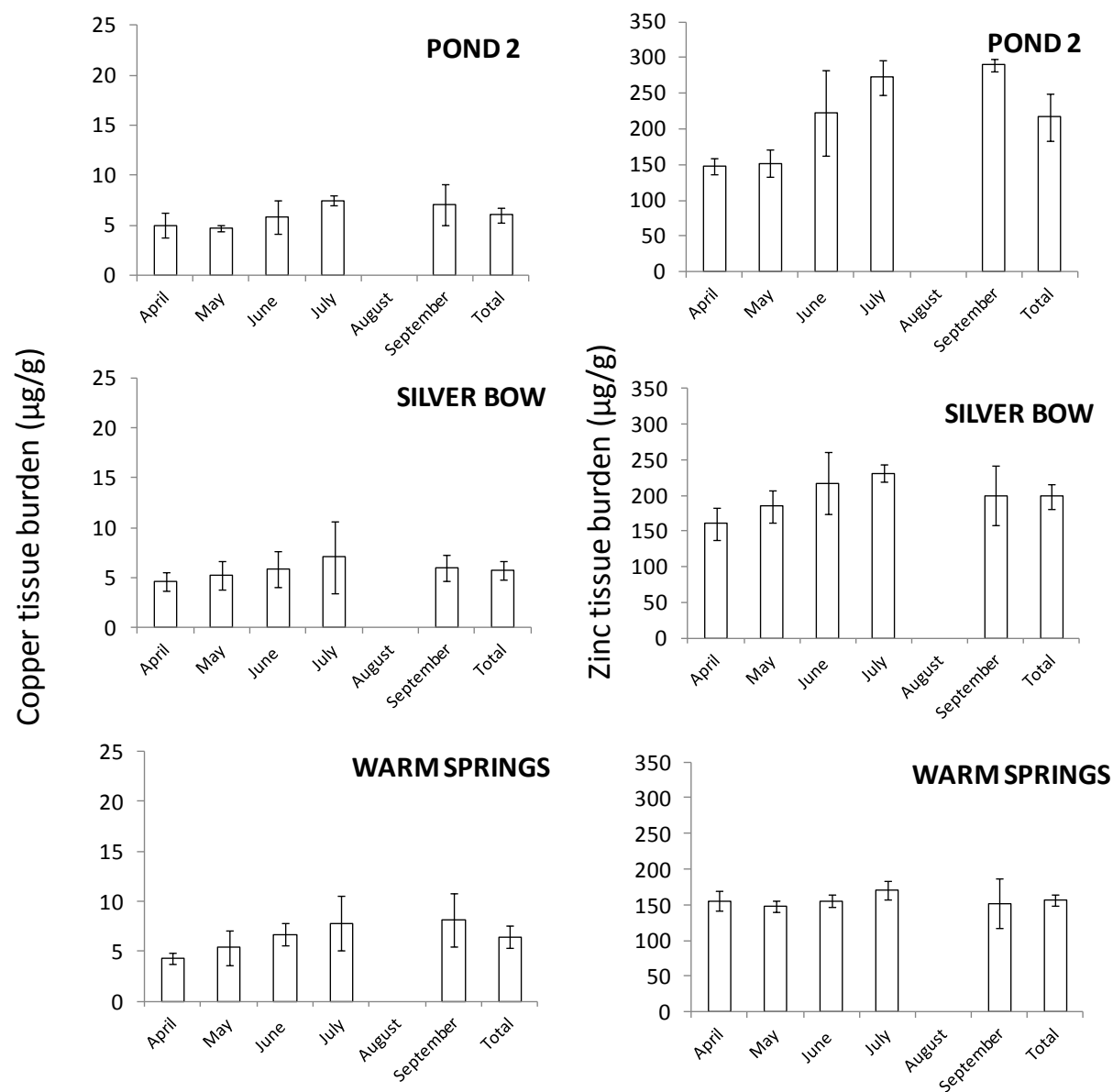


Figure 23a. Mean whole body copper (left panels) and zinc (right panels) tissue burdens for the Pond 2, Silver Bow, and Warm Springs caged fish sites in the Upper Clark Fork River Drainage. Error bars are 95% confidence intervals.

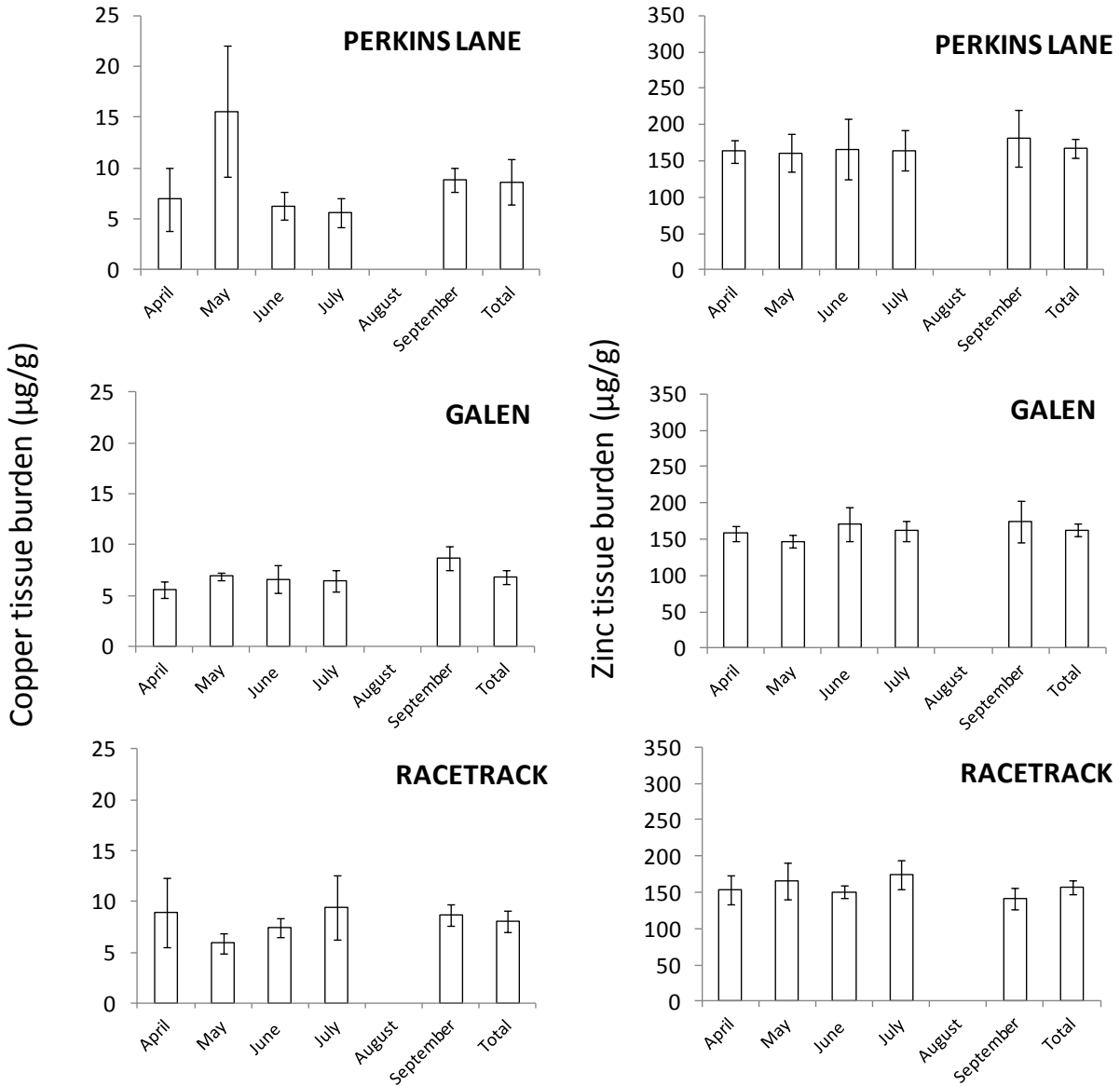


Figure 23b. Mean whole body copper (left panels) and zinc (right panels) tissue burdens for the Perkins Lane, Silver Galen, and Racetrack caged fish sites in the Upper Clark Fork River Drainage. Error bars are 95% confidence intervals.

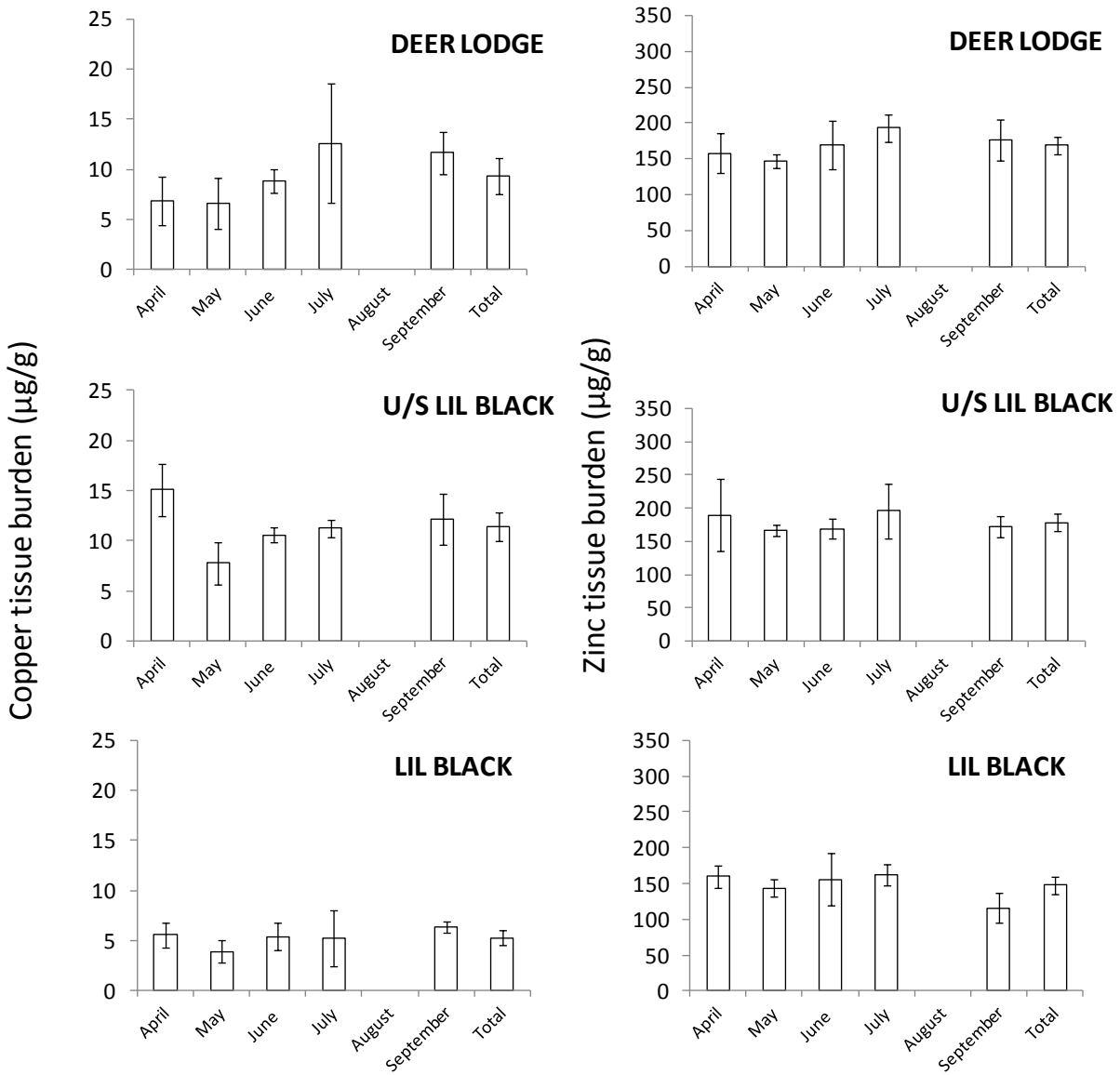


Figure 23c. Mean whole body copper (left panels) and zinc (right panels) tissue burdens for the Deer Lodge, Upstream Lil Black, and Lil Black caged fish sites in the Upper Clark Fork River Drainage. Error bars are 95% confidence intervals.

