Colorado River Aquatic Resource Investigations

Federal Aid Project F-237-R25

Dan A. Kowalski
Aquatic Research Scientist

Job Progress Report

Colorado Parks & Wildlife

Aquatic Research Section

Fort Collins, Colorado

October 2018

STATE OF COLORADO

John W. Hickenlooper, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Bob Randall, Executive Director

COLORADO PARKS & WILDLIFE

Bob Broscheid, Director

WILDLIFE COMMISSION

John V. Howard, Chairman
Michelle Zimmerman, Vice-Chair
James Vigil, Secretary
Robert William Bray
Marie Haskett
VACANT
Carrie Besnette Hauser
Marvin McDaniel
Dale E. Pizel
Robert "Dean" Wingfield
Alexander Zipp

Ex Officio/Non-Voting Members: Don Brown, Bob Randall and Bob Broscheid

AQUATIC RESEARCH STAFF

George J. Schisler, Aquatic Research Leader
Kelly Carlson, Aquatic Research Program Assistant
Pete Cadmus, Aquatic Research Scientist/Toxicologist, Water Pollution Studies
Eric R. Fetherman, Aquatic Research Scientist, Salmonid Disease Studies
Ryan Fitzpatrick, Aquatic Research Scientist, Eastern Plains Native Fishes
Eric E. Richer, Aquatic Research Scientist/Hydrologist, Stream Habitat Restoration
Matthew C. Kondratieff, Aquatic Research Scientist, Stream Habitat Restoration
Dan Kowalski, Aquatic Research Scientist, Stream & River Ecology
Adam G. Hansen, Aquatic Research Scientist, Coldwater Lakes and Reservoirs
Kevin B. Rogers, Aquatic Research Scientist, Colorado Cutthroat Studies
Kevin G. Thompson, Aquatic Research Scientist, 3-Species and Boreal Toad Studies
Andrew J. Treble, Aquatic Research Scientist, Aquatic Data Management and Analysis
Brad Neuschwanger, Hatchery Manager, Fish Research Hatchery
Tracy Davis, Hatchery Technician, Fish Research Hatchery
David Karr, Hatchery Technician, Fish Research Hatchery

Jim Guthrie, Federal Aid Coordinator
Alexandria Austermann, Librarian

Prepared by: ______________________________

Dan Kowalski, Aquatic Research Scientist

Approved by: ______________________________

George J. Schisler, Aquatic Wildlife Research Chief

Date: 10/12/18

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.

Table of Contents

State: Colorado Project Number: F-237-R25

Project Title: Coldwater Stream Ecology Investigations

Period Covered: July 1, 2017 through June 30, 2018

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. Salmonfly Habitat and Ecology Studies

Job Objective: Investigate the habitat use, hatching ecology and limiting factors of the salmonfly *Pteronarcys californica* in Colorado Rivers.

Dams are known to drastically alter river habitat and have many diverse effects on aquatic invertebrates (Ward and Stanford 1979). Those effects can be large and result in long-term changes in invertebrate communities (Vinson 2001). In the upper Colorado River basin, previous work under project F-237 documented that the aquatic invertebrate community below Windy Gap Reservoir has changed dramatically since construction and that these changes may be associated with flow alterations (Nehring 2011). That study documented a 38% reduction in the diversity of aquatic invertebrates below Windy Gap Reservoir from 1980-2011. Nineteen species of mayflies, four species of stoneflies and eight species of caddisflies had been extirpated from the sampling sites since 1982. In addition to the changes over time of the invertebrate community there was a spatial pattern of increasing diversity downstream of Windy Gap that indicated ongoing effects of the reservoir on invertebrate communities. Sensitive species like *Drunella grandis*, *Pteronarcella badia*, and *Pteronarcys californica* were reduced or eliminated from sites close to Windy Gap and replaced by tolerant species like *Ephemerella* sp, *Baetis* sp, and *Hydropsyche* sp.

The salmonfly (*Pteronarcys californica*) is a large aquatic invertebrate that can reach high densities in some Colorado rivers. They play an important ecological role as shredders in stream systems and have been documented to be extremely important to stream dwelling trout as a food resource. Nehring (1987) reported in a diet study of trout in the Colorado River that *P. californica* was the most common food item, comprising 64-75% of the mean stomach content over the four-year study. 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). While ecologically important and found in high abundance at some sites, the salmonfly has relatively specific environmental requirements and is considered intolerant of disturbance in bioassessment protocols (Barbour et al. 1999, Fore et al. 1996, Erickson 1983). Salmonflies are sensitive to habitat alterations in part because of their lifespan; they are one of the longest-lived aquatic insects in the Neararctic (DeWalt and Stewart 1995). Previous work indicates that the range and density of *P. californica* have declined in the Colorado River and that these declines may be associated with flow alterations (Nehring 2011). Once common in the upper Colorado River, the abundance of salmonflies has declined, especially below Windy Gap Reservoir where flow alterations associated with trans-mountain water diversions are the largest. This pattern has been observed in other rivers. Richards (2000) documented 6-8 times lower density of salmonflies in the Madison River below Ennis Reservoir compared to above and found a negative correlation between their density and substrate embeddedness.

Salmonflies have been reported to have a three to five-year life cycle but two studies indicate it is likely to have a three or four-year life cycle in Colorado (DeWalt and Stewart 1995, Nehring 1987). These two studies also identify *P. californica* as one of the most synchronously emerging of all species of stoneflies with emergence at any one site lasting from 5-13 days. The synchronous emergence and hatching behavior allow it to be sampled in unique ways compared to other aquatic invertebrates. Salmonflies 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 nymphal exuvia which is left attached to the structure. If sites are visited soon after emergence, then the density of stoneflies emerging at a site can be estimated by completing multiple pass removal surveys of the exuvia. Nehring (2011) found a high correlation (R^2 = 0.88-0.90) between post emergence exuvia density estimates and more traditional pre-emergent quantitative benthic sampling at 23 sites.

Previous work completed under Project F-237 indicated that the range and density of *P. californica* have declined in the Colorado River and that these declines may be associated with flow alterations (Nehring 2011). Once common in the upper Colorado River, the abundance of salmonflies has declined, especially below Windy Gap Reservoir where flow alterations associated with trans-mountain water diversions are the largest. The objective of this project was to document the distribution, density and habitat use of *P. californica* and measure environmental variables that may be limiting habitat factors in Colorado rivers. Quantifying the preferred habitat characteristics will assist in the restoration of sites where *P. californica* has been extirpated will benefit flow management and river restoration activities.

OBJECTIVES

1. Document the distribution and density of *P. californica* at 18 sites on the Gunnison, Colorado and Rio Grande rivers.
2. Measure physical habitat variables at all 18 sites.
3. Identify the important habitat characteristics that explain their distribution and density.

TABLE 1. Summary of salmonfly habitat sampling sites. Six sites each on three rivers were sampled over four years for exuvia density and surveyed for physical habitat characteristics.

River	#	Site	Side	UTM NAD 83 (Zone 13)
Gunnison	1	Orchard Boat Ramp	River Left	247947, 4295297
Gunnison	2	Cottonwood Campground	River Left	252129, 4295940
Gunnison	3	Goldmine	River Left	253728, 4295747
Gunnison	4	Smith Fork	River Left	253338, 4291889
Gunnison	5	Ute Park	River Left	252376, 4284894
Gunnison	6	Chukar	River Left	253421, 4278775
Colorado	7	State Bridge	River Right	359889, 4414634
Colorado	8	Pumphouse BLM	River Left	370827, 4427300
Colorado	9	Powers BLM	River Right	394914, 4435762
Colorado	10	Byers Canyon	River Left	403335, 4434268
Colorado	11	Hwy 40 Bridge	River Right	408133, 4437708
Colorado	12	Hitching Post	River Left	414589, 4440304
Rio Grande	13	LaGarita	River Left	338264, 4182888
Rio Grande	14	Lower Wason 2	River Right	335653, 4186302
Rio Grande	15	Lower Wason 1	River Right	335353, 4187197
Rio Grande	16	Upper Wason 2	River Right	333668, 4187683
Rio Grande	17	Creede Hatchery	River Left	332145, 4187768
Rio Grande	18	Creede Boat Ramp	River Left	331362, 4187243

METHODS

Locations and description of sites are presented in Table 1. Exuvia estimates were completed by searching 30 meter (98.6 ft) 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 usually last from 7-13 days at our study sites). Stream flow changes and weather conditions also were taken into account when planning surveys to best estimate the total emergence at each site. Three to seven people intensively searched the riparian area from 1-20 meters 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 encountered with the first 3 meters from the water. On a single sampling occasion, each area was searched two to four times with identical search areas, effort and personnel. Each exuvia on the first pass was examined to determine sex. All sites have at least three years of data and a minimum of two years of data collected under favorable flow and weather conditions that did not compromise the estimates.

A multiple pass removal model was used to estimate the total density of exuvia at each site (Zippin 1958). Methods were similar but not identical to previous work (Nehring 2011) and many of the sites on the Colorado and Fraser River were identical to historic sites. More effort (higher number of people) was used compared to earlier studies resulting in higher capture probabilities that better met assumptions of the removal model and likely allowed unbiased estimates of exuvia with two depletion passes.

To evaluate the assumptions of the removal model and evaluate the appropriateness of this sampling technique, three pass removal data was compared to two pass data. The three pass data was analyzed with the Huggins Closed Capture model in Program Mark (Huggins 1989, White and Burnham 1999) and two pass data was analyzed with the simpler two pass removal model of Zippin (1958). In Mark, models were built that varied capture probability by pass, allowing a different capture probability for the first pass then the second and third passes. Declining capture probability with subsequent passes is a common source of bias of removal models in fisheries data (Peterson et al. 2004, Riley and Fausch 1992) and comparing the population estimates and capture probabilities allowed us to evaluate this assumption on the simpler two pass model. The assumptions of demographic and geographic closure were less likely to be violated due to exuvia being stationary and attached to rocks or vegetation and the emergence occurring at night, if good estimates of capture probability could be achieved and they were acceptable high, then the two pass depletion method should be ideal for estimating exuvia density.

Physical habitat surveys were completed at all 18 sites. These surveys included a modified Wolman pebble counts to characterize dominant substrate size (Wolman 1954, Potyondy and Hardy 1994) and two methods to measure substrate embeddedness. The D16 and D84 were calculated for each site to represent the relative size of small particles and larger particles. The D16 is the diameter of the particle that 16% of the sample is smaller than and in a normal distribution, one standard deviation from the median encompasses all data between the D16 and the D84. Embeddedness was visually estimated following the methods of Bain and Stevenson (1999) and was measured following the Weighted Burns Quantitative Method (Burns 1985, Sennatt et al. 2006).

Physical surveys of each site were completed with survey-grade GPS equipment and a HydroSurveyor acoustic Doppler current profiler system (ADCP). The GPS and ADCP surveys were analyzed by CPW aquatic researcher and hydrologist Eric Richer. Examples of the physical habitat survey maps and bathymetric maps produced with the GPS and ADCP surveys are presented in Figures 1 and 2. The data from the physical habitat surveys were analyzed to compile a list of variables that are hypothesized to explain differences in stonefly habitat quality.

To evaluate associations between habitat variables and stonefly density, two different techniques were used, hierarchical partitioning and AIC model selection. Both techniques gave insight into the importance of predictor variables and AIC model selection and model averaging was used to identify the top models and make model average parameter estimates to characterize optimal stonefly habitat. Hierarchical partitioning (Chevan and Sutherland 1991) was performed with the hier.part package in program R (Walsh and Mac Nally 2015). The process of hierarchical partitioning involves computing of the increase in the goodness of fit (R^2 in this case) of all models with a particular variable compared with the equivalent model without that variable (Mac Nally 1996). Therefore, hierarchical partitioning provides an estimate of the independent and joint contribution for each explanatory variable and is especially useful when variable importance ranking is the primary objective rather than prediction in a regression analyses (Mac Nally 2000).

