Colorado River Aquatic Resource Investigations

Federal Aid Project F-237-R26

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.

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State: Colorado Project Number: F-237-R26

Project Title: Coldwater Stream Ecology Investigations

Period Covered: July 1, 2018 through June 30, 2019

Purpose: Improve aquatic habitat conditions and angling recreation in Colorado.

Project Objective: Investigate biological and ecological factors impacting sport fish populations in coldwater streams and rivers in Colorado.

Job No. 1. Quantifying the Habitat Preferences of the Stonefly *Pteronarcys californica* in Colorado

Coauthored by Eric E. Richer, Colorado Parks and Wildlife Aquatic Research Section, Fort Collins, Colorado.

Job Objective: Investigate the habitat use of the salmonfly *Pteronarcys californica* in Colorado rivers.

The salmonfly (*Pteronarcys californica*) is the largest stonefly species in North America and can attain high densities in some western rivers. They play a critical ecological role as shredders in stream ecosystems (Merritt et al. 2008) and can be extremely important to stream dwelling trout as a food resource. Nehring (1987) reported that *P. californica* was the most common food item of trout in the Colorado River, comprising 64-75% of the mean annual stomach contents. Because of their high biomass and hatching behavior, they also play an important role in supplementing terrestrial food webs and riparian communities with stream-derived nutrients (Baxter et al. 2005, Walters et al. 2018). Salmonflies are reported to have a 3-5 year life cycle in various parts of their range, but studies indicate they have a 3-4 year life cycle in Colorado (DeWalt and Stewart 1995, Nehring 1987). Therefore, as one of the longest-lived aquatic insects in the Nearctic, salmonflies are more susceptible to habitat alterations than other taxa (DeWalt and Stewart 1995).

Salmonflies are associated with fast-moving mountain streams and medium to large rivers with clean water and high stream flows (Elder and Gaufin, 1973). Larvae favor fast riffle habitat with medium to large unconsolidated rocky substrates, and rarely inhabit pools or areas with silty substrate (Elder and Gaufin 1973, Freilich 1991, Kauwe et al. 2004). While found in high abundance at some sites, the salmonfly has relatively specific environmental requirements and is classified a sensitive species in bioassessment protocols (Bryce et al. 2010, Fore et al. 1996, Barbour et al. 1999). The sensitivity of *P. californica* to disturbance and habitat alterations has led to declines in range and number in several rivers of the Intermountain West (Anderson et al. 2019), including the Logan and Provo rivers in Utah (Elder and Gaufin 1973, Vinson 2011), and several rivers in Montana (Stagliano 2010, Anderson et al. 2019). In Colorado, the range of salmonflies has declined in both the upper Gunnison and Colorado rivers, primarily due to changes in habitat quality and flow alterations associated with river impoundments (Elder and Gaufin 1973, Nehring et al. 2011).

Salmonflies are one of the most synchronously emerging aquatic invertebrates, with emergence at any one site only lasting from 5-13 days (DeWalt and Stewart 1995). They hatch at night by crawling from the water onto riparian vegetation and other vertical structures, such as rocks, cliff faces, and bridge abutments, where they emerge from the larvae's exoskeleton or exuvia that is left attached to the structure. The synchronous emergence and hatching behaviors allow *P. californica* to be sampled in unique ways compared to other aquatic invertebrates. Nehring et al. (2011) used multiple-pass removal density estimates of the shed exuvia as an index of salmonfly density in rivers in Colorado. This technique was validated and applied to other studies as a cost- and time-efficient index of salmonfly density (Walters et al. 2018). We applied this novel technique to index salmonfly density and explore its relationships with stream habitat variables.

The objective of this study was to document the density and habitat use of *P. californica*, and measure physical habitat variables related to their distribution in rivers in Colorado. Quantifying habitat preferences will assist in the restoration of sites where *P. californica* has declined in range or abundance, and will inform land use, flow management, and river restoration activities to benefit the species.

OBJECTIVES

1. Document the density and habitat use of *P. californica*, and measure physical habitat variables related to their distribution in rivers in Colorado

METHODS

Study Areas

We collected salmonfly density estimates and measured habitat variables at 18 sites on the Colorado, Gunnison, and Rio Grande Rivers (Figure 1). These rivers are sixth-order streams with pool-riffle or pool-riffle/plane bed morphology in the Rocky Mountains of western Colorado, USA (Montgomery & Buffington 1997). A flood-frequency analysis was performed for each watershed to estimate the 1.5-year flood at each study site, which is considered an approximation of the bankfull flow (Dunne and Leopold, 1978). Study sites within the Gunnison River in southwest Colorado have an average 1.5-year flow of 88 m^3/s, an average drainage basin area of 11,711 km^2, and range in elevation from 1,539-1,639 m. Ranging in elevation from 2,070-2,376 m, study sites within the Colorado River in west central Colorado have an average 1.5-year flow of 42 m^3/s, and an average drainage basin area of 3,691 km^2. In the Rio Grande River in south central Colorado, study sites have an average 1.5-year flow of 70 m^3/s, an average drainage basin area of 1,777 km^2, and range in elevation from 2,579-2,613 m.

Approach

This was an observational study exploring the relationship between physical habitat variables and salmonfly density. We followed recommendations of Burnham and Anderson (2002) to identify potential explanatory variables related to salmonfly density *a priori*. The goal was to limit the number of variables due to the time and expense required to collect reliable estimates of the response variable (salmonfly density) in the known range of *P. californica* in large Colorado rivers. We used literature review and biological knowledge of salmonfly habitat preferences to identify a set of habitat features that we hypothesized to be important to *P. californica*. Simple, measureable habitat variables that are commonly used by research scientists and biologists were selected so that study methods could be replicated in future habitat evaluations and restoration projects. Generally, we followed the recommendations of Leopold (1964) and Rosgen and Silvey (1996) to identify basic variables of stream morphology that characterize the hydrology and sediment conditions, and ultimately influence instream habitat for aquatic invertebrates. Five habitat variables were measured and evaluated for their relationship with salmonfly density: width to depth ratio, bed slope, D50, percent fine sediment, and cobble embeddedness. These variables were measured at 18 riffle sites, six each in the Gunnison, Colorado, and Rio Grande Rivers, in habitat known to be occupied by *P. californica*. Sites were chosen in a stratified random fashion to encompass the extent of salmonfly range within the temperature and environmental tolerances of the species.

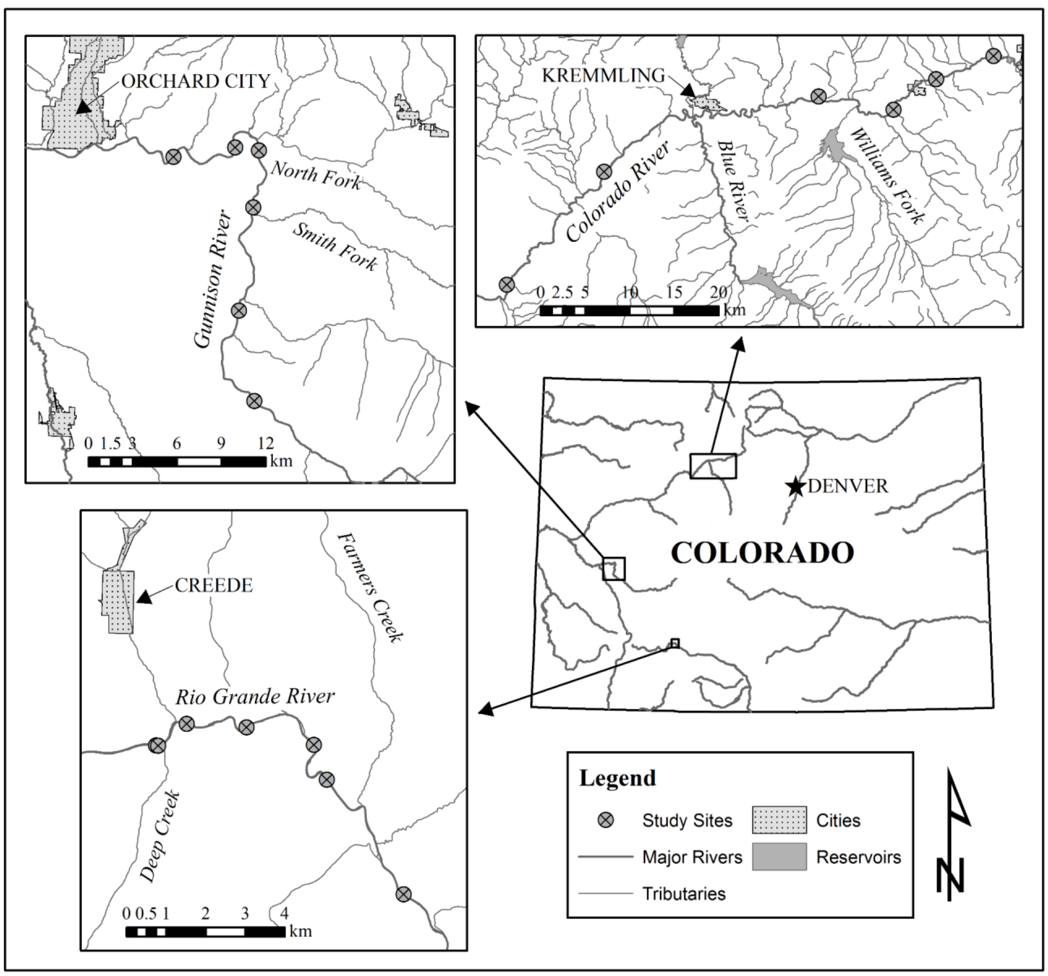

FIGURE 1. Salmonfly habitat study sites on the Gunnison, Colorado, and Rio Grande Rivers.

Habitat Variables
Physical habitat surveys were completed during the late summer base flow period (July-September) in 2013-2016 at all 18 sites. A modified Wolman pebble count was used to characterize dominant substrate size (Wolman 1954, Potyondy and Hardy 1994). Pebble counts consisted of measuring the intermediate axis for ~100 rocks at select cross sections within each study site. Cumulative grain-size distributions were analyzed using the Size-Class Pebble Count Analyzer developed by Potyondy and Bunte (2002) to determine the D50 sediment size, which is the diameter of the median-sized particle at the site.

The embeddedness of cobble-sized particles was measured following the Burns Quantitative Method (Burns 1985). This method was summarized and evaluated by McHugh and Budy (2005) as the "Measurement-Based Technique" for embeddedness, and the field protocols followed the manual produced by Burton and Harvey (1990). In selected riffles, a 60-cm-diameter welded steel hoop was randomly thrown in areas with water depth less than 45 cm, with hoop float times ranging from 0.9-2.5 seconds. Nine hoops were thrown at each riffle site along three transects covering the top, middle, and bottom of the riffle. Within the 60-cm hoop, both the depth of embeddedness (D_e) and particle height (D_t) of each single matrix particle larger than sand (> 2 mm) were measured, and embeddedness for each site was calculated as $(\Sigma D_e)/(\Sigma D_t)*100$.

Fine sediment was measured with the grid toss or sampling frame method (Bunte and Abt 2001, Kershner et al. 2004). Percent fine sediment was visually estimated as 0 or ≥10%, and sampling frames with greater than 10% fine sediment were measured using the scale technique or grid method (Kershner et al. 2004). A metal ruler or welded steel grid similar to the sampling frame of Bunte and Abt (2001) was used to measure 48 points in each of the nine hoops (24 along the vertical axis and 24 along the horizontal axis). At each 2.54 cm interval along those two axes, the presence of fine sediment < 2 mm was determined visually and by feel. Using a sampling frame or grid to quantify fine sediment improves accuracy and reduces bias when compared to traditional pebble counts (Bunte and Abt 2001). The total for each hoop was expressed as a percent of the 48 sampling points that contained fine sediment, and the average of the nine stratified random estimates was used for the value at each riffle site.

Topographic surveys of each site were conducted in 2014-2015 during the same time as the habitat surveys with a Trimble Global Navigation Satellite System (GNSS) Real-Time Kinematic (RTK) surveying system. The SonTek HydroSurveyor Acoustic Doppler Profiler (ADP) was used to collect bathymetric data at sites that were too deep to survey safely by wading. Survey data were used to create triangular irregular networks (TINs) for each site with ArcGIS. Cross sections and longitudinal profiles were then extracted from the TINs to estimate the bankfull width to depth ratio and bed slope, respectively, for each site.

Due to a recent paper suggesting that temperature affects salmonfly density (Anderson et al. 2019), a *post hoc* analysis was conducted to evaluate if our sites were similar enough in temperature regime to accomplish our objectives of comparing only physical habitat variables. To evaluate stream temperature variability at our sites we used modelled stream temperatures from NorWeST, a western United States stream temperature model (Isaak et al. 2017). This model uses extensive thermograph data (>220,000,000 temperature recordings from >22,700 sites) to create a spatial statistical stream network model with 1 km resolution and has been shown to give accurate and unbiased stream temperature predictions ($R^2 \sim 0.90$, RMSE < 1.0 ˚C).

Salmonfly Density

We estimated the density of salmonflies at our sites using the method described in Nehring et al. (2011). This technique is an improvement on the exuvia collection methods of Richards et al. (2000) by applying a multiple-pass removal technique to account for imperfect detection probability. If sites are visited soon after emergence, the density of emerged salmonflies can be estimated by conducting multiple-pass removal sampling of exuvia left attached to riparian vegetation or structure. There is a high correlation ($R^2 = 0.95$) between post-emergence exuvia density estimates and more traditional pre-emergent quantitative benthic sampling (Nehring et al. 2011).

We completed annual salmonfly density estimates in June 2013-2016 by searching 30 m sections of stream bank for *P. californica* exuvia adjacent to riffle habitat. If possible, each site was visited two to three times to encompass the entire emergence. If a site was visited only once, estimates were done as soon as possible after the emergence was complete (emergence lasted from 7-13 days at our sites during this study). Stream flow changes and weather conditions were also taken into account when planning surveys to best estimate the total emergence at each site. Riparian areas were intensively searched by 3-7 people within a search area that extended 1-20 m from the water's edge. The search area varied by site and depended on the thickness and structure of riparian vegetation. The area was extended laterally from the water's edge until no exuvia were encountered, with the exuvia at most sites being found within the first three meters from the water's edge. On a single sampling occasion, each site was searched completely with two to four passes with identical search areas, effort and personnel on each pass. The Huggins Closed Captures model in Program MARK was used to estimate the total density of exuvia at each site (Huggins 1991, White and Burnham 1999). All sites had at least three years of exuvia density estimates, with a minimum of two years of data collected under favorable flow and weather conditions that did not compromise the estimates.

Analysis

As this was an exploratory study with a limited sample size, we focused on a basic analysis of a limited number of variables to produce simple descriptive model(s) and rank top variables. To evaluate associations between habitat variables and salmonfly density, a two-step modelling approach was used. The five habitat variables were first screened with Pearson's product moment correlation coefficient and then analyzed with multiple linear regression. Linear regression modelling was performed with the lm function in Program R (R Core Team 2015). Model assumptions of homogeneity of variance and normality were evaluated by examining residuals of the global model. The response variable, salmonfly exuvia density per m^2, was transformed with the Box Cox procedure due to patterns in the residuals (Box and Cox 1964). The lambda value of 0.3 had a 95% confidence interval that included 0.5 so a square root transformation was used on the salmonfly density data.

Because of the small sample size ($n = 18$), only a limited number of models could be considered without potentially identifying spurious effects and having problems estimating parameters from noisy data (Burnham and Anderson 2002). The three variables with the highest correlation coefficients were evaluated using the information theoretic approach (Burnham and Anderson 2002) to identify the top predictive model(s) using the small sample size version of Akaike's information criterion (AIC*c*). Single variable models with an intercept and an error term were considered as well as a global model of all three top variables. No other additive effect models or interaction models were considered due the sample size restrictions and our main objective of ranking the top variables. Model-averaged parameter estimates were based on model weights, and the sum of weights for each parameter was used to infer variable importance. The analysis was conducted with the AICcmodavg package (Mazerolle 2017) in Program R.

RESULTS

Salmonfly density ranged from 0.17-353 exuvia/m² (mean = 96 exuvia/m²). Pearson's correlation coefficients indicated that percent fines, embeddedness, and D50 were the variables most highly correlated with salmonfly density (Figure 2), which were subsequently used in the model selection analysis. Estimates of fine sediment ranged from 3-22% (mean = 8%). The percent embeddedness of cobble-sized particles at the study sites varied from 10-42% (mean = 23%). The median substrate size (D50) at the study sites ranged from 76-210 mm (mean = 123 mm), so the riffles at our study sites were dominated by particles classified as cobble on the Wentworth scale (Wentworth 1922). Percent fines was the only habitat variable with a significant correlation to salmonfly density at an α level of 0.05 ($p = 0.003$). None of the explanatory variables were correlated with each other at a level that parameter estimation and other problems with multicollinearity would be expected (Graham 2003).

AIC*c* model selection results indicate that the single variable model with percent fines was the top model with a model weight (w_i) of 0.89 (Table 1). The global model with an additive combination of all three variables was 4.7 AIC*c* units behind the top model, and explained 51.4% of the variation in salmonfly density. Akaike weights for each variable were summed across the model set to infer relative variable importance (Burnham and Anderson 2002). Percent fines was the most influential variable with a cumulative weight of 0.94, followed by embeddedness at 0.11 and D50 at 0.10. A null (intercept) models was also evaluated and was 7.6 ΔAICc units behind the top model and would be the lowest ranked model in the set if included.

Mean August water temperature (average = 16.3 C, SE = 0.46) varied little over our study sites (range = 13.8-19.7 C) and exhibited low correlation to salmonfly density at our sites ($R^2 = 0.03$). If mean August temperatures were included as a single variable model in our set of physical habitat models, it would be 10.0 ΔAICc units behind the top model (percent fine sediment).

Salmonfly density increased at sites with low amounts of fine sediment, low embeddedness, and larger median sediment size. We made model predictions to summarize the values of the stream habitat variables associated with the range of salmonfly densities encountered at our sites (Table 2). An average salmonfly density site could be expected with 6% fine sediment, while high densities would be expected only at sites with low amounts of fine sediment (< 3%). Width to depth ratio had the fourth highest correlation coefficient and was left out of the model selection exercise but was still marginally related to salmonfly density ($R^2 = 0.16$, $p = 0.11$). Salmonfly density increased with lower width to depth ratios and an average density site could be expected with a width to depth ratio of 38, while high densities would be expected only at sites with width to depth ratio less than 24.

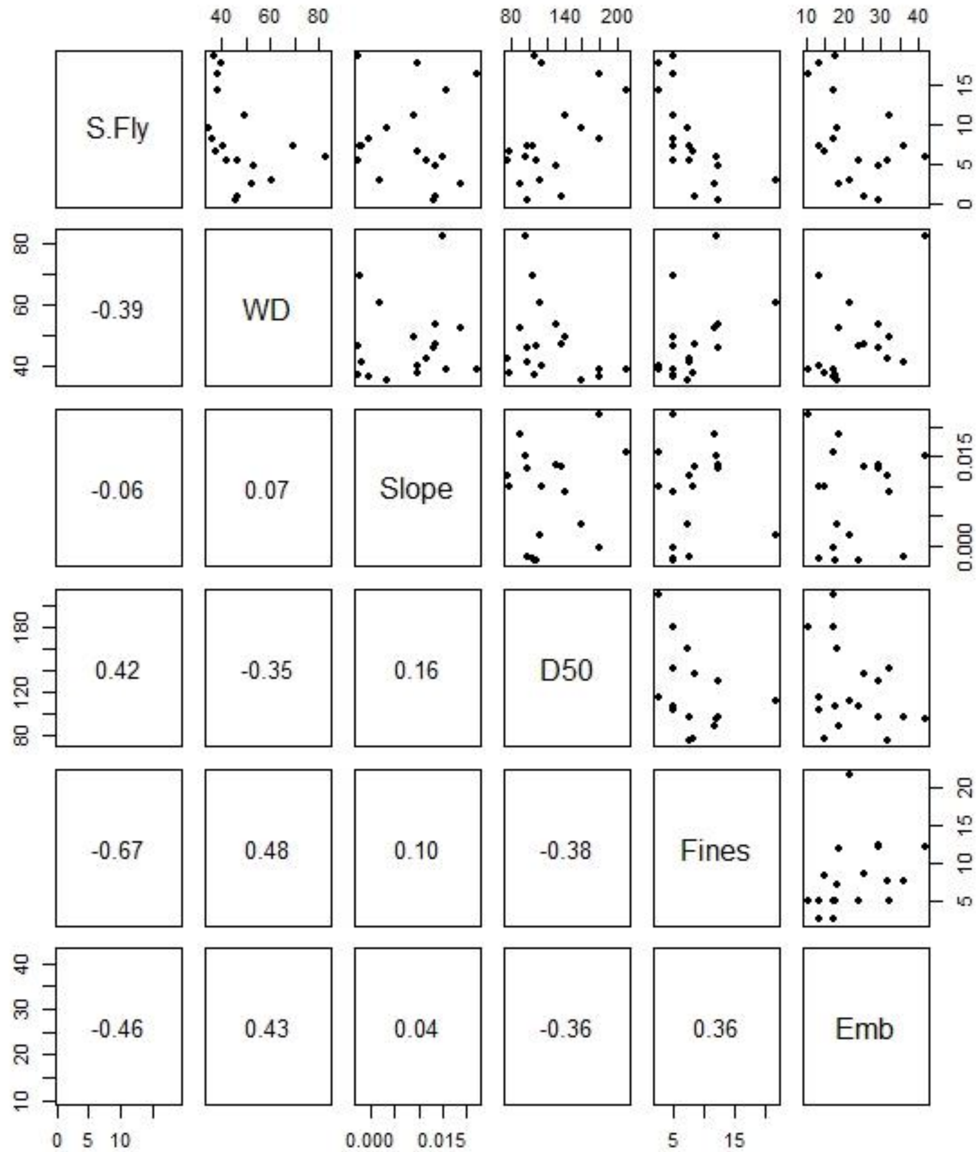

FIGURE 2. Pearson correlation matrix of habitat variables and Box Cox-transformed salmonfly exuvia density (S. Fly). WD is the width to depth ratio, slope is stream bed slope, D50 is the 50% cumulative particle size in mm, fines is percent of sand, silt and clay particles <2 mm, Emb is percent embeddedness. The correlation between salmonfly density and percent fines was significant at the 95% level ($p = 0.003$) while the correlations of embeddedness and D50 with salmonfly density were significant at the 90% level ($p = 0.057$ and 0.082, respectively).

TABLE 1. Model selection results of linear regression models of salmonfly habitat variables, including the number of model parameters (K), Akaike's information criterion corrected for small sample size (AIC_c), ΔAIC_c, AIC_c weight (w_i), and multiple R^2.