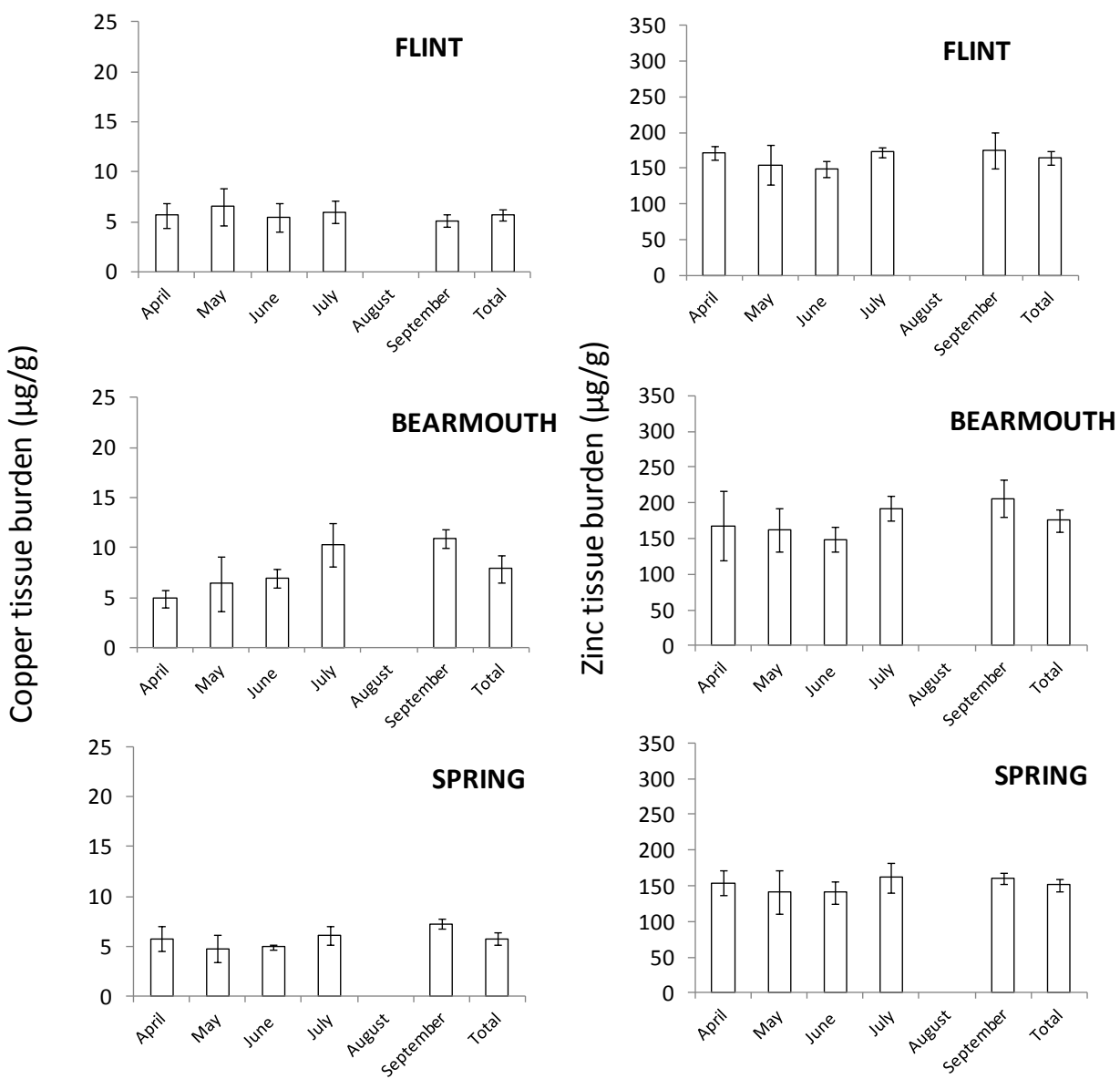


Figure 23d. Mean whole body copper (left panels) and zinc (right panels) tissue burdens for the Flint, Bearmouth, and Spring caged fish sites in the Upper Clark Fork River Drainage. Error bars are 95% confidence intervals.

Tributary vs. Mainstem

For the purposes of the analysis between control tributaries and mainstem treatment sites, Clinton Spring was not included as a control site. For both copper and zinc, tributary sites had significantly lower tissue burdens than mainstem sites and greater tissue burdens than the hatchery controls (Figure 25). The difference in tissue burdens between mainstem and tributary sites was greatest in September for both metals.

Upstream Construction vs. Downstream Construction

For the purposes of the analysis, sites located above and below the Phase 5 and 6 construction area near Galen, Montana were compared. The Galen site was considered above the construction area and the Racetrack site was considered downstream of the construction. The tributary sites were analyzed separately. Generally, upstream sites were found to have lower copper tissue burdens than downstream sites (Figure 26). There were greater differences in copper tissue burdens between upstream sites and downstream sites than zinc tissue burdens (Figure 26).

Annual Comparisons

The number of years with metals tissue burden and fish survival data varied between sites (Figures 27-28). Pond 2, Perkins Lane, Deer Lodge, U/S Lil Black, Lil Black, Flint, and Spring were sampled all four years for tissue burdens and survival. Bearmouth and Turah were sampled for three years. The remaining sites were sampled for fewer than two years. There was generally more variation in metal tissue burdens between sites than between years at a site. The tributary sites (Flint and Lil Black) consistently had lower copper tissue burdens than most mainstem sites. Deer Lodge and U/S Lil Black tended to have higher copper tissue burdens than other sites over the four years of caged fish studies.

The Spring control site consistently had high survival in each year of caged fish studies (Table 12). Deer Lodge had relatively consistent survival from year to year averaging 90% (range 89-91%). Tributary sites (Flint and Lil Black) had inconsistent survival from year to year. The Pond 2 site had the lowest survival of all sites in the 2012 and 2013 studies and the second lowest survival in the 2014 study. Other sites had inconsistent survival from year to year or lacked enough survival estimates to make conclusions about temporal trends.

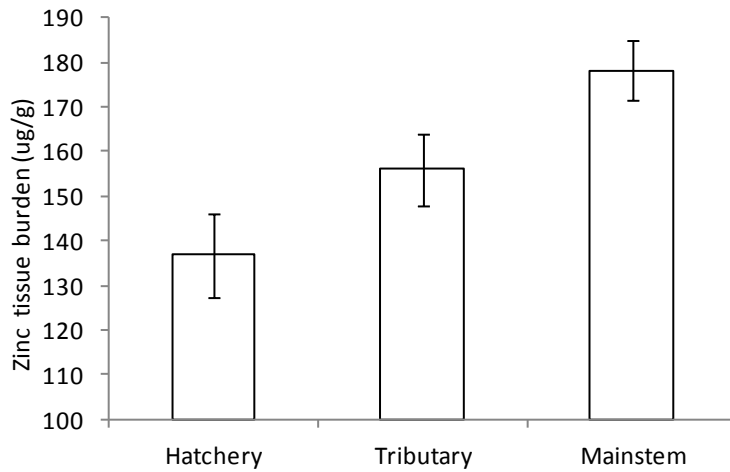
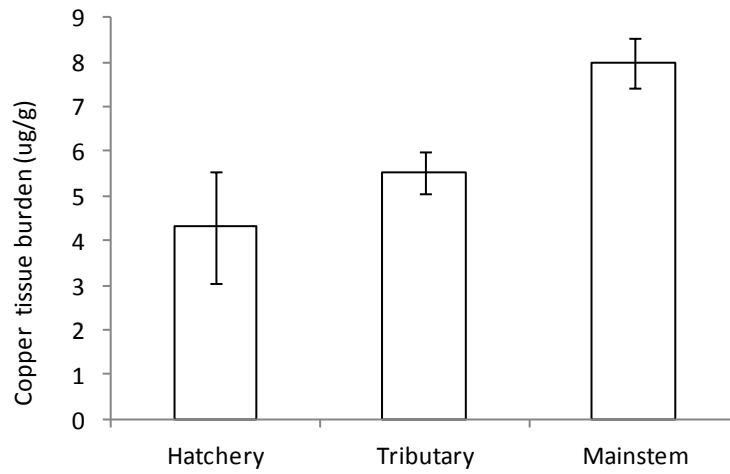


Figure 24. Comparisons between copper and zinc tissue burdens in Brown Trout collected immediately from the hatchery, from cages in tributary sites, and cages in mainstem sites. Error bars are 95% confidence intervals.

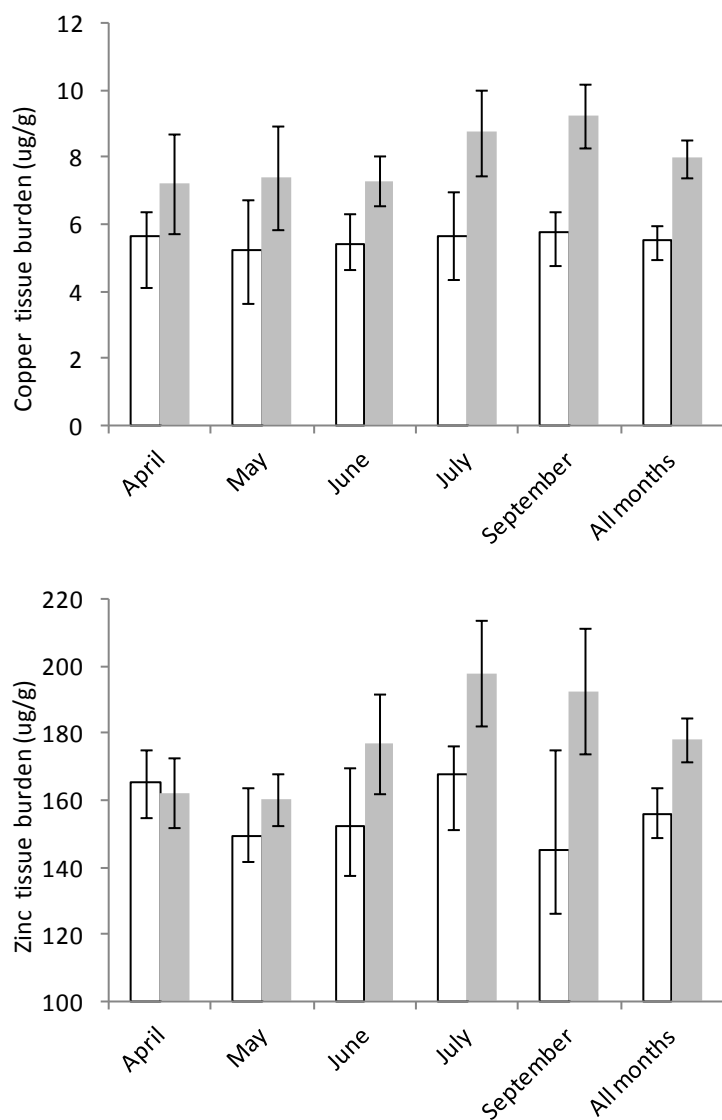


Figure 25. Comparisons between tissue metals burdens of fish from tributary (white bars) and mainstem (grey bars) sites. Error bars are 95 % confidence intervals.