This technique has been shown to be unbiased when applied to fewer than nine explanatory variables (Olea et al. 2010) and addresses collinearity between explanatory variables that are generally problematic in regression analyses (Mac Nally 2000, Grömping 2007). Because of the low sample size (18 sites) we could only explore a limited number of models model selection to identify the best predictive model(s). A candidate set of linear regression models was developed with the top three variables identified by hierarchical partitioning and compared using the information theoretic approach (Burnham and Anderson 2002). Models were evaluated using the small sample size version of AIC with the AICcmodavg package in Program R (R Core Team 2015, Mazerolle 2017).

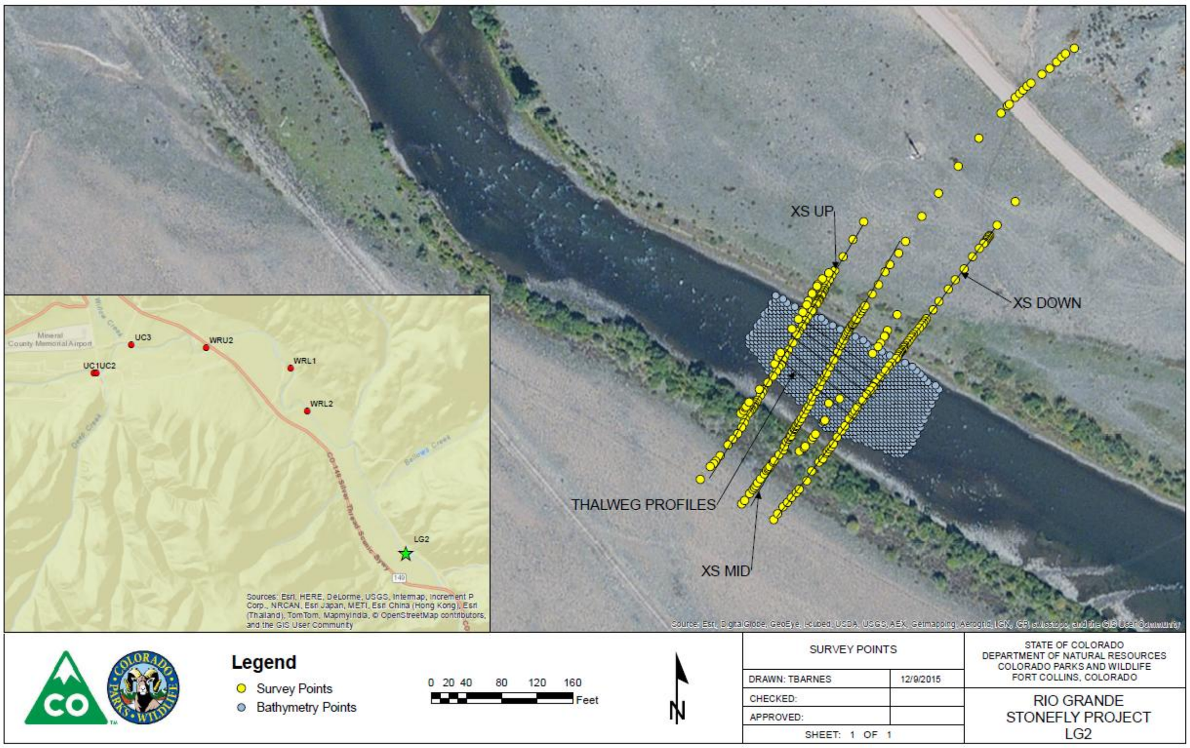

FIGURE 1. Survey points and bathymetry data collected with the survey-grade GPS equipment and Acoustic Doppler Current Profiler of a Rio Grande river stonefly site.

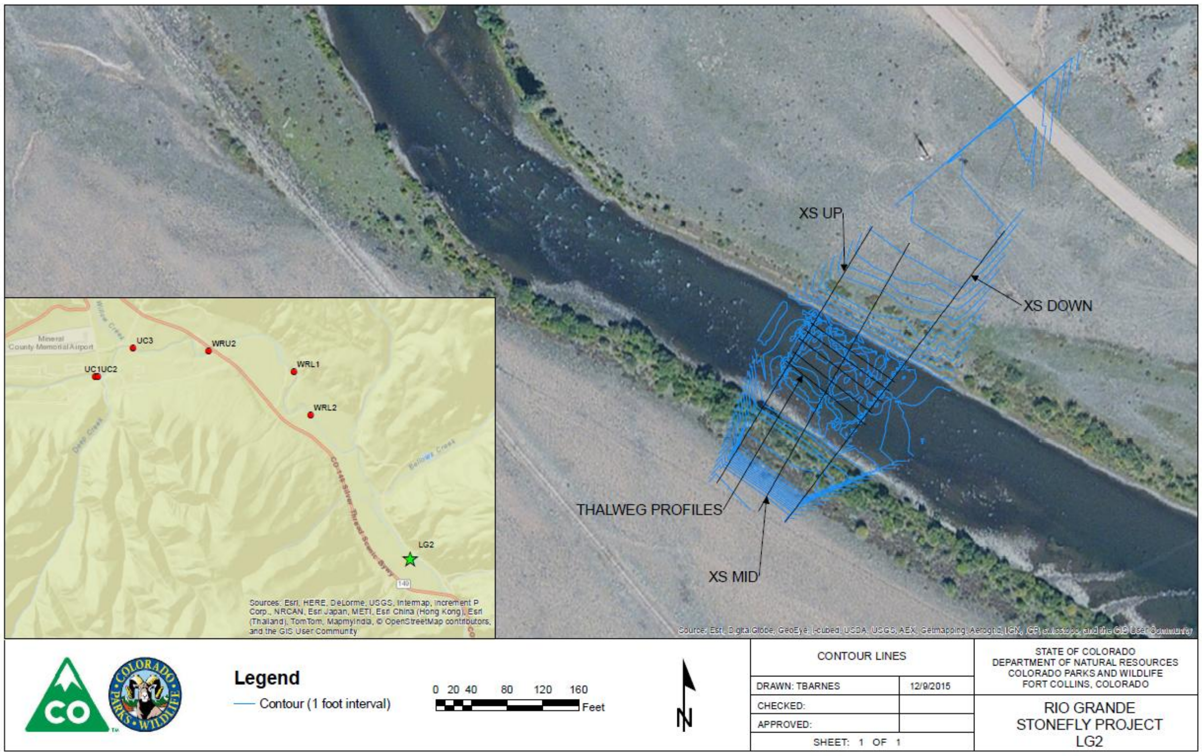

FIGURE 2. Bathymetric map produced by the GPS and ADCP survey used to estimate physical channel characteristics of stonefly study sites.

RESULTS

Stonefly Exuvia Density Estimation

Simple two pass population models were sufficient to get unbiased population estimates of recently emerged stonefly exuvia. Capture probabilities and population estimates were very similar for the Huggins closed capture model three pass estimates and the Zippin two pass estimates (Figure 3). There was some variation in the estimated capture probability at very low densities (<80 exuvia per 30 m) and very high densities (> 6,000 exuvia per 30 m) indicating that the assumption of equal capture probabilities for all passes is violated with the simple two pass model. However, that bias was relatively small and population estimates of the two models were very close. The two pass depletion technique worked well for the vast majority of estimates at our sites where moderate exuvia densities were encountered. Many of the issues with depletion estimates encountered during fish population assessments were not a problem with stoneflies due to the immobile nature of the exuvia, high capture probability, and no size selective gear (Riley and Fausch 1992, Peterson et al. 2004, Saunders et al. 2011).

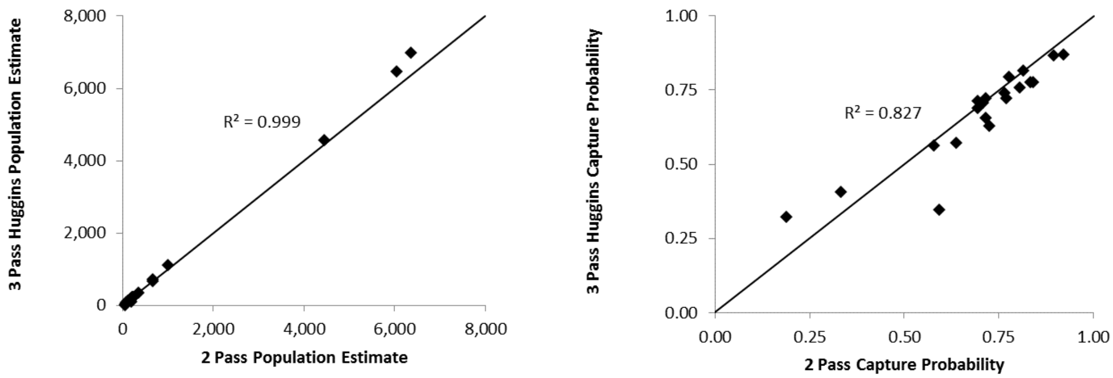

FIGURE 3. Population and capture probability estimates comparing a three pass Huggins Closed Capture model in Program Mark (with time effects that varied capture p̂) to a simple two pass removal model of Zippin (1958).

Stonefly Habitat Preferences

Figure 4 shows the correlation matrix for all habitat variables. The top three variables were Percent Fines, Width to Depth Ratio, and Embeddedness. All of the top three were negatively correlated with exuvia densities and significant at the 95% level. Two explanatory variables, D16 and D84, were highly correlated (as expected) and D16, a better predictor of exuvia density, was positively correlated. The results of the hierarchical partitioning exercise are summarized in Figure 5 and reveal the same patterns. Percent Fines had the highest independent contribution (24.7%) followed by Width to Depth Ratio (19.6%), and Embeddedness (15.0%).

The AIC*c* model selection results are presented in Table 2. All of the models were within 3.1 ΔAIC_c units of each other and the single variable model with Percent Fines as the top model. Summing AIC weights across the model set, Percent Fines had 0.71 of the weight, while Width to Depth Ratio had 0.43 and Embeddedness had 0.37 of the weight. The single variable model of Percent Fines (with intercept and error terms) explained 35% of the variation in exuvia density. More work is needed to investigate other factors that contribute to salmonfly density as our best models explained less than half of the variability in exuvia density.

The results of this study identify that a low amount of fine sediment, a low width to depth ratio, and low embeddedness are associated with river sites in Colorado with the highest stonefly density. If conservation or restoration of salmonfly habitat is a goal of river managers or biologists, then flow management activities and habitat restoration should strive for riffle sites with percent fine sediment between 2.5-8%, percent embeddedness less than 23% and a width to depth ratio between 34 and 57.

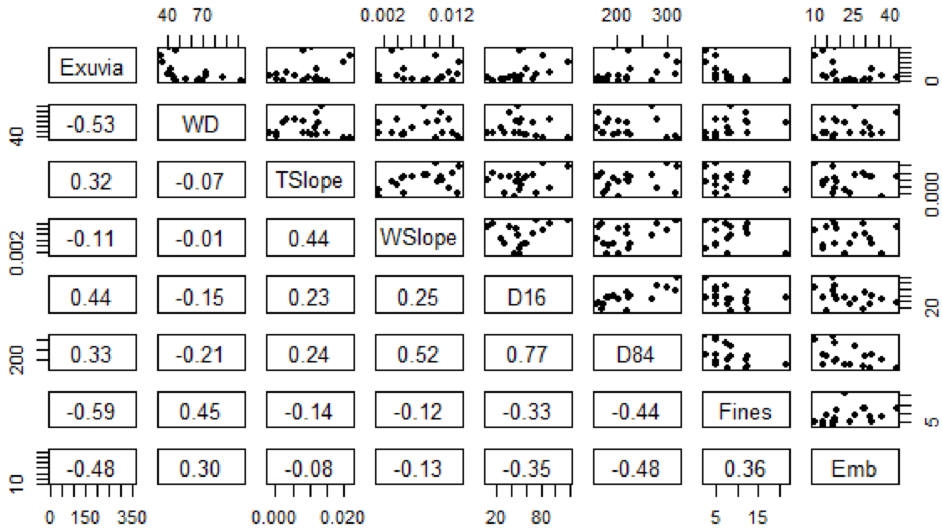

FIGURE 4. Pearson correlation matrix of habitat variables and exuvia density. WD is the width to depth ratio, TSlope is the thalweg slope, WSlope is the water surface slope, D16 and D84 are cumulative particle size 16% and 84%, Fines is % particles <6.4 mm), and Emb is % embeddedness. Three variables were significantly correlated at the 95% level with exuvia density; % Fines (p=0.009), Width to Depth Ratio (p=0.024), and Embeddedness (p=0.043).