Model	K	AIC_c	ΔAIC_c	w_i	R^2
Fines	3	109.1	0	0.89	0.44
Fines+Embeddedness+D50	5	114.0	4.7	0.06	0.51
Embeddedness	3	115.4	6.3	0.03	0.21
D50	3	116.1	7.0	0.02	0.18

DISCUSSION

The correlation and model selection analyses indicated that salmonfly density was highest at sites with low amounts of fine sediment, low embeddedness, and larger median substrate size, and that fine sediment was the single best predictor of salmonfly density. The sensitivity of *P. californica* to fine sediment has been reported previously. Bryce et al. (2010) considered the salmonfly as "sediment sensitive" and reported optimum sediment tolerance values of 2.6% for fines ≤ 0.06 mm and 8.2% for sand ≤ 2 mm, which corresponds with results of this study. Our results also agree with conventional understanding of the impacts of fine sediment on aquatic invertebrates. Sedimentation is the largest cause of stream degradation in the United States affecting over 40% of streams and rivers (USEPA 2000). Excessive sedimentation is known to impair the habitat of aquatic invertebrates in a multitude of ways (Waters 1995, Wood and Armitage 1997). Fine sediment changes the species richness of invertebrate communities and reduces the density of sensitive species (Waters 1995). The principal mechanism for these effects was filling of interstitial spaces, increasing cobble embeddedness and thereby reducing the available habitat for Ephemeroptera, Plecoptera and Trichoptera species (Waters 1995).

TABLE 2. Model-estimated values of important habitat variables across a range of salmonfly densities observed in the Gunnison, Colorado, and Rio Grande rivers.

Relative Density	Exuvia/m²	% Fines	D50 (mm)	% Embeddedness
Moderate (Q1)	20	13	64	36
Median	48	10	104	27
Average	96	6	150	17
High (Q3)	147	3	187	9
Maximum Observed	353	0	295	0

There are many biotic and abiotic factors that affect the distribution and abundance of invertebrate species and more research is needed to investigate other factors that influence salmonfly density. Water temperature is an abiotic factor recently reported to influence salmonfly abundance (Anderson et al. 2019). We purposely restricted our sampling sites to river reaches well within the known range of *P. californica* to achieve our objective of exploring physical habitat characteristics within a stream reach where temperature (and other environmental factors) was not likely to limit distribution. The results of the *post hoc* modelled stream temperature analyses indicated that our sites varied relatively little in summer water temperatures and that we were successful in limiting the range of our study sites to river reaches of similar temperature regimes. All of our habitat variables except bed slope explained more variability in salmonfly densities than mean August water temperature at our sites on three different rivers in Colorado, and fine sediment was much more influential in explaining salmonfly densities than temperature.

Salmonfly distribution and abundance are likely driven by many factors, and may be limited by different environmental and habitat factors at different scales. Our objective was to describe relationships of physical habitat variables to salmonfly density at the reach scale (Frissel et al. 1986). Within the range of sites we studied, aspects of substrate composition like percent fine sediment and cobble embeddedness, and the geomorphic characteristic median sediment size were related to salmonfly density. While different abiotic factors influence invertebrate distribution at different scales, many are likely to be difficult for land managers to influence at the river reach level. River geomorphology and sedimentation in rivers, however, can be influenced by land use practices, alterations to stream flows and sediment supplies, and even direct physical river restoration (Leopold et al. 1964, Rosgen and Silvey 1996, Wood and Armitage 1997). If conservation of salmonfly habitat is a goal of resource managers, then flow management, land-use decisions, and habitat restoration activities should focus on reducing the input of fine sediment in rivers and encouraging flow regimes and channel morphology that maintain low cobble embeddedness and larger median substrate size in riffles.

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Job No. 2. Effects of Whitewater Park Development on Trout, Aquatic Invertebrates, and Mottled Sculpin *Cottus bairdii*

Job Objective: Investigate the effects of whitewater parks on trout, aquatic invertebrates, and Mottled Sculpin.

Artificial whitewater parks (WWP) are increasingly common throughout Colorado and there are concerns about how they affect fish and aquatic invertebrates (Kolden et al. 2015, Fox et al. 2016). Over 30 whitewater parks exist in Colorado or are in the construction planning stages (Figure 3). Many of the rivers throughout the state with whitewater parks are also some of the best wild trout fisheries. The construction of whitewater parks involves replacing natural riffles with concrete or grouted rock grade control structures to produce hydraulic waves for recreational boating. Natural riffles serve many important physical and ecological roles in rivers. Ecologically, riffles serve as the most productive areas of a stream for periphyton and invertebrate production that form the foundation of the aquatic food web. Physically, riffles serve as grade control structures for streams and their location and frequency are main drivers of stream geomorphology. Artificial pools created below WWP waves have been found to hold a lower biomass of trout than natural pools, and have more dynamic and higher magnitude flows and velocities (Kolden et al. 2015). Whitewater parks have also been documented to cause a suppression of fish movement that is related to fish length (Fox et al. 2016). Concerns have been raised that whitewater parks not only impact fish habitat and fish passage but could affect some aquatic invertebrates that are primary diet items for trout (Kondratieff 2012).

In addition to sportfish concerns, native non-game fish are also common at many whitewater park sites. Sculpin are an ecologically important part of freshwater ecosystems because they can occur in high densities in depauperate coldwater mountain streams (Adams and Schmetterling 2007). They also can exert a large influence on aquatic food webs through their diverse trophic positions. The Mottled Sculpin *Cottus bairdi* is common in coldwater western Colorado streams where they occur in sympatry with important sport and native trout species. They prefer cool, high gradient mountain streams with cobble habitat and are rarely found in stream reaches where substrate is embedded with silt (Sigler and Miller 1973, Woodling 1985, Nehring et al. 2011). Their habitat preferences for cobble substrate and high quality riffle-run habitat make them a good ecological indicator of stream health (Adams and Schmetterling 2007, Nehring et al. 2011). Because the function of riffle and run habitat is generally impacted when stream flows are altered or instream habitat is manipulated, Mottled Sculpin may be affected by habitat driven alterations before fish in higher trophic positions like trout. Sculpin not only indicate ecological problems that could eventually affect sport fish, but they serve as an important food source, especially for Brown Trout *Salmo trutta* common in many Colorado rivers.

The objective of this study was to investigate the effects of building whitewater parks on Mottled Sculpin, aquatic invertebrates, and trout. Two whitewater parks were constructed in western Colorado in 2014, on the Uncompahgre River in Montrose and at the Pumphouse Recreation site on the Colorado River. Their construction provided an opportunity for the first comprehensive study of potential impacts to fish and invertebrates. A before-after control-impact (BACI) study design was used to evaluate changes in salmonid and catostomid populations, Mottled Sculpin density, and aquatic invertebrate density and diversity at these two sites.

OBJECTIVES

1. Investigate the effects of building whitewater parks on aquatic invertebrate density and diversity at two whitewater park sites on the Colorado and Uncompahgre Rivers before and after construction.

2. Investigate the effects of building whitewater parks on the Colorado and Uncompahgre Rivers on the density of salmonids, catostomids, and Mottled Sculpin before and after construction.

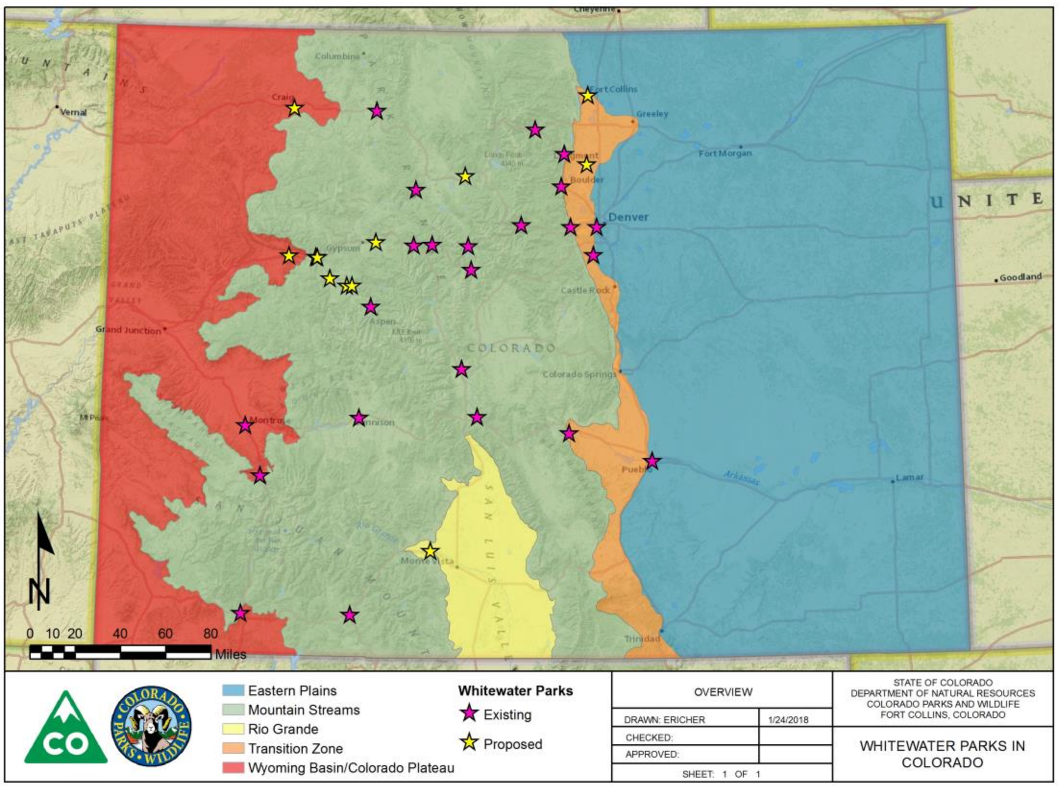

FIGURE 3. Whitewater parks existing and proposed in Colorado.

FIGURE 4. Before and after photos of the whitewater park feature at Pumphouse on the Colorado River. The whitewater park feature replaced a natural run with a drop structure featuring two hydraulic waves.

METHODS

Uncompahgre River

On the Uncompahgre River, aquatic invertebrate samples were taken at five sites, one below the planned WWP, three within the park, and one above. The WWP on the Uncompahgre River consists of six drop structures over 0.2 miles of river. Of the three sites within the WWP, one was converted from a natural riffle to a run (WWP3) while the other two contained functioning (but shorter) riffles between drop structures.

Five replicate macroinvertebrate samples were collected at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. Samples were collected in November of 2014 (pre-construction), 2015, 2016, and 2017. Replicates were collected from the same riffle with predominantly cobble substrate by disturbing the streambed to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and preserved in 80% ethanol. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited (Moulton et al. 2000). Approximately 300 individual organisms were identified from each replicate and a 15 minute search for large or rare organisms was conducted on the entire sample. All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to family and non-insects were identified to class. Each replicate sample was processed separately so that more individual specimens were identified from each site to ensure rare organism were sampled and to increase the power of the comparisons between riffle sites in close proximity (Vinson and Hawkins 1996). All taxonomic identifications followed recommendations by Moulton et al. (2000) and were completed by a single CPW invertebrate taxonomist.

To monitor Mottled Sculpin and Brown Trout, three electrofishing stations were established concurrent with the invertebrate sites, one below the WWP, one within (that encompassed two invertebrate sampling riffles), and one above. The upstream and downstream control sites (sites 1 and 3) had habitat improvement projects completed in 2007 aimed at improving fish habitat. The electrofishing stations averaged 704.3 ft (512-849 ft) long. Block nets were not used due to high discharge and velocity of the Uncompahgre River but natural stream features like shallow riffles were used as endpoints to best insure closure. Three pass removal electrofishing was completed at each site with a Smith Root VVP15 truck mounted electrofisher and five to seven anodes. All fish were weighed, measured, and population estimates were made with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999). To reduce the bias associated with the size selectivity of electrofishing, capture probabilities were modeled with fish length as a covariate similar to the approach described in Saunders et al. 2011. Four models were built for each species estimating capture probabilities by length, time, time + length, as well as a constant capture probability for all fish and all three passes. The time models allowed for different capture probabilities for the 2nd and 3rd passes to address a common source of bias in electrofishing removal models. Model selection was conducted with the small sample size corrected version of Akaike's information criterion (AIC_c), population and parameter estimates were made by model averaging across all four models with AIC*c* weights (Burnham and Anderson 2002).

Colorado River
On the Colorado River, aquatic invertebrate samples were taken at three sites in a 0.4 mile reach, one below, one within and one above the WWP. The upper site is two riffles above the WWP site and the lower site is the first downstream riffle. The WWP on the Colorado River consists of a single cross channel wave structure so fewer sampling sites were necessary than on the Uncompahgre River. Unlike the Uncompahgre where post construction riffles remained in the WWP, at Pumphouse a natural run was converted to a drop structure with pools above and below (Figure 4). Five replicate macroinvertebrate samples were collected at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net, samples were collected and processed using the same protocols as on the Uncompahgre River.

To monitor sportfish populations around the WPP, mark recapture electrofishing was conducted with a 16 ft aluminum jet boat and a Smith Root 2.5GPP electrofisher. The sampling reach was 6,451 ft long, averaged 171 ft wide and was centered on the WWP structure. The sampling reach was divided into four sub-reaches to evaluate fish density with the study reach. Station 1 is from bottom of Gore Canyon to the riffle above Launch #1, Station 2 is from the riffle above Launch #1 to the whitewater park feature, Station 3 is from the whitewater park feature to Launch #3, and Station 4 is from Launch #3 to the bottom of the sampling reach. Fish population estimates were made with the Huggins Closed Capture Model in Program Mark (Huggins 1989, White and Burnham 1999). Four models were built by estimating capture probabilities by length, species, species + length, as well as a constant capture probability for all fish (but varying by time), identical to a Lincoln Petersen model (Seber 1982). Model selection was done with AIC*c* and population and parameter estimates were made by model averaging across all four models with AIC*c* weights (Burnham and Anderson 2002).

To evaluate fish movement through the WWP structure, fish were marked in 2016 and 2017 above and below the WWP structure with different caudal punches. Any movement upstream or downstream through the structure was documented on the recapture pass 48 hours after the marking event. To evaluate longer-term fish movement, 142 trout sampled in Station 2 (above the structure) were marked with an adipose clip and moved below the structure in 2016. These included 13 Rainbow Trout from 244-427 mm and 129 Brown Trout from 182-510mm. During the 2017 sampling all fish were inspected for marks to document long (one year) and short term (48 hours) passage upstream through the WWP structure.

Mottled Sculpin were sampled from representative sites above, at, and below the whitewater park structure. The sampling reaches were concurrent with the invertebrate sampling riffles in the invertebrate study and were 101, 154, and 152 feet long with an average width of 17.7 ft. Three pass removal electrofishing was conducted, fish were measured to the nearest millimeter, and density estimates were made for each site with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999). Four models were built for each species estimating capture probabilities by length, time, time + length, as well as a constant capture probability for all fish and all three passes. The time models allowed for different capture probabilities for the 2^{nd} and 3^{rd} passes to address a common source of bias in electrofishing removal models (Riley and Fausch 1992, Peterson et al. 2004).

RESULTS

Uncompahgre River Aquatic Invertebrates

There were no large scale changes in the density of aquatic invertebrates in the Uncompahgre River relative to annual and spatial variability but some subtle changes in invertebrate diversity occurred post WWP construction (Figures 5-9). Canonical discriminant analysis, a multivariate statistical technique, was used to investigate separation and overlap of stations based on abundance of the 13 dominant species of taxa in 2017 (Figure 10). Most of the stations were relatively similar except for the most upstream whitewater park site, WWP3. This station was separated significantly from the rest with the two canonical variables. This pattern was also evident in the Shannon diversity index of the sites, WWP#3 site had a lower diversity score than the other sites (Shannon 1948). The Shannon index was 2.4 for Downstream Control, 2.2 for WWP1, 2.5 for WWP2, 1.5 for WWP3 and 2.3 for the Upstream Control site. The WWP3 site is immediately above the 2nd whitewater park structure and was transformed from a riffle to a run. Because the first two structures are the most closely spaced together, the pool created below the first structure runs all the way to the second structure. Due to farther spacing of the drop structures, good quality riffles formed above the structures at WWP sites 1 and 2. These riffles are not functionally different from the upstream and downstream control sites in density, diversity, or community structure.

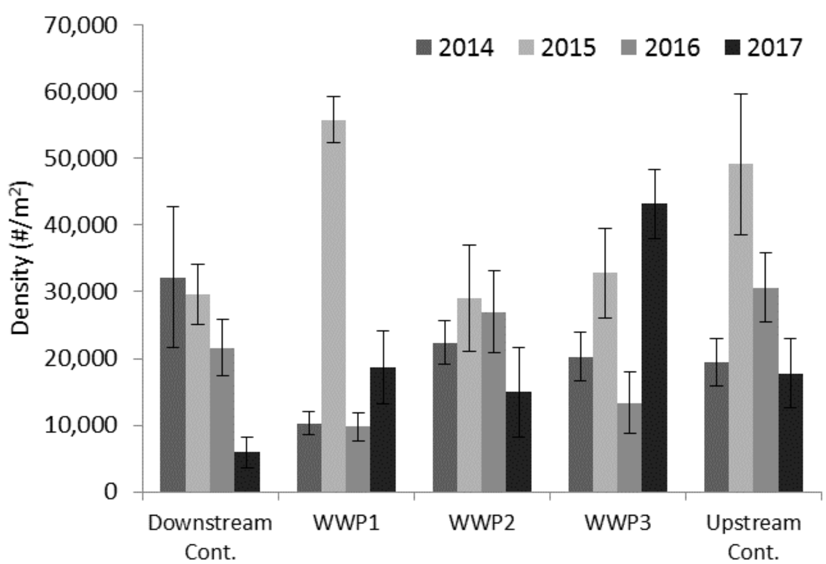

FIGURE 5. Density of all species of aquatic invertebrates with standard error bars on the Uncompahgre River 2014-2017.

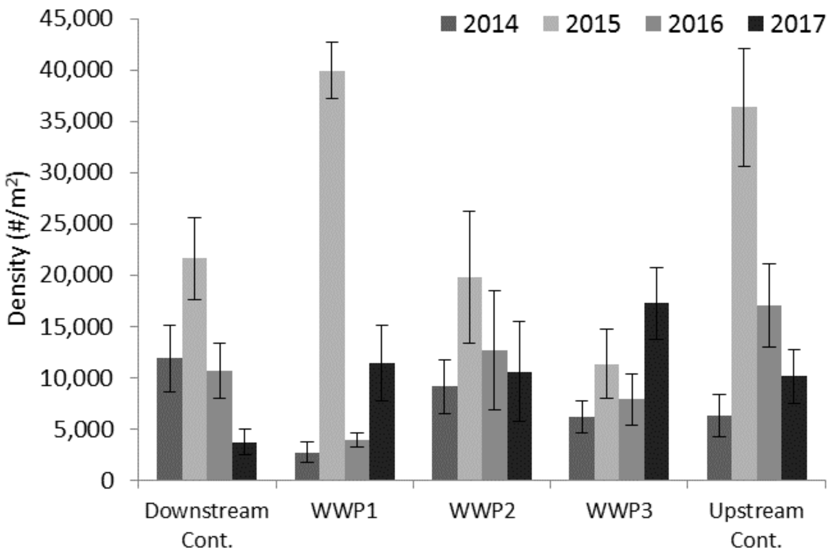

FIGURE 6. Density of ephemeroptera, plecoptera, and trichoptera fauna with standard error bars on the Uncompahgre River 2014-2017.

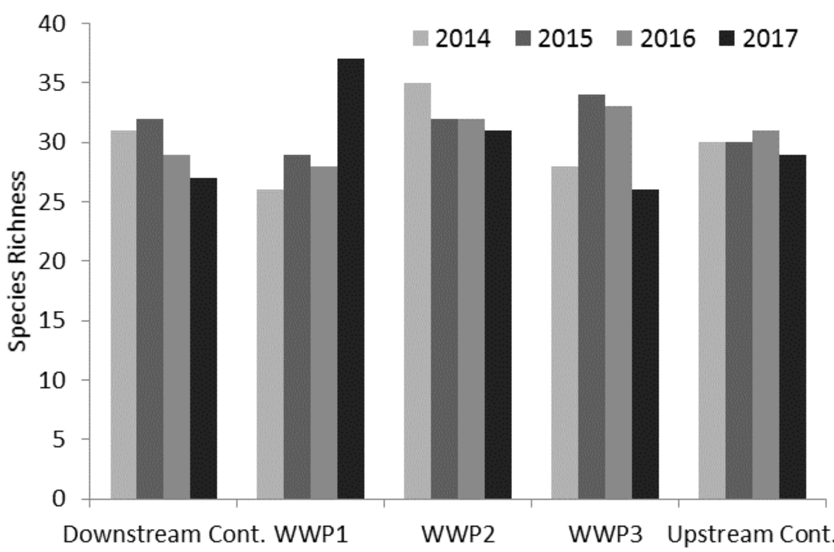

FIGURE 7. Total species richness on the Uncompahgre River 2014-2017.

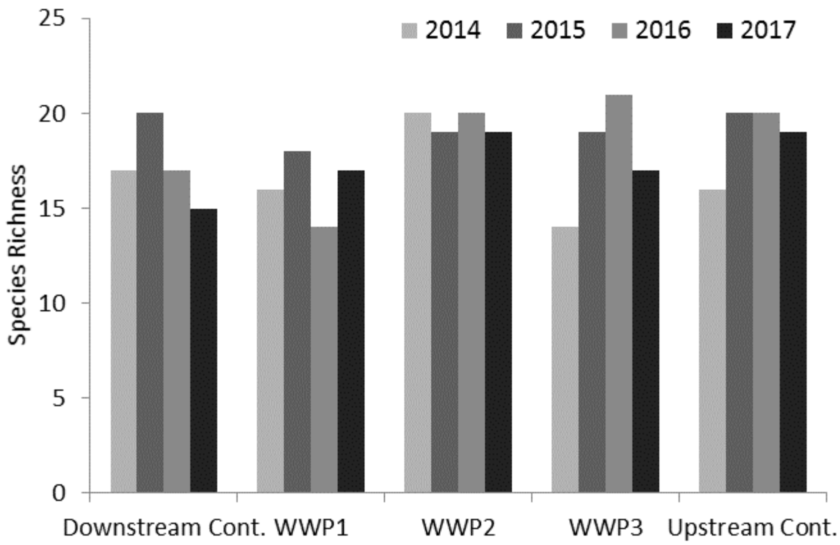

FIGURE 8. Species richness of ephemeroptera, plecoptera, and trichoptera (EPT) fauna on the Uncompahgre River 2014-2017.