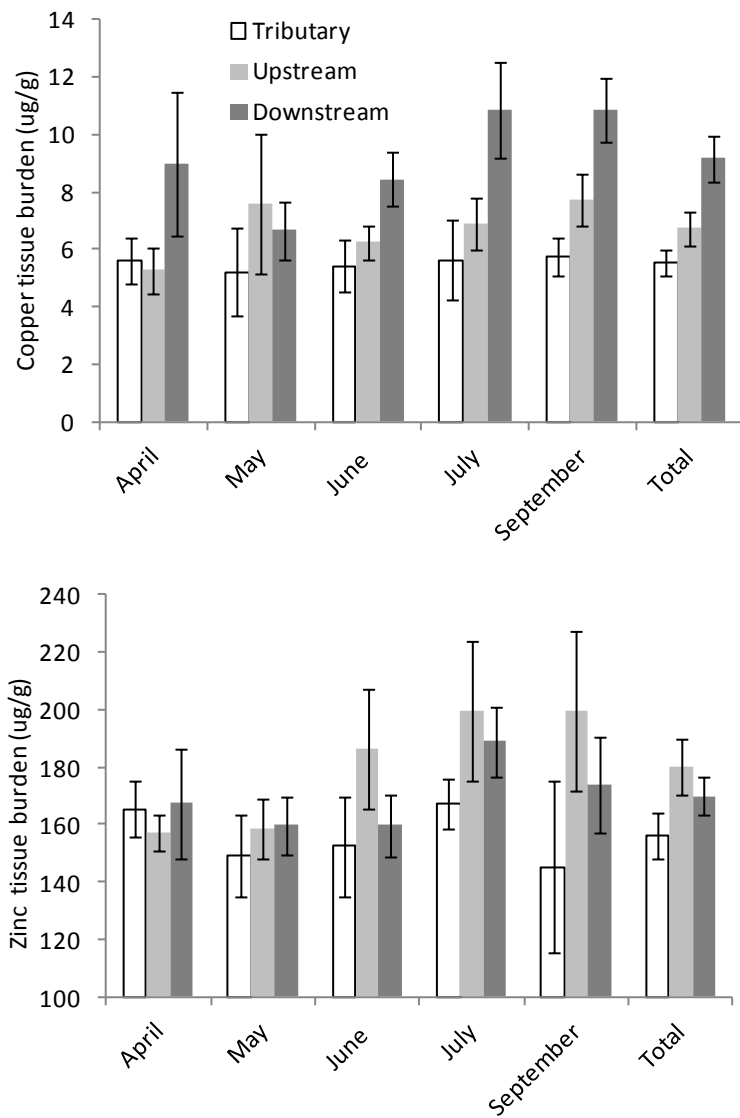


Figure 26. Comparisons between tissue metals burdens of fish from sites upstream of construction and downstream of construction. Error bars are 95 % confidence intervals.

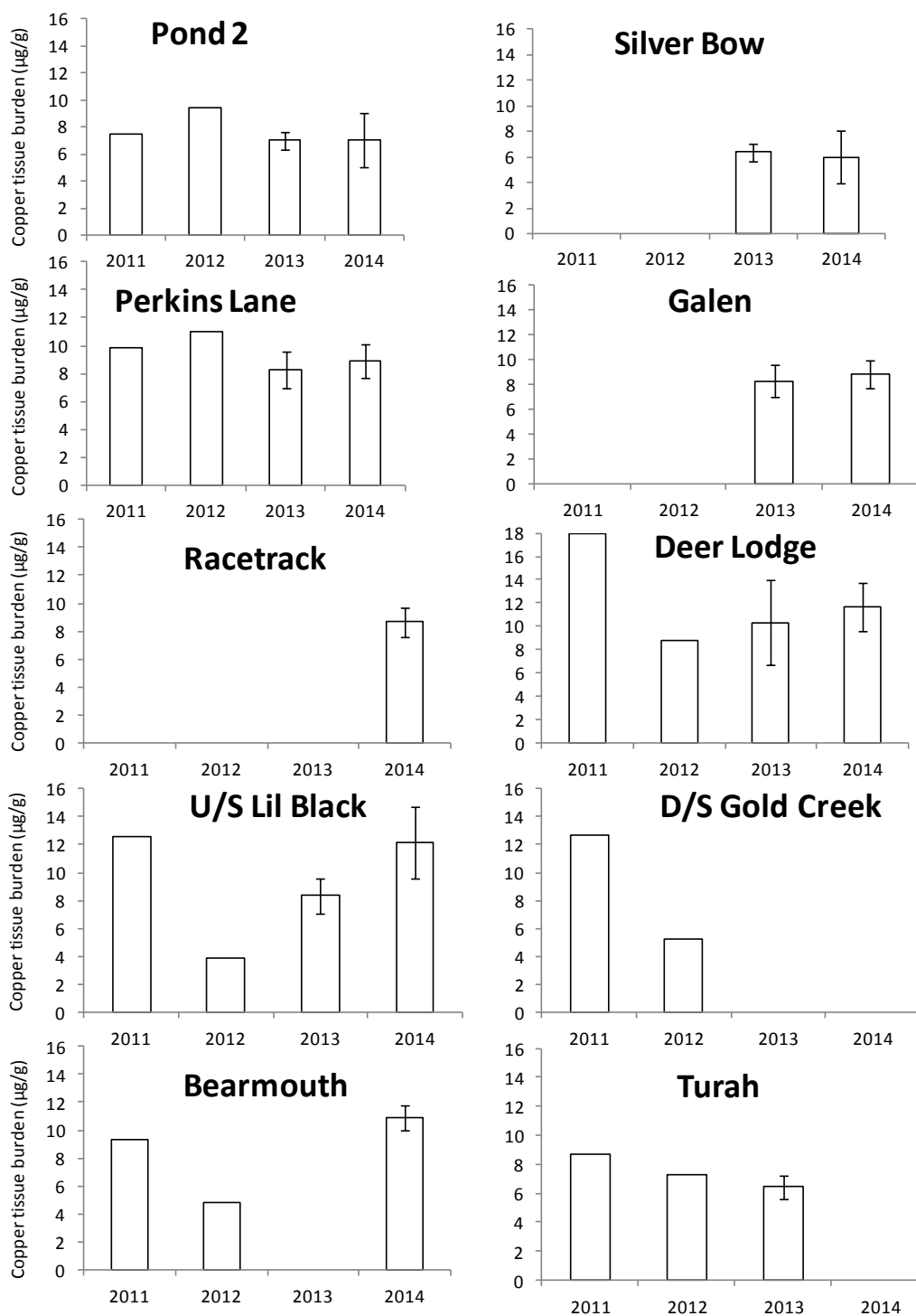


Figure 27a. Annual mean whole body Brown Trout copper tissue burdens for fish collected at the end of the season from fish cages at mainstream sites in the Upper Clark Fork River Basin, 2011-2014. Location of fish cage sites was dependent on the year; not all sites were sampled each year. Error bars are 95% confidence intervals. Fish samples were combined into composites for tissue burden analysis in 2011 and 2012, so error bars are not available for those years. In 2013 and 2014, individual fish were submitted for tissue burden analysis.

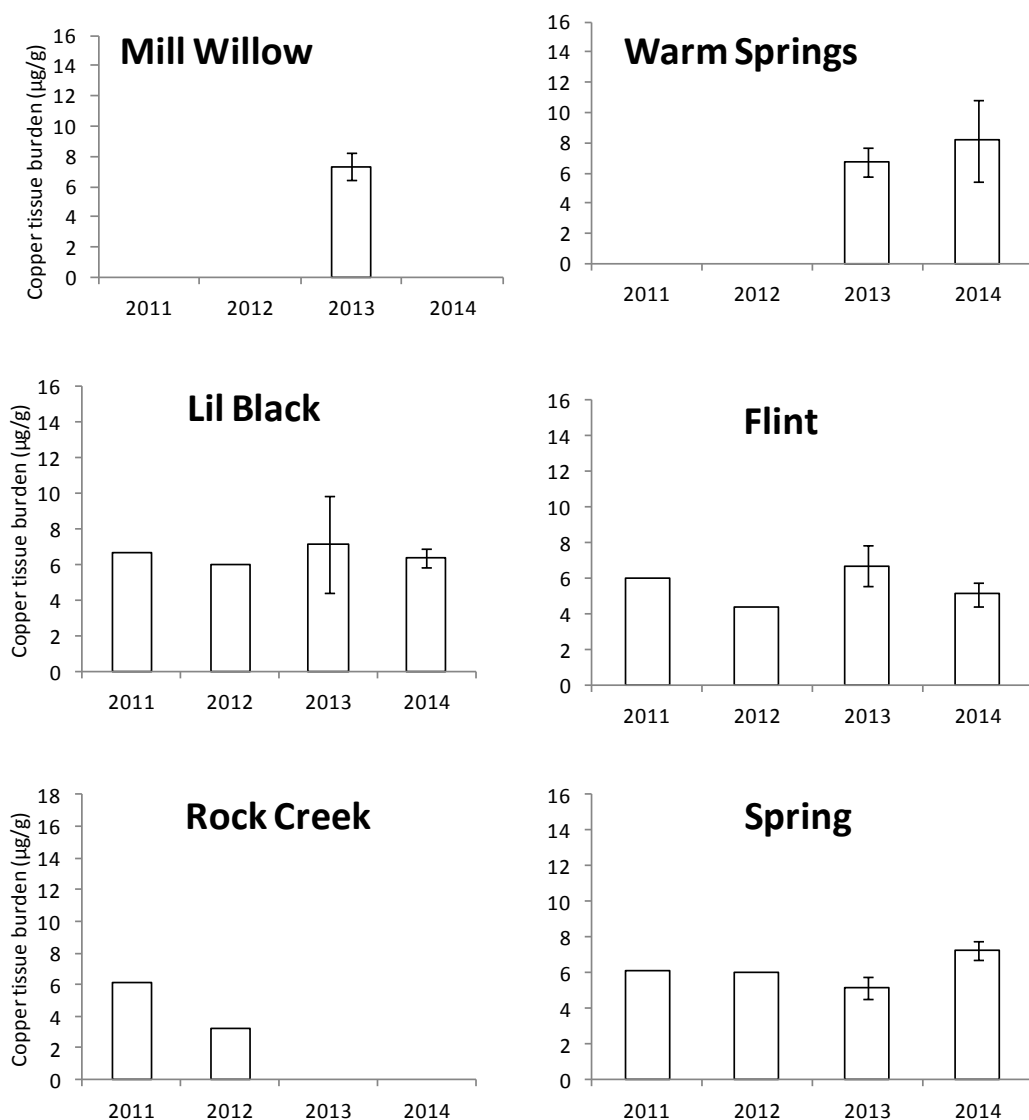


Figure 27b. Annual mean whole body Brown Trout copper tissue burdens for fish collected at the end of the season from fish cages in tributary sites in the Upper Clark Fork River Basin, 2011-2014. Location of fish cage sites was dependent on the year; not all sites were sampled each year. Error bars are 95% confidence intervals. Fish samples were combined into composites for tissue burden analysis in 2011 and 2012, so error bars are not available for those years. In 2013 and 2014, individual fish were submitted for tissue burden analysis.