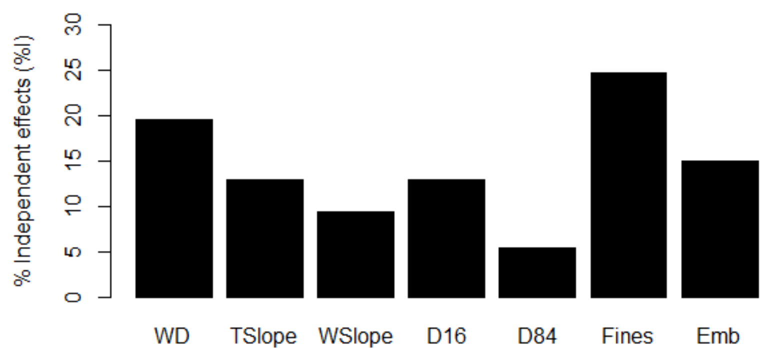

FIGURE 5. Independent effects of each of the habitat variables from a hierarchical partitioning analysis. WD is the width to depth ratio, TSlope is the thalweg slope, WSlope is the water surface slope, D16 and D84 are cumulative particle size 16% and 84%, Fines is % particles <6.4 mm), and Emb is % embeddedness.

TABLE 2**.** Model selection results for linear regression models for stonefly habitat variables. 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), multiple R^2, and sum of Akaike weights (Σw_i) for individual parameters.

Model	**K**	**AIC_c**	**ΔAIC_c**	**w_i**	**R^2**	**Σw_i**
%Fines	3	220.11	0	0.31	0.35	0.71
%Fines + Width/Depth	4	220.94	0.83	0.20	0.44	
%Fines + Embeddedness	4	221.00	0.89	0.20	0.44	
Width/Depth	3	222.05	1.93	0.12	0.28	0.43
Width/Depth + Embeddedness	4	222.32	2.21	0.10	0.39	
Embeddedness	3	223.21	3.10	0.07	0.23	0.37

REFERENCES

Bain, M.B., and Stevenson, N.J., eds. 1999. Aquatic habitat assessment: common methods. American Fisheries Society, Bethesda, MD.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. Second edition. EPA 841– D–97–002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Baxter, C.V., K.D. Fausch, and W.C. Saunders. 2005. Tangled webs: reciprocal flows of Invertebrate prey link streams and riparian zones. Freshwater Biology. 50: 201-220.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag, New York.

Burns, D. C., and Edwards, R. E. 1985. Embeddedness of salmonid habitat of selected streams on the Payette National Forest. USDA Forest Service, Payette National Forest, McCall, ID.

Chevan, A. and M. Sutherland. 1991. Hierarchical Partitioning. The American Statistician, 45(2): 90–96.

Dewalt, R. E. and K.W. Stewart. 1995. Life-histories of stoneflies (Plecoptera) in the Rio Conejos of southern Colorado. Great Basin Naturalist. 55: 1-18.

Erickson, R.C. 1983. Benthic field studies for the Windy Gap study reach, Colorado River, Colorado, fall, 1980 to fall, 1981. Prepared for The Northern Colorado Water Conservancy District, Municipal Sub-District.

Fore, L.S., J.R. Karr, and R.W. Wisseman. 1996. Assessing invertebrate responses to human activities: Evaluating alternative approaches. Journal of the North American Benthological Society 15(2):212-231.

Grömping, U. 2007. Estimators of relative importance in linear regression based on variance decomposition. The American Statistician 61(2):139-147.

Huggins, R. M. 1989. On the statistical analysis of capture-recapture experiments. Biometrika 76:133–140.

Nehring, R.B. 1987. Stream fisheries investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-51-R, Progress Report, Fort Collins.

Nehring, R.B. 2011. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237-R-18, Final Report, Fort Collins.

Olea, P.P., P. Mateo-Tomas, and A. de Frutos. 2010. Estimating and Modelling Bias of the Hierarchical Partitioning Public-Domain Software: Implications in Environmental Management and Conservation. PLoS ONE 5(7).

Peterson, J.T., R.F. Thurow, and J.W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 133:462-475.

Potyondy, J.P. and T. Hardy. 1994. Use of pebble counts to evaluate fine sediment increase in stream channels. Water Resources Bulletin 30:509-520.

Mac Nally, R. 1996. Hierarchical partitioning as an interpretative tool in multivariate inference. Australian Journal of Ecology 21:224–228.

Mac Nally, R. 2000. Regression and model-building in conservation biology, biogeography and ecology: The distinction between and reconciliation of predictive and explanatory models. Biodivers Conserv 9: 655–671.

Mazerolle, M.J. 2017. Package "AICcmodavg", http://cran.r-project.org/web/packages/ AICcmodavg.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Richards, D.C., M.G. Rolston, and F.V. Dunkle. 2000. A comparison of salmonfly density upstream and downstream of Ennis Reservoir. Intermountain Journal of Sciences 6(1)1-9.

Riley, S.C., and K.D. Fausch. 1992. Underestimation of trout population size by maximum likelihood removal estimates in small streams. North American Journal of Fisheries Management 12:768–776.

Saunders, W.C., K.D. Fausch and G.C. White. 2011. Accurate estimation of salmonid abundance in small streams using nighttime removal electrofishing: an evaluation using marked fish. North American Journal of Fisheries Management, 31:403-415.

Sennatt, K.M., N.L. Salant, C.E. Renshaw, and F.J. Magilligan. 2006. Assessment of Methods for Measuring Embeddedness: Application to Sedimentation in Flow Regulated Streams. Journal of the American Water Resources Association 42(6):1671-1682.

Vinson, M. R. 2001. Long-term dynamics of an invertebrate assemblage downstream from a large dam. Ecological Applications. 11: 711-70.

Walsh, C. and R. Mac Nally. 2015. Package ‘hier.part’, http://cran.r-project.org/web/packages /hier.part/hier.part.

Walters, D.M., J.S. Wesner, R.E. Zuellig, D.A. Kowalski, M.C. Kondratieff. 2018. Holy flux: spatial and temporal variation in massive pulses of emerging insect biomass from wester U.S. rivers. Ecology 99(1): 238-240.

Ward, J.V. and J.A. Stanford. 1979. The ecology of regulated streams. Plenum Press, New York.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(Supplement): 120–139.

Wolman, M.G. 1954. A method of sampling coarse river-bed material. Transactions American Geophysical Union. Volume 35. Number 6. Pp. 951-956.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

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

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 (Fox 2013, Kolden 2013). Over 30 whitewater parks exist in Colorado or are in the construction planning stages (Figure 6). 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 2013). Whitewater parks have also been documented to cause a suppression of fish movement that is related to fish length (Fox 2013). 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 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 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 impacted by habitat related changes before higher predators like trout. Sculpin could not only indicate ecological problems that will eventually affect sport fish like trout, but they serve as an important food source, especially for Brown Trout common in many Colorado rivers.

The objective of this study is to investigate the effects of building whitewater parks on Mottled Sculpin, aquatic invertebrates, and trout by sampling before and after construction with control sites. 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 before/after impacts to fish and invertebrates. To meet the objectives of this project a before, after, control, impact

(BACI) study design was used to evaluate changes in trout population, Mottled Sculpin density and aquatic invertebrates 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 trout and Mottled Sculpin before and after construction.

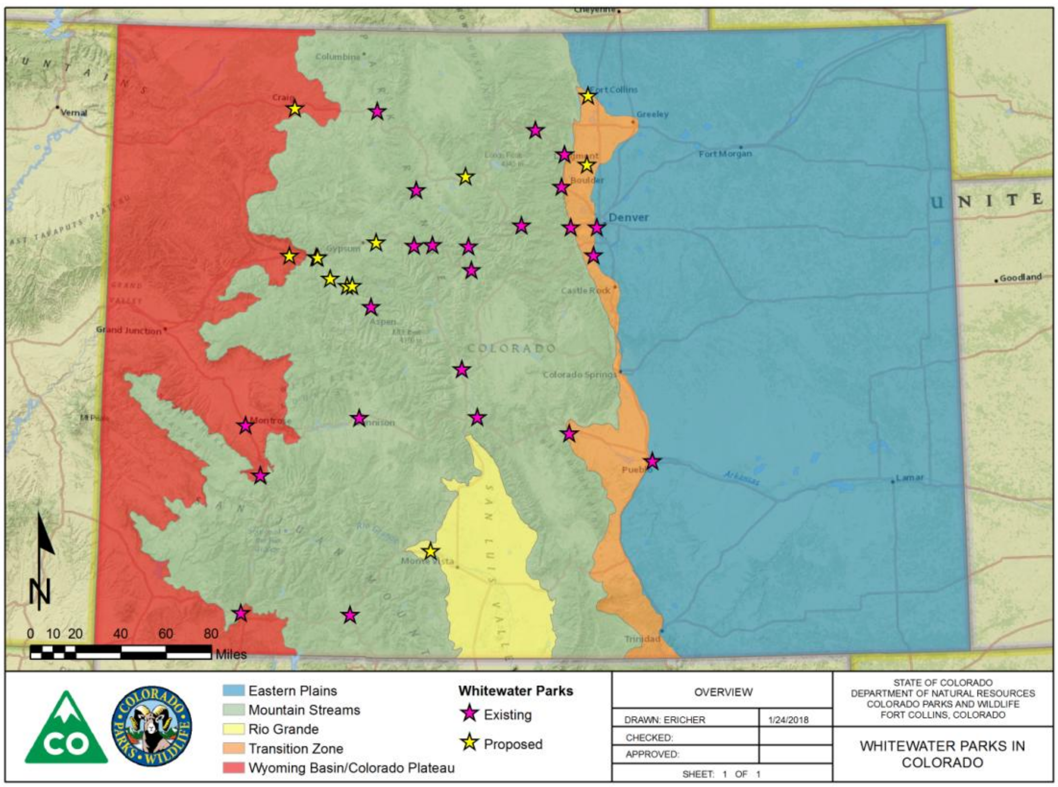

FIGURE 6. Whitewater parks existing and proposed in Colorado.

FIGURE 7. 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 consist of six drop structures over about 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 remained functioning (but smaller) 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. Samples 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 organisms preserved in 80% ethanol. Velocity and depth were taken at each Hess sample site to ensure samples were taken from similar riffle habitat. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited and each one underwent the protocol (Moulton et al. 2000). All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to subfamily and non-insects (e.g., oligochaetes, amphipods) were identified to class. Each replicate sample was processed separately so an average of 1,670 individual specimens were identified at each riffle site. Many more individual specimens were identified from each site compared to standard methods to ensure rare organism were sampled and to increase the power of the comparisons between riffles sites in close proximity within the same stream (Vincent and Hawkins 1996).