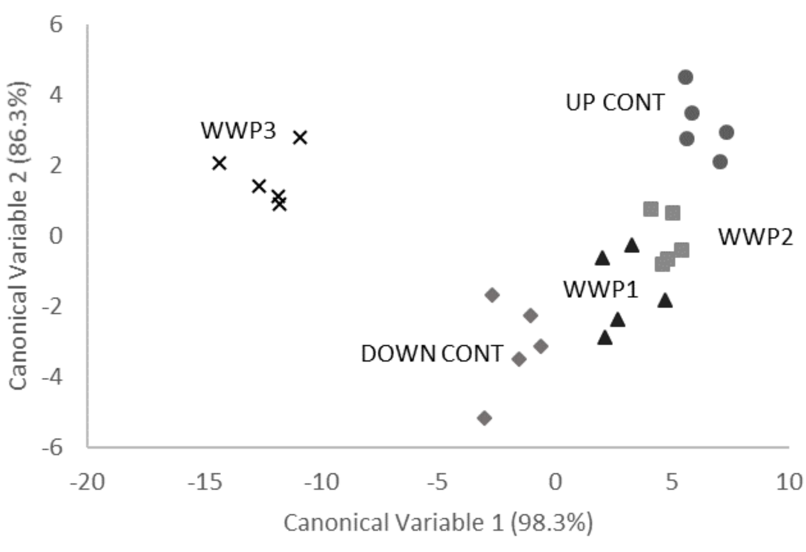

FIGURE 9. Canonical correlation analysis and percent of variation explained by the top two canonical variables of invertebrate community at Uncompahgre River sites in 2017.

Uncompahgre River Sportfish and Mottled Sculpin Populations

Trends in the Brown Trout and Mottled Sculpin in the Uncompahgre River were difficult to detect due to considerable annual variability and poor precision in some population estimates due to low capture probability (Figures 10 and 11). High flows and the steep gradient of the Uncompahgre River led to difficult sampling conditions most years and led to low capture probabilities. The whitewater park site always had the lowest number of Brown Trout of the three sites in all years. The number of Brown Trout at all three sites increased 2014-2017. In the final year of sampling, the flow conditions were low enough to have a capture probability sufficient for reliable estimates and the WWP site had significantly lower Brown Trout population at the 95% level than the upstream and downstream control sites. However, because that site began with the lowest Brown Trout numbers, differences at the end of the study were not significant considering pre-construction sampling. The Uncompahgre River has a relatively modest wild Brown Trout population (380-772 fish per mile in 2017) and the low density contributes to higher sampling variation and imprecise population estimates. Low trout densities are likely because of limited adult trout habitat due to high water velocities and low depths in most locations. Decreasing velocities and increasing depth by any means, including WWP pool construction, may improve habitat for Brown Trout but this response is not likely to be seen on rivers with better trout populations that are not limited by available pool and run habitat. Mottled Sculpin numbers increased over time at both the WWP site and the upstream control site while high variability and low capture probability did not reveal any trends at the downstream site. In 2017, there was no statistical difference at the 95% level in Mottled Sculpin densities between any of the sites. Overall, the whitewater park site on the Uncompahgre River does not appear to have impacted the fish population at a detectable level.

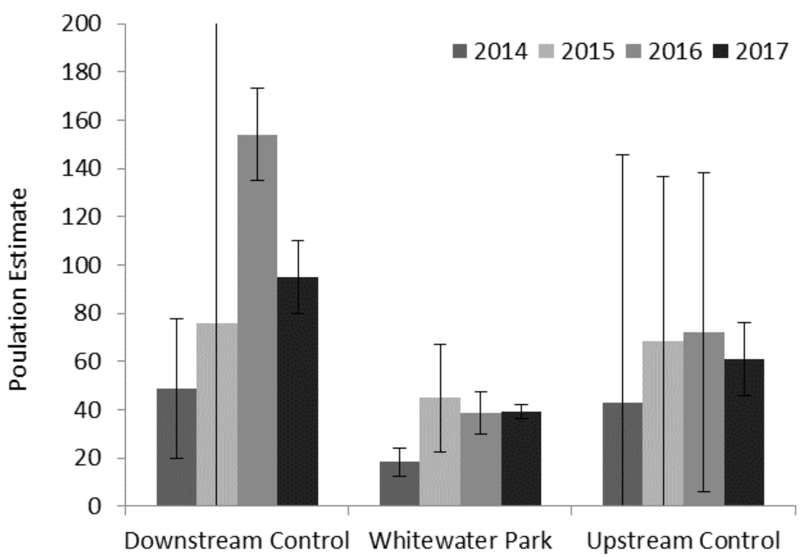

FIGURE 10. Brown Trout population estimates from the three sampling reaches of the Uncompahgre River 2014-2017.

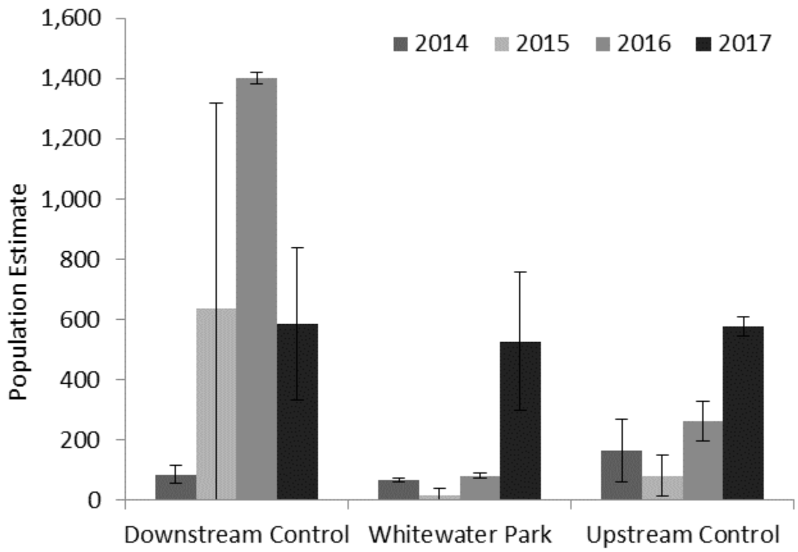

FIGURE 11. Mottled Sculpin population estimates from the three sampling reaches of the Uncompahgre River 2014-2017.

Colorado River Aquatic Invertebrates

Trends in the aquatic invertebrate sampling data show moderate impacts to aquatic invertebrate community from the WWP, mostly on species richness (Figures 12-15). Density of ephemeroptera, plecoptera, and trichoptera (EPT) fauna, as well as overall invertebrate density declined at the WWP immediately after construction but have since recovered and are similar to the other sites. However, species richness has declined at the WWP site from the highest of the three sites pre-construction to the lowest in 2017. The density and diversity of EPT species has also declined at the WWP site (Figures 16 and 17). Six species of aquatic invertebrates (four species of EPT) are no longer present at that site. This pattern was also reflected in the Shannon diversity index of the sites. The downstream site diversity score was 2.7, the WWP site was 2.2, and the upstream site was 2.5. Generally, while diversity is lower at that site, the invertebrate community is similar at coarse scales. When canonical discriminant analysis was used to investigate separation and overlap of stations based on abundance of the eight dominant species of taxa there was not much evidence for large community differences between the sites. There were some small differences like large numbers of Elmidae (riffle beetles) at the upstream site, but there was little separation of the three sites from each other.

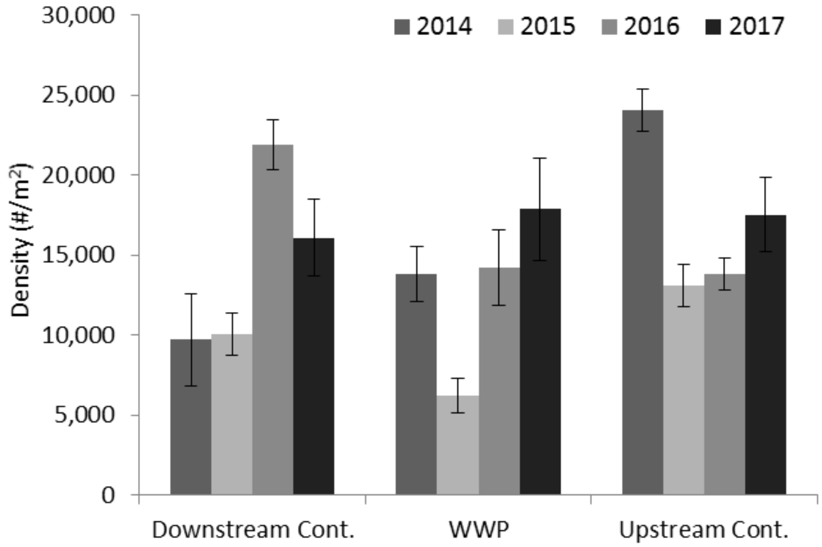

FIGURE 12. Density of all invertebrates with standard error bars at sites on the Colorado River at Pumphouse 2014-2017.

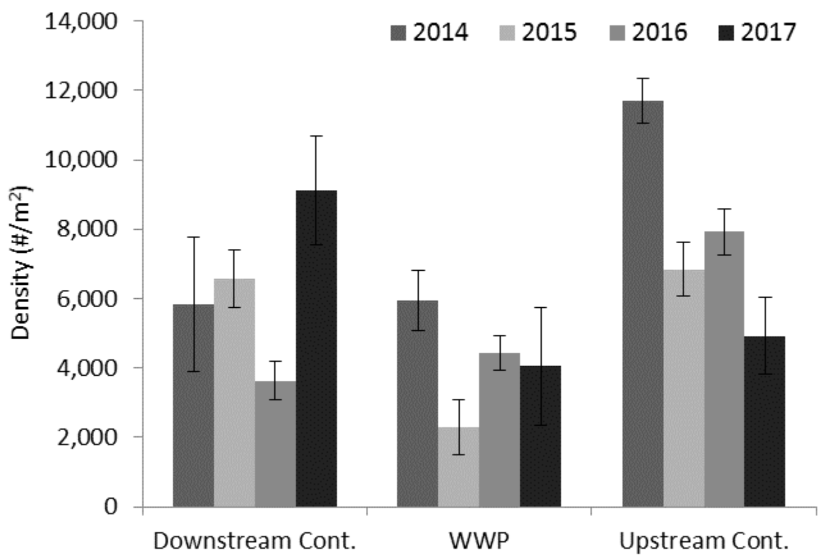

FIGURE 13. Density of EPT fauna with standard error bars at sites on the Colorado River at Pumphouse 2014-2017.

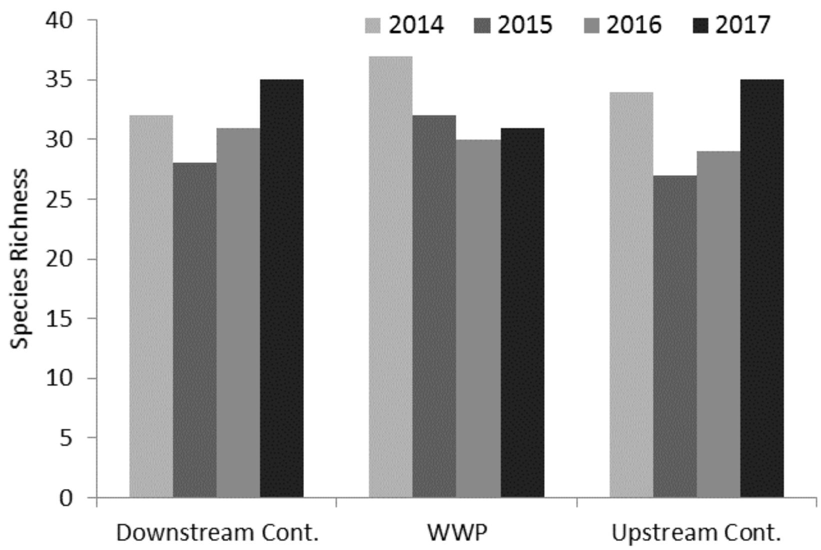

FIGURE 14. Total species richness at sites on the Colorado River at Pumphouse 2014-2017.

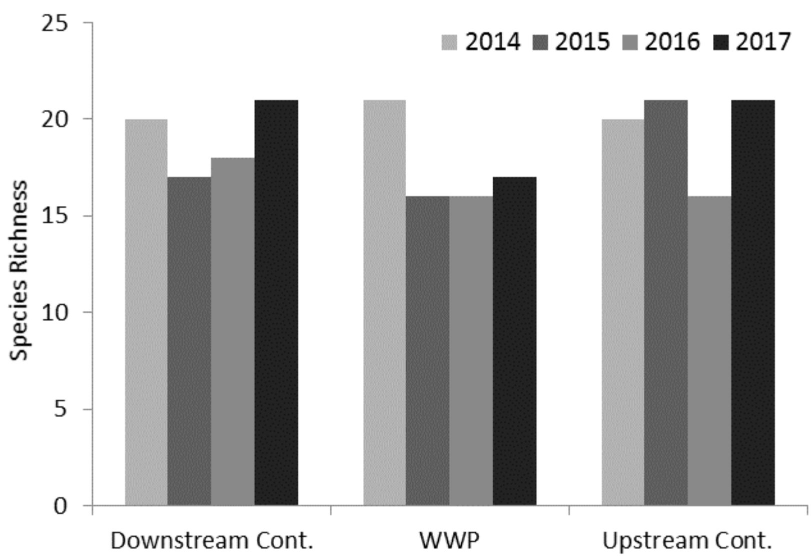

FIGURE 15. Species richness of EPT fauna on the Colorado River 2014-2017.

Colorado River Sportfish Populations

On the Colorado River, Brown Trout and Mountain Whitefish *Prosopium williamsoni* populations have remained relatively stable throughout this study and there is no evidence of population level effects of the whitewater park structure on gamefish populations in the study reach (Figure 18). Rainbow Trout *Oncorhynchus mykiss* numbers have increased in the study reach from 2014 to 2017 from an estimated 98±41 to 649 ±469. This trend in Rainbow Trout numbers has been observed in upstream reaches of the Colorado River as well (Fetherman and Schisler 2017). However, the WWP structure may have affected fish habitat and distribution in the river immediately around the structure. The sampling reach below the structure has significantly more Longnose Sucker *Catostomus catostomus* and White Suckers *C. commersonii* ($\alpha = 0.05$) and significantly fewer trout than the reach above it and the reach below (Figure 19). It is also the only reach where the sucker species outnumber trout.

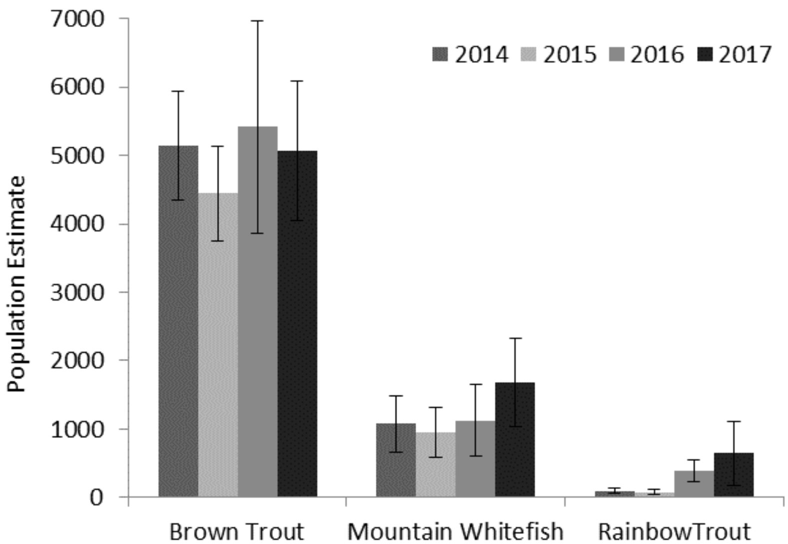

FIGURE 16. Fish population estimates and 95% confidence intervals before and after construction of the whitewater park structure on the Colorado River at Pumphouse.

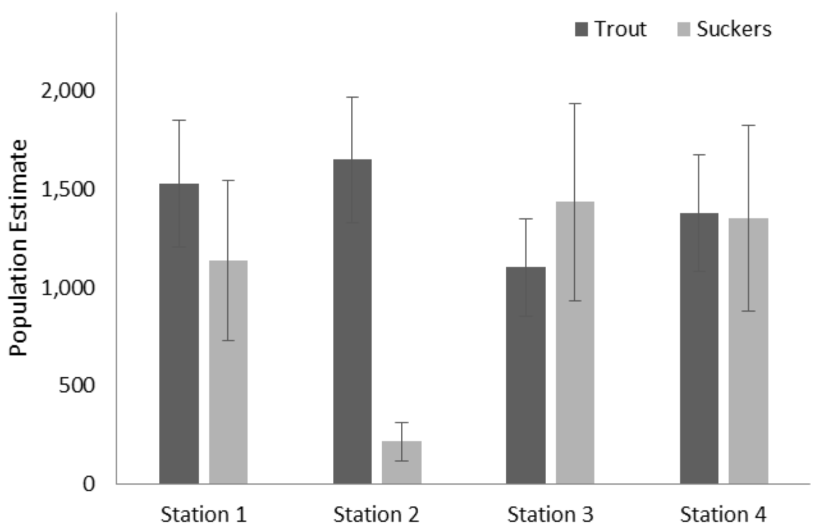

FIGURE 17. Fish population estimates and 95% confidence intervals for each sampling station on the Colorado River at Pumphouse for each sampling station in 2017. Trout estimates combine Brown Trout and Rainbow Trout and sucker estimates combine Longnose Sucker and White Sucker. Station 1 is from bottom of Gore Canyon to the riffle above Launch #1, Station 2 is from the riffle above Launch #1 to the whitewater park feature, Station 3 is from the whitewater park feature to Launch #3, and Station 4 is from Launch #3 to the bottom of the sampling reach.

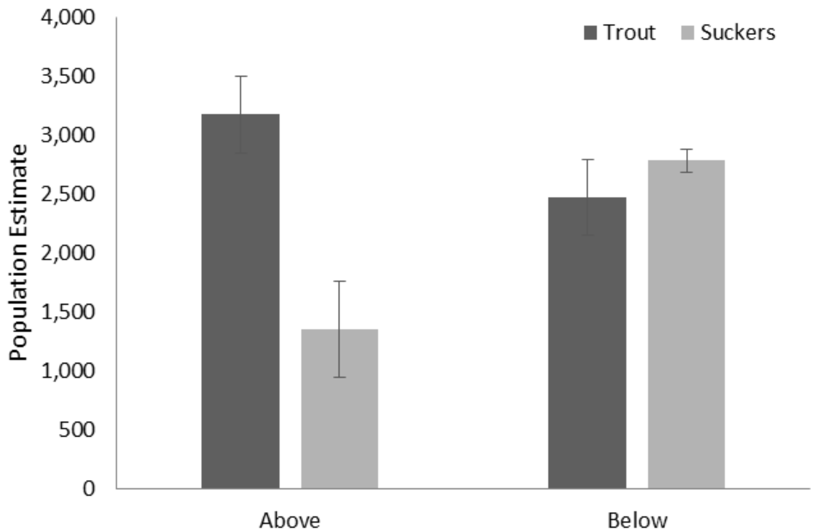

FIGURE 18. Fish population estimates and 95% confidence intervals above and below the WWP structure on the Colorado River at Pumphouse in 2017. Trout estimates combine Brown Trout and Rainbow Trout and sucker estimates combine Longnose Sucker and White Sucker.

Fish Passage

The structure does not appear to be a complete migration barrier for adult Brown or Rainbow Trout but is likely impeding movement of juvenile salmonids. Over the short term, four Brown Trout 371-422 mm were documented passing above the structure between the first and second passes in 2016. In 2017, four Brown Trout 204-430 mm and one Longnose Sucker 296 mm were documented passing above the structure between the first and second passes.

In the long term fish passage evaluation, 26 of the 142 adipose fin clipped trout that were moved below the structure in 2016 were recaptured above the structure, including three Rainbow Trout (312-395 mm) and 23 Brown Trout (274-526 mm). Adult Rainbow Trout and Brown Trout have been documented passing the structure but to date smaller fish are not passing the structure proportionate with the numbers of marked fish. Two Brown Trout measuring 204 mm and 212 mm were the smallest fish documented passing upstream through the WWP of 151 fish marked (250 mm and smaller).

Mottled Sculpin

Mottled Sculpin densities at the WWP structure have declined significantly (at the 95% confidence level) from 2014 to 2017, and the WWP site has the lowest Mottled Sculpin densities of the three sites (Figure 19). However, Mottled Sculpin densities were down at all sites in 2017 and while densities have declined 39% at the WWP site, that difference is not significant at the 95% level due to the high annual variability (Figure 20).

CONCLUSIONS

The whitewater park on the Uncompahgre River has not impacted fish at the population level at a detectable scale. Aquatic invertebrate communities were not impacted on a large scale but one site in the WWP has declined in invertebrate diversity and now has a functionally different invertebrate community than other riffle sites in the river. That site has the lowest diversity of total species and EPT species of any sampling site. The impacted site occurred where WWP structures were placed close together given the geomorphology of the river and has converted a riffle into a run.

The Gore Canyon whitewater park structure has impacted fish distribution and suppressed the movement of juvenile trout, but no population level impacts were documented. Habitat changes after construction of the whitewater park are likely reducing the habitat suitability for trout around the structure and increasing densities of white and longnose suckers. The WWP also affected the diversity of aquatic invertebrates with declines in total species richness and EPT species richness, both post construction and compared to reference sites.

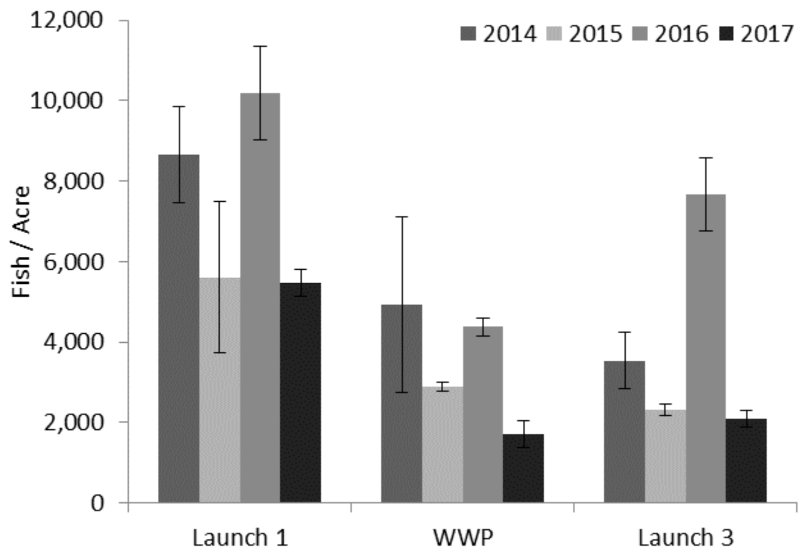

FIGURE 19. Mottled Sculpin density estimates and 95% confidence intervals on the Colorado River at Pumphouse 2014-2017.