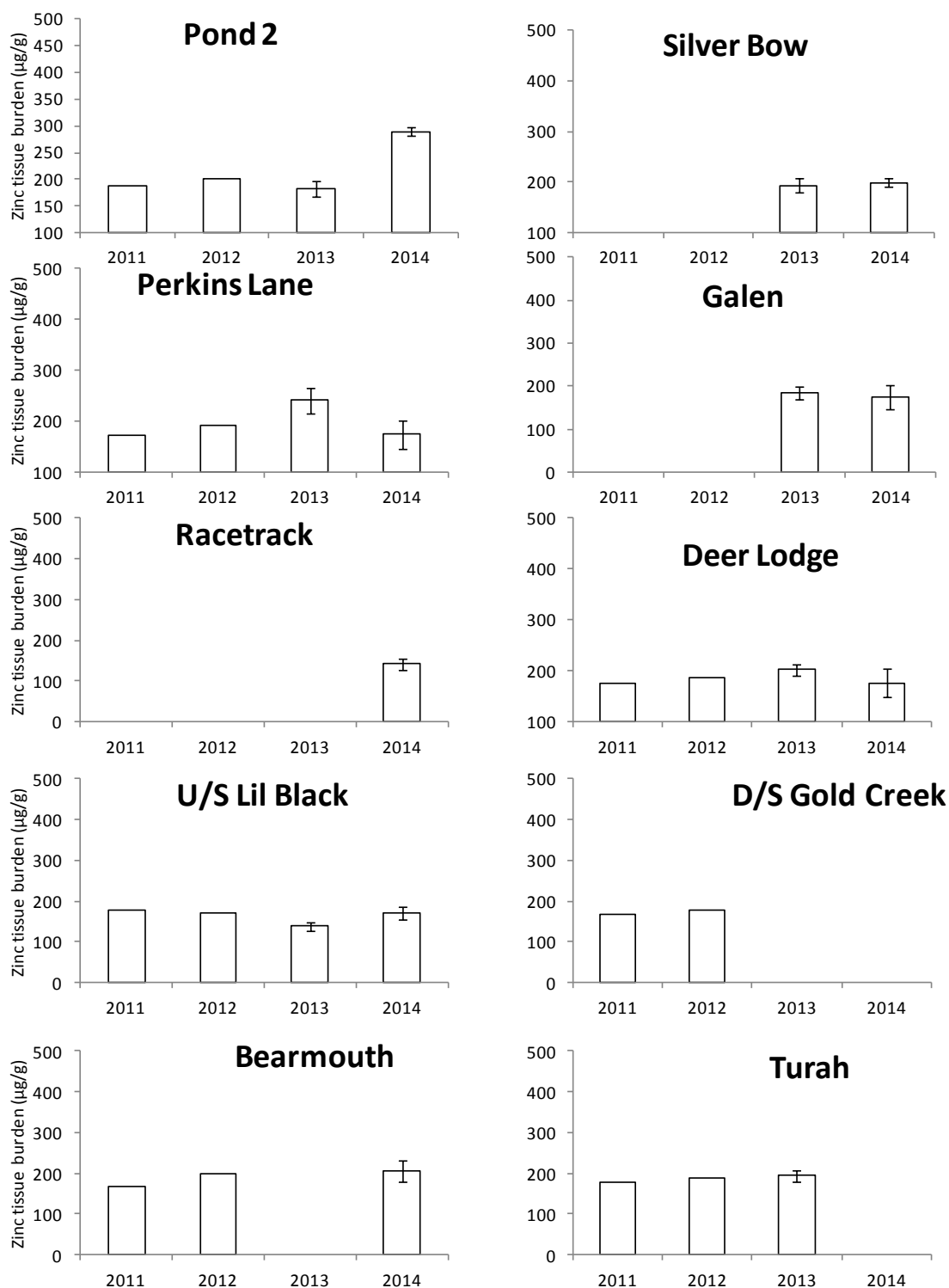


Figure 28a. Annual mean whole body Brown Trout zinc tissue burdens for fish collected at the end of the season from fish cages at mainstem sites in the Upper Clark Fork River Basin, 2011-2014. Location of fish cage sites was dependent on the year; not all sites were sampled each year. Error bars are 95% confidence intervals. Fish samples were combined into composites for tissue burden analysis in 2011 and 2012, so error bars are not available for those years. In 2013 and 2014, individual fish were submitted for tissue burden analysis.

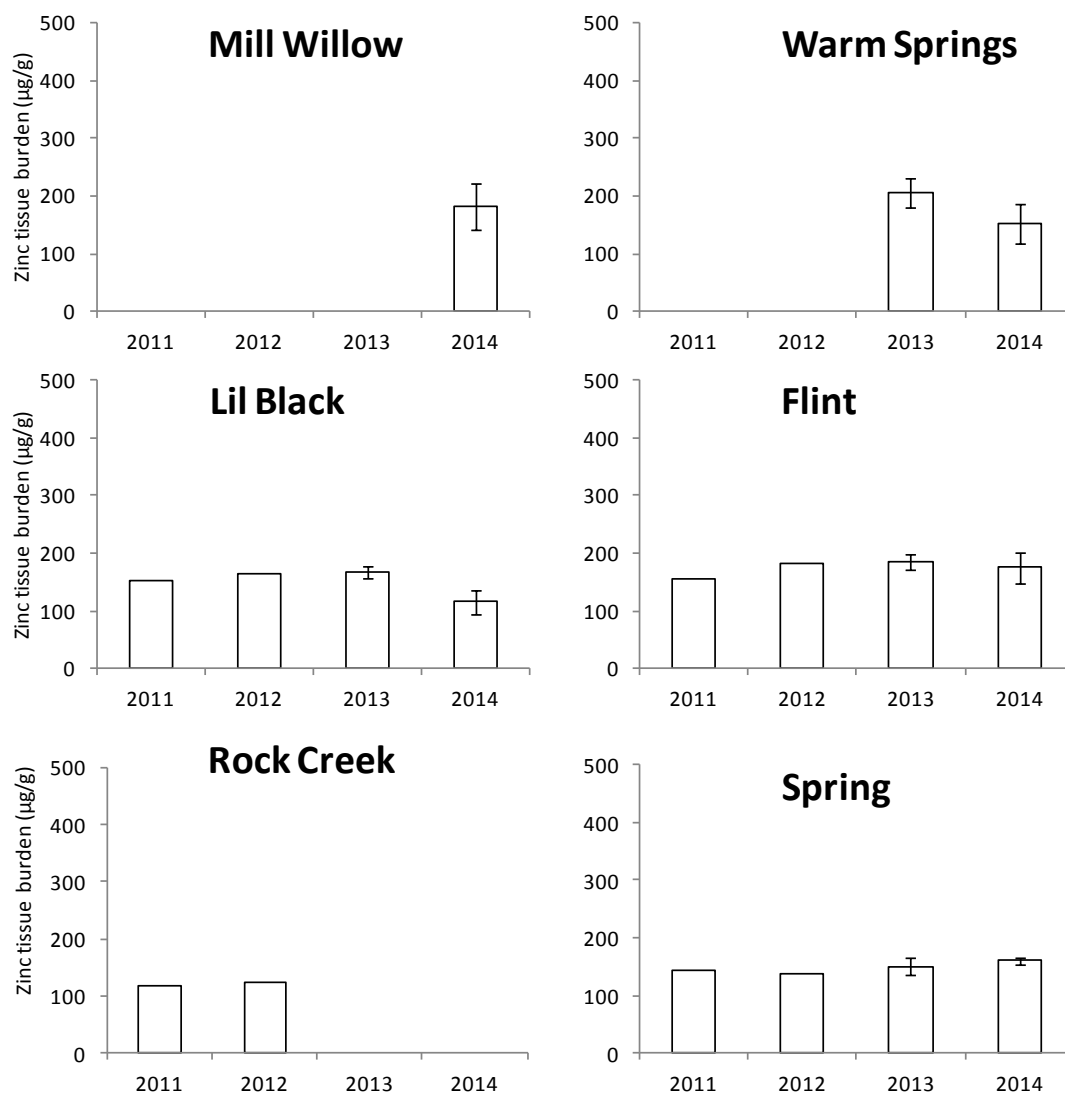


Figure 28b. Annual mean whole body Brown Trout zinc tissue burdens for fish collected at the end of the season from fish cages at tributary sites in the Upper Clark Fork River Basin, 2011-2014. Location of fish cage sites was dependent on the year; not all sites were sampled each year. Error bars are 95% confidence intervals. Fish samples were combined into composites for tissue burden analysis in 2011 and 2012, so error bars are not available for those years. In 2013 and 2014, individual fish were submitted for tissue burden analysis.

Table 12. Mean annual survival at in caged fish studies conducted in the Upper Clark Fork Drainage, 2011-2014.

Site	2011	2012	2013	2014	Mean by site	<i>SD</i>
Turah	69	89	94		84.0	13.2
Spring	100	100	88	95	95.8	5.7
Bearmouth	100	88		70	86.0	15.1
Rock Creek	86	89			87.5	2.1
Flint	93	88	68	72	80.3	12.1
Gold Creek	100	89			94.5	7.8
Lil Black	88	91	75	89	85.8	7.3
U/S Lil Black	89	83	93	44	77.3	22.5
Deer Lodge	89	91	89	90	89.8	1.0
Racetrack				88	88.0	
Galen				86	86.0	
Perkins Lane	73	83	82	85	80.8	
Mill Willow			89		89.0	
Warm Springs			83	60	71.5	16.3
Silver Bow			83	50	66.5	23.3
Pond 2*	96	78	58	51	70.8	20.4
Mean by year	89.4	88.1	82.0	73.3		
<i>SD</i>	10.5	5.6	11.1	18.1		

*The Pond 2 site was referred to as “Warm Springs” in previous years (Richards et al 2013). The Warm Springs site in this study refers to a site in Warm Springs Creek near the confluence with Silver Bow Creek.

Water Contaminants

Chronic freshwater ALS values for metals in surface water are evaluated based upon the analysis of samples following a total recoverable method (MTDEQ 2012b); therefore discussion of water sampling results will focus on total recoverable levels. Ammonia nitrogen ($\text{NH}_3\text{-N}$) was only detected at four sites during two days in March. On March 18, 2014 (prior to fish cage deployment) concentrations of $\text{NH}_3\text{-N}$ were 1.08 and 0.11 mg/L at Silver Bow and Perkins Lane, respectively. On March 19, 2014 concentrations of $\text{NH}_3\text{-N}$ were 0.06 mg/L at both Racetrack and Deer Lodge.

Total recoverable concentrations of arsenic did not exceed the chronic ALS in any water sample collected at caged fish sites in 2014 (Figure 30). Across all sites, the highest concentrations of arsenic occurred at Pond 2 (mean = 0.030 mg/L, SD = 0.016) followed by the Silver Bow site (mean = 0.025 mg/L, SD = 0.008). Arsenic concentrations were lowest at Spring (mean = 0.001 mg/L, SD = 0.001), followed by the tributary sites at Lil Black (mean = 0.005 mg/L, SD = 0.001), Warm Springs (mean = 0.007 mg/L, SD = 0.001), and Flint (mean = 0.011 mg/L, SD = 0.002).

The cadmium chronic ALS was exceeded at the Pond 2 site on April 21, 2014 (Figure 31), and nearly exceeded at Silver Bow, Perkins Lane, and Galen on the same date. The site at U/S Lil Black had a near exceedance event on July 21, 2014. U/S Lil Black had the highest average cadmium concentration (mean = 0.0006 mg/L, SD = 0.0010) while the non-mainstem sites (Lil Black, Spring, Flint, and Warm Springs) had the lowest concentrations (means < 0.0002 mg/L, SD < 0.0002).

The chronic ALS for copper was exceeded at least once during the 2014 caged fish study at all sites except Lil Black and Spring (Figure 32). The chronic copper ALS was exceeded in all eight samples taken at Deer Lodge and all seven samples taken at U/S Lil Black. Mean copper concentrations were highest at U/S Lil Black (mean = 0.047, SD = 0.031) followed by Deer Lodge (mean = 0.043 mg/L, SD = 0.022). Copper concentrations were lowest at the non-mainstem sites (means 0.001-0.009 mg/L, SD = 0.001-0.004).