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. 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 compared to the first to address a common source of bias in electrofishing removal models. Model selection was conducted with 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, 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 next downstream riffle, all sites are with a 0.4-mile reach. The WWP on the Colorado River consists of a single large cross channel wave structure so fewer sites were necessary. Unlike the Uncompahgre where post construction riffles remained in the WWP, at Pumphouse the middle site was converted from a run to a drop structure with pools above and below (Figure 12). 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 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 differentially marked in 2016 and 2017 above and below the WWP structure with upper caudal punches used in Station 1 and Station 2 and lower caudal punches used in Stations 3 and Station 4. With 48 hours between mark and recapture events, any movement upstream or down through the structure was documented on the recapture pass. To evaluate longer-term fish movement, 142 trout were marked with an adipose clip in 2016 that were sampled in Station 2 (above the structure) and moved below the structure. 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 structures. The sampling reaches were concurrent with the invertebrate sampling riffles in the invertebrate study and were 80, 125, and 100 feet long with an average width of 17.7 ft. Three pass removal electrofishing with a concurrent mark recapture estimate was conducted to evaluate assumptions on capture probabilities between passes. Fish were measured to the nearest millimeter and density estimates were made for each site with the Huggins Closed Capture model in Program Mark and are presented in Table 4 (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 (Riley and Fausch 1992, Peterson et al. 2004).

RESULTS

Uncompahgre River Aquatic Invertebrates

Trends in the aquatic invertebrate density and diversity on the Uncompahgre River are displayed in Figures 8-11. Overall invertebrate density and diversity has not changed much at the study sites relative to annual and spatial variability. 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. Most of the stations were relatively similar except 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 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. The other two WWP sites are at good quality riffles that formed above each of the drop structures. These riffles are not functionally different from the upstream and downstream control sites in density, diversity, or community structure.

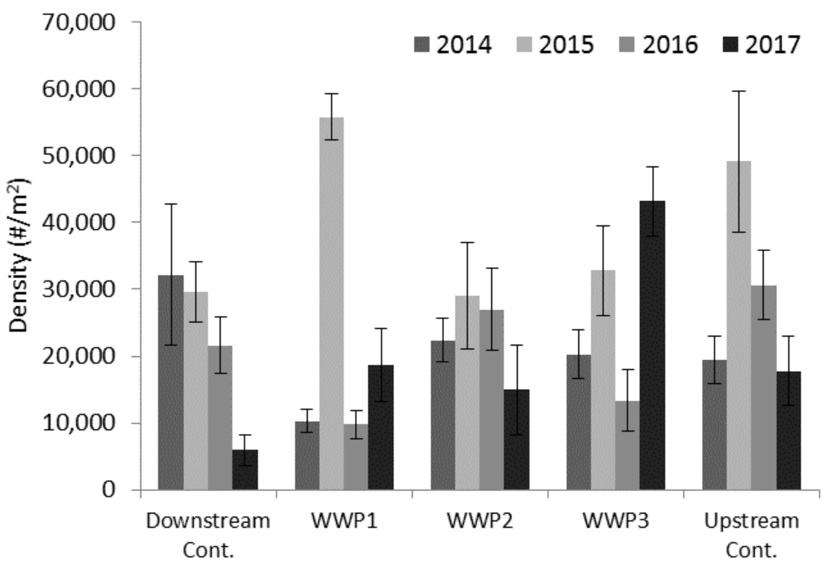

FIGURE 8. Density of all species of aquatic invertebrates with standard error bars on the Uncompahgre River 2014-2017.

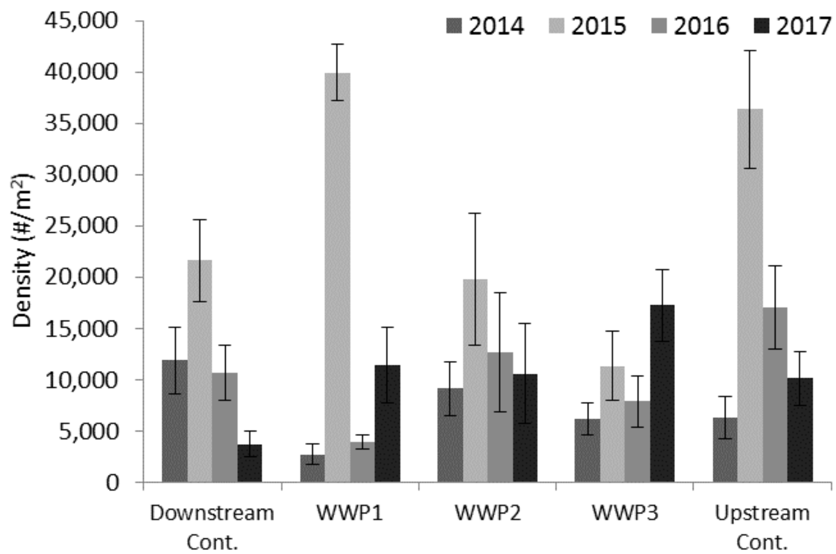

FIGURE 9. Density of ephemeroptera, plecoptera, and trichoptera fauna with standard error bars on the Uncompahgre River 2014-2017.

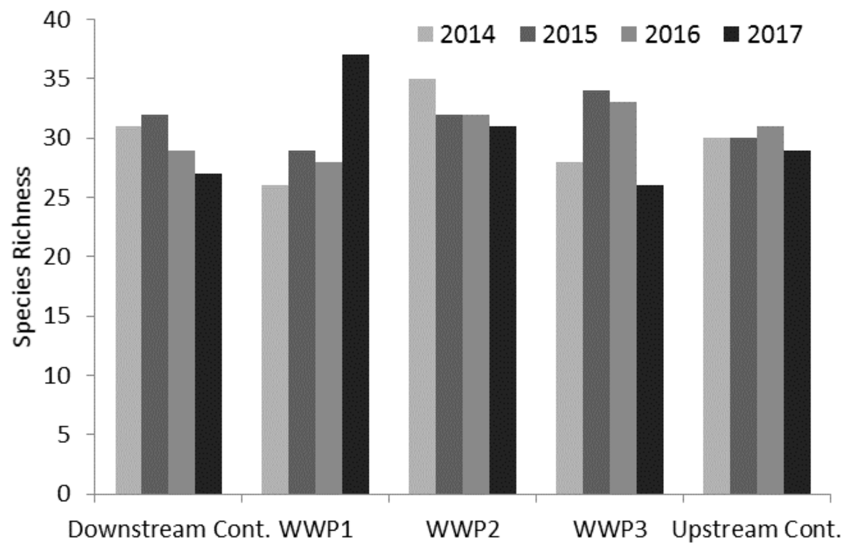

FIGURE 10. Total species richness on the Uncompahgre River 2014-2017.

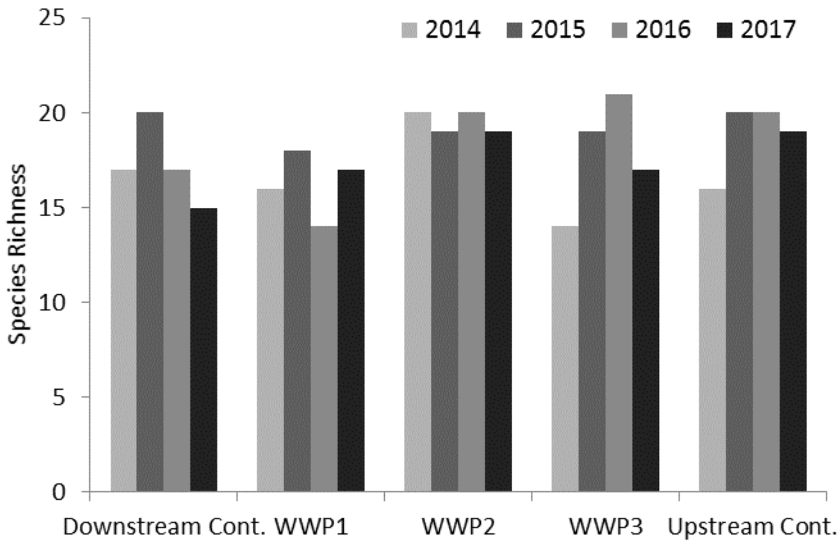

FIGURE 11. Species richness of ephemeroptera, plecoptera, and trichoptera (EPT) fauna on the Uncompahgre River 2014-2017.

Uncompahgre River Sportfish and Mottled Sculpin Populations

Trends in the Brown Trout population of the Uncompahgre River 2014-2107 are displayed in Figure 12 and trends in Mottled Sculpin density are displayed in Figure 13. Difficult sampling conditions most years led to low capture probabilities and imprecise estimates. 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 from 2014 to 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 has relatively poor trout habitat due to the high water velocities in most locations. Decreasing velocities and increasing depth by any means may improve habitat for Brown Trout. The low numbers, high variability, and challenging sampling limited the ability to detect many trends over time and space. 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 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 and a detectable scale.

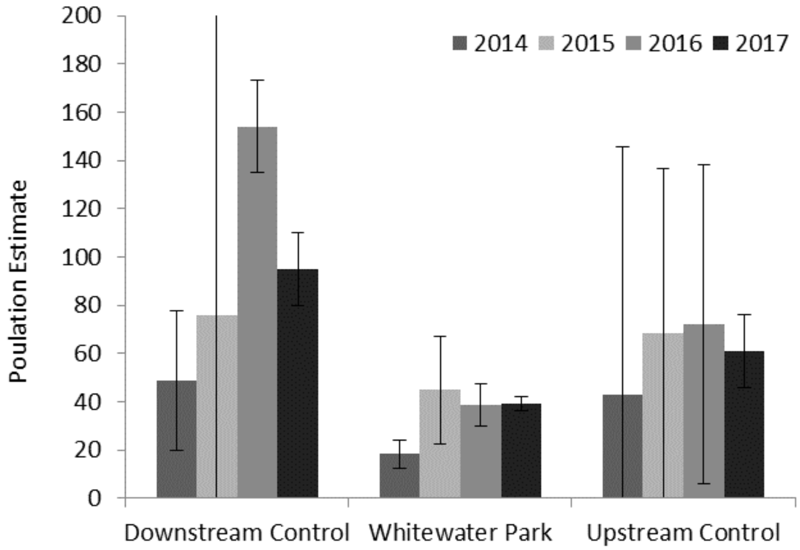

FIGURE 12. Brown Trout population estimates from the three sampling reaches of the Uncompahgre River 2014-2017.

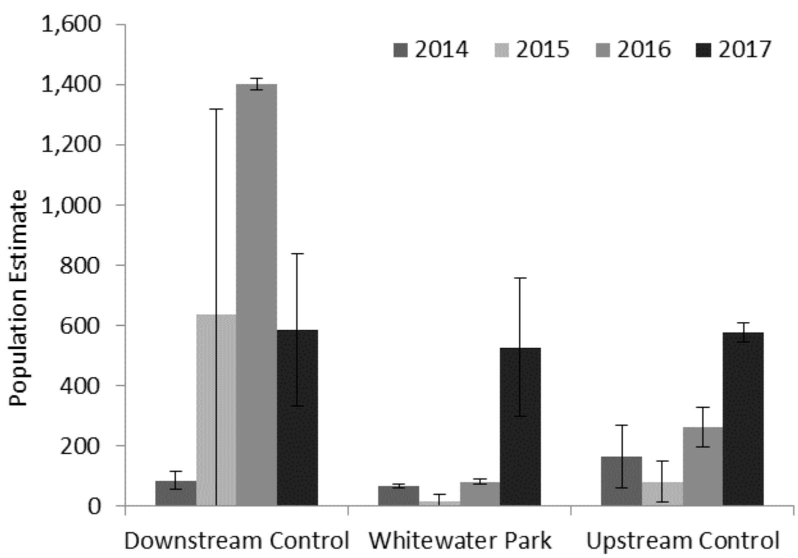

FIGURE 13. Mottled Sculpin population estimates from the three sampling reaches of the Uncompahgre River 2014-2017.

Colorado River Aquatic Invertebrates
Trends in the aquatic invertebrate density and diversity are displayed in Figures 14-17. 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. 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 not much separation of the three sites from each other.