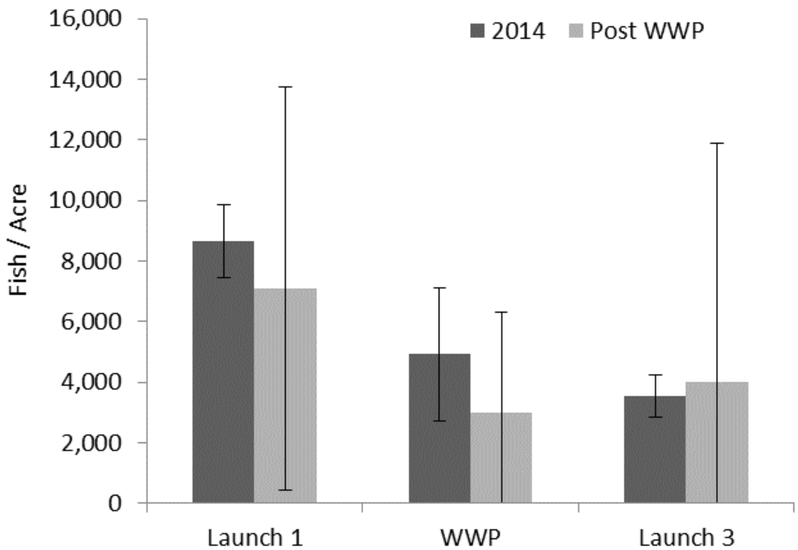

FIGURE 20. Mottled Sculpin density estimates and 95% confidence intervals on the Colorado River at Pumphouse before and after construction of the whitewater park structure.

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Job No. 3. Colorado River Ecology and Water Project Mitigation Investigations

Coauthored by Brian D. Heinold, Colorado Parks and Wildlife Aquatic Research Section, Fort Collins, Colorado.

Job Objective: Investigate the ecological impacts of stream flow alterations on aquatic invertebrates and fish of the Colorado River and evaluate the mitigation efforts associated with Windy Gap Firming project.

Dams are known to drastically alter the habitat of rivers and have a multitude of impacts on fish and aquatic invertebrates (Ward and Stanford 1979). Trans-basin water diversions remove approximately 67% of the annual flow of the upper Colorado River and future projects will deplete flows further. Previous work under Project F-237 identified ecological impacts of streamflow reductions and a main stem reservoir on the invertebrates and fish of the river. Native Mottled Sculpin *Cottus bairdii*, once common, are now rare or extirpated immediately below Windy Gap Reservoir (Dames and Moore 1977, Nehring et al. 2011). The health of the invertebrate community also declined after the construction of Windy Gap Reservoir: there has been a 38% reduction in the diversity of aquatic invertebrates from 1980 to 2011. A total of 19 species of mayflies, four species of stoneflies, and eight species of caddisflies had been extirpated from the sampling site below Windy Gap Reservoir (Erickson 1983, Nehring et al. 2011). Historically, the stonefly *Pteronarcys californica* was common in the upper Colorado River but have become rare immediately below Windy Gap Reservoir (USFWS 1951, Nehring et al. 2011).

In addition to impacts on the aquatic invertebrate community, Windy Gap Reservoir has altered the fish community of the upper Colorado River. Stream reaches below many of dams and water projects in Middle Park have reduced density of Mottled Sculpin (Nehring et al. 2011). The decline in sculpin distribution appears both temporally and spatially related to impoundments (Kowalski 2014). A survey in 1975-1976, before Windy Gap Reservoir construction, documented sculpin at all sampling sites (Dames and Moore 1977). In 2010, a project investigating the distribution of sculpin in the upper Colorado River revealed that their density was 15 times higher in sites above impoundments compared to downstream sites (Nehring et al. 2011). In the main stem Colorado River between Windy Gap Reservoir and the Williams Fork, a single fish was sampled in 3,200 ft of river sampled by electrofishing. This study attributed the decline of sculpin in the upper Colorado River to habitat and flow changes below the reservoir. Surveys in 2013 confirmed these patterns finding sculpin common above impoundments on the upper Colorado River but rare or absent downstream. No sculpin were found at three sites between Windy Gap Reservoir and the Williams Fork River (Kowalski 2014).

The planned Windy Gap Firming Project will increase trans-basin water diversions from the upper Colorado River. There are ongoing efforts to implement mitigation measures to reduce the impact of the new projects. A large component of the mitigation plan is the construction of a bypass channel around the reservoir. This would reconnect the Colorado River and address various impacts of a large main channel impoundment but the firming project overall could exacerbate flow depletions from the system. The planned bypass channel offers a unique opportunity to evaluate the effects of reconnecting the river and investigate if mitigation measures can offset the impacts of large flow depletions on the ecology of the river.

OBJECTIVES

1. Continue monitoring invertebrate and fish populations of the upper Colorado River.
2. Evaluate the effectiveness of mitigation measures in restoring the ecological function of the Colorado River in Middle Park.
3. Compare aquatic invertebrate sampling methods common in Colorado.

METHODS

Aquatic invertebrate samples were taken at seven sites on the Colorado River in 2018 and fish sampling occurred at four sites (Table 3, Figures 21-22). Invertebrate samples were collected by two different protocols commonly used in Colorado, the standard USGS method used by the National Water Quality Monitoring Laboratory (Moulton et al. 2000) and the MMI method used by Water Quality Control Division of the Colorado Department of Public Health and Environment (CDPHE). Samples were taken by both methods from the same riffle at each site.

The USGS method involved taking five replicate macroinvertebrate samples at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. Because a known and exact area of stream bottom is sampled by the Hess sampler, true density estimates can be made. Samples were collected September 11-12, 2018. All replicates were collected from the same riffle with predominantly cobble substrate by disturbing the streambed to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and preserved in 80% ethanol. Samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol (Moulton et al. 2000). Approximately 300 individual organisms were identified from each replicate and a 15 minute search for large or rare organisms was conducted on the entire sample. All organisms were identified to genus or species except chironomids were identified to family and non-insects were identified to class. Each replicate was processed separately so that more individual specimens were identified from each site to ensure rare organism were identified and to increase the power of the comparisons between riffle sites in close proximity (Vinson and Hawkins 1996). All taxonomic identifications followed recommendations by Moulton et al. (2000) and were completed by a single CPW invertebrate taxonomist. Recommended quality control and quality assurance procedures were followed and at least 10% of all individual identifications were verified by Dr. Boris Kondratieff at Colorado State University (Moulton et al. 2000). All invertebrates and material remaining after the subsampling procedure was also checked for the presence of non-represented species. Four individual identifications were raised from Genus to Family level, but no misidentifications occurred and no additional species were identified in the remaining material from each subsample.

The MMI is a multimetric index that is that standard regulatory method used by the state of Colorado to determine stream impairment under the Colorado Water Quality Control Act and the Federal Clean Water Act (CDPHE 2010a). Multimetric indices combine invertebrate community information with expected species composition and community metrics from reference sites.

They have been shown to be an effective and cost-efficient method for invertebrate bioassessment (Hughes and Noss 1992, Barbour et al. 1995, Karr 1998). The Colorado MMI is made up of metrics that represent various aspects of the community structure and function and are grouped into five categories: taxa richness, composition, pollution tolerance, functional feeding groups, and habit. Combining metrics from these categories into a multi-metric index transforms invertebrate sampling data into a unit-less score that ranges from 0-100 that indicates the community health and stream condition (CDPHE 2010a).

Sampling protocols followed standard methods and involved collecting a semi-quantitative kick net sample from each site (CDPHE 2010b). Approximately one square meter of stream bottom was disturbed for a timed one minute and all organisms were preserved in 80% ethanol. Sampling occurred on the same day and from the same riffles as the USGS method. Samples were sent to the Colorado Department of Public Health and Environment, Denver, CO and processed using the same methods, taxonomists, and facilities as CDPHE-collected samples. Processing the MMI samples involves subsampling and identifying a fixed count of 300 individual organisms from the entire sample, including chironomids to species. Because the area of stream bottom sampled is approximated and sampling time is restricted, the MMI method cannot provide true density estimates but instead is a community index of invertebrate quality collected by standardized methods where sites can be compared to each other as well as to reference sites of similar stream types.

Fish sampling occurred at four of the invertebrate sites. The objective was to monitor the composition of the fish community of the Colorado River and specifically to monitor for Mottled Sculpin. Fish sampling focused on the habitat of small-bodied fish (<150 mm). Larger trout were captured incidentally and measured but the focus was on young-of-year trout and other small-bodied fish. Fish sampling consisted of single or multiple pass electrofishing with three Smith Root 20B backpack electrofishers. A 30.5 m (100 ft) reach of stream was sampled along a randomly selected bank and approximately 1/3 the stream channel was covered with three backpack electrofishers (mean width 6.5 m). If sculpin were found in the first 30.5 m then three pass removal sampling was completed to estimate density. If no sculpin were found the sampling continued upstream for a total of 91.4 m (300 ft). All fish were counted and measured to the nearest millimeter. At sites where Mottled Sculpin were found, three pass density estimates were made with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999).

TABLE 3. Aquatic invertebrate sampling sites 2018. UTMs are in zone 13. Fish sampling occurred at sites CR1, CR2, CR5, and CR6.

Site Number	Site Name	UTM East	UTM North
CR1	Below Fraser Confluence	416914	4439457
CR2	Hitching Post Bridge	414652	4440330
CR3	Chimney Rock, Upper Red Barn	412703	4439648
CR4	Sheriff Ranch	408973	4438004
CR5	Pioneer Park SWA	405504	4436635
CR6	Hot Sulphur SWA, Gerrans Unit	403440	4434141
CR7	Breeze Bridge	398319	4435421

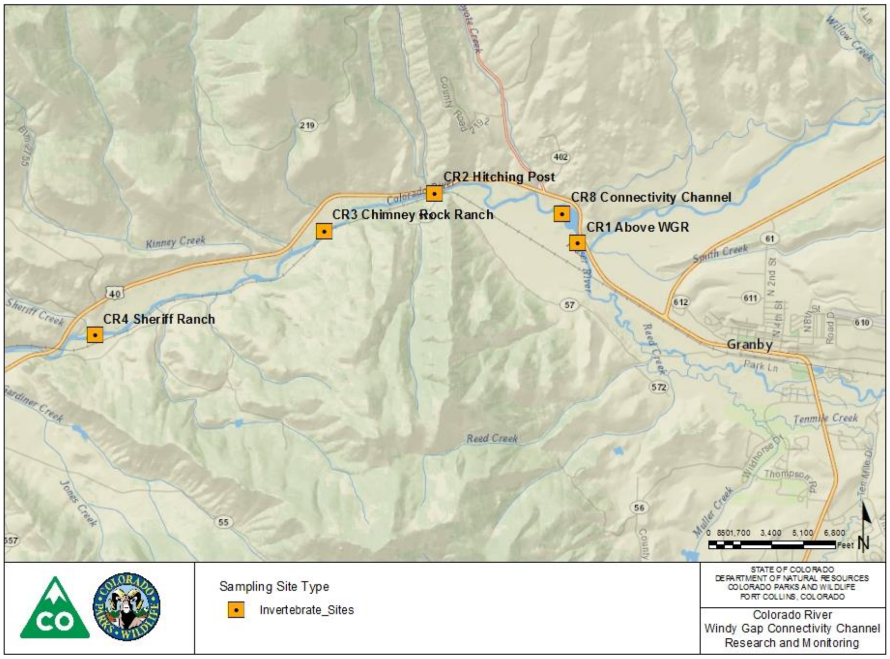

FIGURE 21. Map of the upper benthic macroinvertebrate sampling sites on the Colorado River. Site CR8 will be sampled in the future after construction of the connectivity channel. Fish sampling occurred at CR1 and CR2.

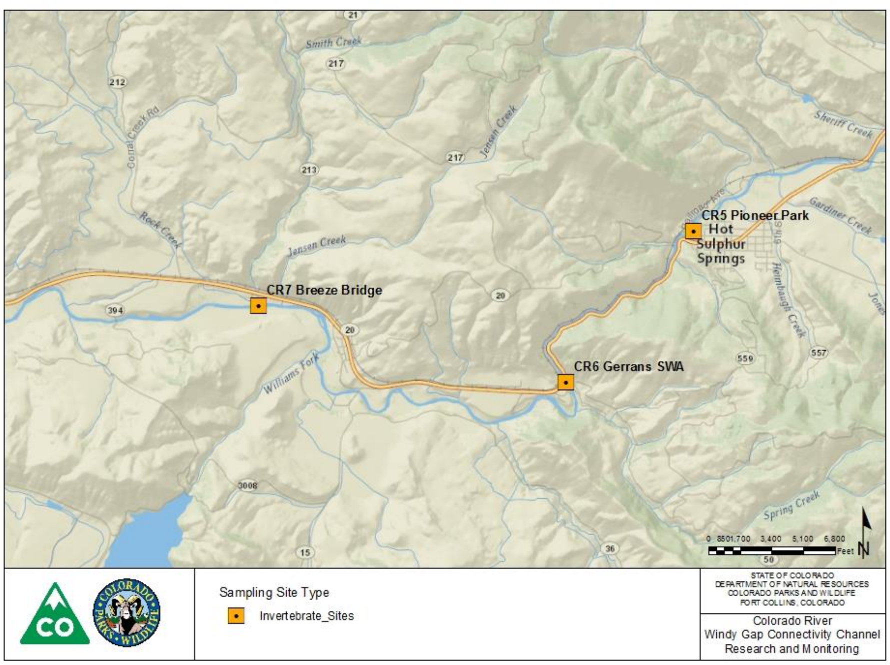

FIGURE 22. Map of the lower benthic macroinvertebrate sampling sites on the Colorado River. Fish sampling occurred at CR5 and CR6.

RESULTS

Invertebrate Sampling

There were no large differences in total invertebrate density between the sites but the density of stonefly species declined below Windy Gap Reservoir and the diversity of the invertebrate community decreased as well. Generally, species diversity was highest at the most upstream site and declined at sites below the reservoir. Site CR1 above Windy Gap Reservoir consistently had the most diverse invertebrate community and scored the highest regardless of method or index (Figures 23-26). Sites CR4 (Sheriff Ranch) and CR7 (Breeze Bridge) were particular low scoring in species diversity and community health indices. Site CR1 had the highest MMI score (67.6) while three of the six sites below Windy Gap Reservoir were below the state impairment standard (Table 4). Plecoptera diversity was lower below Windy Gap Reservoir; there were six species of stoneflies at CR1 and three to four species at all of the sites below the reservoir. Plecoptera density was also much lower at sites below Windy Gap Reservoir (Figure 27). Density was estimated at 1,122 per m^2 (SE 274) at site CR1 while the average of all the sites below Windy Gap Reservoir was 346 per m^2 (SE 70).

Site CR6, Gerrans Unit of the Hot Sulphur Springs SWA, was the most diverse site below the reservoir and ranked second in community health indices behind only site CR1. This site is below Byers canyon, a narrow higher gradient reach of Colorado River that has been identified as having the largest population of salmonflies of sites below the reservoir (but above Gore Canyon) in the salmonfly habitat study (Job 1) and previous work (Nehring et al. 2011). It appears that the increased velocity and gradient of the river in the confined reach in Byers Canyon leads to improved invertebrate community below, potentially due to decreased fine sediment, lower cobble embeddedness and lower width to depth ratio (Job 1).

While previous work identified declines in the range of some species of aquatic invertebrates, the 2018 sampling did document the presence of several species of interest at some sites below Windy Gap Reservoir. Salmonflies were sampled with the USGS method at sites CR3, CR4, CR5, CR6 and CR7 and by the MMI method at sites CR1, CR4, CR5, and CR6. Densities of salmonflies were low at all sites except CR6. While it was encouraging to document their presence at five of the seven sites, they remain rare or absent immediately below Windy Gap Reservoir. The mayfly *Drunella grandis*, which has declined in range in the Colorado River, was documented at sites CR1, CR2, CR5, and CR6 by the MMI method and CR1, CR2, CR4, CR5, CR6, and CR7 by the USGS method. This species was rare or absent at sites immediately below Windy Gap Reservoir in 2010 so its presence at CR2 is a positive sign for the upper Colorado River. However, several sensitive invertebrate species that were present in the river in the early 1980's before Windy Gap Dam was constructed continue to be absent from sites below the reservoir. Mayflies in the genus *Rhithrogena* were reported pre-construction and are found at downstream sites (CR4, CR5, and CR6) but not found in 2018 at sites immediately below the reservoir. Mayflies in the genus *Heptagenia* were reported at multiple sites in the early 1980s but were absent in 2010 and 2018. Stoneflies in the genus *Isogenoides* and *Pteronarcella* are also no longer found at sites below Windy Gap Reservoir though they were documented there before construction.

TABLE 4. MMI scores for invertebrate sampling sites on the upper Colorado River in 2018. A score of greater than 48 is needed to attain the aquatic life standard for cold water class I waters and a score less than 40 indicates impairment.

	CR1	CR2	CR3	CR4	CR5	CR6	CR7
MMI Score	67.6	57.8	38.1	37.9	59.0	62.3	35.7

The two sampling methods generally showed similar trends between the sites but the USGS methods almost always detected more species of invertebrates at each site (Table 5). At site CR7 the CDPHE methods detected more total species than the USGS method but at all other sites and families the USGS method found more species. The CDPHE method samples approximately $1 m^2$ while the USGS method samples a total of $0.43 m^2$. Despite sampling less than half of the streambed area of the CDPHE method, the USGS method identifies more individual insects from each site and those individuals come from a broader spatial area due to the replicate samples. A subsample of 300 individual invertebrates were identified per sample with the CDPHE methods while an average of 1,631 were identified with the USGS method and each replicate was entirely searched for large and rare organisms. Because of the larger number of identified invertebrates, the replicate samples, and the large/rare search of the entire sample, the USGS method appears to do a better job of representing more of the species present at each site. However, considering that the CDPHE method identifies less than 20% of the individual insects per site, it still detects on average 94.8% of the total number of species and 64.3% of the EPT species of the USGS method. Within each method the same trends were shown between sites and generally the methods produced similar conclusions about aquatic invertebrates at the community level.

TABLE 5. The number of species of invertebrates collected by the two sampling methods.

	Ephemeroptera		Plecoptera		Trichoptera		Total Taxa	
Site	CDPHE	USGS	CDPHE	USGS	CDPHE	USGS	CDPHE	USGS
CR1	6	7	5	6	6	9	36	38
CR2	5	8	1	4	6	10	35	37
CR3	3	7	1	4	6	9	31	34
CR4	7	7	2	4	4	8	34	36
CR5	6	8	2	4	7	9	33	37
CR6	5	7	3	4	5	10	28	37
CR7	7	8	0	3	5	7	41	32

The CDPHE method is a superior method for information on Chironomidae and non-insect taxa like Oligochaeta due to the higher level of taxonomic identification for those families. Chironomidae can be useful for evaluating the presence of pollution-tolerant midge species and species level identification of Oligochaetes has utility if concern exists about the secondary host of Salmonid whirling disease *Tubifex tubifex*. The CDPHE method took less time to collect at each site and the sample processing costs were considerably less. The main benefit of the CDPHE method is that it is specifically calibrated to the native invertebrate communities in Colorado and stratified by stream type. If the sampling objective is to generally characterize invertebrate community health, then the multimetric index of the CDPHE method (MMI) is a cost-efficient tool that also has the weight of regulatory authority behind it. However, the more time and labor intensive USGS method is superior for detecting rare species and giving real density estimates.

The results of the 2018 benthic sampling reflect the patterns in invertebrate community of the Colorado River presented in previous work (Nehring et al. 2011). Generally, while healthy and diverse invertebrate communities exist above the reservoir, sites below Windy Gap Reservoir are less diverse, have lower numbers of sensitive species, and are lower in the density and diversity of stonefly species.

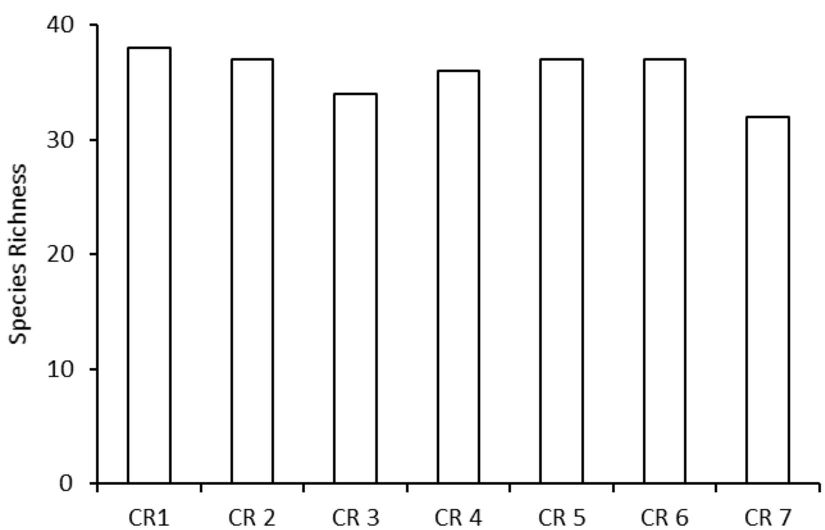

FIGURE 23. Total species richness from Colorado River invertebrate sampling with the USGS method in 2018.

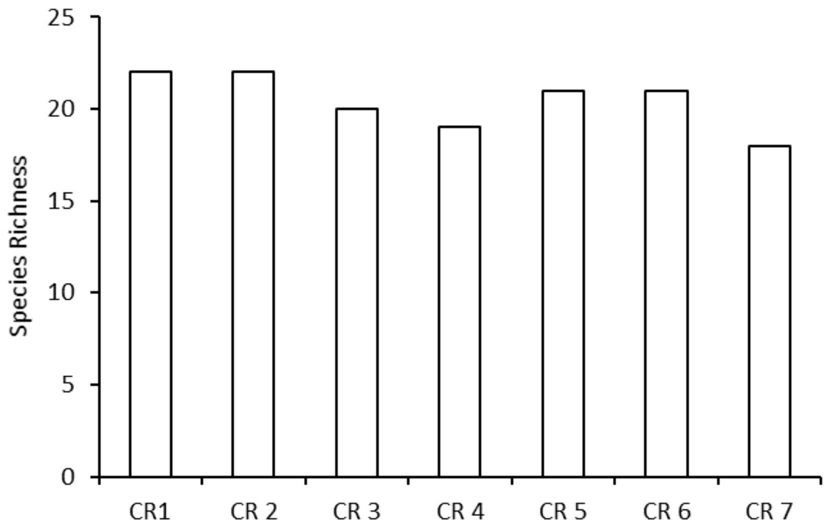

FIGURE 24. Ephemeroptera, Plecoptera, and Trichoptera species richness from Colorado River invertebrate sampling with the USGS method in 2018.

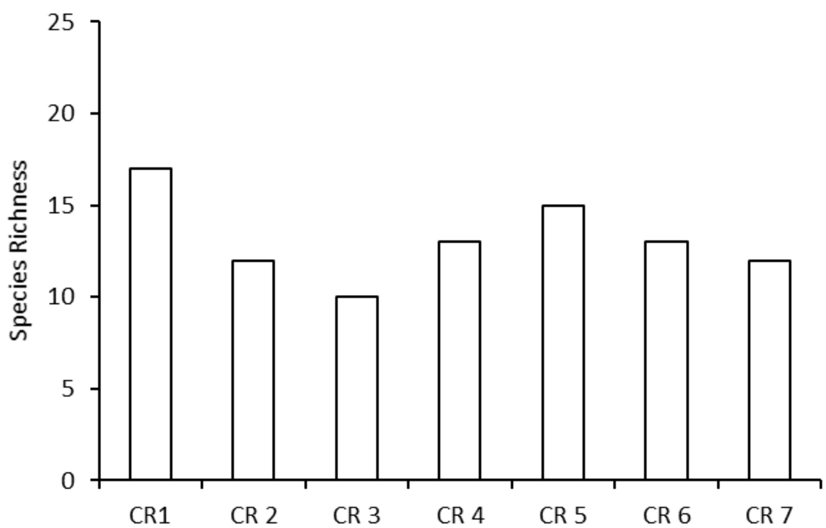

FIGURE 25. Ephemeroptera, Plecoptera, and Trichoptera species richness from Colorado River invertebrate sampling with the CDPHE method in 2018.