Chronic lead ALS values were exceeded at least once at the Deer Lodge, U/S Lil Black, and Bearmouth mainstem sites as well as the Flint tributary site (Figure 33). Lead concentrations were highest on average at U/S Lil Black (mean = 0.006 mg/L, SD = 0.005) followed by Deer Lodge (mean = 0.005 mg/L, SD = 0.003). With the exception of the Flint site, the non-mainstem sites tended to have relatively low lead concentrations (means < 0.001).

Total recoverable zinc concentrations in 2014 did not exceed the chronic ALS value at any site at any time (Figure 34). Zinc concentrations tended to be relatively high at U/S Lil Black site (mean = 0.042 mg/L, SD = 0.033) and Deer Lodge (mean = 0.036 mg/L, SD = 0.020). Lil Black, Warm Springs, and Spring had the lowest zinc concentrations (means = 0.001-0.012 mg/L, SD = 0.004-0.004).

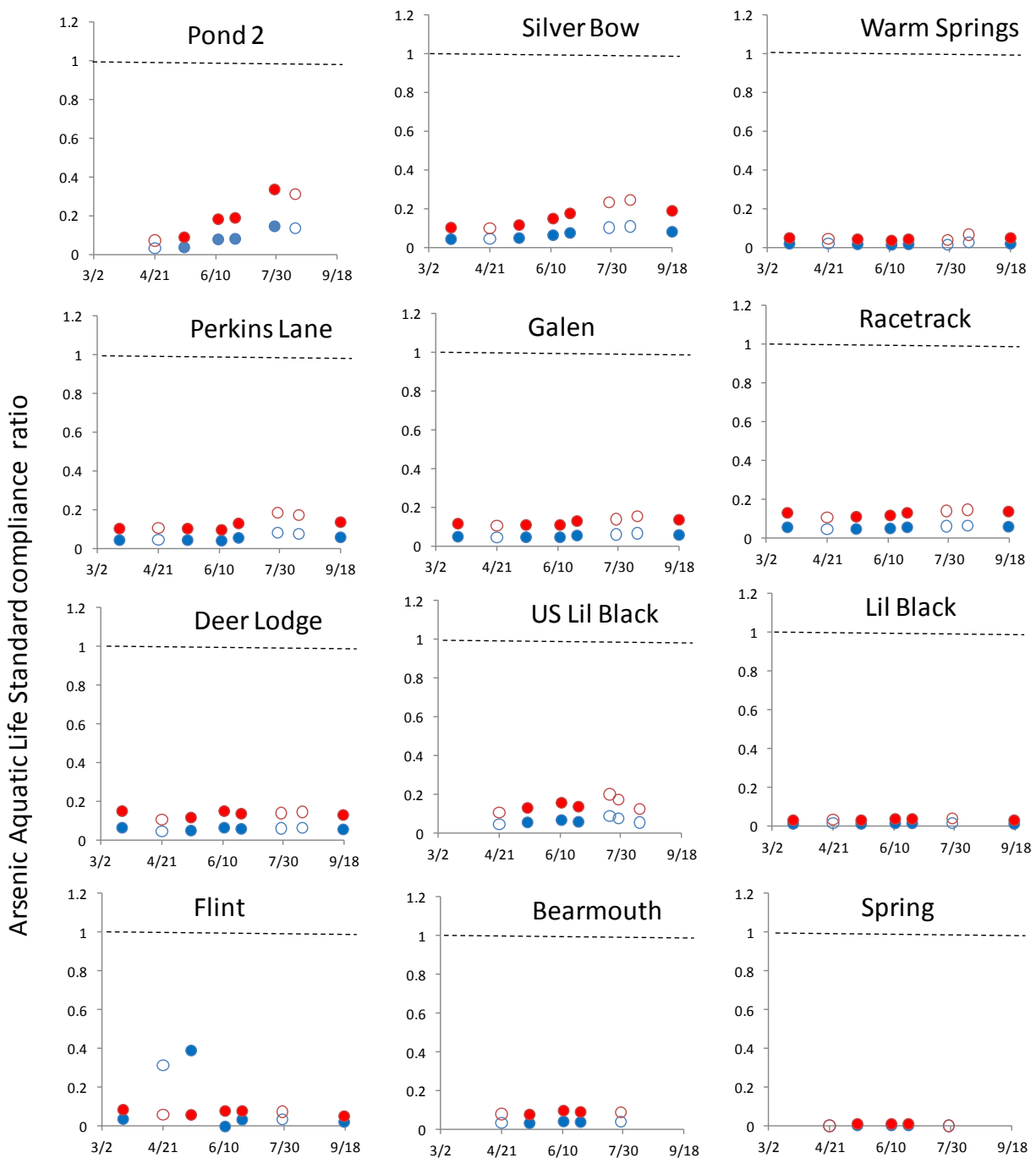


Figure 29. Acute (blue dots) and chronic (red dots) compliance ratios for total recoverable arsenic at the 2014 caged fish sites. Compliance ratios were calculated by dividing the measured arsenic concentration by the Aquatic Life Standard value (MTDEQ 2012b). Water samples collected by MTFWP are depicted by the open dots and samples collected by RESPEC are depicted with solid dots. Compliance ratio values <1 indicate arsenic levels below the aquatic life standard while values >1 indicate levels above the standard.

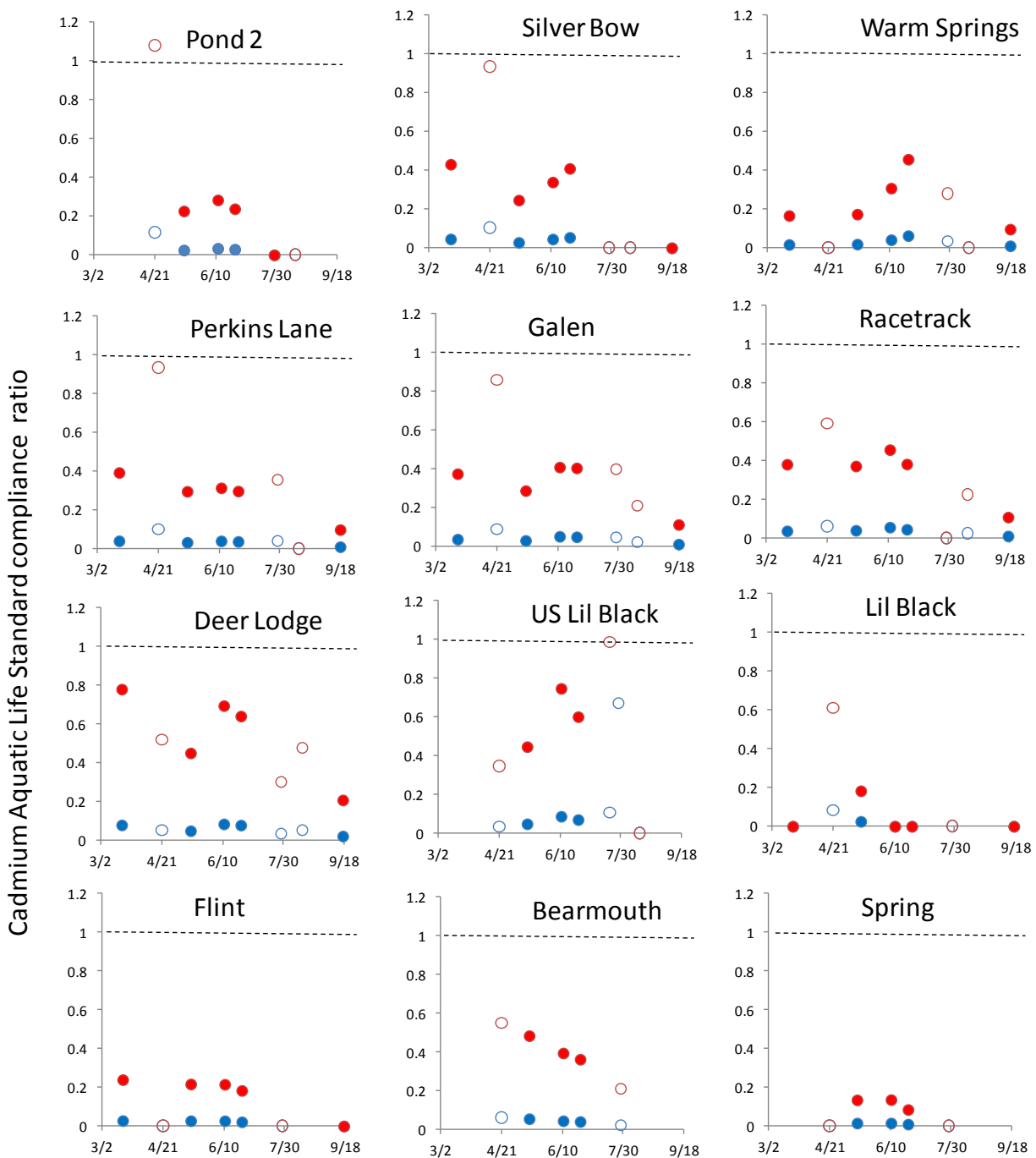


Figure 30. Acute (blue dots) and chronic (red dots) compliance ratios for total recoverable cadmium at the 2014 caged fish sites. Compliance ratios were calculated by dividing the measured cadmium concentration by the Aquatic Life Standard value (MTDEQ 2012b). Water samples collected by MTFWP are depicted by the open dots and samples collected by RESPEC are depicted with solid dots. Compliance ratio values <1 indicate cadmium levels below the aquatic life standard while values >1 indicate levels above the standard.