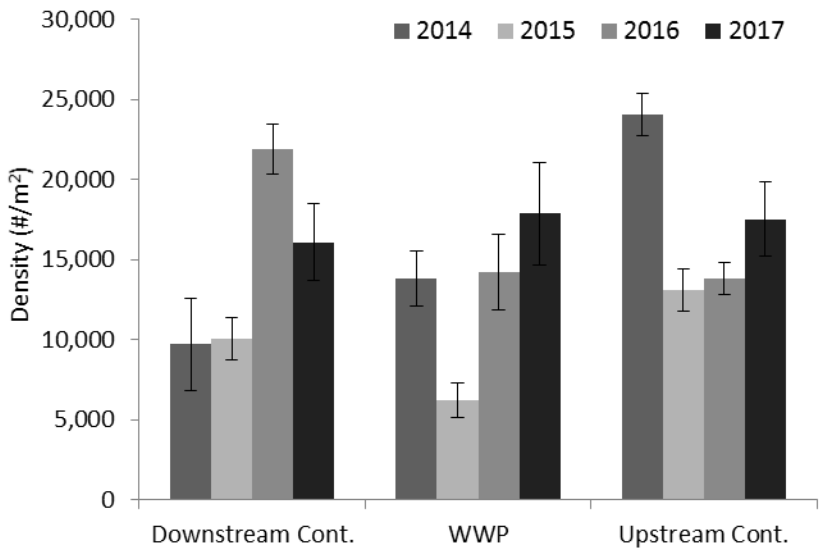

FIGURE 14. Density of all invertebrates with standard error bars at sites on the Colorado River at Pumphouse 2014-2017.

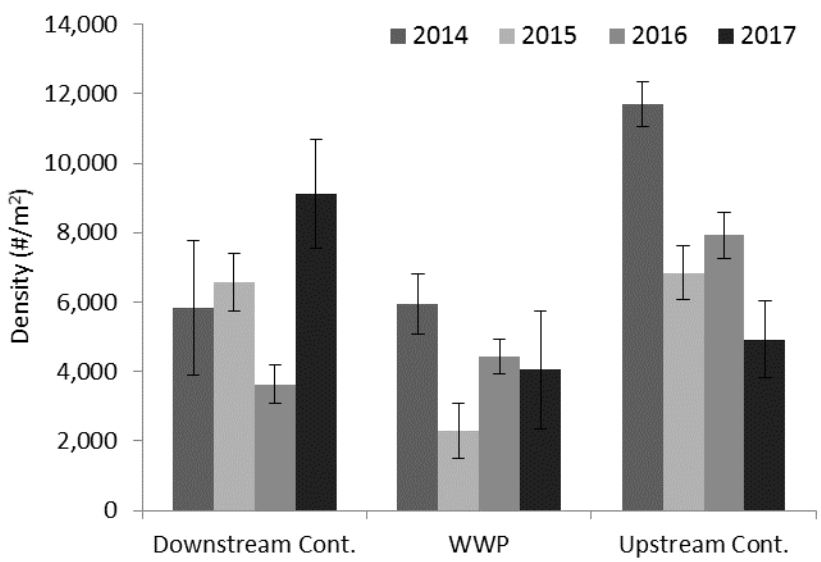

FIGURE 15. Density of EPT fauna with standard error bars at sites on the Colorado River at Pumphouse 2014-2017.

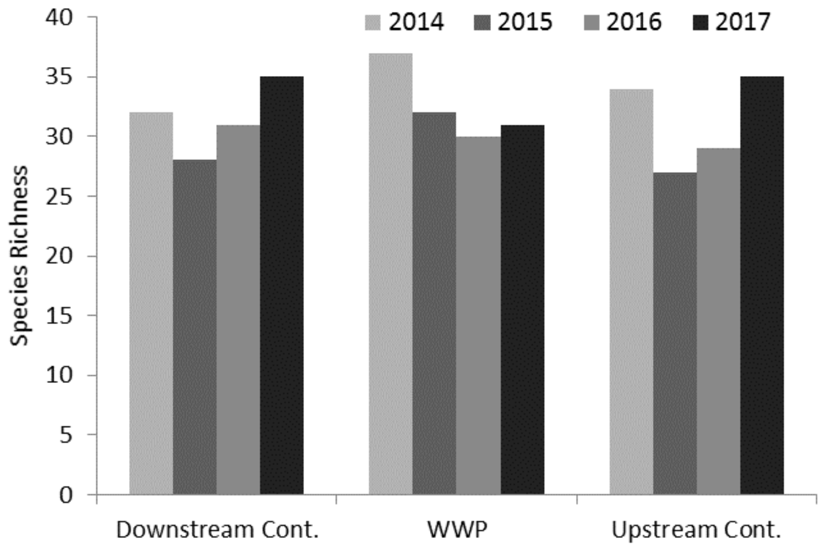

FIGURE 16. Total species richness at sites on the Colorado River at Pumphouse 2014-2017.

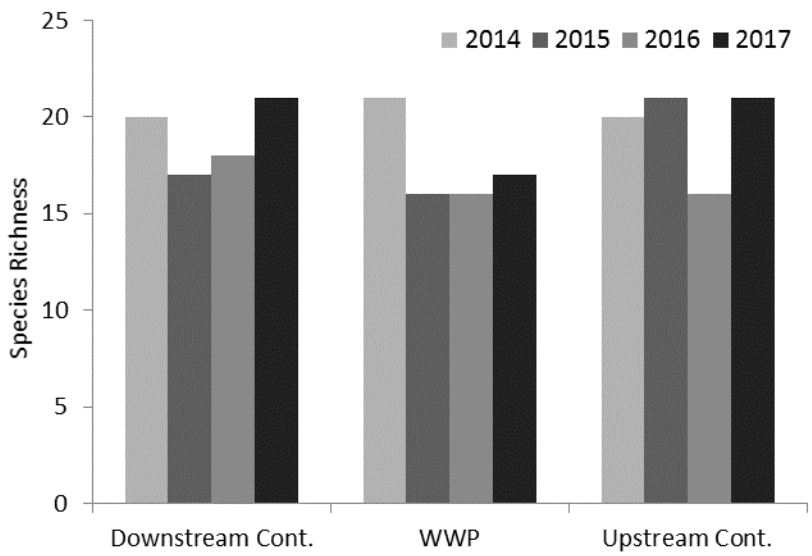

FIGURE 17. Species richness of EPT fauna on the Colorado River 2014-2017.

Colorado River Sportfish Populations

On the Colorado River, Brown Trout and Mountain Whitefish 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 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 (at the 95% level) longnose and white suckers and significantly fewer trout than the reach above it (Figure 19) and the reach immediately below the structure is the only reach where suckers outnumber trout.

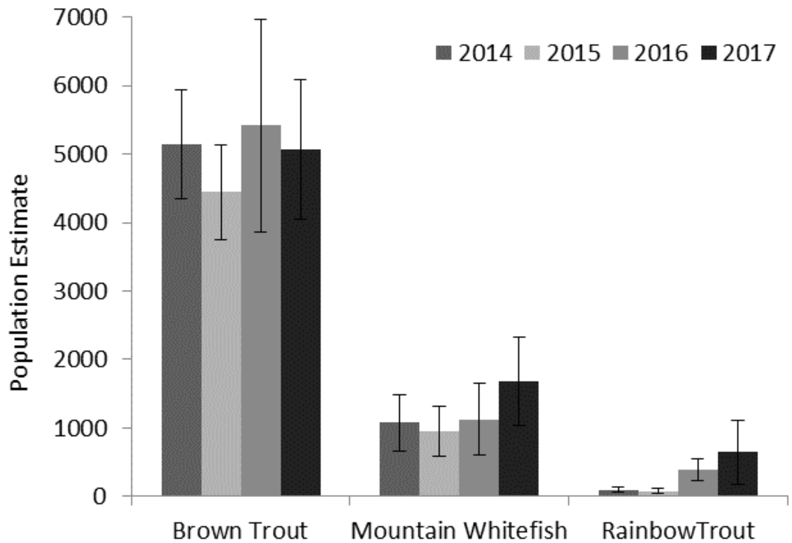

FIGURE 18. Fish population estimates and 95% confidence intervals before and after construction of the whitewater park structure on the Colorado River at Pumphouse.

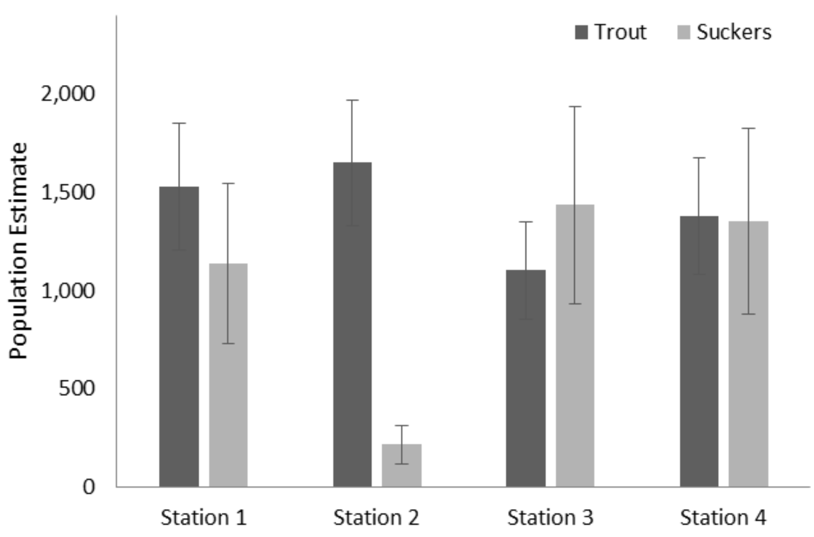

FIGURE 19. Fish population estimates and 95% confidence on the Colorado River at Pumphouse for each sampling station in 2017. Station 1 is from bottom of Gore Canyon to the riffle above Launch #1, Station 2 is from the riffle above Launch #1 to the whitewater park feature, Station 3 is from the whitewater park feature to Launch #3, and Station 4 is from Launch #3 to the bottom of the sampling reach.

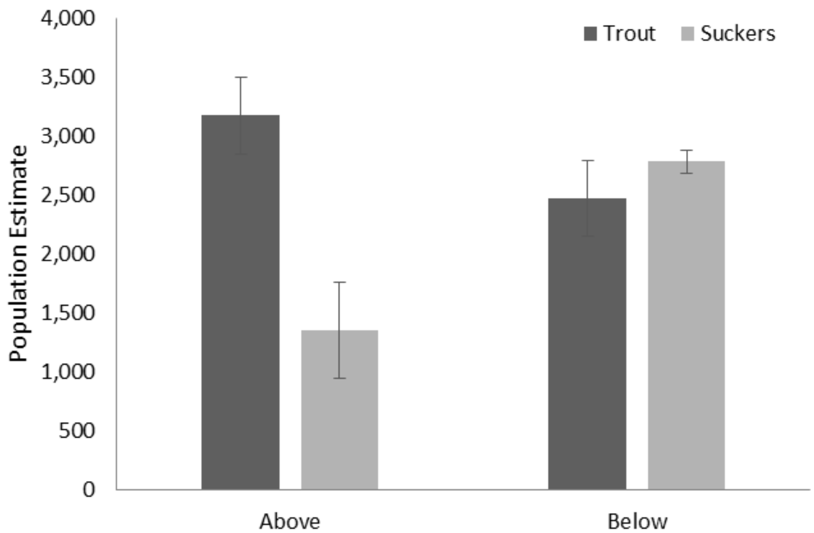

FIGURE 20. Fish population estimates and 95% confidence intervals above and below the WWP structure on the Colorado River at Pumphouse in 2017.

Fish Passage

The structure does not appear to be a complete migration barrier for adult Brown Trout or Rainbow Trout. In 2016, four Brown Trout 371-422 mm were documented passing above the structure between the first and second passes. 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. Twenty-six 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 have not been observed passing the structure proportionate with the large 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

Trends in the Mottled Sculpin densities at the three sampling sites are shown in Figure 21. 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 sculpin densities of the three sites. However, sculpin densities were down at all sites in 2017 and while sculpin densities have declined 39% at the WWP site, that difference is not significant at the 95% level due to the high annual variability of sculpin densities (Figure 22).