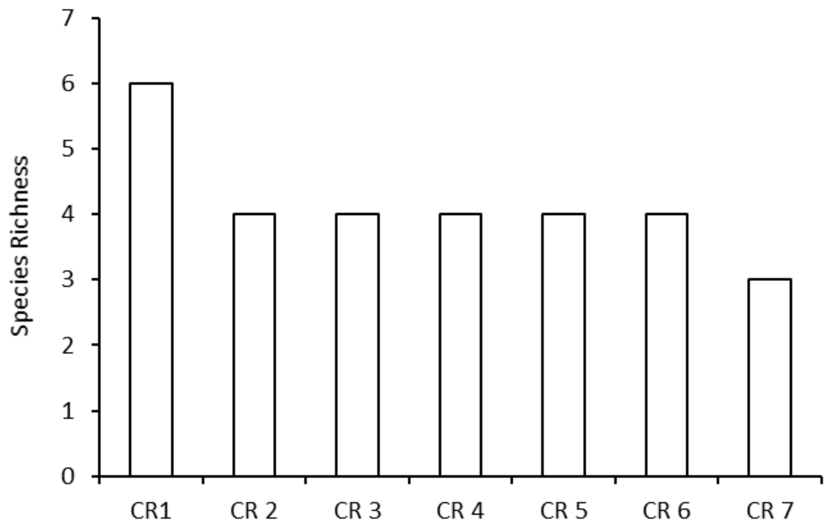

FIGURE 26. Plecoptera species richness from Colorado River invertebrate sampling with the USGS method 2018.

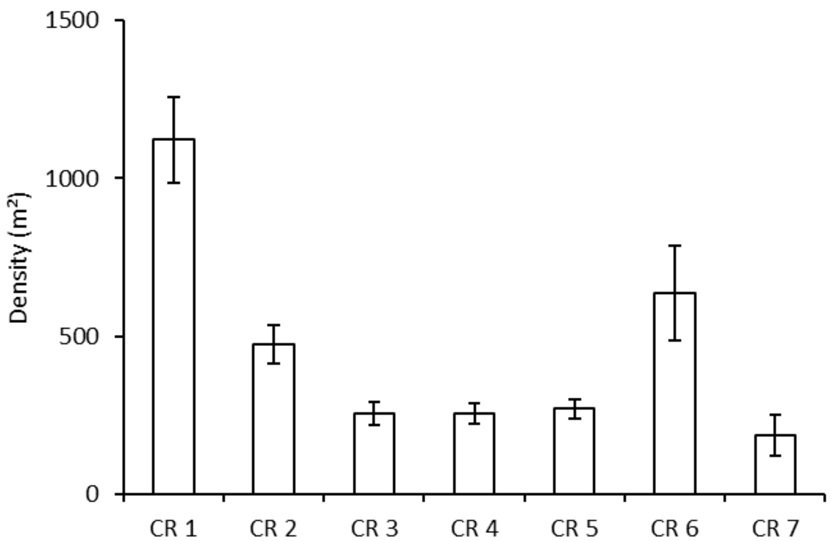

FIGURE 27. Plecoptera density and standard error bars from Colorado River invertebrate sampling 2018.

Fish Sampling
Mottled Sculpin were found at a single site on the Colorado River (CR1) immediately above Windy Gap Reservoir (Table 6). At that site there was an estimated density of Mottled Sculpin of 0.20 per m^2 (95% confidence interval 0.18-0.32). This density is similar to what has been observed in the Colorado River above the Fraser confluence but lower than the Fraser River itself (J. Ewert, Colorado Parks and Wildlife unpublished data). No Mottled Sculpin were observed at the three sampling sites below Windy Gap Reservoir despite the sampling of 1,806 m^2 of stream and the capture of 237 other individual small-bodied fish. Extensive sampling near our study sites on the Colorado River for trout fry (multiple pass electrofishing at five sites sampled four times annually) also failed to find Mottled Sculpin in 2018 below Windy Gap Reservoir (E. Fetherman, Colorado Parks and Wildlife personal communication). These results reflect the pattern of Mottled Sculpin distribution reported in previous work; this native fish species continues to be absent in formerly occupied habitat in the Colorado River below Windy Gap Reservoir (Erickson 1983, Nehring et al. 2011, Kowalski 2014).

CONCLUSIONS

Fish and aquatic invertebrate sampling results from the upper Colorado River in 2018 reflect the patterns presented in previous work (Nehring et al. 2011). Generally, while healthy and diverse invertebrate communities exist above the reservoir, sites below Windy Gap Reservoir are less diverse, have fewer sensitive species, and are lower in density and diversity of stonefly species. Several sites below Windy Gap Reservoir fall below the state standard for coldwater stream impairment. Several species of disturbance-sensitive aquatic invertebrates that were rare or absent below Windy Gap Reservoir in 2010 were confirmed to be present, although in low numbers and not at all sites. Fish sampling results from 2018 also reflect patterns previously observed in the upper Colorado River, native Mottled Sculpin continue to be absent from sites below Windy Gap Reservoir while they are common above the reservoir and in tributaries.

Both the USGS method and CDPHE method were informative in evaluating the aquatic invertebrate community of the sampling sites and generally gave similar information on the trends between sites. The USGS method was superior for detecting rare species, fully characterizing the diversity at each site, and giving true density estimates. The CDPHE method was faster, more cost-effective, superior for identifying midges and oligochaete worms, and has the added benefit of being able to produce standard metric scores comparable to the state water quality standards and to other locations in western Colorado.

TABLE 6. Summary of fish sampled at four monitoring sites on the upper Colorado River in 2018. MTS is Mottled Sculpin, LOC is Brown Trout, RBT is Rainbow Trout, WHS is White Sucker, LGS is Longnose Sucker, LND is longnose Dace, and JOD is Johnny Darter.

Site	CR1 Below Fraser	CR2 Hitching Post	CR5 Pioneer Park	CR6 Gerrans SWA
Stream Area Sampled	193.5 m²	599.2 m²	603.7 m²	603.1 m²
Species (# sampled)	MTS (33)	LOC (30)	LOC (14)	LOC (28)
	LOC (2)	RBT (2)	LND (57)	LND (63)
	RBT (2)	LND (33)	WHS(5)	LGS(3)
	WHS (1)		LGS(2)	
	JOD (13)			

ACKNOWLEDGMENTS

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Job No. 4. Bacterial Kidney Disease Investigations

Job Objective: Investigate the distribution and prevalence of *Renibacterium salmoninarum*, the causative agent of Bacterial Kidney Disease, in Colorado's wild trout and stocked sport fisheries.

Native and sport fish populations across Colorado are impacted by many factors including habitat alterations, depleted stream flows, changes in temperature regime, water quality impacts, and host of less obvious biological threats from diseases and parasites. While the prevalence of many fish diseases has declined in recent years due to good management practices, cases of bacterial kidney disease (BKD) seem to be increasing. The causative agent of bacterial kidney disease is *Renibacterium salmoninarum*, a gram-positive intracellular parasite. The disease is characterized by the presence of gray-white necrotic abscesses in the kidney and can cause mortality in both wild and cultured salmonids. Unlike other common fish pathogens, this bacterium can be transmitted horizontally between fish through contaminated water and vertically from adult to egg due to its intracellular nature. This likely plays a major role in the persistence of this bacterium in susceptible fish populations.

Renibacterium salmoninarum and bacterial kidney disease is a regulated fish disease in the state of Colorado. Fish production facilities that test positive are generally prohibited from stocking fish in state waters except in specific instances (Colorado Parks and Wildlife Regulations Chapter 0, Article VII, #14). From 1970 to 1999 the bacteria was detected at least 16 times at state or federal fish hatcheries during routine fish health inspections. A reported 14,159,445 fish were stocked from those hatcheries into all counties in Colorado and all major river drainages (Kingswood 1996). After going undetected for in the state hatchery system for 18 years, four state hatcheries, one federal fish hatchery, and a wild broodstock lake have tested positive for the disease since 2015. Clinical disease has been documented at least two times since 2016 and an outbreak at one hatchery cost over $2.1 million and impacted fish management statewide with the loss of over 675,000 sport fish. The recent detections of *R. salmoninarum* in hatcheries and wild fish populations in Colorado has generated questions about its prevalence in feral trout populations and caused managers to revisit best management practices in hatcheries. Despite the large body of knowledge about *R. salmoninarum* in anadromous Pacific salmonids, relatively little is known about the bacteria in resident trout of the interior western U.S.

The objective of this study was to document the distribution and prevalence of *R. salmoninarum* in Colorado's wild and stocked sport fisheries and investigate if fish stocking practices have influenced that distribution.

OBJECTIVES

1. Investigate the distribution and prevalence of *R. salmoninarum* in Colorado's wild trout fisheries and stocked sport fisheries.

2. Survey a stratified random sample of wild trout streams in all major river basins in Colorado to determine the distribution and prevalence of *R. salmoninarum*.

3. Survey sport fisheries recently stocked with fish from hatcheries that tested positive for *R. salmoninarum* to determine if stocking has affected the prevalence and distribution.

METHODS

To investigate the prevalence of *R. salmoninarum* in wild trout streams in Colorado, Colorado aquatic data management system was used to randomly select third to fifth order streams in CPW management codes 302, 303, 405, and 406 in each major river basin. Streams were vetted by area fish biologists for inclusion and waters were removed for reasons such as lack of salmonid populations or ephemeral stream flow and they were replaced by the next randomly selected water. A total of 68 streams were sampled. To investigate if both recent and/or historical stocking practices have affected prevalence and distribution of the bacteria, we took two approaches. To investigate recent stocking practices, stocking records were compiled for all of the hatcheries that tested positive for *R. salmoninarum* in the last 20 years. Waters that received more than 1,000 stocked trout from these hatcheries ("suspect waters") were paired with nearby waters of the same or similar management code that had no recorded stocking in the last 20 years from positive hatcheries ("control waters"). A total of 75 different suspect or control stocked sport fisheries were sampled. To investigate historical practices, records were compiled for all study waters for two ten year time periods. The first time period was from 1987 to 1997 when positive tests in CPW hatcheries for *R. salmoninarum* were common, and the second time period was 1998-2008 when most state hatcheries were thought to be free from the bacteria. Forty-nine additional waters around the state were sampled opportunistically including waters that have specific management needs relating to BKD, waters around positive hatcheries, and waters with observed fish health issues. A total of 194 waters were sampled during this study (Figure 28). While some warmwater fish were sampled in the stocked waters sampling, the summaries and modeling results presented here are for salmonid species only due to the established susceptibility of trout species and the objectives of this project. Previous progress reports for Federal Aid Project F-237 contain data summaries that include non-salmonid results.

Disease samples were taken from up to 60 individuals of the dominant salmonid species present and up to 60 of the dominant warmwater game fish if present, with the number of samples varying by water and dictated by fish populations. In 2016, fish were sampled individually but in 2017 fish were combined into five fish lots by species and age class to reduce processing time.

Diagnostic Assays

Samples were tested by enzyme-linked immunosorbent assay (ELISA) at the Colorado Parks and Wildlife Aquatic Animal Health Laboratory and by real-time PCR (qPCR), nested PCR (nPCR), and direct fluorescent antibody test (DFAT) at the U.S. Fish and Wildlife Service Bozeman Fish Health Center. All assays followed American Fisheries Society Blue Book standard operating procedures (Elliot 2016, Elliot et al. 2016a, Elliot et al. 2016b).

The ELISA assay used a negative-positive threshold for optical density values (OD) of 0.100 following Munson et al. (2010) and the considerations outlined in Elliot et al. (2013) and Myers et al. (1993). Because of the unknown status of waters in this study for *R. salmoninarum*, we used a conservative threshold to reduce the probability of false positive results. The mean OD value for all negative controls was 0.071 (SD=0.0111) so the negative-positive threshold was conservative and the risk of false positive results was very low. The tiered classification system of Elliot et al. (2013) was used to characterize antigen levels. Optical density values between the negative threshold and 0.199 considered as low antigen levels, those between 0.200 and 0.999 as moderate antigen levels, and values greater than 1.000 as high antigen levels.

All samples with sufficient kidney tissue were screened by ELISA and qPCR. Positive results from qPCR tests were confirmed with nPCR and samples were considered PCR positive if they tested positive by both qPCR and nPCR. We compared lots of fish (single species from a single water) to compare the sensitivity of the assays and considered a water positive by a specific assay of any lots from that water were positive. To confirm a waters status as positive for management purposes it is recommended that results be confirmed by multiple assays (Elliot 2016).

Statistical Analysis
Experimental groups (wild trout, suspected, and control) were compared by the percent positive for a particular assay by chi-squared tests or Fisher's exact test for small sample sizes. Exact binomial confidence intervals for each group were calculated with an α level of 0.05.

To explore the relationship of ELISA OD values with historical stocking practices, linear regression models were built with explanatory variables for total trout stocked from 1987 to 2016, fish stocked from 1987 to 1997, fish stocked 1998-2008, and stream order or lake elevation. These models represented specific *a priori* hypotheses about how stocking could have affected prevalence and severity. The first ten-year period represents a time when many CPW hatcheries were likely positive for the *R. salmoninarum* and the second ten-year period when there were no positive inspections at CPW hatcheries. If stocking fish from positive hatcheries influenced bacteria levels in receiving waters then we hypothesized that fish stocked from 1987 to 1997 would better explain antigen levels.

To investigate how stream or lake size and location may affect antigen levels, models for streams included variables for stream order and lakes included elevation. We hypothesized that bacteria levels would increase in lower order streams and lower elevation lakes due to higher stocking rates, larger fish populations, and potentially more exposure to fish carrying the bacteria.

Linear regression modelling was performed with the lm function in Program R (R Core Team 2015). Model assumptions of homogeneity of variance and normality were evaluated by examining residuals of the global model. The response variable, OD value, was transformed with the Box Cox procedure due to patterns in the residuals (Box and Cox 1964). The lambda value had 95% confidence interval that included -1 so an inverse transformation was used. Model selection was done with the small sample size version of Akaike's information criterion (AIC_c) following Burnham and Anderson (2002). Program R was used for analysis including packages MASS and AICcmodav (R Core Team 2015). Relative variable importance of the explanatory variables was evaluated with correlation analysis, comparing standardized regression coefficients, and by comparing the cumulative model weights.

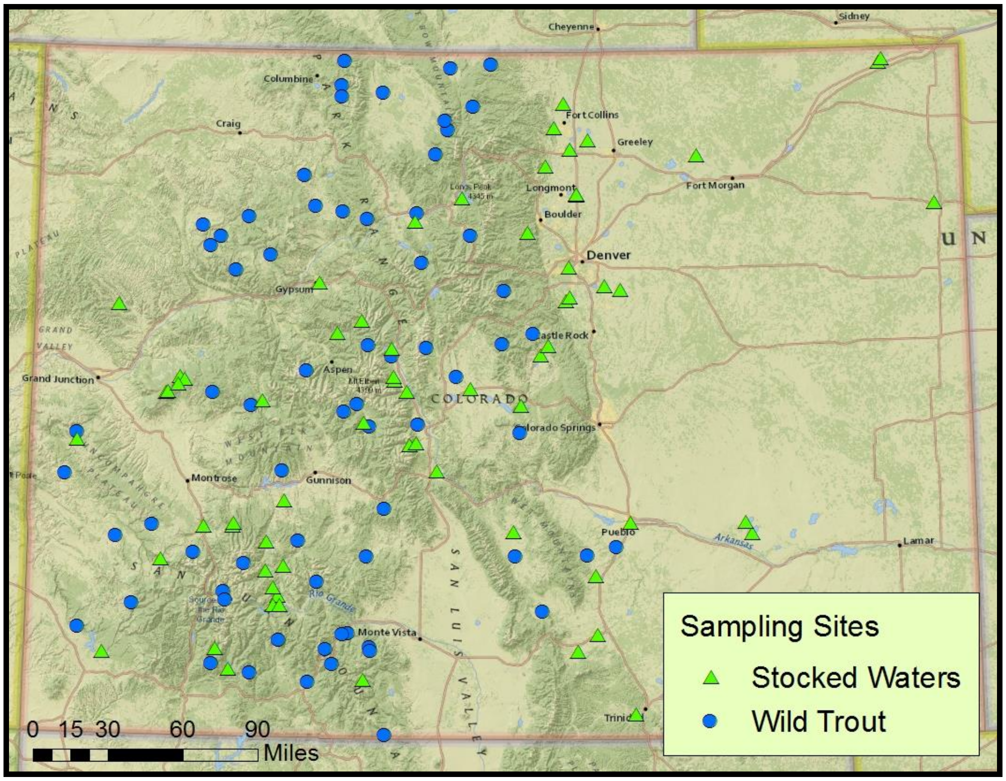

FIGURE 28. Waters sampled 2016-2017 and tested for *R. salmoninarum*.

RESULTS

A total of 194 waters were sampled during the two years of this study, 68 wild trout waters, 75 stocked sportfish waters, and 49 additional waters (Figure 28). Ninety-three percent of all waters had some samples that tested positive by ELISA with an OD threshold of 0.100. Thirty-seven percent had samples that tested positive by qPCR, 12% tested positive by both qPCR and nPCR, and 13% tested positive by DFAT. Positive cases by all assays were found throughout Colorado in all major drainages (Figures 29 and 30). Testing results of all waters in this study are summarized in Appendix A at the end of this report.

Stocked Sportfish Waters
Eighty-seven percent of stocked sportfish waters had some lots test positive by ELISA, 20% tested positive by DFAT, 45% tested positive by qPCR and 12% were confirmed positive by nPCR (Figure 31). There was no significant difference ($\alpha = 0.05$) between prevalence of *R. salmoninarum* in stocked or control waters by ELISA, PCR, or DFAT. There was also not a significant difference ($\alpha = 0.05$) between stocked and control waters' average OD value (Figure 32).

The modeling exercise, standardized regression coefficients, and simple correlation analysis supported this conclusion as well (Figure 33, Tables 7 and 8). Fish stocking from the time period where *R. salmoninarum* was common in hatcheries was negatively correlated with OD values and the relationship was weak (Figure 33). Lake elevation was the best predictor of OD values. Lake elevation was also the only significant correlation ($\alpha = 0.05$) and was the single explanatory variable in the top model (Table 8). Contrary to our hypothesis, lake elevation was positively correlated with OD values, higher elevation waters generally had higher levels of antigen of *R. salmoninarum* (Figure 34).

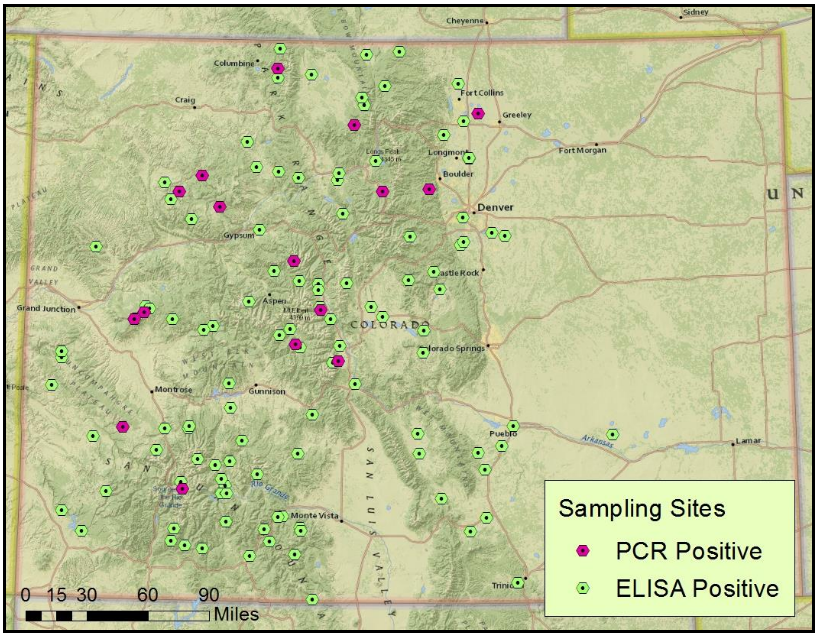

FIGURE 29. Study sites that tested positive for *R. salmoninarum* with qPCR and confirmed with nPCR.

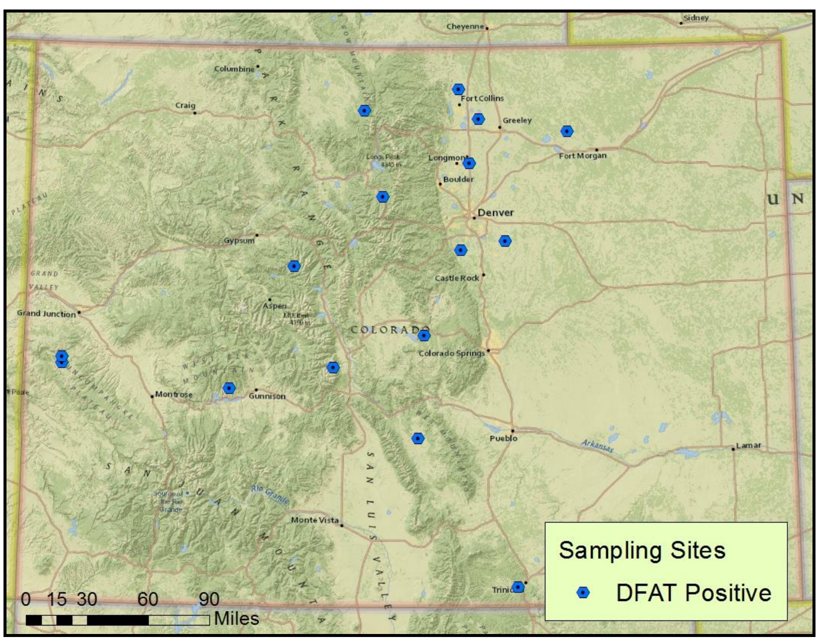

FIGURE 30. Study sites that tested positive for *R. salmoninarum* with DFAT.