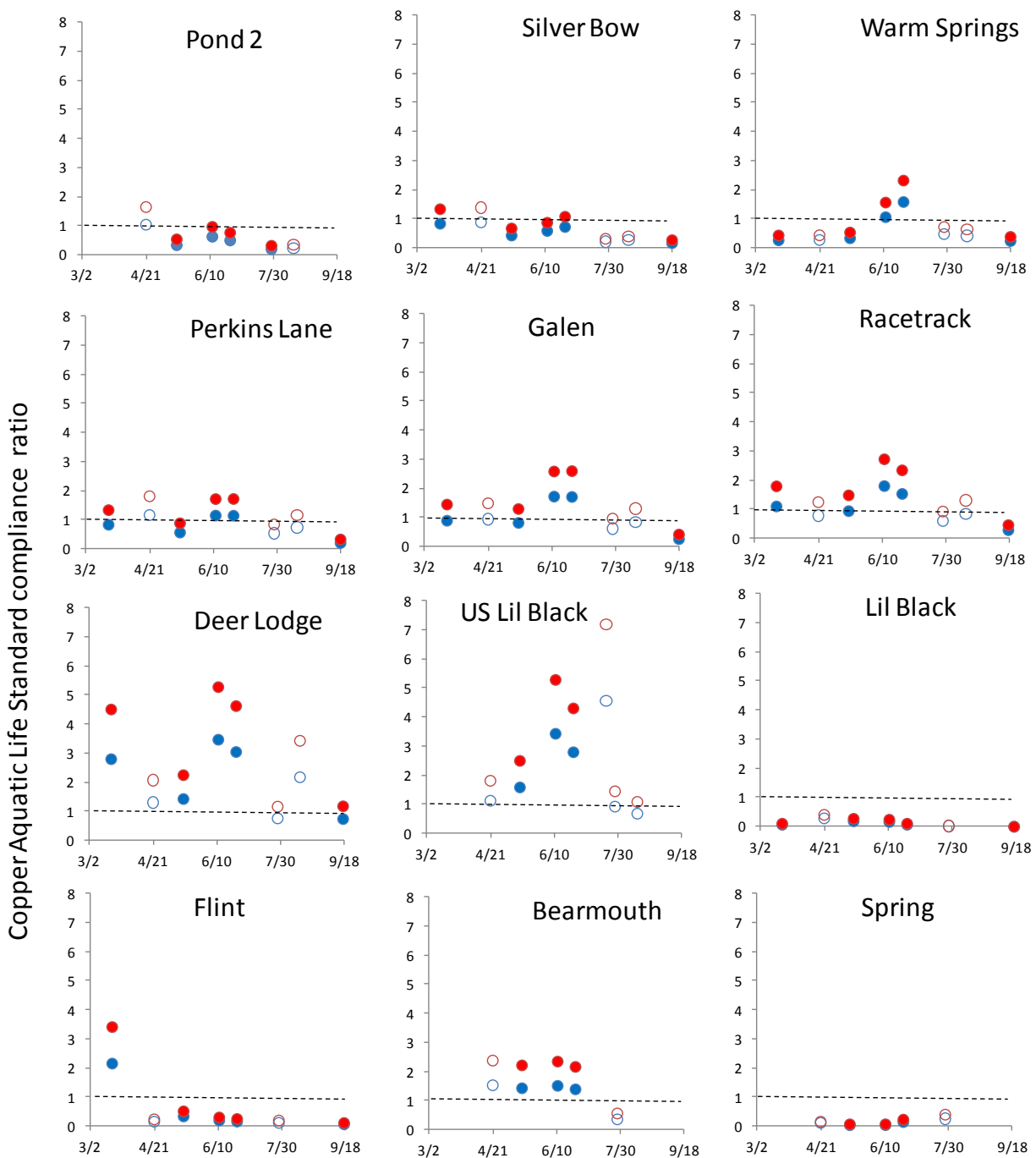


Figure 31. Acute (blue dots) and chronic (red dots) compliance ratios for total recoverable copper at the 2014 caged fish sites. Compliance ratios were calculated by dividing the measured copper concentration by the Aquatic Life Standard value (MTDEQ 2012b). Water samples collected by MTFWP are depicted by the open dots and samples collected by RESPEC are depicted with solid dots. Compliance ratio values <1 indicate copper levels below the aquatic life standard while values >1 indicate levels above the standard.

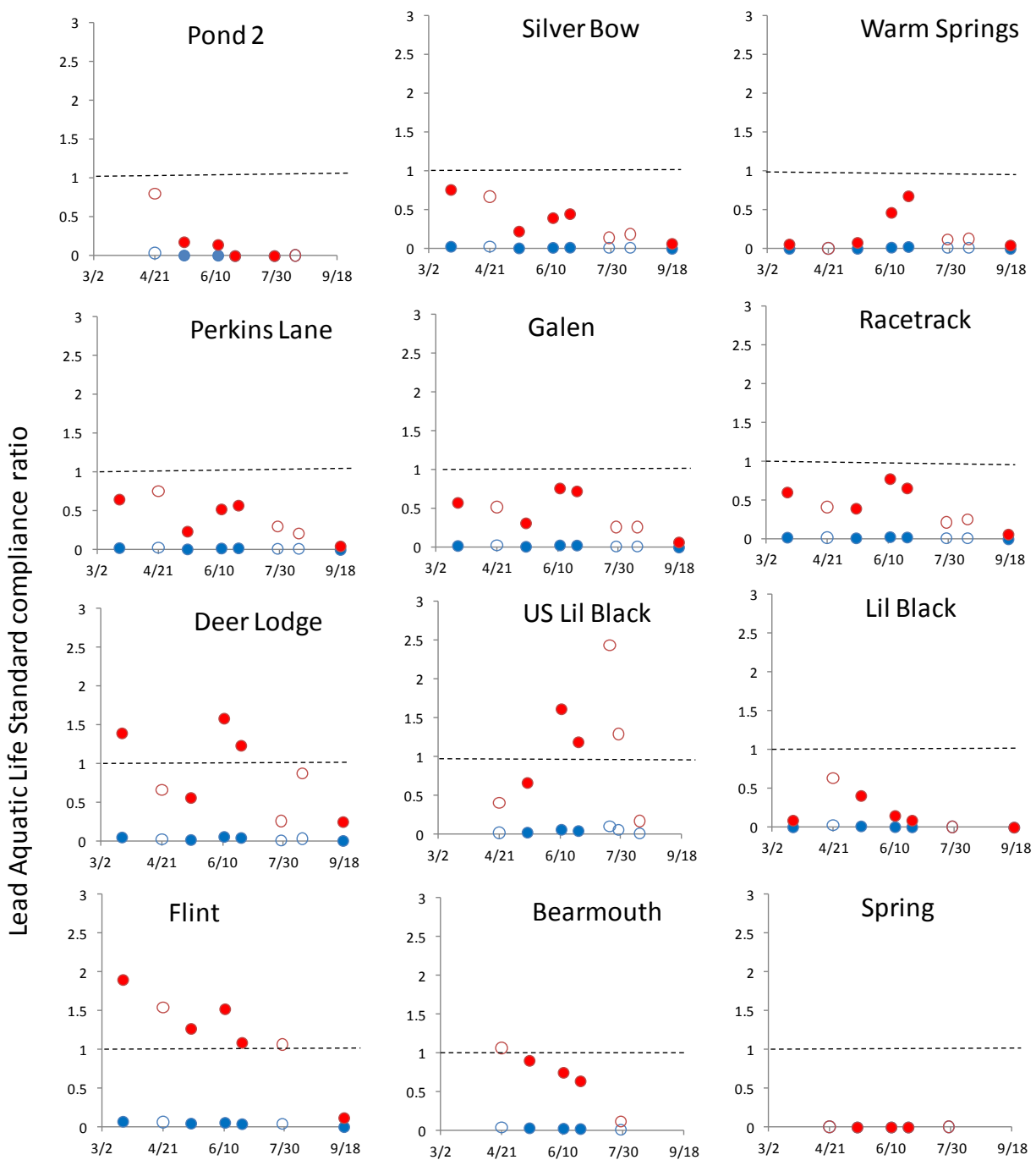


Figure 32. Acute (blue dots) and chronic (red dots) compliance ratios for total recoverable lead at the 2014 caged fish sites. Compliance ratios were calculated by dividing the measured lead concentration by the Aquatic Life Standard value (MTDEQ 2012b). Water samples collected by MTFWP are depicted by the open dots and samples collected by RESPEC are depicted with solid dots. Compliance ratio values <1 indicate lead levels below the aquatic life standard while values >1 indicate levels above the standard.

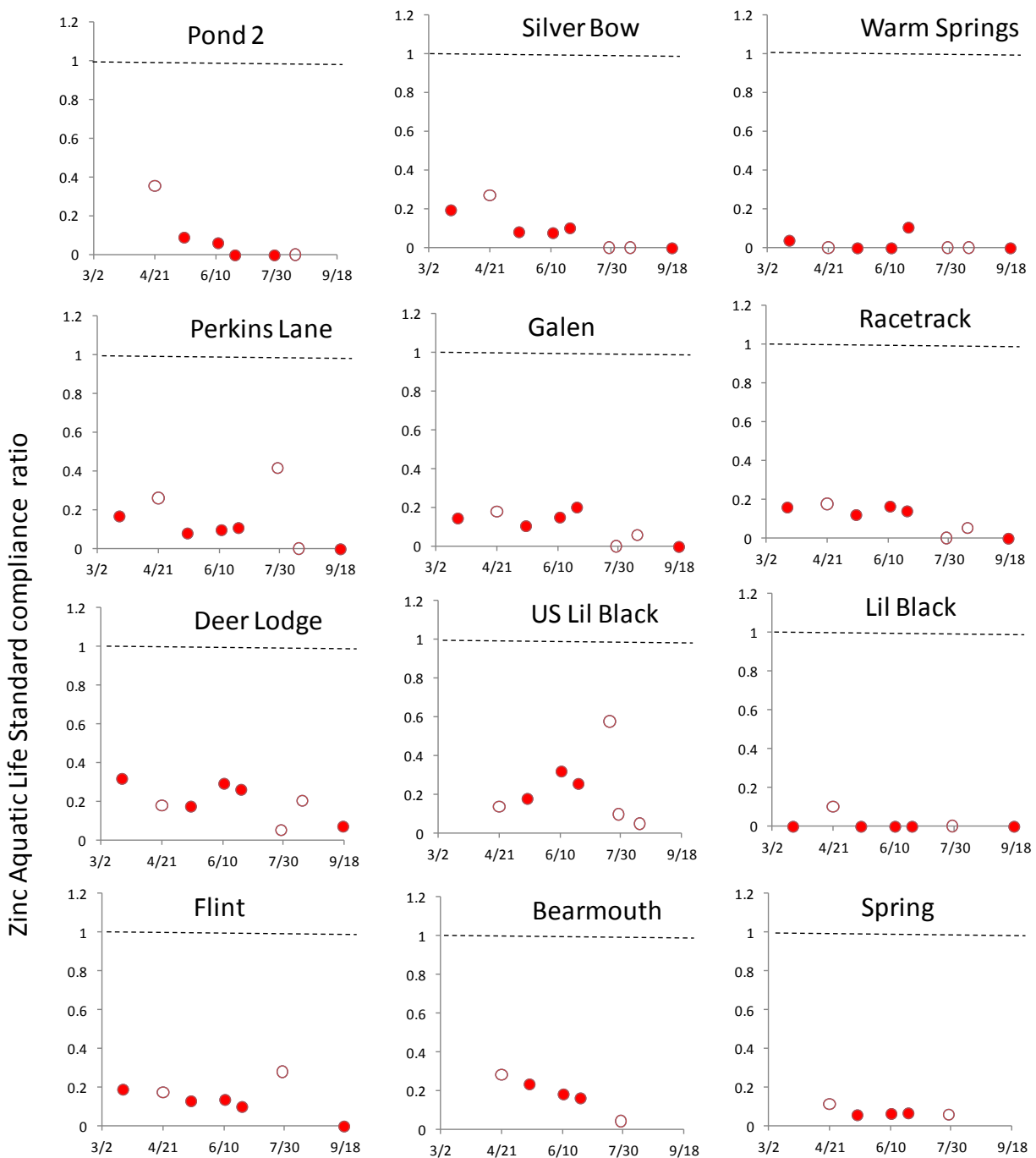


Figure 33. Compliance ratios for total recoverable zinc at the 2014 caged fish sites. Compliance ratios were calculated by dividing the measured zinc concentration by the Aquatic Life Standard value (MTDEQ 2012b). The acute and chronic standards for zinc are identical. Water samples collected by MTFWP are depicted by the open dots and samples collected by RESPEC are depicted with solid dots. Compliance ratio values <1 indicate zinc levels below the aquatic life standard while values >1 indicate levels above the standard.

Water Quality

Water quality parameters were recorded on continuously recording Hydrolab ® MS5 water quality probes at Pond 2, Silver Bow, Galen, Racetrack, and U/S Lil Black in 2014. Due to spurious readings in past years, particularly ammonia readings, the Hydrolab was calibrated several times over the course of the field season. Despite recalibration, abnormal data revealed that the specific conductivity probe and dissolved oxygen sensor at Racetrack, dissolved oxygen sensor at Galen, specific conductivity probe at U/S Lil Black, and ammonia sensor at Pond 2 failed for various length of time in 2014. As a result, spurious data were removed from Figures 35-38.

pH

Elevated pH was observed at the Pond 2 and at Silver Bow sites (Figure 35). Extended exposure to pH > 9 may be harmful to trout (Colt et al. 1979) and results in higher ammonia toxicity (DEQ-7). Mean daily values for pH exceeded 9 in early April, late May, June, July, and August at Pond 2, and at Silver Bow in late June, early July, and much of August. In contrast, mean daily pH at the remaining mainstem sites with probes deployed did not exceed 9 and generally varied from 7.0 to 8.8 (Figure 35), which is considered within the ranges suitable for trout (Colt et al. 1979). For comparison, pH periodically measured with a handheld probe at the tributary sites ranged from 6.6 to 7.9.