The Gore Canyon whitewater park structure has had subtle effects on the invertebrate and fish communities of the Colorado River but no population level impacts were documented. The largest concerns raised in this study include fish passage through the structure of smaller fish, and localized impacts to the fish habitat below the structure that may reduce the habitat suitability for trout and increase densities of white and longnose suckers.

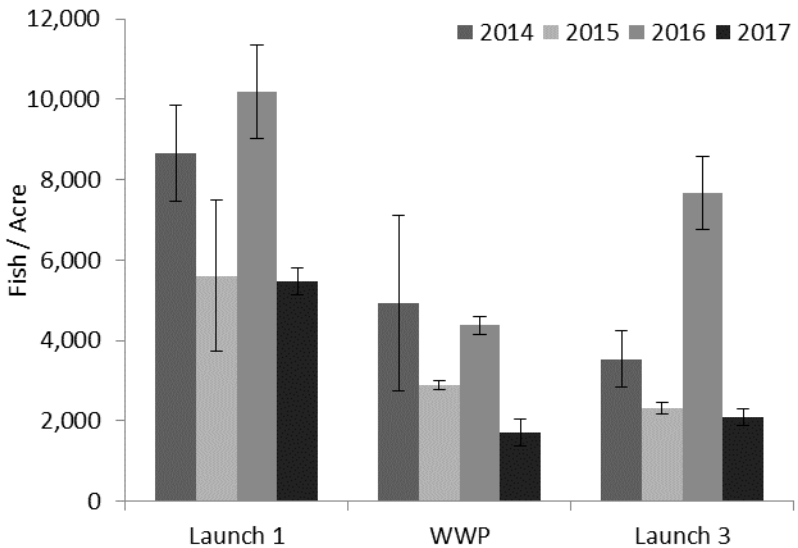

FIGURE 21. Mottled Sculpin density estimates and 95% confidence intervals on the Colorado River at Pumphouse 2014-2017.

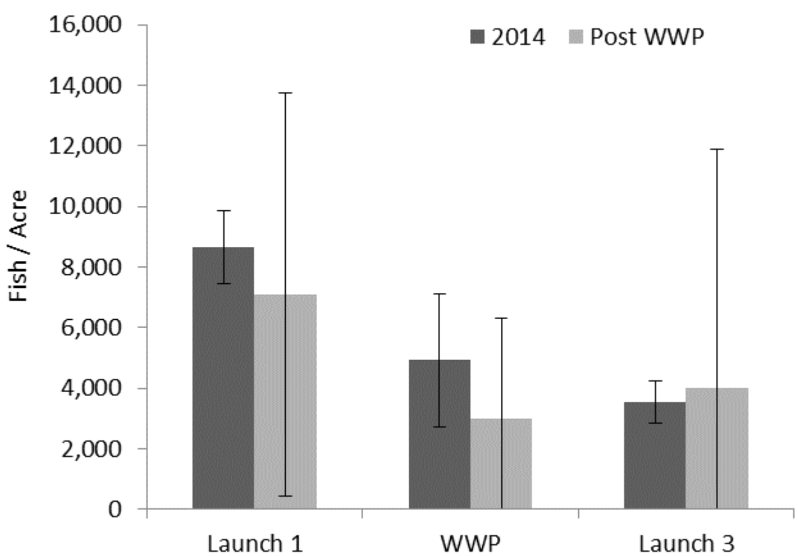

FIGURE 22. Mottled Sculpin density estimates and 95% confidence intervals on the Colorado River at Pumphouse before and after construction of the whitewater park structure.

REFERENCES

Adams, S.B. and D.A. Schmetterling. 2007. Freshwater sculpins: phylogenetics to ecology. Transactions of the American Fisheries Society 136:1763-1741.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag, New York.

Fetherman, E.R and G.J. Schisler. 2017. Sport fish research studies. Colorado Parks and Wildlife Progress Report, Federal Aid in Sportfish Restoration F-394-R16 Progress Report, Fort Collins.

Fox, B. 2013. Eco-evaluation of whitewater parks as fish passage barriers. Master's thesis. Colorado State University, Fort Collins, Colorado.

Huggins, R. M. 1989. On the statistical analysis of capture-recapture experiments. Biometrika 76:133–140.

Kolden, E. 2013. Modeling in a three-dimensional world: whitewater park hydraulics and their impact on aquatic habitat in Colorado. Master's thesis. Colorado State University, Fort Collins, Colorado.

Kondratieff, M.C. 2012. Stream habitat investigations and assistance. Colorado Parks and Wildlife Progress Report, Federal Aid in Sportfish Restoration F-161-R18n Progress Report, Fort Collins.

Kowalski, D.A. 2014. Colorado River aquatic resources investigations. Colorado Parks and Wildlife, Federal Aid in Sport Fish Restoration, Project F-237-R-21, Progress Report, Fort Collins.

Moulton, S.R., II, Carter, J.L., Grotheer, S.A., Cuffney, T.F. & Short, T.M. 2000. Methods of analysis by the U. S. Geological Survey national water quality laboratory: processing, taxonomy, and quality control of benthic macroinvertebrate samples. Open-File Report 00-212, U.S. Geological Survey, Washington D.C.

Nehring, R.B. 2011. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237R-18, Final Report, Fort Collins.

Peterson, J.T., R.F. Thurow and J.W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 133:2, 462-475.

Riley, S. C., and K. D. Fausch. 1992. Underestimation of trout population size by maximum likelihood removal estimates in small streams. North American Journal of Fisheries Management 12:768–776.

Saunders W.C., K.D. Fausch, and G.C. White. 2011. Accurate estimation of salmonid abundance in small streams using nighttime removal electrofishing: an evaluation using marked fish. North American Journal of Fisheries Management 31:403-415.

Seber, G. A. 1982. The estimation of animal abundance and related parameters, Second edition. Charles Griffin and Company, Ltd, London.

Shannon, C. E. 1948. A mathematical theory of communication. The Bell System Technical Journal, 27, 379–423.

Sigler, F.F. and R.R. Miller. 1963. Fishes of Utah. Utah Department of Fish and Game, Salt Lake City, Utah.

Vinson, M.R. and C.P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15(3): 392-399.

Woodling, J. 1985. Colorado's little fish, a guide to the minnows and other lesser known fishes in the state of Colorado. Colorado Division of Wildlife, Denver, Colorado.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46(Supplement): 120–139.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

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

Job Objective: Investigate the ecological impacts of stream flow alterations on aquatic invertebrates and fish of the Colorado River and assist in the planning and evaluation of mitigation efforts to address those impacts.

Dams are known to drastically alter river habitat and have many diverse effects on fish and invertebrate habitat and populations (Ward and Stanford 1979). Trans-basin and local water use divert approximately 67% of the 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 (Windy Gap) on the invertebrates and fish of the river. Native Mottled Sculpin, once common, are now rare or extirpated immediately below the reservoir (Dames and Moore 1951, Nehring 2011). The health of the invertebrate community declined after the construction of Windy Gap. A 38% reduction has occurred in the diversity of aquatic invertebrates from 1980 to 2011. In addition, 19 species of mayflies, four species of stoneflies and eight species of caddisflies had been extirpated from the sampling site below Windy Gap (Erickson 1983, Nehring 2011). Historically, salmonflies were common in the upper Colorado River but have become rare immediately below Windy Gap (USFWS 1951).

In the upper Colorado River basin, stream reaches below many dams and water projects have been observed to have reduced density of Mottled Sculpin (Nehring 2011). The decline in sculpin distribution appears both temporally and spatially related to impoundments. Mottled Sculpin were common in the main stem Colorado River before Windy Gap Reservoir was built but are rare or absent in later years (Erickson 1983, Nehring 2011). A survey in 1975-1976, before Windy Gap construction, documented Mottled Sculpin at all sampling sites (Dames and Moore 1977). In 2010, a project investigating the sculpin distribution and density around the upper Colorado River revealed that sculpin density on average was 15 times higher in sites above impoundments compared to downstream sites (Nehring 2011). In the main stem Colorado River between Windy Gap and the Williams Fork, a single fish was sampled in 3,200 ft of river sampled by electrofishing. This study attributed the decline of Mottled Sculpin in the upper Colorado River below 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 (Kowalski 2014). Three sites were sampled on the Colorado between Windy Gap and the Williams Fork confluence and no Mottled Sculpin were found.

Increased trans-basin water diversions are planned and there are ongoing efforts to implement mitigation measures to reduce the impact of the new projects. A large component of the mitigation plan is constructing a bypass around the reservoir. This would reconnect the river and address various impacts of a large main channel impoundment but would not reduce the impacts of water withdrawals from the system. The planned bypass channel offers a unique opportunity to evaluate the effects reconnecting the river around the reservoir as well as investigate if mitigation measures can offset the impacts of large water diversions on the ecology of the river.

OBJECTIVES

1. Assist CPW staff as needed in planning of mitigation efforts.
2. Continue monitoring invertebrate and fish populations of the upper Colorado and Fraser Rivers.
3. Evaluate the effectiveness of mitigation measures in restoring and improving the ecological function of the Colorado River in Middle Park (if they are completed).

Approach

Coordination is continuing among project stakeholders including CPW personnel, the Upper Colorado River Learning by Doing Management Committee, Windy Gap Technical Assistance Committee (TAC), Trout Unlimited, and private landowners downstream of Windy Gap. The two most relevant efforts to this research are the bypass channel planning and construction being handled mostly by the TAC and the planned stream habitat improvement that CPW will be heavily involved with. Coordination with all of the stakeholders will continue under project F237 and increase as projects move from the planning stage to implementation.

A large amount of baseline data has been collected previously under Project F-237. If mitigation measures are finalized and implementation appears eminent, routine sampling will continue at historic sites. The exact sampling protocols and sampling sites will depend on the specifics of mitigation measures and will be defined in cooperation with other researchers. Currently, it appears that the largest mitigation measure, a bypass channel around Windy Gap Reservoir, could be constructed as early as 2021. Invertebrate and fish sampling is planned to resume in 2018-2019 to collect pre-construction data above and below Windy Gap.

REFERENCES

Erickson, R.C. 1983. Benthic field studies for the Windy Gap study reach, Colorado River, Colorado, fall, 1980 to fall, 1981. Prepared for The Northern Colorado Water Conservancy District, Municipal Sub-District.

Dames and Moore. 1977. Environmental assessment report Windy Gap project Grand County, Colorado for municipal subdistrict, Northern Water Conservancy District. Denver, CO.

Kowalski, D.A. 2014. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237-R21, Progress Report, Fort Collins.

Nehring, R.B. 2011. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237R-18, Final Report, Fort Collins.

USFWS (U.S. Fish and Wildlife Service). 1951. Recreational use and water requirements of the Colorado River fishery below Granby Dam in relation to the Colorado-Big Thompson diversion project. U.S. Fish and Wildlife Service, Region 2, Albuquerque, NM.

Ward, J.V. and J.A. Stanford. 1979. The ecology of regulated streams. Plenum Press, New York.

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 associated with changes in stream flows, temperature, and water quality, 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. 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 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 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 across Colorado, third to fifth order streams in CPW management codes 302, 303, 405, and 406 were randomly selected in each major river basin. Streams were vetted by area biologists to validate that they were appropriate for this study. A total of 67 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. 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, stocking 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-eight 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. Waters sampled as part of this study are shown in Figure 23.

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 S.O.P.'s (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, with OD 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 alpha 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 rivers and 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 rate, more fish and potentially more exposure to fish carrying the bacteria.

To evaluate the response variable (OD value) for normality we used the Box Cox procedure, which indicated the inverse of OD values was the appropriate transformation. 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).