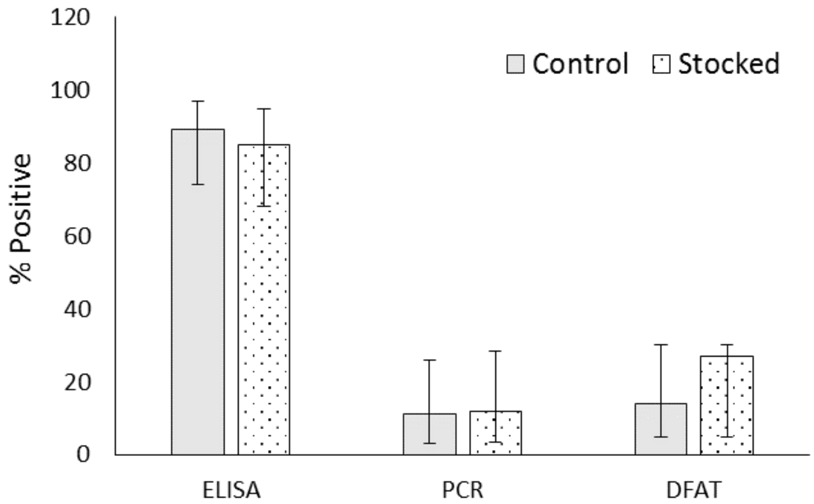

FIGURE 31. Positive test results and 95% binomial confidence intervals of waters stocked with suspect fish with nearby similar waters not stocked with fish from suspect hatcheries.

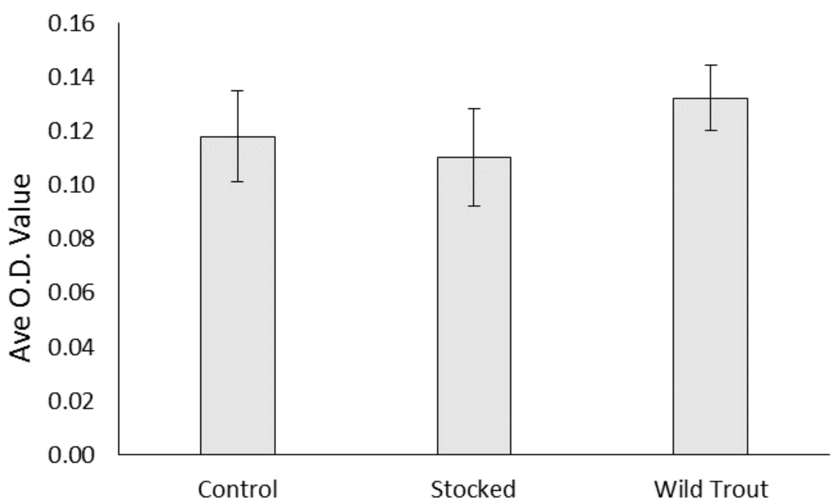

FIGURE 32. Average OD values of study waters and 95% binomial confidence intervals.

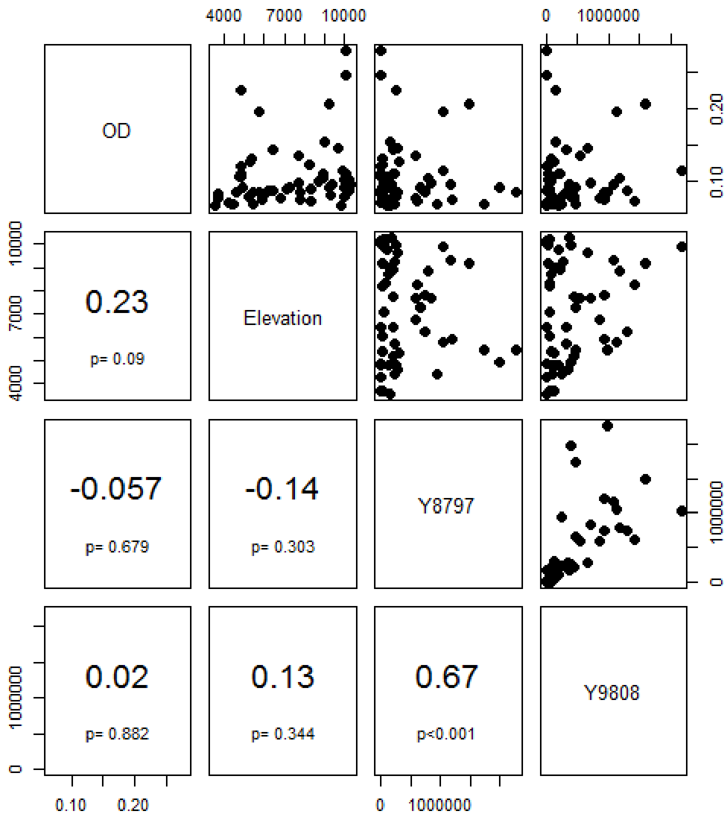

FIGURE 33. Pearson correlation coefficents and *p* values of lake variables with untransformed OD values to dsplay the correlaton patterns. Lake elevation was the most highly correlated variable and contrary to our hypothesis OD values increased with increasing elevation.

TABLE 7. Standardized regression coefficients and correlation coefficients of variables used in the linear regression modelling for lakes.

Variable	Standardized Regression Coefficient	Pearson Correlation Coefficient
Elevation	-0.26	-0.28
Stocked 1987-1997	0.10	0.09
Stocked 1998-2008	-0.07	-0.04

TABLE 8. Model selection results for linear regression models for study lakes. Presented are the number of model parameters (K), Akaike's information criterion corrected for small sample size (AIC_c), ΔAIC_c, AIC_c weight (w_i), and multiple R^2.

Model	K	ΔAIC_c	w_i	R^2
Elevation	3	0	0.67	0.08
Elevation x Stocked 1987-1997	5	3.64	0.11	0.10
Stocked 1987-1997	3	4.18	0.08	0.01
Stocked 1998-2008	3	4.53	0.07	0.08
Elevation x Stocked 1998-2008	5	4.67	0.07	0.08

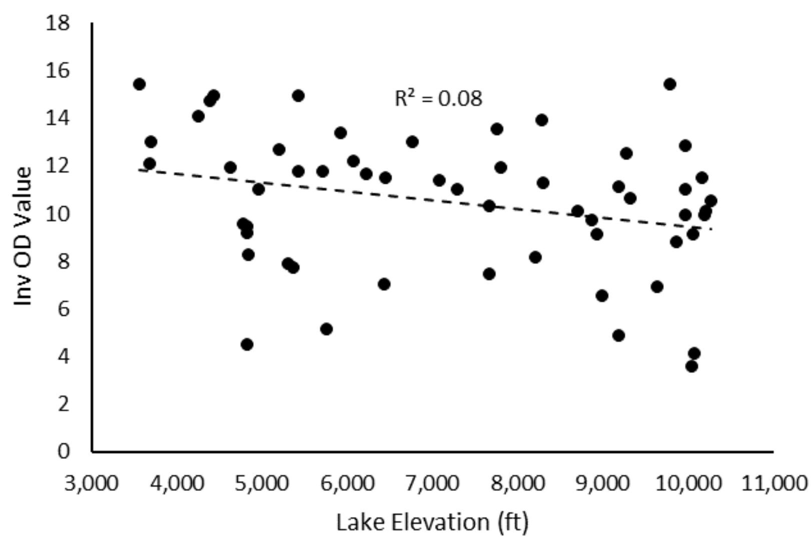

FIGURE 34. Top linear model for inverse transformed OD values for lakes.

Wild Trout Streams

All wild trout streams had some fish that tested positive by ELISA and 84% percent of individual lots tested positive by ELISA (Figure 35). Six percent of all waters tested positive by DFAT, 24% tested positive by qPCR and 13% were confirmed positive by nPCR. While prevalence of *R. salmoninarum* was high (100%) among wild trout waters, most of the samples from wild trout waters (as well as stocked waters) had relatively low antigen levels (Figure 32). Of the 116 lots tested from wild trout waters, 16% were negative, 45% had low antigen levels (OD < 0.199), 31% had moderate antigen levels (OD 0.200-0.999), and 8% had high antigen levels (OD > 1.000). Wild trout waters had significantly higher ($\alpha = 0.05$) average OD values and percent positives than stocked waters by ELISA but stocked waters had a higher percent positive than wild trout waters by qPCR and DFAT.

More than half (54%) of the wild trout waters were stocked at some point historically, but the prevalence and average OD values for those waters were very similar to wild trout waters with no stocking records (Table 9). None of the differences between the stocked and unstocked waters were significant at the $\alpha = 0.05$ level.

Simple correlations, standardized regression coefficients, and the linear modeling exercise confirmed trends in the prevalence data (Tables 10 and 11). While stocking during the time period that *R. salmoninarum* was common in hatcheries was the most highly correlated variable, that correlations was relative weak ($R^2 = 0.12$) and stocking was negatively correlated with OD values, OD values were higher at lower stocking levels.

The top model for stream data was the interaction model of stream order and fish stocking 1998-2008 (Table 11). Optical density values declined with increased stocking for lower order streams but the relationship is positive for higher order streams (Figure 36). While it was the top model, it only explained 20% of the variability in transformed OD values and there were very few samples collected from higher order streams so more sampling in large rivers is necessary to explore this relationship. Overall, the stocking and environmental variables that we explored in this study explained relatively little variation in OD values so more work is needed to investigate factors that are related to *R. salmoninarum* antigen levels in trout in Colorado.

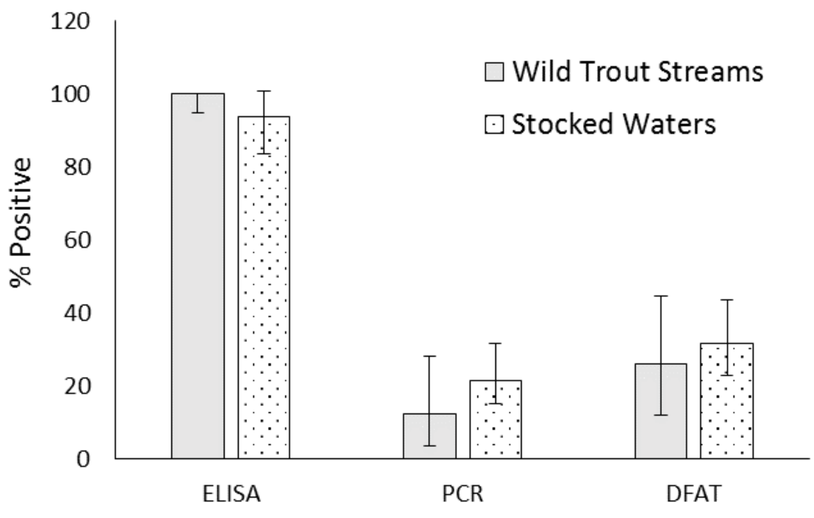

FIGURE 35. Positive test results of all waters in the stocked waters study and all wild trout waters.

TABLE 9. Comparison of wild trout waters with historical stocking records and those without.

	No Stocking Records (n=31)	Historical Stocking (n=37)
ELISA Ave OD	0.135	0.134
% Pos. ELISA	100	100
% Pos. qPCR	26	22
% Pos. nPCR	10	14
% Pos. DFAT	3	8

TABLE 10**.** Standardized regression coefficients and correlation coefficients of variables used in the linear regression modelling for streams.

Variable	Standardized Regression Coefficient	Pearson Correlation Coefficient
Stocked 1987-1997	0.18	0.34
Stream Order	0.20	0.33
Stocked 1998-2008	0.08	0.33

TABLE 11**.** Model selection results for linear regression models for study streams. Presented are the number of model parameters (K), Akaike's information criterion corrected for small sample size (AIC_c), ΔAIC_c, AIC_c weight (w_i), and multiple R^2.

Model	K	ΔAIC_c	w_i	R^2
Order x Stocked 1998-2008	5	0	0.59	0.20
Stocked 1987-1997	3	3.14	0.12	0.12
Stocked 1998-2008	3	3.52	0.10	0.11
Order x Stocked 1987-1997	5	3.64	0.10	0.16
Order	3	3.67	0.09	0.11

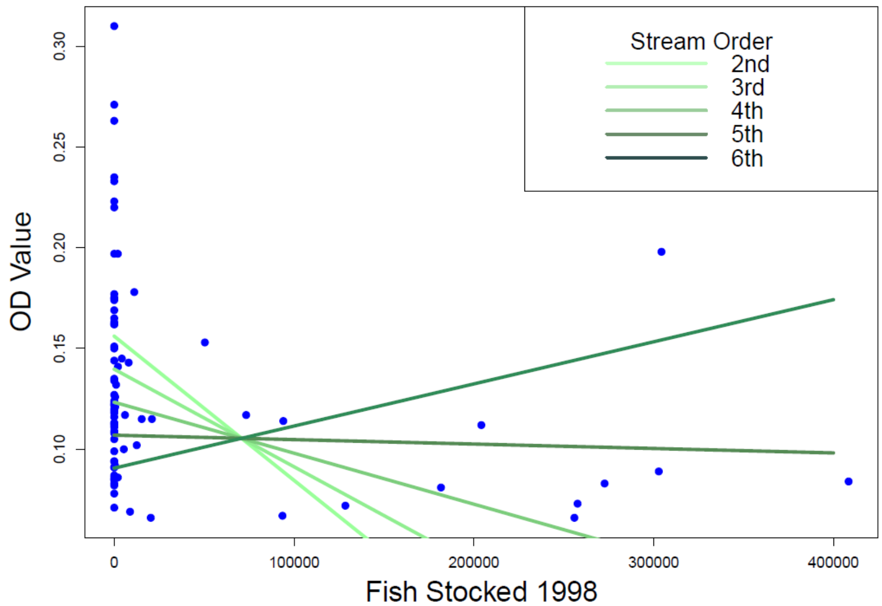

FIGURE 36. Plot of the top model for stream data, an interaction model of stream order and fish stocking 1998-2008, with untransformed OD values for trend interpretation purposes.

Diagnostic Assays

As reported in other work, ELISA was the most sensitive assay and detected the most positive cases. With a sample of size of 349-399 individual lots, qPCR detected 27.6% of the cases ELISA did, DFAT detected 11.2%, and qPCR confirmed with nPCR detected 8.8%. Using the tiered classification system on all individual lots, the ELISA low category had a 23% agreement with PCR, ELISA moderate had 67% agreement, and ELISA high had 90% agreement. This level of concordance is similar to previous work and should not be viewed as ambiguous test results. The different assays not only have varying diagnostic sensitivity but are testing for different endpoints (antigen vs. DNA) and can reflect different states of infection *R. salmoninarum* infection when kidney samples are tested (Elliot et al. 2013, Nance et al. 2010). Generally it is recommended that to confirm the presence of *R. salmoninarum* multiple assays should be used and antigen (DFAT, ELISA) and molecular test (qPCR, nPCR) tests should be used. We had 49 waters that tested positive by both a DNA and antigen test (Table 12).

One of the few studies published on *R. salmoninarum* in resident trout populations in Alaska reported that the standard DFAT assay would not detect *R. salmoninarum* in positive fish samples with OD values less than 0.173 and inconsistentely detected the bacteria at OD values less than 0.978 (Meyers et al. 1993). Of all our wild trout samples tested (n=1,616), 87.4% had OD values less than 0.17 and 99.6% were less than 0.98 (Figure 37). The vast majority of fish samples in our study would be unlikely to test positive by DFAT but actually have low *R. salmoninarum* anitgen levels.

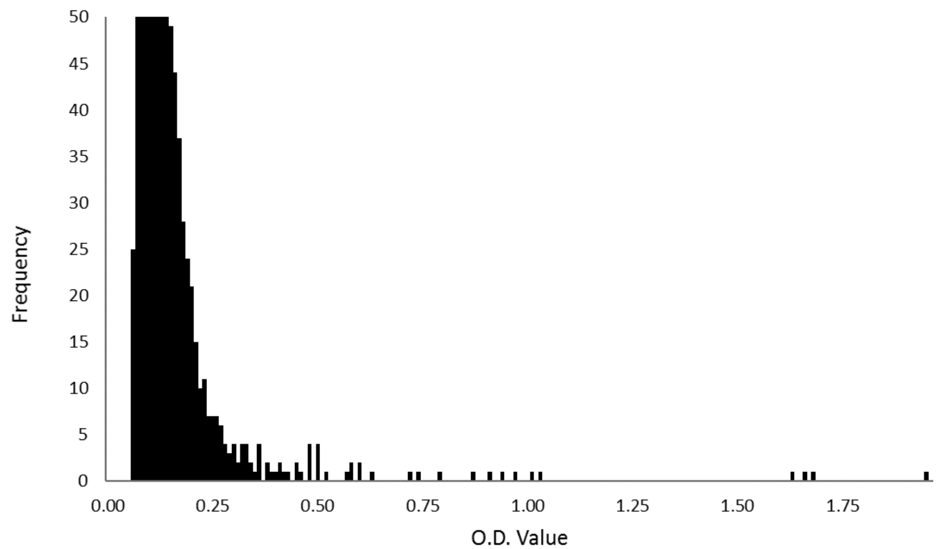

FIGURE 37. Distribution of OD values for all samples tested. Samples with OD values greater than 0.100 were considered positive. DFAT does not detect *R. salmoninarum* in positive fish samples with OD values less than 0.173 and inconsistentely detected the bacteria at OD values less than 0.978 (Meyers et al. 1993). Of all the samples tested in this study, 99.6% were less than 0.98 indicating DFAT is not a reliable tool to identify the presence of the bacteria's antigen at levels common in Colorado.

CONCLUSIONS AND RECOMMENDATIONS

The bacteria *R. salmoninarum*, causative agent of bacterial kidney disease, is widespread throughout Colorado's wild trout and stocked sport fisheries. While common and widespread, bacteria levels are generally low and clinical disease is very rare. After sampling over 12,800 individual fish from 194 waters thought out the state, only two clinical cases of bacterial kidney disease were observed in this study. Historical and recent stocking practices have little correlation with antigen levels or detection of *R. salmoninarum* DNA and fish stocking during periods where the bacteria was common in state hatcheries was actually negatively correlated with antigen levels. The elevation of lakes was a better predictor than any of the stocking variables we explored in stocked sport fisheries. In streams (both stocked and wild trout) stream order and the stocking variables were all similar in their correlation with OD values. They were all negatively related to OD values; as stream order increased and stocking increased, OD values declined. Bacteria levels generally increased at higher elevations and lower stream orders, contrary to our hypotheses, some of the highest average OD values we observed were in unstocked high elevation wild brook trout waters.

These findings agree with 1996 project at Colorado State University that found *R. salmoninarum* was widespread in Rocky Mountain National Park (Kingswood 1996). They sampled nine different waters and 100% were positive by ELISA. Eighty-two percent of all fish tested by ELISA were positive by ELISA and all samples were taken from wild self-sustaining populations with no clinical signs of disease. Our results also agree with studies outside of Colorado that found *R. salmoninarum* common in inland trout which were seen as common carriers of the bacteria and more resistant than anadromous salmonids (Meyers 1993).

The results of this study have some important ramifications for using the various screening assays on resident trout in Colorado. ELISA detected far more cases and detected much lower bacteria levels than the other assays. Using only the DFAT or PCR assay to screen resident trout populations or hatcheries in Colorado is likely to vastly underestimate the prevalence of *R. salmoninarum* and only identify rare cases with high bacteria levels. We recommend using a quantitative tool like ELISA to estimate bacteria levels of trout in Colorado, knowing that it is likely common but at low levels. Results should be confirmed with a molecular test for *R. salmoninarum* DNA in cases of high OD values or waters of high management or conservation importance.

ACKNOWLEDGEMNTS

The Colorado Parks and Wildlife Aquatic Animal Health Lab completed much of the sample collection and all of the ELISA testing. John Drennan and Vicki Milano were integral in project conception, planning, and completion. Tawni Riepe completed all of the ELISA testing. April Kraft, Victoria Vincent, Weston Niep, and Cody Minor were responsible for some of the sample collection and processing. This work was a close collaboration with U.S. Fish and Wildlife Service Bozeman Fish Health Center and the National Wild Fish Health Survey. Lacey Hopper, Rick Cordes, and Molly Bensley completed all of the PCR and DFAT testing. Andy Treble with Colorado Parks and Wildlife was integral in the sampling design and results presentation.

TABLE 12. Waters sampled 2016-2017 that tested positive for both the antigen and DNA of *R. salmoninarum*.

Water	Water Code	Study	qPCR	nPCR	ELISA	DFAT
Buck Creek	19340	Wild Trout	POS	POS	POS	NEG
Buffalo Creek	10380	Wild Trout	POS	NEG	POS	NEG
Cunningham Creek	39506	Wild Trout	POS	POS	POS	NEG
Elk River, North Fork #1	20189	Wild Trout	POS	POS	POS	NEG
Elk River, South Fork	20191	Wild Trout	POS	NEG	POS	NEG
Encampment River	10861	Wild Trout	POS	NEG	POS	NEG
Fraser River	20355	Wild Trout	POS	POS	POS	POS
Gunnison River, North Fork #2	40509	Wild Trout	POS	NEG	POS	NEG
Horsefly Creek	44507	Wild Trout	POS	POS	POS	NEG
Illinois River #4	13881	Wild Trout	POS	POS	POS	NEG
Lost Creek	14023	Wild Trout	POS	NEG	POS	NEG
Marvine Creek #1	21092	Wild Trout	POS	POS	POS	NEG
North Elk Creek	20139	Wild Trout	POS	POS	POS	NEG
North Fork Mesa Creek	41537	Wild Trout	POS	NEG	POS	NEG
Pinos Creek, West Fork	42161	Wild Trout	POS	NEG	POS	NEG
Rio de los Pinos #1	40173	Wild Trout	POS	NEG	POS	NEG
Chalk Creek Lake	81909	Stocked	POS	NEG	POS	POS
Chatfield Reservoir	54306	Stocked	POS	NEG	POS	POS
Clear Creek Reservoir	81719	Stocked	POS	NEG	POS	NEG
DeWeese Reservoir	81729	Stocked	POS	NEG	POS	POS
DeWeese Reservoir	81729	Stocked	POS	NEG	POS	POS
Douglas Lake	58695	Stocked	POS	NEG	POS	POS
Eagle Lake	66363	Stocked	POS	POS	POS	POS
Eagle Watch Lake	60210	Stocked	POS	NEG	POS	NEG
Gross Reservoir	55043	Stocked	POS	POS	POS	NEG
Hotel Twin Lake	90578	Stocked	POS	POS	POS	NEG
Lake San Cristobal	92130	Stocked	POS	NEG	POS	NEG
Little Battlement Reservoir	88472	Stocked	POS	POS	POS	NEG
Mallard Pond, St. Vrain State Park	58099	Stocked	POS	NEG	POS	N/A
Paonia Reservoir	91657	Stocked	POS	NEG	POS	NEG
Pelican, St. Vrain State Park	52388	Stocked	POS	NEG	POS	POS
Platoro Reservoir	91758	Stocked	POS	NEG	POS	NEG
Ridgway Reservoir	96695	Stocked	POS	NEG	POS	NEG
Roan Creek	21701	Stocked	POS	NEG	POS	NEG
Sand Piper, St. Vrain State Park	58087	Stocked	POS	NEG	POS	POS
South Platte River 1A	32641	Stocked	POS	NEG	POS	NEG
Taylor Reservoir	92510	Stocked	POS	POS	POS	NEG
Twin Lakes	80022	Stocked	POS	POS	POS	NEG
Windsor Reservoir	53645	Stocked	POS	POS	POS	POS
Wrights Lake	83128	Stocked	POS	POS	POS	NEG
Cap K Ranch	69528	Extra	POS	POS	POS	NEG
Chartiers Pond	52578	Extra	POS	POS	POS	NEG
Cuates Creek	38141	Extra	POS	NEG	POS	NEG
Cunningham Creek	23957	Extra	POS	POS	POS	POS
Fall Creek	40131	Extra	POS	NEG	POS	NEG
Jaroso Creek	48066	Extra	POS	NEG	POS	NEG
Jerry Creek Reservoir #1	66160	Extra	POS	POS	N/A	POS
Quartz Creek (upper)	42262	Extra	POS	NEG	POS	NEG
Torcido Creek	38137	Extra	POS	NEG	POS	POS

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Job No. 5 Technical Assistance

Job Objective: Provide information and assistance to aquatic biologists, aquatic researchers and managers in a variety of coldwater ecology applications.