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 $\mu\text{S}/\text{cm}$, but may exceed 1000 $\mu\text{S}/\text{cm}$ in polluted waters or waters receiving large quantities of land runoff (Chapman 1996). Mean daily specific conductivities at all sites were within normal ranges in 2014 (Figure 36). Specific conductivities ranged from 95 to 711 $\mu\text{S}/\text{cm}$.

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 never went below this threshold at any site in 2014 (Figure 37). The overall trend in mean daily dissolved oxygen levels was values > 11.0 mg/L at all sites up to mid-April then a decrease to between 8-11 mg/L for the remainder of the study. One exception was the U/S Lil Black site that had mean DO values in late August between 7-8 mg/L.

Total Ammonia

Water ammonia levels were below the detection limit (0.05 mg/L N) in water samples collected by Montana Fish, Wildlife, and Parks and RESPEC during the time period that the Hydrolabs were installed at Pond 2 and Silver Bow. The Hydrolab recorded mean daily ammonia concentrations of 0.17 mg/L at Silver Bow and 1.45 mg/L at Pond 2 on July 28, and 0.17 mg/L at Silver Bow and 2.84 mg/L at Pond 2 on August 14. The reason for the discrepancy between the

Hydrolab and water sample data is likely the result of the ammonia probe not being as reliable as the more common water quality parameters noted above. The precision with which the Hydrolab® MS5 records total ammonia levels has been questionable in the past (T. Selch, Montana, Fish, Wildlife, and Parks, personal communication, 2014). As a result of the questionable reliability of the ammonia sensors, ammonia data as recorded by the Hydrolabs are not presented in this report.

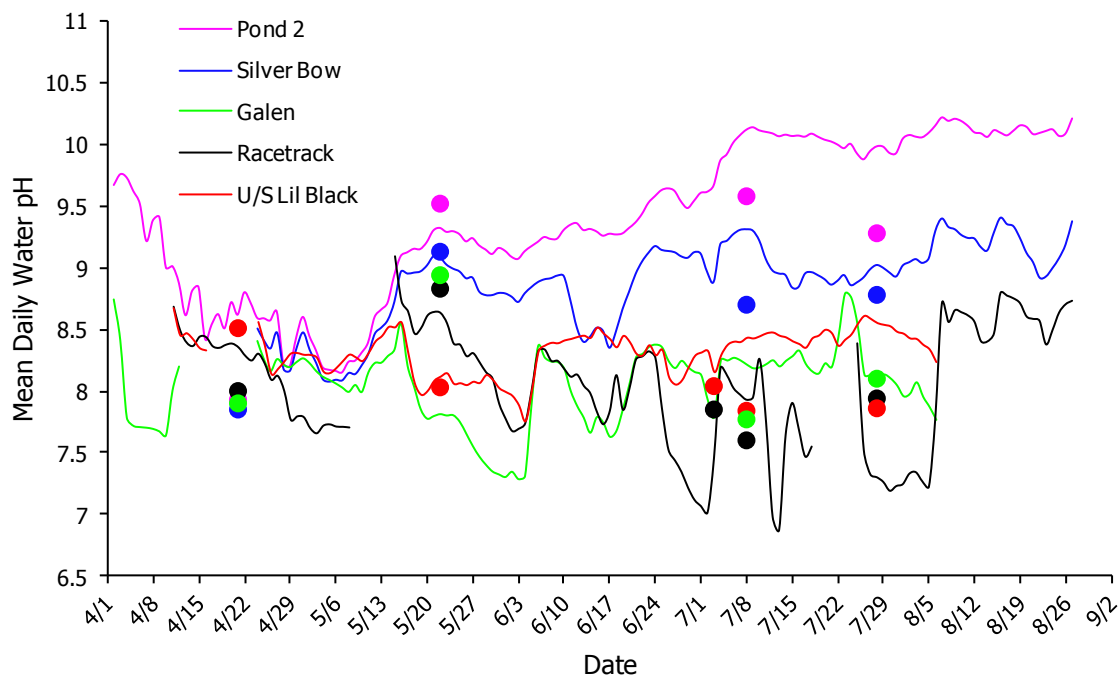


Figure 34. Mean daily water pH at sites with probes deployed in 2014. Lines represent Hydrolab data and circles represent handheld multiprobe data.

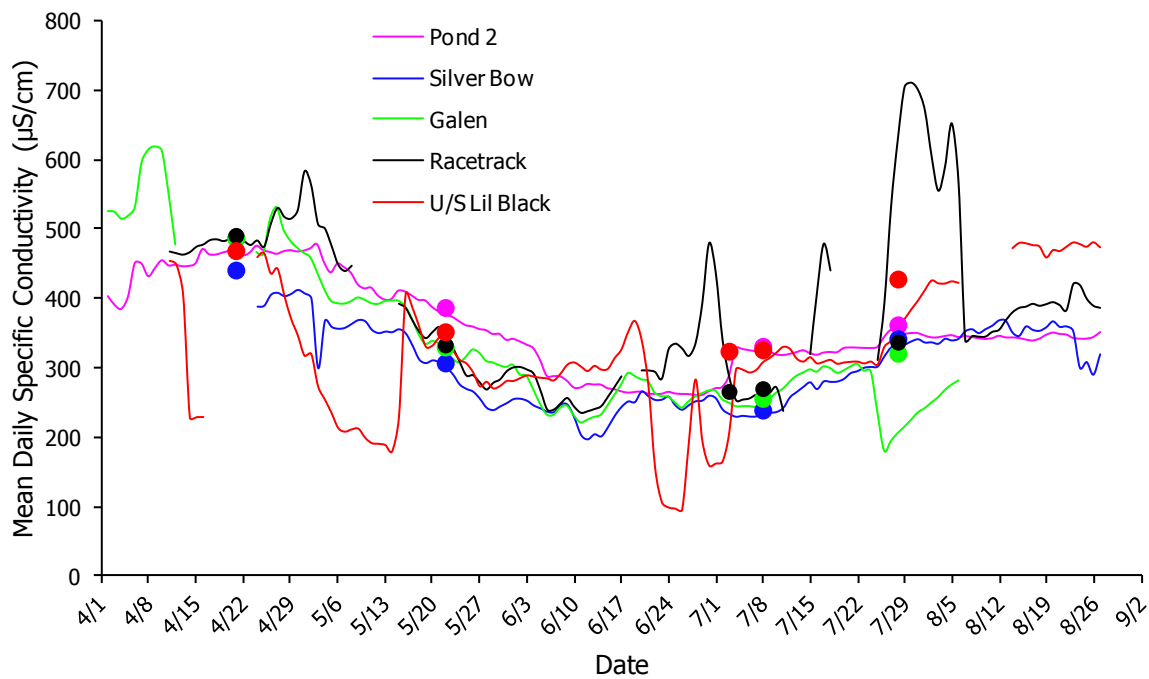


Figure 35. Mean daily specific conductivity at sites with probes deployed in 2014. Lines represent Hydrolab data and circles represent handheld multiprobe data.

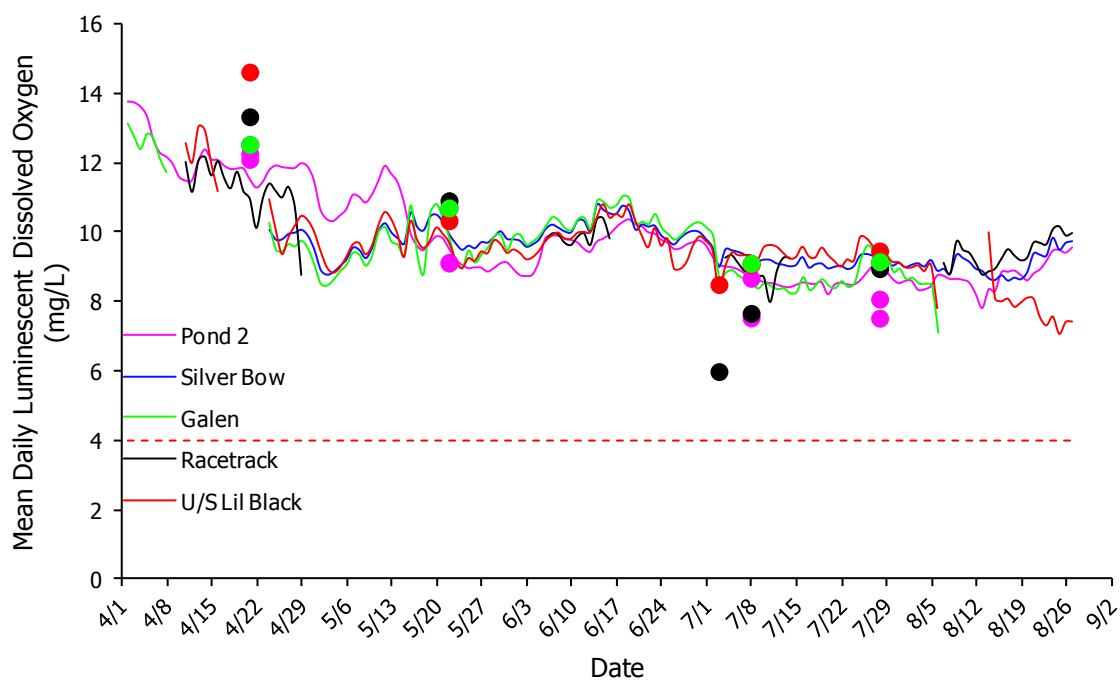


Figure 36. Mean daily luminescent dissolved oxygen at sites with probes deployed in 2014. Lines represent Hydrolab data and circles represent handheld multiprobe data. The red dashed horizontal line denotes the freshwater ALS one day minimum.

Discussion

Trout Population Monitoring

Brown trout population estimates have been generally increasing since 2011 at monitoring sites in the mid- and upper- reaches of the Clark Fork River. Estimates for 2013 and 2014 at the Flint Creek mouth were also slightly higher than previous estimates from this site. The Bearmouth reach consistently supports low numbers of Brown Trout. It is possible that above average discharge in 2011 increased the quality and quantity of Brown Trout spawning and/or rearing habitat in the upper Clark Fork River and tributaries. Based on a telemetry study, most spawning activity in the Upper Clark Fork River drainage takes place in and upstream of the Little Blackfoot River, although a few radio tagged Brown Trout did make spawning related movements into Rock and Flint creeks (Mayfield 2013). There are many potential reasons for low densities of brown trout in the reach between Flint and Rock Creeks (see RESPEC 2014), but the lack of spawning observed in this reach by Mayfield (2013) may indicate that low recruitment into this reach is an issue.

Fish species composition is dependent on the environmental conditions in the water in which fish live. Heavy metal contamination will tend to favor more tolerant fish species and have more negative effects (reduced survival, growth, or reproduction) for sensitive species (Klerks and Levinton 1989). Conversely, as heavy metal contamination in the Upper Clark Fork River is reduced through ongoing remediation efforts, the abundance of sensitive species may increase. There have been numerous studies on the effects of heavy metals on the trout species present in the Upper Clark Fork River, but relatively little is known about how the impacts of heavy metals (or the subsequent cleanup efforts) will affect non-trout species. Therefore, the data collected in 2014 at the two CPUE sections will provide valuable baseline information about the relative abundance of all fish species present in the Clark Fork River.

Results of this study, as in previous studies in the UCFR (Phillips and Spoon 1990; Richards et al 2013; Leon et al. 2014), revealed variation in fish mortality across space and time. Most of the mortality in 2014 in caged fish occurred in April, July, and August. This bimodal pattern is consistent with previous caged fish studies (Richards et al. 2013; Leon et al. 2014) where mortality tended to be highest during spring runoff and on the descending limb of the hydrograph as water temperatures increase. Heavy metal exposure increases in the spring as the concentrations of these metals increase due to the flushing of contaminated soils in the flood plain and river banks (Sando 2014). Also, hatchery fish used in this study may not have enough time to acclimate to high concentrations of metals in the water. This lack of acclimation could significantly increase their susceptibility to the negative effects of substances such as copper (e.g., Dixon and Sprague 1981).