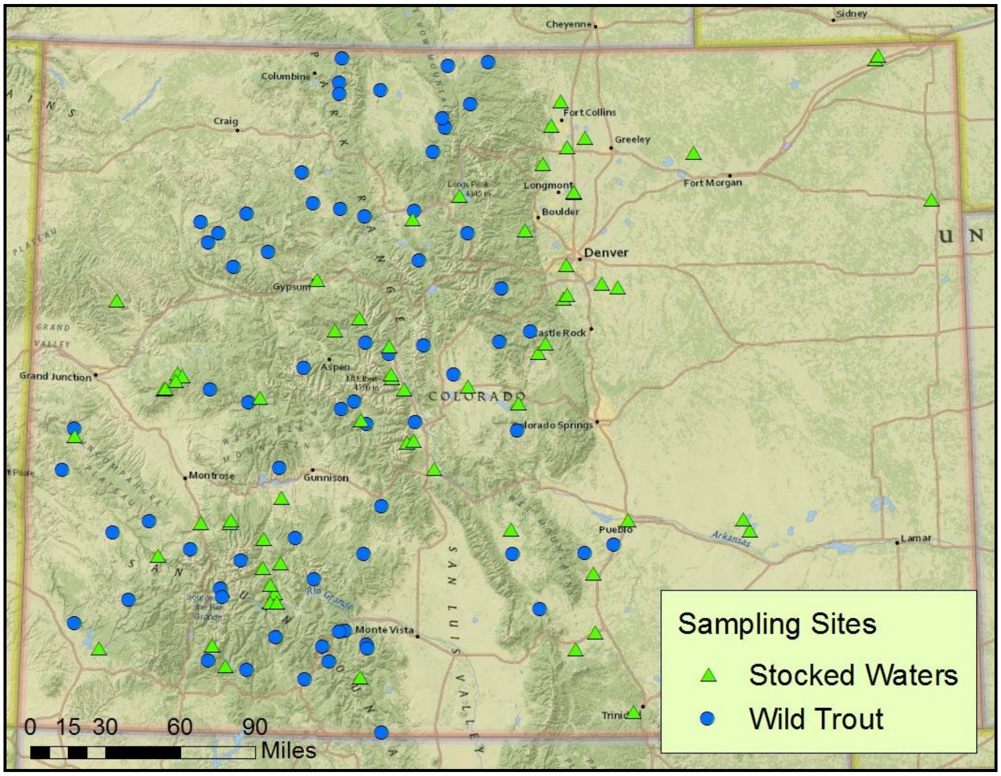

FIGURE 23. Waters sampled 2016-2017 and tested for *R. salmoninarum*.

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. Ninety-three percent of all waters tested positive by ELISA, 37% tested positive by qPCR, 12% tested positive by nPCR and 13 % tested positive by DFAT. Positive cases by all assays were found throughout Colorado in all major drainages (Figures 24 and 25). Testing results of all waters in this study are contained in Appendix A.

Stocked Sportfish Waters

Eighty-seven percent of stocked sportfish waters tested positive by ELISA, 20% tested positive by DFAT, 45% tested positive by qPCR and 12% were confirmed positive by nPCR (Figure 26). Figure 27 displays the average OD values and 95% confidence intervals of the suspect stocked and control waters while Figure 28 shows the percent positive for all assays. There was no difference at the 95% level by ELISA, PCR, or DFAT between the suspect and control waters. The modeling exercise and simple correlation analysis supported this conclusion as well. 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 30). Lake elevation was the best predictor of OD values and was the only significant correlation at the 95% level.

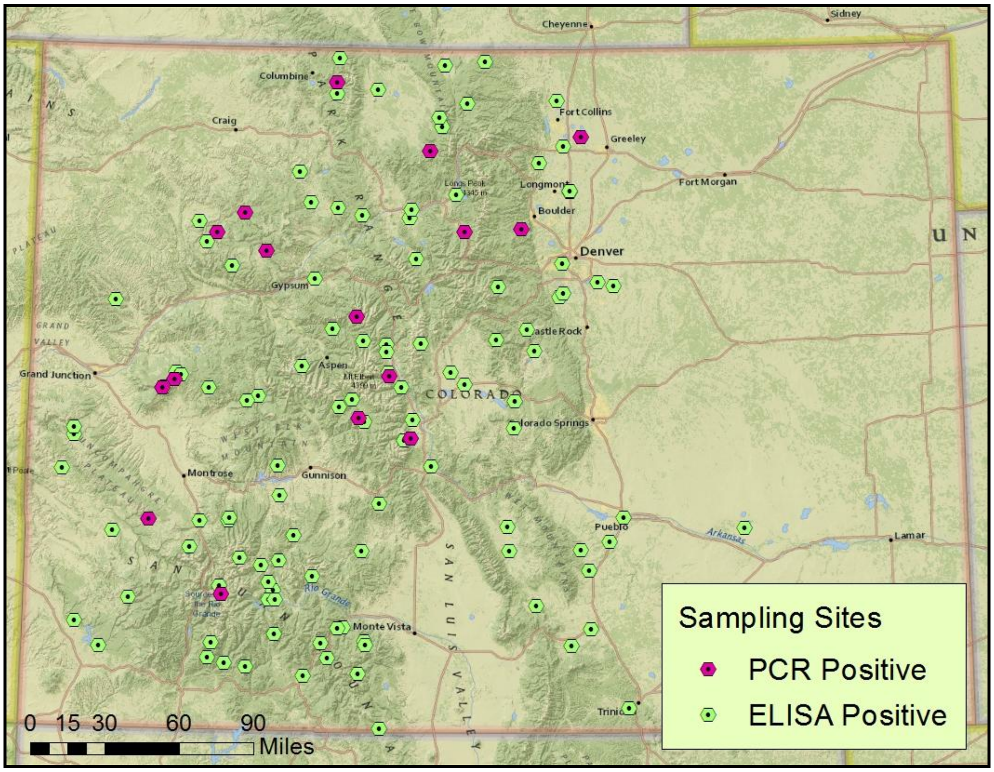

FIGURE 24. Study sites that tested positive for *R. salmoninarum* with qPCR and confirmed with nPCR.

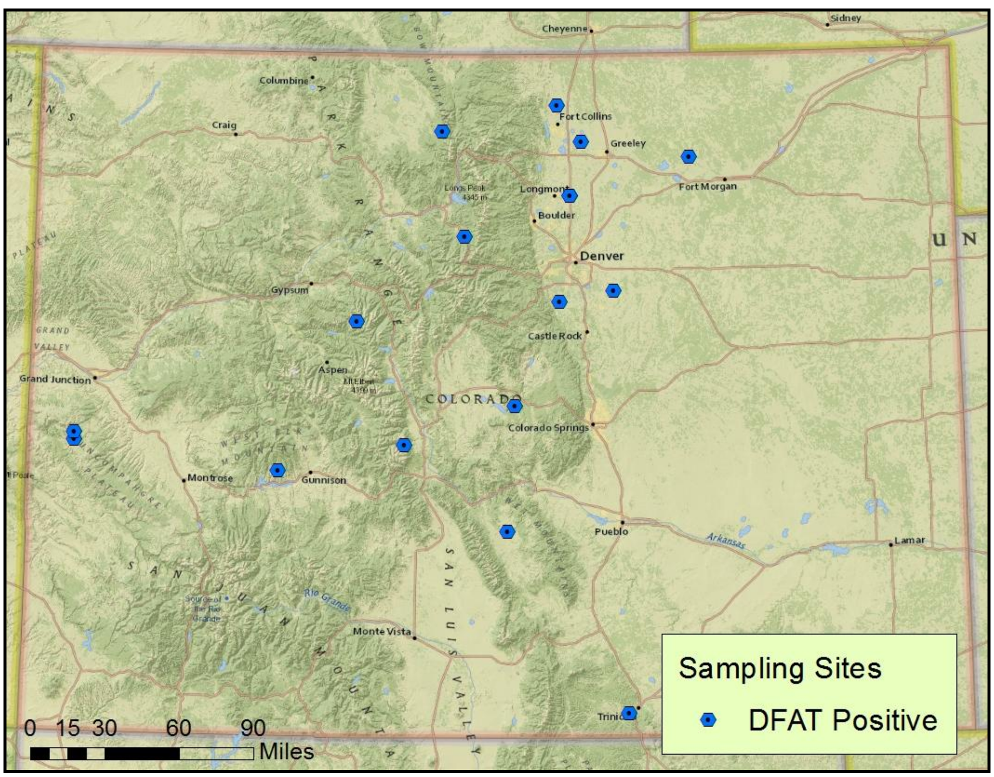

FIGURE 25. Study sites that tested positive for *R. salmoninarum* with DFAT.

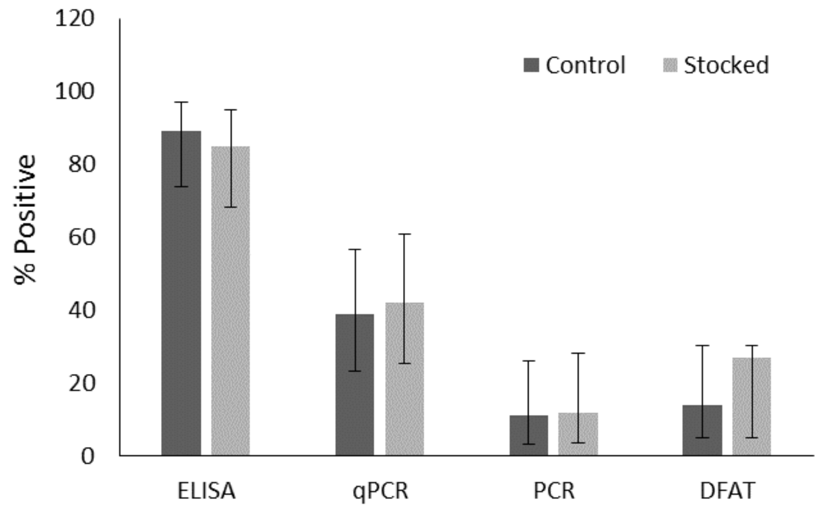

FIGURE 26. Positive test results and 95% binomial confidence intervals of waters stocked with suspect fish with nearby similar waters not stocked with fish from suspect hatcheries.

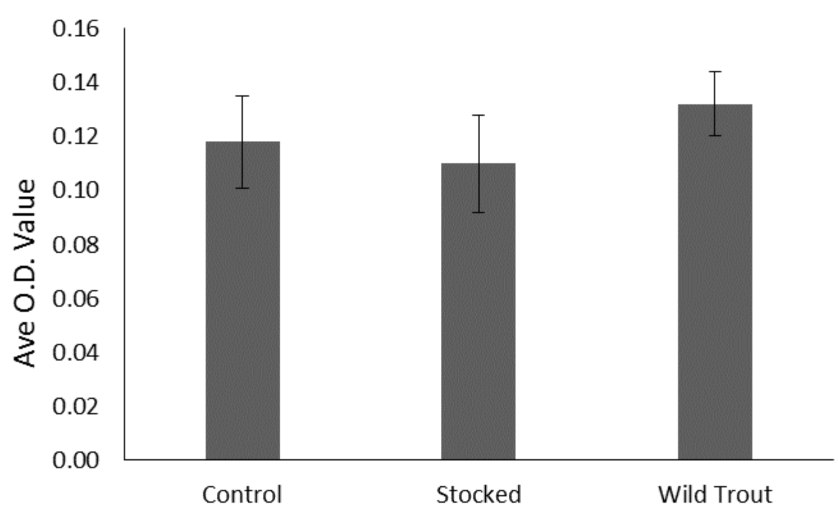

FIGURE 27. Average OD values of study waters and 95% binomial confidence intervals.