Aquatic researchers and aquatic biologist work closely to investigate and manage the aquatic resources of Colorado. The need for this job is to cooperate closely with biologist and other stakeholders to disseminate results from aquatic research projects and to more effectively and efficiently conduct meaningful research that addresses management needs.

Objectives

1. Provide technical assistance to biologists, managers, researchers, and other internal and external stakeholders as needed.

Fishery managers, hatchery personnel, administrators, and CPW Field Operations personnel often need fishery ecology information or technical consulting on specific projects. Effective communication between researchers, fishery managers and other internal and external stakeholders is essential to the management coldwater stream fisheries in Colorado. Technical assistance projects are often unplanned and are addressed on an as-needed basis.

Accomplishments

One fact sheet was produced to summarize and disseminate information from the coldwater stream ecology research projects;

Kowalski, D. A., D. Drennan, V. M. Milano. 2018. Bacterial Kidney Disease Research. Colorado Parks and Wildlife Fact Sheet. Denver, Colorado.

Three external presentations were given to disseminate results of aquatic ecology projects to fishery scientists, ecologists, and other external audiences;

Kowalski, D. A., A. J. Treble, J. Drennan, V. M. Milano, R. Cordes. 2019. Surveying Colorado's sport fisheries for R. salmoninarum, the causative agent of bacterial kidney disease. Colorado Aquaculture Association Meeting, Nathrop, Colorado. February 1, 2019.

Kowalski, D. A., A. J. Treble, J. Drennan, V. M. Milano, R. Cordes. 2019. Prevalence and distribution of *R. salmoninarum*, the causative agent of bacterial kidney disease, in Colorado's wild trout and stocked sport fisheries. Colorado Wyoming American Fisheries Society Meeting, Fort Collins, Colorado. February 28, 2019.

Kowalski, D. A., E. E. Richer, B. Heinold, and M. C. Kondratieff. 2018. Quantifying the habitat preferences and emergence ecology of the Salmonfly, *Pteronarcys californica*. Rocky Mountain Biological Laboratory. August 15, 2018, Gothic, Colorado

Five internal presentations were given to disseminate results of aquatic ecology projects to CPW staff;

Kowalski, D. A., A. J. Treble, J. Drennan, V. M. Milano, R. Cordes. 2018. Prevalence and distribution of *R. salmoninarum* in Colorado's wild trout and stocked sport fisheries. Colorado Parks and Wildlife Aquatic Section and Aquatic Animal Health Lab, Denver, Colorado. July 5, 2018.

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Kowalski, D. A., E. E. Richer, A. B. Brubaker, and M. C. Kondratieff. 2019. Effects of Whitewater Parks on Fisheries. Colorado Parks and Wildlife Area 9 Meeting, Hot Sulphur Springs, Colorado. March 12, 2019.

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APPENDIX A. Testing results for all waters tested for *Renibacterium salmoninarum* 2016-2017.

Waters	Water Code	Study	AAHL CASE#	Species	ELISA	ELISA Ave OD	ELISA # POS	qPCR	nPCR	DFAT
Animas River #4	38011	Wild Trout	17-278	BRK	POS	0.121	7/12	NEG		NEG
Arkansas River #7	29012	Wild Trout	16-328	LOC	POS	0.081	4/60	NEG		NEG
Arkansas River Lake Fork #1 Lower	31954	Wild Trout	16-254	LOC	POS	0.146	29/59	NEG		NEG
Arkansas River Lake Fork #1 Upper	31954	Wild Trout	16-253	BRK	POS	0.092	15/60	NEG		NEG
Bear Creek #4	60073	Wild Trout	16-283	LGS	NEG	0.066	0/4	NEG		NEG
Bear Creek #4	60073	Wild Trout	16-283	RBT	NEG	0.070	0/7	NEG		NEG
Bear Creek #4	60073	Wild Trout	16-283	LOC	POS	0.072	3/17	NEG		NEG
Bear River	21212	Wild Trout	17-242	BRK	NEG	0.071	0/1	NEG		NEG
Bear River	21212	Wild Trout	17-242	RBT	NEG	0.088	0/2	NEG		NEG
Bear River	21212	Wild Trout	17-242	LOC	POS	0.128	4/9	NEG		NEG
Beaver Creek #1	38299	Wild Trout	17-198	LOC	POS	0.096	4/10	NEG		NEG
Beaver Creek #1	38299	Wild Trout	17-198	RBT	POS	0.118	1/2	NEG		NEG
Blacktail Creek	19225	Wild Trout	17-205	BRK	POS	0.087	1/12	NEG		NEG
Blue River #2	19249	Wild Trout	16-255	LOC	POS	0.083	6/60	NEG		NEG
Buck Creek	19340	Wild Trout	17-196	BRK	POS	0.144	8/12	POS	POS	NEG
Buffalo Creek	10380	Wild Trout	16-217	WHS	POS	0.109	1/2	NEG		NEG
Buffalo Creek	10380	Wild Trout	16-217	LOC	POS	0.223	40/46	POS	NEG	NEG
Cebolla Creek #2	38895	Wild Trout	16-281	LOC	POS	0.069	1/60	NEG		NEG
Cottonwood Creek	29480	Wild Trout	17-182	LOC		0.235	9/12	NEG		NEG
Cunningham Creek	39506	Wild Trout	17-280	BRK	POS	0.145	3/12	POS	POS	NEG
Dolores River #3B	48179	Wild Trout	17-288	LOC	POS	0.084	1/12	NEG		NEG
East Dallas Creek	39568	Wild Trout	17-237	CRN	POS	0.105	1/1	NEG		NEG
East Dallas Creek	39568	Wild Trout	17-237	BRK	POS	0.184	10/12	NEG		NEG
Elk Creek	20115	Wild Trout	17-326	RBT	POS	0.105	1/1	NEG		NEG
Elk Creek	20115	Wild Trout	17-326	LOC	POS	0.108	5/11	NEG		NEG
Elk Creek, East	39962	Wild Trout	17-244	RBT	POS	0.112	4/4	NEG		NEG
Elk Creek, East	39962	Wild Trout	17-244	LOC	POS	0.125	1/2	NEG		NEG
Elk Creek, East	39962	Wild Trout	17-244	BRK	POS	0.186	6/6	NEG		POS
Elk River, North Fork #1	20189	Wild Trout	17-250	LOC	NEG	0.077	0/7	NEG		NEG
Elk River, North Fork #1	20189	Wild Trout	17-250	RBT	NEG	0.090	0/1	NEG		NEG
Elk River, North Fork #1	20189	Wild Trout	17-250	BRK	POS	0.542	1/4	POS	POS	NEG
Elk River, South Fork	20191	Wild Trout	17-238	RBT	POS	0.131	1/1	NEG		NEG
Elk River, South Fork	20191	Wild Trout	17-238	BRK	POS	0.200	7/7	POS	NEG	NEG
Elk River, South Fork	20191	Wild Trout	17-238	LOC	POS	0.209	4/4	NEG		NEG
Encampment River	10861	Wild Trout	17-290	LOC	POS	0.078	1/2	POS	NEG	NEG
Florida River #2	40256	Wild Trout	17-235	BRK	NEG	0.078	0/1	NEG		NEG
Florida River #2	40256	Wild Trout	17-235	RBT	NEG	0.080	0/1	NEG		NEG
Florida River #2	40256	Wild Trout	17-235	LOC	POS	0.106	6/10	NEG		NEG
Fourmile Creek, West	33186	Wild Trout	17-197	BRK	POS	0.175	8/9	NEG		NEG
Fraser River	20355	Wild Trout	16-265	BRK	POS	0.115	19/60	POS	POS	POS
Gill Creek	40333	Wild Trout	16-300	BRK	POS	0.112	18/33	NEG		POS
Gill Creek	40333	Wild Trout	16-300	RBT	POS	0.142	21/27	NEG		NEG
Grape Creek #2	29913	Wild Trout	17-224	SNF	NEG	0.079	0/1	NEG		NEG
Grape Creek #2	29913	Wild Trout	17-224	WHS	NEG	0.081	0/1	NEG		NEG
Grape Creek #2	29913	Wild Trout	17-224	FMW	POS	0.104	2/5	NEG		NEG
Grape Creek #2	29913	Wild Trout	17-224	BRK	POS	0.109	1/1	NEG		NEG
Grape Creek #2	29913	Wild Trout	17-224	LND	POS	0.119	2/5	NEG		NEG
Groundhog Creek #1	40410	Wild Trout	17-231	BRK	POS	0.120	1/1	NEG		NEG
Groundhog Creek #1	40410	Wild Trout	17-231	RBT	POS	0.120	5/8	NEG		NEG
Groundhog Creek #1	40410	Wild Trout	17-231	LOC	POS	0.127	2/3	NEG		NEG
Gunnison River, North Fork #2	40509	Wild Trout	17-243	LOC	NEG	0.083	0/2	NEG		NEG
Gunnison River, North Fork #2	40509	Wild Trout	17-243	RBT	POS	0.092	4/12	POS	NEG	NEG
Henson Creek	40612	Wild Trout	16-279	BRK	POS	0.105	28/60	NEG		NEG

Horsefly Creek	44507	Wild Trout	17-190	RBT	POS	0.162	9/12	POS	POS	NEG
Huefano River #2	30130	Wild Trout	17-236	LOC	POS	0.132	9/12	NEG		NEG
Illinois River #4	13881	Wild Trout	17-279	LOC	POS	0.149	3/5	NEG		NEG
Illinois River #4	13881	Wild Trout	17-279	BRK	POS	0.323	2/7	POS	POS	NEG
Ivanhoe Creek	20761	Wild Trout	16-274	BRK	POS	0.135	28/60	NEG		NEG
Laramie River #2	11407	Wild Trout	16-286	LOC	POS	0.111	13/60	NEG		NEG
Leroux Creek, East Fork	38849	Wild Trout	17-240	BRK	POS	0.101	1/1	NEG		NEG
Leroux Creek, East Fork	38849	Wild Trout	17-240	RBT	POS	0.117	7/11	NEG		NEG
Long Branch Creek	41210	Wild Trout	16-278	LOC	POS	0.127	35/60	NEG		NEG
Lost Creek	14023	Wild Trout	16-223	BRK	POS	0.113	28/60	POS	NEG	NEG
Marvine Creek #1	21092	Wild Trout	17-204	RBT	NEG	0.087	0/1	NEG		NEG
Marvine Creek #1	21092	Wild Trout	17-204	BRK	POS	0.177	8/10	POS	POS	NEG
Michigan River North Fork #2	11615	Wild Trout	17-289	BRK	NEG	0.074	0/7	NEG		POS
Michigan River North Fork #2	11615	Wild Trout	17-289	LOC	POS	0.080	1/3	NEG		NEG
Michigan River North Fork #2	11615	Wild Trout	17-289	RBT	POS	0.115	1/2	NEG		POS
Miller Creek, East	25761	Wild Trout	17-207	LOC	POS	0.310	12/12	NEG		NEG
Mosquito Creek	30445	Wild Trout	16-224	BRK	POS	0.123	27/60	NEG		NEG
Naturita Creek	41804	Wild Trout	17-199	RBT	POS	0.099	2/5	NEG		NEG
North Elk Creek	20139	Wild Trout	17-209	BRK	POS	0.263	11/12	POS	POS	NEG
North Fork Canadian River	13259	Wild Trout	17-291	LOC	NEG	0.068	0/3	NEG		NEG
North Fork Canadian River	13259	Wild Trout	17-291	BRK	POS	0.088	2/9	NEG		NEG
North Fork Mesa Creek	41537	Wild Trout	17-192	RBT	POS	0.174	6/6	POS	NEG	NEG
North Fork North Platte #A	10836	Wild Trout	17-305	LOC	POS	0.091	3/12	NEG		NEG
Parachute Creek, East Fork	21460	Wild Trout	17-189	BRK				POS	POS	NEG
Piedre River, First Fork	42109	Wild Trout	17-286	LOC	POS	0.092	2/12	NEG		NEG
Pinos Creek, East	44951	Wild Trout	17-284	LOC	POS	0.133	9/10	NEG		NEG
Pinos Creek, East	44951	Wild Trout	17-284	BRK	POS	0.137	2/3	NEG		NEG
Pinos Creek, West Fork	42161	Wild Trout	17-234	LOC	POS	0.165	10/12	POS	NEG	NEG
Poudre River #4B Bliss	11923	Wild Trout	16-327	LOC	POS	0.114	28/60	NEG		NEG
Rio de los Pinos #1	40173	Wild Trout	17-201	LOC	POS	0.118	8/10	POS	NEG	NEG
Rio de los Pinos #1	40173	Wild Trout	17-201	RBT	POS	0.129	1/2	NEG		NEG
Rio Grande South Fork #2	48959	Wild Trout	17-245	RBT	POS	0.118	5/6	NEG		NEG
Rio Grande South Fork #2	48959	Wild Trout	17-245	BRK	POS	0.129	5/7	NEG		NEG
Rio Grande, South Fork #1	42565	Wild Trout	17-247	LOC	POS	0.114	8/11	NEG		NEG
Rio Grande, South Fork #1	42565	Wild Trout	17-247	RBT	POS	0.146	1/1	NEG		NEG
Saguache Creek #2	42793	Wild Trout	17-206	LOC	POS	0.197	8/8	NEG		NEG
Saguache Creek #2	42793	Wild Trout	17-206	WHS	POS	0.198	4/4	NEG		NEG
San Juan River #2	42919	Wild Trout	17-248	WHS	NEG	0.072	0/1	NEG		NEG
San Juan River #2	42919	Wild Trout	17-248	RBT	POS	0.110	1/2	NEG		NEG
San Juan River #2	42919	Wild Trout	17-248	LOC	POS	0.113	2/7	NEG		NEG
Sheep Creek	12257	Wild Trout	16-212	LOC	POS	0.069	1/21	NEG		NEG
Sheep Creek	12257	Wild Trout	16-212	BRK	POS	0.095	9/38	NEG		NEG
Snow Mass Creek #2	23444	Wild Trout	16-284	RBT	POS	0.081	1/5	NEG		NEG
Snow Mass Creek #2	23444	Wild Trout	16-284	BRK	POS	0.095	19/55	NEG		NEG
South Platte River #1B	31390	Wild Trout	16-311	LOC	POS	0.115	30/60	NEG		NEG
Spring Creek #2	43264	Wild Trout	17-241	LOC	POS	0.220	11/12	NEG		NEG
St. Charles River	33275	Wild Trout	17-222	LND	NEG	0.062	0/3	NEG		NEG
St. Charles River	33275	Wild Trout	17-222	WHS	POS	0.136	2/2	NEG		NEG
St. Charles River	33275	Wild Trout	17-222	LOC	POS	0.150	2/2	NEG		NEG
St. Charles River, North	31475	Wild Trout	17-223	LOC	POS	0.113	1/2	NEG		NEG
St. Charles River, North	31475	Wild Trout	17-223	LND	POS	0.342	3/6	NEG		NEG
St. Charles River, North	31475	Wild Trout	17-223	WHS	POS	0.388	4/4	NEG		NEG
Taylor River #2	43543	Wild Trout	17-281	LOC	POS	0.163	12/12	NEG		NEG
Toponas Creek	22400	Wild Trout	17-329	RXN	POS	0.094	5/12	NEG		NEG
Trout Creek #2	23533	Wild Trout	17-233	BRK	POS	0.112	5/12	NEG		NEG
Waterfall Creek	38575	Wild Trout	17-230	LOC	NEG	0.088	0/2	NEG		NEG
Waterfall Creek	38575	Wild Trout	17-230	BRK	POS	0.129	3/9	NEG		NEG
White River #4	37659	Wild Trout	17-287	LOC	POS	0.077	0/1	NEG		NEG

White River #4	37659	Wild Trout	17-287	MWF	POS	0.081	1/9	NEG		NEG
White River #4	37659	Wild Trout	17-287	RXN	POS	0.107	1/2	NEG		NEG
Williams Creek #2	44418	Wild Trout	17-285	RBT	NEG	0.073	0/3	NEG		NEG
Williams Creek #2	44418	Wild Trout	17-285	BRK	POS	0.090	2/9	NEG		NEG
Willow Creek	44064	Wild Trout	17-246	LOC	POS	0.162	11/12	NEG		NEG
Willow Creek East	44103	Wild Trout	17-292	BRK	POS	0.085	2/12	NEG		NEG
Aurora Reservoir	56420	Stocked	16-172	YPE	POS	0.073	1/24	NEG	NEG	NEG
Aurora Reservoir	56420	Stocked	16-172	SMB	POS	0.076	3/36	NEG	NEG	POS
Beckwith Reservoir	82026	Stocked	16-209	BGL	NEG	0.067	0/4	NEG		NEG
Beckwith Reservoir	82026	Stocked	16-209	SGR	POS	0.075	2/28	NEG		NEG
Beckwith Reservoir	82026	Stocked	16-209	CCF	POS	0.088	3/22	NEG		NEG
Beckwith Reservoir	82026	Stocked	16-209	YPE	POS	0.101	3/6	NEG		NEG
Big Creek Reservoir	88573	Stocked	16-301	CRN	POS	0.123	37/60	NEG		POS
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	WAL				NEG		NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	CPP				NEG		NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	WHS				POS	NEG	NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	GSD				NEG		NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	BCR				NEG		NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	BBH				NEG		NEG
Blue Heron Reservoir, St. Vrain	58083	Stocked	16-184	YPE				NEG		NEG
Blue Mesa Reservoir	88748	Stocked	16-190	RBT				POS	NEG	NEG
Blue Mesa Reservoir	88748	Stocked	16-191	YPE				POS	NEG	NEG
Boyd Lake	52491	Stocked	16-288	YPE	NEG	0.060	0/10	NEG		NEG
Boyd Lake	52491	Stocked	16-288	WAL	NEG	0.063	0/30	NEG		NEG
Boyd Lake	52491	Stocked	16-288	CPP	POS	0.148	12/20	NEG		NEG
Brown Lake Upper	88802	Stocked	17-187	RBT	NEG	0.062	0/2	NEG		NEG
Brown Lake Upper	88802	Stocked	17-187	BRK	NEG	0.070	0/1	NEG		NEG
Brown Lake Upper	88802	Stocked	17-187	WHS	POS	0.177	8/9	NEG		NEG
Carter Lake Reservoir	54255	Stocked	16-343	WAL	POS	0.195	52/60	NEG		NEG
Cebolla Creek #1	38883	Stocked	17-277	LOC	POS	0.118	7/12	NEG		NEG
Cebolla Creek #3	38908	Stocked	16-280	LOC	POS	0.175	36/60	NEG		NEG
Chalk Creek Lake	81909	Stocked	17-200	BRK	POS	0.074	0/10	NEG		NEG
Chalk Creek Lake	81909	Stocked	17-200	RBT	POS	0.223	2/2	POS	NEG	POS
Chatfield Reservoir	54306	Stocked	16-174	SMB	POS	0.085	10/60	POS	NEG	POS
Cherry Creek Reservoir	52580	Stocked	16-044	GSD				POS	POS	
Cherry Creek Reservoir	52580	Stocked	16-044	WAL				POS	POS	
Clear Creek Reservoir	81719	Stocked	17-184	KOK	NEG	0.069	0/1	NEG		NEG
Clear Creek Reservoir	81719	Stocked	17-184	LOC	NEG	0.074	0/1	POS	NEG	NEG
Clear Creek Reservoir	81719	Stocked	17-184	WHS	NEG	0.081	0/2	NEG		NEG
Clear Creek Reservoir	81719	Stocked	17-184	RBT	POS	0.113	2/6	NEG		NEG
Colorado River #8	19718	Stocked	16-292	LOC	POS	0.198	29/60	NEG		NEG
Continental Reservoir	89107	Stocked	17-327	SPL	NEG	0.067	0/1	NEG		NEG
Continental Reservoir	89107	Stocked	17-327	BRK	NEG	0.074	0/2	NEG		NEG
Continental Reservoir	89107	Stocked	17-327	RBT	POS	0.129	2/2	NEG		NEG
Continental Reservoir	89107	Stocked	17-327	WHS	POS	0.237	8/8	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	WHS	NEG	0.089	0/1	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	CCF	POS	0.094	2/14	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	YPE	POS	0.095	1/5	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	BCR	POS	0.106	1/5	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	BGL	POS	0.107	1/1	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	LMB	POS	0.124	4/4	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	WAL	POS	0.124	11/12	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	GSD	POS	0.146	3/9	NEG		NEG
Coot Pond, St. Vrain State Park	58091	Stocked	16-203	CPP	POS	0.170	5/7	NEG		NEG
Cottonwood Lake #4	66008	Stocked	16-271	LXB	POS	0.098	6/30	NEG		NEG
Cottonwood Lake #4	66008	Stocked	16-271	RBT	POS	0.118	5/6	NEG		NEG
Cottonwood Lake #5	66010	Stocked	16-269	LOC	POS	0.088	5/5	NEG		NEG
Cottonwood Lake #5	66010	Stocked	16-269	BRK	POS	0.091	1/4	NEG		NEG