The highest mortality rates did not consistently occur at sites with the highest water temperatures or tissue metals burdens. For example Deer Lodge had relatively high survival, but the site also had high copper tissue burdens and 50 days of maximum water temperatures above 19 °C. The U/S Lil Black site also had high copper tissue burdens, 52 days above 19 °C, and the lowest survival of any site in 2014. The site in Warm Springs Creek had relatively low copper tissue burdens, cooler water temperatures, but low survival in 2014. It is clear that environmental factors in the UCFR interact in complex ways to affect fish survival. As such, site-specific survival has not been a clear-cut measure of water quality in caged fish studies in the UCFR (Leon 2013).

Overall, survival was lower (mortality was higher) in 2014 than in previous years. Across all sites, average survival was 89% in 2011, 88% in 2012, 82% in 2013, and 73% in 2014. The reason for the decreased survival is not entirely clear, but could be related to infections of *Saprolegnia* fungus (*Saprolegnia* sp.). *Saprolegnia* is an opportunistic fish parasite that feeds on diseased flesh of injured, diseased, or stressed fish. *Saprolegnia* is present in most freshwaters and infections are more common during spawning, high water temperatures, or other stressful events. A review of notes from caged fish studies 2011-2014 suggests a possible outbreak of the fungus in 2014. Fungal infections were noted on three Brown Trout mortalities in 2011. There were no noted cases *saprolegnia* in 2012 or 2013. Fourteen cases were noted in 2014. Cases occurred in every month of the 2014 study from April until July, although July alone had 11 cases. The site at the Pond 2 outflow accounted for 5 of the cases, with other sites having one or two. Fungus was noted at sites from Pond 2 downstream to U/S Little Blackfoot, and was not noted at Bearmouth or in any of the tributaries.

High water temperatures and exposure to copper have been shown to reduce trout growth (Woodward et al. 1995a; Marr et al. 1996; Elliot and Hurley 2001). Of all the sites in the 2014 study, the Pond 2 site had the most days with water temperatures above the upper critical threshold of 19 ° C. Based only on water temperature, the fish at the Pond 2 site were predicted to have the lowest growth of any site in this study. Surprisingly, fish at this site displayed the largest increase in weight of any site. The high rate of growth below Pond 2 can be attributed to the “tail water” effect, which results in increased primary and secondary productivity below the ponds. Apparently, food availability has a more significant effect on weight gain than temperature at this site.

Tissue Burdens

Brown Trout used in this study accumulated both copper and zinc in their tissues after they were stocked in cages in both the mainstem Clark Fork River and its tributaries. Tissue burdens of fish straight from the hatchery were low compared to fish sampled from cages in the UCFR drainage. Fish from cages in the mainstem had significantly higher metals burdens compared to fish from tributaries, but the difference was much less for zinc than it was for copper. Higher ratios of copper:zinc in fish tissue in the mainstem versus tributaries is a result consistent with copper:zinc ratios in water sampling conducted in these waters (Leon 2014, Sando 2014, this study).

Copper and zinc tissue burdens of fish collected in tributaries remained relatively stable from month to month over the course of the 2014 study. On the other hand, copper tissue burdens of fish from most mainstem sites appeared to increase over the 2014 field season. Tissue burdens of zinc from Pond 2 and Silver Bow displayed a increasing similar pattern.

From a spatial perspective, copper tissue burdens generally increased upstream to downstream from the Pond 2 site to US Lil Black, an observation consistent with tissue burdens in previous caged fish studies (Leon et al. 2014) and copper concentrations in UCFR water (Sando et al. 2014). Sando et al. (2014) concluded that suspended sediment and copper concentrations are reduced below Warm Springs Ponds by settling and liming operations within the ponds. Our study supports this conclusion and indicates that less copper is being taken up by fish at sites directly below the ponds. While the Warm Springs Ponds do reduce copper concentrations in the section of the Clark Fork River directly downstream, our results suggest

that other water quality factors such as temperature, pH, and ammonia have the potential to negatively affect fisheries downstream. Sando et al. (2013) identified the reach from Galen to Deer Lodge as a major source of additional copper and suspended sediment to the Clark Fork River, a conclusion supported by the increase in copper tissue burdens from the Galen to Deer Lodge sites in this study. The decrease in copper tissue burdens in the Clark Fork River downstream of the Little Blackfoot River indicate that flow from the Little Blackfoot River is important for diluting contaminants and improving water quality.

Comparisons of tissue burdens at sites that were sampled in multiple years indicated relatively consistent values between years. For instance Deer Lodge and U/S Lil Black tended to have high copper tissue burdens from year to year compared to other sites. Pond 2 had copper tissue burdens from year to year that were relatively low compared to other mainstem sites. The Lil Blackfoot site had consistently low copper burdens, whereas the other tributary site in Flint Creek, was more variable from year to year. The Spring control had consistently the lowest copper tissue burdens of all the sites. For zinc, the Spring and Lil Blackfoot sites had consistently low tissue burdens from year to year. Based on the two years that it was sampled, Rock Creek also displayed low tissue burdens. Other sites tended to be more variable in zinc tissue burdens from year to year. Differences in zinc tissue burdens between fish from mainstem and tributary sites were not as apparent as the difference of copper tissue burdens between tributaries and the mainstem.

The consistency in copper tissue burdens from year to year is informative in several ways. First, the technique used to determine tissue metals burdens in this study is repeatable from year to year. Second, sites such as Deer Lodge and U/S Lil Black suggest that the fish in the reach of the Clark Fork River immediately upstream of the Little Blackfoot have the highest potential to be impacted by copper contamination. This conclusion is consistent with concentrations of metals in water samples (Leon 2014, Sando 2014, this study). Thirdly, reductions in copper tissue burdens following remediation efforts initiated in 2013 are not yet apparent. As remediation efforts continue and remediated sites become revegetated, significant declines in tissue burdens will hopefully become apparent.

Water Contaminants

High pH was observed for much of the study period at the Pond 2 and Silver Bow sites. Liming operations in the Warm Springs Ponds are designed to reduce toxicity of copper, zinc, lead and other cationic metals. However, waters with pH above 9 are considered harmful to trout (Colt 1979). High pH also causes relatively harmless ammonium (NH_4) to convert to highly toxic ammonia (NH_3) at very low concentrations ($< 0.885 \text{ mg/L}$). As measured by a continuously logging Hydrolab, ammonia reached highly toxic levels in July and August at Pond 2. However, these values were not supported by periodic water sampling conducted at the site. This discrepancy, coupled with the fact that most caged fish survived through July and August suggest an error in instrumentation occurred. Pond 2 is thought to discharge ammonia when the pond mixes after ice out in March. Water sampling indicates that a pulse of ammonia occurred at the Pond 2 outflow in mid-March of 2014, but this pulse occurred before the caged fish study was initiated for the season.

Periodic water sampling of heavy metal concentrations demonstrated exceedances of the copper ALS at all mainstem sites. Overall, there were more exceedances of copper ALSs than

any other contaminant measured in this study. Lack of exceedances of arsenic and zinc are consistent with sampling done in previous years (Leon et al. 2014). Of all metals measured in this study, copper is present in the Clark Fork River at the highest concentrations relative to its toxicity. The fact that no zinc exceedances were documented in water sampling is interesting considering the elevated levels of zinc in fish tissues. Because zinc is an essential nutrient, it is commonly added to commercial hatchery fish pellets. It is possible that fish in this study obtained at least some of their whole body zinc concentrations from the hatchery food that we used.

Conclusion

Caged fish studies have provided valuable data on fish survival and tissue burdens. These data can be used as baselines to evaluate the efficacy of remediation efforts in the future. For example, post-remediation monitoring may reveal reduced tissue metals burdens and fish mortality as well as changes in the spatial pattern of tissue burdens and water contaminants. Caged fish studies have also highlighted the complex interactions of multiple factors that affect survival of young Brown Trout in the UCFR.

Because sufficient baseline data has been collected, caged fish studies in the next few years will shift to focusing specifically on monitoring potential impacts that remediation activities may have on the UCFR. Better understanding of the processes occurring at the Warm Springs Ponds and the impact that discharge from these ponds have on fish in the UCFR is also needed. We will deploy fish cages earlier in the spring and monitor ammonia concentrations during the period of time that Pond 2 experiences turnover. More information on the influences of mortality, recruitment, and role of water contaminants on wild fish in the UCFR is also needed. Age and growth, mortality, and recruitment studies of wild fish in the UCFR will be completed in coming years. This data will serve as a baseline to assess changes in fish population metrics as remediation and restoration activities continue in both the mainstem and tributaries of the UCFR.

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Appendices

Appendix I: Published Electrofishing Data from Lindstrom (2011)

Table A1-1. Electrofishing data collected on the Upper Clark Fork River at the pH Shack Section from 2008 through 2010. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Number following the population estimate (in parentheses) represents the 95 % confidence interval. Cutt x Rbow represents a phenotypic hybrid between a Cutthroat and Rainbow trout.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# of Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2008	Brown	708 (+/- 102)	26	567	318	88-461	99
	Rainbow	-	-	5	388	296-502	< 1
	Cutthroat	-	-	3	365	355-381	< 1
2009	Brown	185 (+/- 73)	22	116	357	96-500	95
	Rainbow	-	-	5	362	302-560	4
	Cutthroat	-	-	1	383	-	1
2010	Brown	421 (+/- 149)	15	232	300	111-615	95
	Rainbow	-	-	5	478	312-565	2
	Cutthroat	-	-	3	260	252-276	1
	Cutt x Rbow	-	-	3	357	338-392	1

Table A1-2. Electrofishing data collected on the Upper Clark Fork River at the Below Sager Lane Section in 2010. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Number following the population estimate (in parentheses) represents the 95 % confidence interval.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# of Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2010	Brown	262 (+/- 85)	14	383	293	93-525	99
	Brook	-	-	3	232	125-293	< 1
	Rainbow	-	-	1	645	-	< 1

Table A1-3. Electrofishing data collected on the Upper Clark Fork River at the original Williams-Tavener Section from 2008 through 2010. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Number following the population estimate (in parentheses) represents the 95 % confidence interval.

Year	Trout Species	Population Estimate -fish/mile-	Capture Efficiency -%-	# of Fish Handled	Mean Length -mm-	Length Range -mm-	Species Composition -%-
2008	Brown	324 (+/- 84)	28	194	349	118-524	100
2009	Brown	158 (+/- 77)	19	77	341	132-527	99
	Cutthroat	-	-	1	279	-	1
2010	Brown	206 (+/- 59)	27	146	332	114-509	99
	Cutthroat	-	-	1	285	-	< 1
	Brook	-	-	1	145	-	< 1

Table A1-4. Electrofishing data collected on the Upper Clark Fork River at the Phosphate Section from 2008 through 2010. Population estimates and capture efficiencies are for Brown Trout greater than 175 mm (~7") in total length. Number following the population estimate (in parentheses) represents the 95 % confidence interval.

Year	Trout Species	Population Estimate (fish/mile)	Capture Efficiency (%)	# of Fish Handled	Mean Length (mm)	Length Range (mm)	Species Composition (%)
2008	Brown	316 (+/- 58)	31	343	333	97-468	99
	Cutthroat	-	-	3	325	256-380	1
2009	Brown	292 (+/- 143)	13	159	334	125-465	99
	Cutthroat	-	-	1	274	-	1
2010	Brown	233 (+/- 46)	35	279	308	97-478	99
	Cutthroat	-	-	3	291	242-345	1