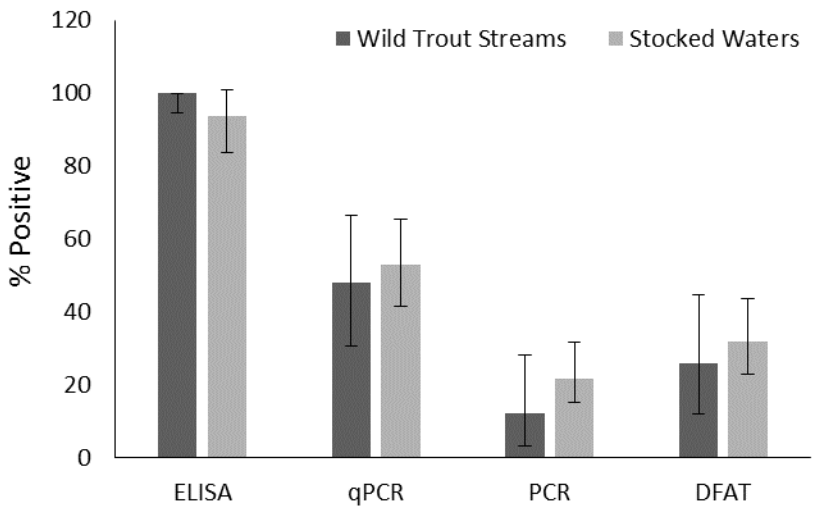

FIGURE 28. Positive test results of all waters in the stocked waters study and all wild trout waters.

Wild Trout Streams

One hundred percent of all wild trout streams tested positive by ELISA and 84% percent of individual lots of single species tested positive by ELISA. Six percent of all waters tested positive by DFAT, 24% tested positive by qPCR and 13% were confirmed positive by nPCR. Figure 27 displays the average OD values and 95% confidence intervals for wild trout waters while Figure 28 compares the percent positive of wild trout and all stocked waters for all assays. Wild trout waters had significantly higher (at the 95% level) 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.

While prevalence of *R. salmoninarum* was high (100%) among wild trout waters, most of the samples had relatively low antigen levels. 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).

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 3). None of the differences between the stocked and unstocked waters were significant at the 95% level.

TABLE 3. 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

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). Table 6 contains a list of all waters that tested positive by both an antigen (DFAT, ELISA) and molecular test (qPCR, nPCR).

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 29). 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.

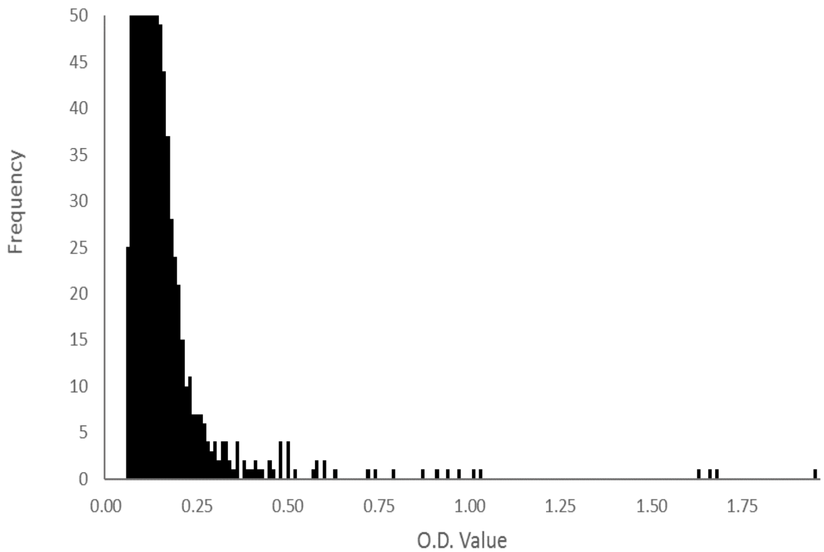

FIGURE 29. Distribution of OD values for all samples tested. Samples with OD values greater than 0.100 were considered positive. DFAT reportedly does not detect *R. salmoninarum* in positive fish samples with OD values less than 0.173 and inconsistentely detected the bacteria at OD values less than 0.978 (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.

TABLE 4**.** 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**	**AIC_c**	**ΔAIC_c**	**w_i**	**R^2**
Order x Stocked 1998-2008	5	401.73	0	0.48	0.18
Stocked 1987-1997	3	403.31	1.59	0.22	0.12
Stocked 1998-2008	3	403.78	2.06	0.17	0.11
Order x Stocked 1987-1997	5	405.22	3.50	0.08	0.14
Order	3	406.01	4.28	0.06	0.09

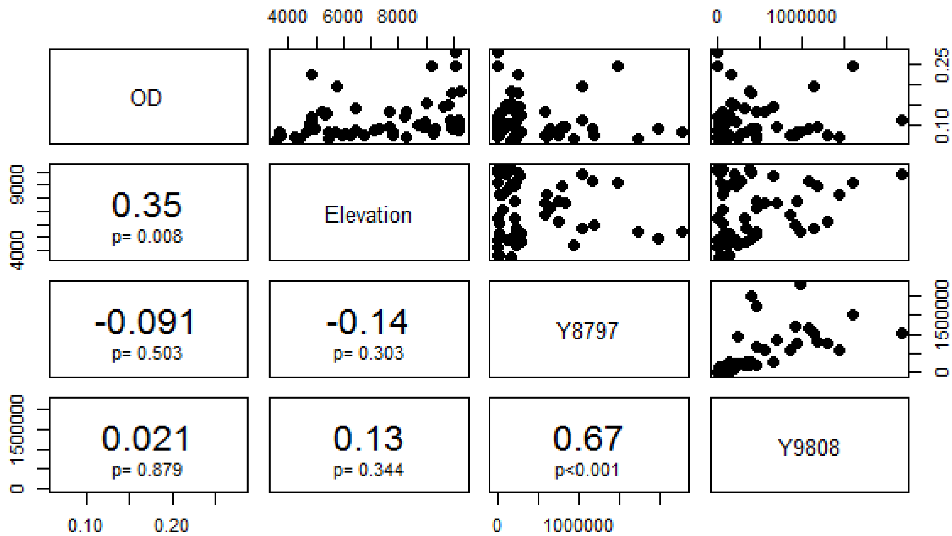

FIGURE 30. Pearson correlation coefficent table for lakes with un-transformed response variables.

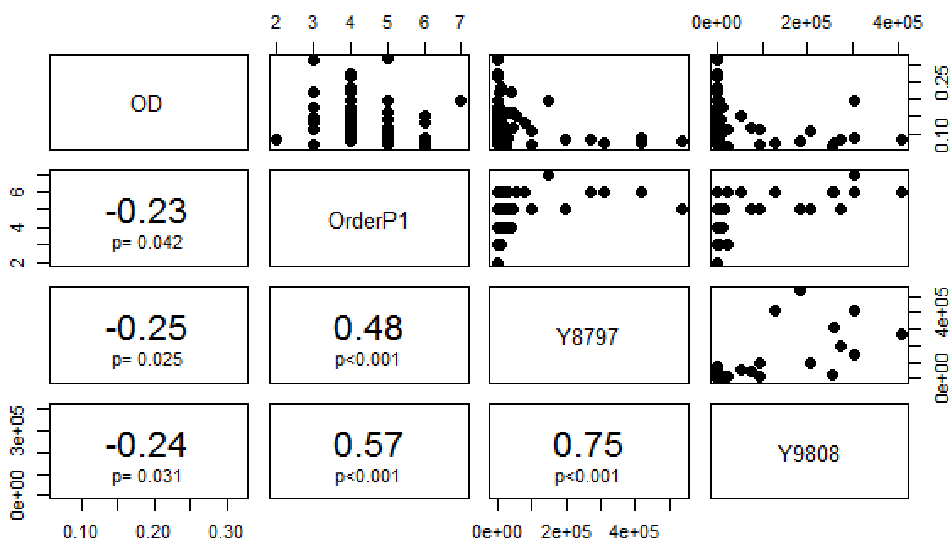

FIGURE 31. Pearson correlation coefficent table for streams with un-transformed response variable.

TABLE 5**.** 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**	**AIC_c**	**ΔAIC_c**	**w_i**	**R^2**
Elevation	3	277.66	0	0.76	0.18
Elevation x Stocked 1987-1997	5	281.07	3.41	0.14	0.20
Elevation x Stocked 1998-2008	5	281.95	4.30	0.09	0.18
Stocked 1987-1997	3	286.91	9.26	0.01	0.03
Stocked 1998-2008	3	288.50	10.84	0	0

CONCLUSIONS AND RECCOMENDATIONS

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. 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 high elevation brook trout streams.

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.

TABLE 6. 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

REFERENCES

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag, New York.

Elliott, D.G. 2016. Bacterial Kidney Disease. *In* AFS–FHS (American Fisheries Society–Fish Health Section). FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition. AFS–FHS, Bethesda, Maryland.

Elliott, D.G., L.J. Applegate, A.L. Murray, M.K. Purcell, and C.L. McKibben. 2013. Benchtop validation testing of selected immunological and molecular *Renibacterium salmoninarum* diagnostic assays by comparison with quantitative bacteriological culture. Journal of Fish Diseases 36:779-809.

Elliott, D.G., D.M. Chase and M.K. Purcell. 2016a. Bacterial Kidney Disease Appendix 1 ELISA for Detection of *Renibacterium salmoninarum*. *In* AFS–FHS (American Fisheries Society–Fish Health Section). FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition. AFS–FHS, Bethesda, Maryland.

Elliott, D.G., D.M. Chase and M.K. Purcell. 2016b. Bacterial Kidney Disease Appendix 2 Quantitative PCR for Detection of *Renibacterium salmoninarum*. *In* AFS–FHS (American Fisheries Society–Fish Health Section). FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition. AFS–FHS, Bethesda, Maryland.

Kingswood, R.W. 1996. The range and effect of *Renibacterium salmoninarum* on trout in Colorado. Master's thesis. Colorado State University, Fort Collins, Colorado.

Meyers, T.R., S. Short, C. Farrington, K. Lipson, H.J. Geiger, and R. Gates. 1993. Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of *Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, USA. Diseases of Aquatic Organisms 16:181-189.

Munson, D.A., D.G. Elliot, and K. Johnson. 2010. Management of bacterial kidney disease in Chinook Salmon hatcheries based on broodstock testing by enzyme-linked immunosorbent assay: a multiyear study. North American Journal of Fisheries Management 30:940–955.

Nance, S.L, M. Riederer, T. Zubkowski, M. Trudel, and L.D. Rhodes. 2010. Interpreting dual ELISA and qPCR data for bacterial kidney disease of salmonids. Diseases of Aquatic Organisms 91:113-119.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

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

Collaboration with federal and university researchers resulted in one peer reviewed publication in the journal Ecology;

Walters, D.M., J.S. Wesner, R.E. Zuellig, D.A. Kowalski, M.C. Kondratieff. 2018. Holy flux: spatial and temporal variation in massive pulses of emerging insect biomass from wester U.S. rivers. Ecology 99(1): 238-240.

Several fact sheets and special reports were produced to summarize and disseminate information from the coldwater stream ecology research projects;

Kowalski, D.A. 2017a. Electric fish barrier research. Colorado Parks and Wildlife Fact Sheet. Denver, Colorado.

Kowalski, D.A. 2017b. Evaluation of an electric fish barrier on the south canal, an irrigation ditch on the lower Gunnison River, Colorado. Final Report, Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado

Kowalski, D.A. 2017c. Bacterial kidney disease research. Colorado Parks and Wildlife Fact Sheet. Denver, Colorado.

One external presentation was given at the American Fisheries Society Western Fish Disease Workshop;

Kowalski, D.A. 2018. Prevalence and distribution of *R. salmoninarum* in Colorado's Wild Trout and Stocked Sport Fisheries. Western Fish Disease Workshop, Bozeman, Montana. June 21, 2018.

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

Kowalski, D.A. 2018. Surveying Colorado's sport fisheries for *R. salmoninarum*, the causative agent of bacterial kidney disease. Colorado Parks and Wildlife Aquatic Biologist Meeting, Gunnison, Colorado. January 17, 2018.

Kowalski, D.A. 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.

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