Cottonwood Lake #5	66010	Stocked	16-269	CRN	POS	0.096	1/4	NEG		NEG
DeWeese Reservoir	81729	Stocked	16-316	SMB	NEG	0.064	0/13	NEG		NEG
DeWeese Reservoir	81729	Stocked	16-316	TGM	POS	0.080	1/8	NEG		NEG
DeWeese Reservoir	81729	Stocked	16-317	RBT	POS	0.092	13/60	POS	NEG	POS
DeWeese Reservoir	81729	Stocked	16-316	WHS	POS	0.128	20/28	NEG		NEG
Dolores River #4	39796	Stocked	16-320	KOK	POS	0.073	2/60	NEG		NEG
Douglas Lake	58695	Stocked	16-176	LMB	NEG	0.071	0/1	NEG		NEG
Douglas Lake	58695	Stocked	16-176	SXW	POS	0.072	2/18	NEG		POS
Douglas Lake	58695	Stocked	16-176	WAL	NEG	0.072	0/3	NEG		NEG
Douglas Lake	58695	Stocked	16-176	SGR	POS	0.073	1/7	NEG		POS
Douglas Lake	58695	Stocked	16-176	RBT	NEG	0.079	0/1	NEG		NEG
Douglas Lake	58695	Stocked	16-176	GSD	POS	0.083	1/16	POS	NEG	POS
Douglas Lake	58695	Stocked	16-176	HGC	POS	0.325	14/14	POS	NEG	NEG
Eagle Lake	66363	Stocked	16-235	BRK	POS	0.245	45/60	POS	POS	POS
Eagle River #1	20026	Stocked	16-208	LOC	POS	0.153	51/60	NEG		
Eagle Watch Lake	60210	Stocked	16-216	LMB	POS	0.082	2/13	NEG		NEG
Eagle Watch Lake	60210	Stocked	16-216	WAL	POS	0.088	1/3	NEG		NEG
Eagle Watch Lake	60210	Stocked	16-216	SMB	POS	0.129	8/18	POS	NEG	NEG
Eagle Watch Lake	60210	Stocked	16-216	YPE	POS	0.160	15/26	NEG		NEG
Florida River #3	40268	Stocked	17-232	LOC	POS	0.175	11/11	NEG		NEG
Florida River #3	40268	Stocked	17-232	RBT	POS	0.194	1/1	NEG		NEG
Forty Acre Lake	66666	Stocked	16-270	BRK	POS	0.087	4/50	NEG		NEG
Granby Reservoir	66969	Stocked	16-353	KOK	POS	0.072	2/60	NEG		NEG
Granby Reservoir #12	90201	Stocked	17-185	CRN	POS	0.101	6/12	NEG		NEG
Gross Reservoir	55043	Stocked	16-287	BRK	NEG	0.071	0/1	NEG		NEG
Gross Reservoir	55043	Stocked	16-287	RBT	POS	0.077	1/29	POS	POS	NEG
Gross Reservoir	55043	Stocked	16-287	LOC	POS	0.105	11/29	NEG		NEG
Gross Reservoir	55043	Stocked	16-287	MAC	POS	0.119	1/1	NEG		NEG
Horseshoe Reservoir	79803	Stocked	16-151	SGR	NEG	0.066	0/1	NEG		
Horseshoe Reservoir	79803	Stocked	16-151	SMB	POS	0.096	3/43	NEG		
Horseshoe Reservoir	79803	Stocked	16-151	CCF	POS	0.109	1/1	NEG		
Horseshoe Reservoir	79803	Stocked	16-151	HGC	POS	0.287	14/15	NEG		
Horsetooth Reservoir	55168	Stocked	16-206	SMB	NEG	0.067	0/60	POS	NEG	NEG
Hotel Twin Lake	90578	Stocked	17-183	BRK	NEG	0.062	0/1	NEG		NEG
Hotel Twin Lake	90578	Stocked	17-183	RBT	POS	0.105	1/6	POS	POS	NEG
Hotel Twin Lake	90578	Stocked	17-183	WHS	POS	0.142	3/5	POS	NEG	NEG
Jackson Reservoir	53037	Stocked	16-341	WAL	NEG	0.067	0/60	NEG		POS
Jumbo Annex	53051	Stocked	16-266	BCR	NEG	0.060	0/31	POS	NEG	NEG
Jumbo Annex	53051	Stocked	16-266	GSD	NEG	0.067	0/8	NEG		NEG
Jumbo Annex	53051	Stocked	16-266	WAL	NEG	0.124	18/21	NEG		NEG
Jumbo Reservoir	53063	Stocked	16-313	WAL	NEG	0.077	0/60	POS	NEG	NEG
Lake Fork Gunnison River #2	40484	Stocked	17-297	LOC	NEG	0.066	0/6	NEG		NEG
Lake Fork Gunnison River #2	40484	Stocked	17-297	RBT	NEG	0.066	0/2	NEG		NEG
Lake Fork Gunnison River #2	40484	Stocked	17-297	BRK	NEG	0.072	0/1	NEG		NEG
Lake San Cristobal	92130	Stocked	17-194	LOC	POS	0.153	9/12	POS	NEG	NEG
Little Battlement Reservoir	88472	Stocked	17-188	LXB	NEG	0.072	0/1	NEG		NEG
Little Battlement Reservoir	88472	Stocked	17-188	CRN	NEG	0.072	0/1	NEG		NEG
Little Battlement Reservoir	88472	Stocked	17-188	BRK	POS	0.325	8/9	POS	POS	NEG
Mallard Pond, St. Vrain State Park	58099	Stocked	16-179	TGM	NEG	0.070	0/6	NEG		
Mallard Pond, St. Vrain State Park	58099	Stocked	16-179	CPP	POS	0.103	4/10	NEG		
Mallard Pond, St. Vrain State Park	58099	Stocked	16-179	GSD	POS	0.117	8/24	POS	NEG	
Mallard Pond, St. Vrain State Park	58099	Stocked	16-179	BRC				NEG		
Mallard Pond, St. Vrain State Park	58099	Stocked	16-179	WHS				NEG		
Meredith Reservoir	79586	Stocked	17-264	SAG	NEG	0.059	0/4	NEG		NEG
Meredith Reservoir	79586	Stocked	17-264	GSD	NEG	0.063	0/6	NEG		NEG
Meredith Reservoir	79586	Stocked	17-264	WHS	NEG	0.087	0/1	NEG		NEG
Meredith Reservoir	79586	Stocked	17-264	CPP	POS	0.145	1/1	NEG		NEG
Mt. Elbert Forebay	82684	Stocked	17-195	RBT	POS	0.142	3/3	NEG		NEG

Mt. Elbert Forebay	82684	Stocked	17-195	LOC	POS	0.146	7/8	NEG		NEG
Mt. Elbert Forebay	82684	Stocked	17-195	MAC	POS	0.148	1/1	NEG		NEG
North Sterling Reservoir	53328	Stocked	16-111	WAL				NEG		
North Sterling Reservoir	53328	Stocked	16-111	GSD				POS		
Ordway Reservoir	79649	Stocked	17-283	SAG	NEG	0.060	0/6	POS	NEG	NEG
Ordway Reservoir	79649	Stocked	17-283	SXW	NEG	0.069	0/2	NEG		NEG
Ordway Reservoir	79649	Stocked	17-283	CCF	NEG	0.072	0/2	NEG		NEG
Ordway Reservoir	79649	Stocked	17-283	GSD	NEG	0.088	0/2	NEG		NEG
Paonia Reservoir	91657	Stocked	17-117	BRK	NEG	0.070	0/1	NEG		NEG
Paonia Reservoir	91657	Stocked	17-117	RBT	POS	0.089	3/7	POS	NEG	NEG
Pelican, St. Vrain State Park	52388	Stocked	16-180	CPP	POS	0.103	4/10	NEG		POS
Pelican, St. Vrain State Park	52388	Stocked	16-180	GSD	POS	0.285	12/20	POS	NEG	POS
Platoro Reservoir	91758	Stocked	17-328	RBT	NEG	0.060	0/1	NEG		NEG
Platoro Reservoir	91758	Stocked	17-328	SPL	NEG	0.063	0/1	NEG		NEG
Platoro Reservoir	91758	Stocked	17-328	LOC	NEG	0.067	0/1	NEG		NEG
Platoro Reservoir	91758	Stocked	17-328	KOK	POS	0.084	1/7	NEG		NEG
Platoro Reservoir	91758	Stocked	17-328	WHS	POS	0.349	6/6	POS	NEG	NEG
Quincy Reservoir	57198	Stocked	16-237	YPE	POS	0.085	11/60	NEG		NEG
Regan Lake	91948	Stocked	17-116	BRK	POS	0.108	6/9	NEG		NEG
Regan Lake	91948	Stocked	17-116	RBT	POS	0.127	1/1	NEG		NEG
Ridgway Reservoir	96695	Stocked	17-191	RBT	POS	0.077	1/12	POS	NEG	NEG
Road Canyon Reservoir	92003	Stocked	17-180	RBT	NEG	0.071	0/4	NEG		NEG
Road Canyon Reservoir	92003	Stocked	17-180	BRK	POS	0.084	1/8	NEG		NEG
Roan Creek	21701	Stocked	17-249	BRK	POS	0.141	10/12	POS	NEG	NEG
Rowdy Reservoir	96708	Stocked	17-202	LXB	POS	0.090	1/4	NEG		NEG
Ruedi Reservoir	69535	Stocked	16-272	RBT	POS	0.068	1/29	NEG		NEG
Ruedi Reservoir	69535	Stocked	16-272	MAC	NEG	0.071	0/6	NEG		NEG
Ruedi Reservoir	69535	Stocked	16-273	YPE	POS	0.084	2/14	NEG		NEG
Ruedi Reservoir	69535	Stocked	16-272	LOC	POS	0.099	2/8	NEG		NEG
Runyon Lake	79714	Stocked	16-295	YPE	NEG	0.058	0/3	NEG		NEG
Runyon Lake	79714	Stocked	16-295	BCR	NEG	0.059	0/3	NEG		NEG
Runyon Lake	79714	Stocked	16-295	LMB	NEG	0.060	0/6	NEG		NEG
Runyon Lake	79714	Stocked	16-295	SGR	NEG	0.074	0/21	NEG		NEG
Runyon Lake	79714	Stocked	16-295	BGL	NEG	0.089	0/1	NEG		NEG
Runyon Lake	79714	Stocked	16-295	GSD	POS	0.091	6/20	NEG		NEG
Runyon Lake	79714	Stocked	16-295	WHS	POS	0.128	3/5	NEG		NEG
Runyon Lake	79714	Stocked	16-295	CPP	POS	0.232	1/1	NEG		NEG
San Miguel River #3	46844	Stocked	17-282	LOC	NEG	0.082	0/3	NEG		NEG
San Miguel River #3	46866	Stocked	17-282	RBT	NEG	0.091	0/9	POS	NEG	NEG
Sand Piper, St. Vrain State Park	58087	Stocked	16-178	BCR	NEG	0.061	0/9	POS	NEG	POS
Sand Piper, St. Vrain State Park	58087	Stocked	16-178	GSD	POS	0.115	21/47	NEG		POS
Sand Piper, St. Vrain State Park	58087	Stocked	16-178	CPP	POS	0.165	2/3	NEG		NEG
Silverjack Reservoir	92255	Stocked	17-203	RBT	POS	0.109	5/11	NEG		NEG
Silverjack Reservoir	92255	Stocked	17-203	CRN	POS	0.119	1/1	NEG		NEG
Sloans Lake	53493	Stocked	16-215	BCR	POS	0.127	45/60	NEG		NEG
South Platte River #3C	14706	Stocked	16-364	LOC	NEG	0.066	0/60	NEG		NEG
South Platte River #4	11837	Stocked	16-365	LOC	POS	0.066	1/1	NEG		NEG
South Platte River #6	30849	Stocked	16-345	LOC	POS	0.072	5/60	NEG		POS
South Platte River 1A	32641	Stocked	16-310	LOC	POS	0.143	26/60	POS	NEG	NEG
Spinney Mountain Reservoir	82583	Stocked	16-183	NPK				NEG	NEG	NEG
Spinney Mountain Reservoir	82583	Stocked	16-183	RBT				NEG	NEG	NEG
Spinney Mountain Reservoir	82583	Stocked	16-183	YPE				NEG	NEG	NEG
Spinney Mountain Reservoir	82583	Stocked	16-183	LOC				POS	NEG	NEG
Stalker Lake	56590	Stocked	16-115	BGL	NEG	0.065	0/60	NEG		
Taylor Reservoir	92510	Stocked	17-186	RBT	POS	0.092	2/10	POS	POS	NEG
Taylor Reservoir	92510	Stocked	17-186	NPK	POS	0.103	1/2	NEG		NEG
Trinidad Reservoir	81911	Stocked	16-314	YPE	NEG	0.061	0/3	NEG		NEG
Trinidad Reservoir	81911	Stocked	16-314	BCR	NEG	0.065	0/5	NEG		NEG

Trinidad Reservoir	81911	Stocked	16-314	SXW	NEG	0.073	0/1	NEG		NEG
Trinidad Reservoir	81911	Stocked	16-314	SAG	POS	0.077	4/41	NEG		POS
Trinidad Reservoir	81911	Stocked	16-314	GSD	NEG	0.080	0/1	NEG		NEG
Trinidad Reservoir	81911	Stocked	16-315	RBT	POS	0.086	2/14	NEG		NEG
Trinidad Reservoir	81911	Stocked	16-314	SMB	POS	0.087	3/9	NEG		NEG
Turquoise Reservoir	80010	Stocked	17-193	LOC	POS	0.100	5/11	NEG		NEG
Turquoise Reservoir	80010	Stocked	17-193	RBT	POS	0.272	1/1	NEG		NEG
Twin Lakes	80022	Stocked	17-181	RBT	POS	0.105	1/2	NEG		NEG
Twin Lakes	80022	Stocked	17-181	LOC	POS	0.119	1/2	NEG		NEG
Twin Lakes	80022	Stocked	17-181	WHS	POS	0.299	4/5	POS	POS	NEG
Twin Lakes	80022	Stocked	17-181	MAC	POS	0.331	3/3	NEG		NEG
Vallecito Reservoir	92902	Stocked	16-362	KOK	POS	0.134	2/5	NEG		NEG
Wahatoya	82406	Stocked	16-256	RBT	POS	0.088	9/60	NEG		NEG
Williams Fork Reservoir	70881	Stocked	16-333	KOK	POS	0.084	4/60	NEG		NEG
Windsor Reservoir	53645	Stocked	16-238	WHS	POS	0.084	3/27	NEG		NEG
Windsor Reservoir	53645	Stocked	16-238	YPE	POS	0.094	6/17	NEG		POS
Windsor Reservoir	53645	Stocked	16-238	GSD	POS	0.151	8/16	POS	POS	POS
Wrights Lake	83128	Stocked	17-208	RBT	NEG	0.080	0/8	POS	POS	NEG
Wrights Lake	83128	Stocked	17-208	BRK	POS	0.157	1/1	NEG		NEG
Wrights Lake	83128	Stocked	17-208	WHS	POS	0.334	2/2	POS	NEG	NEG
Barker Reservoir	53772	Extra	16-282	RBT	NEG	0.072	0/33	NEG		NEG
Barker Reservoir	53772	Extra	16-282	LGS	NEG	0.072	0/12	NEG		NEG
Barker Reservoir	53772	Extra	16-282	KOK	NEG	0.073	0/9	NEG		NEG
Barker Reservoir	53772	Extra	16-282	LOC	POS	0.105	3/6	NEG		NEG
Bear Creek	29157	Extra	16-294	BRK	POS	0.135	43/60	NEG		POS
Bennet Creek	10203	Extra	16-289	RBT	POS	0.102	22/60	NEG		NEG
Black Canyon Creek	29212	Extra	16-251	BRK	POS	0.133	6/20	NEG		NEG
Boulder Creek Estates East Pond	81103	Extra	16-062	CPP				POS	POS	
Cap K Ranch	69528	Extra	16-350	BRK	POS	0.094	14/60	POS	POS	NEG
Chartiers Pond	52578	Extra	16-136	GSF	POS	0.081	1/23	POS	NEG	NEG
Chartiers Pond	52578	Extra	16-136	LMB	POS	0.099	4/4	POS	NEG	NEG
Chartiers Pond	52578	Extra	16-136	GSD	POS	0.136	9/9	POS	POS	NEG
Cuates Creek	38141	Extra	16-142	RGN	POS	0.290	26/27	POS	NEG	NEG
Cunningham Creek	23957	Extra	16-348	LOC	POS	0.128	8/11	POS	POS	POS
Cunningham Creek	23957	Extra	16-348	BRK	POS	0.384	30/49	POS	POS	POS
Dry Gulch	10877	Extra	16-240	CRN	POS	0.126	34/60	NEG		NEG
Eagle River S.F.	20076	Extra	16-285	BRK	POS	0.075	3/26	NEG		NEG
Eagle River S.F.	20076	Extra	16-285	LOC	POS	0.079	4/34	NEG		NEG
Fall Creek	40131	Extra	16-262	CRN	POS	0.276	20/20	POS	NEG	NEG
Fall Creek	40131	Extra	16-262	LOC	POS	0.313	14/15	NEG		NEG
Fall Creek	40131	Extra	16-262	BRK	POS	0.314	24/25	NEG		NEG
Harvey Gap Reservoir	67226	Extra	16-263	LMB	NEG	0.062	0/10	NEG		NEG
Harvey Gap Reservoir	67226	Extra	16-263	YPE	POS	0.078	4/40	NEG		NEG
Harvey Gap Reservoir	67226	Extra	16-263	BLG	POS	0.079	1/10	NEG		NEG
Highline Reservoir	67315	Extra	16-048	LMB				NEG		
Highline Reservoir	67315	Extra	16-048	BGL				POS	NEG	
Jaroso Creek	48066	Extra	16-144	RGN	POS	0.157	23/27	POS	NEG	NEG
Jeff's Pond	52887	Extra	16-112	GSF				NEG		
Jeff's Pond	52887	Extra	16-112	LMB				POS	NEG	
Jerry Creek Reservoir #1	66160	Extra	16-131	LMB				POS	POS	NEG
Jerry Creek Reservoir #1	66160	Extra	16-131	BGL				POS	POS	POS
Joe Wright Creek	11306	Extra	16-162	GRA	POS	0.095	20/60	NEG	NEG	
John Martin Reservoir	79524	Extra	16-234	WBA	POS	0.071	1/60	NEG		NEG
Lake Nighthorse	91672	Extra	16-360	KOK	NEG	0.074	0/60	NEG		NEG
Lower Rock Creek, Leadville	30659	Extra		BRK				NEG		NEG
May Creek	12978	Extra	16-299	CRN	POS	0.084	2/10	NEG		NEG
Nanita Lake	72897	Extra	16-182	CRN	POS	0.144	53/60	NEG		POS
Neota Creek	13007	Extra	16-196	GBN				NEG		NEG

North Delaney Butte	54609	Extra	16-307	LOC	NEG	0.071	0/60	NEG		NEG
Pawnee Power Plant Reservoir	61250	Extra	16-134	GSF	NEG	0.058	0/15	POS	NEG	NEG
Pawnee Power Plant Reservoir	61250	Extra	16-134	LMB	NEG	0.060	0/15	POS	NEG	NEG
Pike View Reservoir	79663	Extra	16-244	RBT	POS	0.084	6/50	NEG		NEG
Pike View Reservoir	79663	Extra	16-245	CCF				NEG		NEG
Pike View Reservoir	79663	Extra	16-245	SGR				NEG		NEG
Pike View Reservoir	79663	Extra	16-245	SXW				NEG		NEG
Poudre River #1B	11887	Extra	16-318	LOC	POS	0.078	5/60	NEG		NEG
Poudre River #3 Kelly Flats	11902	Extra	16-326	LOC	POS	0.083	10/60	NEG		POS
Pueblo Reservoir	81783	Extra	16-050	WAL				POS	NEG	
Pueblo Reservoir	81783	Extra	16-050	GSD				NEG		
Quartz Creek	42262	Extra	17-239	RBT	NEG	0.098	0/1	NEG		NEG
Quartz Creek	42262	Extra	17-239	BRK	POS	0.155	1/2	NEG		NEG
Quartz Creek	42262	Extra	17-239	LOC	POS	0.174	10/11	NEG		NEG
Quartz Creek (lower)	42262	Extra	16-297	LOC	POS	0.123	25/50	NEG		NEG
Quartz Creek (lower)	42262	Extra	16-297	RBT	POS	0.165	3/10	NEG		NEG
Quartz Creek (upper)	42262	Extra	16-297	LOC	POS	0.098	12/60	POS	NEG	NEG
Red Tail Pond, St. Vrain State Park	58085	Extra	16-204	CCF	NEG	0.079	0/1	NEG		NEG
Red Tail Pond, St. Vrain State Park	58085	Extra	16-204	LMB	POS	0.094	1/2	NEG		NEG
Red Tail Pond, St. Vrain State Park	58085	Extra	16-204	BLG	POS	0.103	1/2	NEG		NEG
Red Tail Pond, St. Vrain State Park	58085	Extra	16-204	BCR	POS	0.105	1/1	NEG		NEG
Red Tail Pond, St. Vrain State Park	58085	Extra	16-204	RBT	POS	0.146	2/2	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	BCR	NEG	0.062	0/5	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	YPE	NEG	0.063	0/30	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	LMB	NEG	0.063	0/7	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	SMB	NEG	0.069	0/8	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	SNF	NEG	0.070	0/2	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	WAL	NEG	0.073	0/5	NEG		NEG
Rifle Gap Reservoir	69422	Extra	16-335	BGL	POS	0.085	1/3	NEG		NEG
Roaring Creek	12081	Extra	16-195	GBN	POS	0.109	27/59	NEG		NEG
Rock Creek, Jefferson	30661	Extra	16-249	BRK	POS	0.095	13/55	NEG		NEG
San Isabel Lake	79980	Extra	16-127	YPE				POS	NEG	NEG
Sheep Creek	12245	Extra	16-298	CRN	POS	0.082	6/60	NEG		NEG
South Platte River #13, Proctor	12663	Extra	16-088	FHM				NEG		
South Platte River #13, Proctor	12663	Extra	16-088	CAP				NEG		
South Platte River #13, Proctor	12663	Extra	16-088	BYM				NEG		
South Platte River #13, Proctor	12663	Extra	16-088	GSF				POS	NEG	
South Platte River #13, Proctor	12663	Extra	16-088	LMB				NEG		
South Platte River #13, Proctor	12663	Extra	16-088	BCR				NEG		
Stagecoach Reservoir	73902	Extra	16-098	NPK				POS	NEG	
Sweetwater Lake	70425	Extra	16-336	RBT	POS	0.075	2/34	NEG		NEG
Sweetwater Lake	70425	Extra	16-336	BRK	POS	0.085	2/12	NEG		NEG
Sweetwater Lake	70425	Extra	16-336	LOC	POS	0.104	2/4	NEG		NEG
Sweetwater Lake	70425	Extra	16-336	KOK	POS	0.128	7/10	NEG		NEG
Synder Pond	75494	Extra	16-117	NPK				NEG		
Synder Pond	75494	Extra	16-117	GSF				POS	NEG	
Synder Pond	75494	Extra	16-117	LMB				POS	NEG	
Torcido Creek	38137	Extra	16-146	RGN	POS	0.186	27/27	POS	NEG	POS
Trap Creek	12423	Extra	16-198	GBN				POS	NEG	NEG
Trappers Lake	70552	Extra	16-340	BRK	POS	0.135	48/60	NEG		NEG
Upper Rock Creek, Leadville	30659	Extra		BRK				NEG		NEG
West Plum Creek	13122	Extra	16-139	FHM				NEG	NEG	
West Plum Creek	13122	Extra	16-139	CHS				POS	NEG	
Willow Creek	12675	Extra	16-081	PTM				NEG		
Woldford Reservoir	70989	Extra	16-322	KOK	POS	0.078	1/1	NEG		NEG
Zimmerman Lake	57059	Extra	16-160	GBN	POS	0.163	58/60	NEG	NEG	NEG