

Colorado Coldwater Stream Ecology Investigations
Project Summary

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2021 Progress Report

Colorado Parks and Wildlife

Aquatic Research Section

Fort Collins, Colorado

August 2021

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

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COLORADO COLDWATER STREAM ECOLOGY INVESTIGATIONS

PROJECT SUMMARY

Period Covered: July 1, 2020 to June 30, 2021

PROJECT OBJECTIVE

Improve aquatic habitat conditions and angling recreation in Colorado by investigating biological and ecological factors affecting sport fish populations in coldwater streams and rivers in Colorado.

RESEARCH PRIORITY

Bacterial Kidney Disease and *Renibacterium salmoninarum* in Colorado Trout Fisheries

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OBJECTIVE

Document the distribution and prevalence of *R. salmoninarum* in Colorado's wild trout fisheries, investigate the influence of environmental variables and historic fish stocking practices on that distribution, and evaluate common testing methods under conditions found in wild trout populations

INTRODUCTION

Native and sport fish populations across Colorado are impacted by many factors including habitat alterations, depleted stream flows, changes in temperature regime, water quality impacts, and a host of less obvious biological threats from diseases and parasites. While the prevalence of many fish diseases has declined in Colorado in recent years, cases of bacterial kidney disease (BKD) seem to be increasing in fish hatcheries. Bacterial kidney disease is caused by *Renibacterium salmoninarum*, a gram-positive intracellular bacterium. The disease is characterized by the presence of gray-white, necrotic abscesses in the kidney and causes mortality in both wild and cultured salmonids. The bacterium can be transmitted horizontally among fish and vertically from adult to egg due to its intracellular nature (Austin and Austin 2016).

Overt bacterial kidney disease is most frequently observed in cultured salmonid fishes where it continues to be a serious concern worldwide in hatchery and aquaculture facilities (Fryer and Lannan 1993). The disease is less common in wild fish populations but has been reported (Mitchum et al. 1979, Fryer and Lannan 1993, Austin and Austin 2016). Most of the documented disease outbreaks in wild fish are in anadromous Pacific salmonids. Resident trout are common carriers of the bacterium but are more resistant to disease than anadromous salmonids (Meyers et al. 1993b, Suzuki and Sakai 2007, Guðmundsdóttir et al. 2017). Wild trout commonly carry *R. salmoninarum*

without showing any clinical signs of disease and without testing positive for the bacteria with methods like fluorescent antibody tests frequently used in Pacific salmonids (Meyers et al. 1993b).

Several different assays are commonly used to test for *R. salmoninarum* and using multiple tests is necessary to properly screen for the bacteria (Elliott et al. 2013, Austin and Austin 2016). Molecular, serology, and culture methods have all been developed and evaluated but there is no consensus on a “gold standard” method (Bruno et al. 2007, Elliott et al. 2013, Austin and Austin 2016). For detecting subclinical infections, the American Fisheries Society Fish Health Section Blue Book recommends using an enzyme-linked immunosorbent assay (ELISA) or a direct fluorescent antibody test (DFAT) for screening and a PCR method for confirmation of *R. salmoninarum* in kidney tissues (Elliott 2012, AFS-FHS 2014). The Blue Book recommendation for fish health inspections is to use DFAT for screening and culture or PCR for confirmation (Elliott 2012, AFS-FHS 2014). Much of the evaluation of various testing procedures has been conducted in Pacific salmonid hatcheries where bacteria levels are generally higher and disease is more common. Some standard methods may not be ideal for resident trout populations that commonly have subclinical infections with low levels of bacterial DNA or antigen (Meyers et al. 1993b, Guðmundsdóttir et al. 2017). Results of DFAT in particular have been found to have low agreement with other assays in resident trout, while agreement is better at the higher bacterial loads in anadromous salmonids (Meyers et al. 1993b). Different assays may be better suited for certain objectives like screening fish of unknown status or investigating disease in symptomatic fish (Elliott 2012, Elliott et al. 2013). Different testing assays are also used to indicate various stages of infection; using a molecular test with an antigen test can reveal if infections are recent, ongoing, or resolving (Faisal and Eissa 2009, Nance et al. 2010, Elliott et al. 2013).

In Colorado, *R. salmoninarum* is a regulated pathogen and hatcheries that test positive are restricted from stocking fish into most state waters that are quality wild trout habitat. The bacterium and associated disease outbreaks were a large problem in Colorado’s hatchery system in the 1950s and 1960s and it was detected at least 16 times from 1970 to 1997 at state or federal fish hatcheries (Kingswood 1996, Fetherman et al. 2020). Annual fish health inspections did not detect the bacterium in Colorado hatcheries between 1997 and 2015, but since 2016, six hatcheries and a wild broodstock lake have tested positive for *R. salmoninarum* (Fetherman et al. 2020). Clinical bacterial kidney disease outbreaks have been documented at least twice in Colorado hatcheries since 2016. One outbreak cost over \$2.1 million in depopulation and disinfection efforts, which affected fish management statewide with the loss of over 675,000 sport fish. That outbreak may have originated from bacteria in fertilized eggs brought into a hatchery from a wild spawn take. In addition, a BKD epizootic in Wyoming was spread from hatchery fish to wild fish (Mitchum et al. 1979). Ongoing concern about the transfer of bacteria between hatcheries and wild fish emphasized the need for more information on the prevalence of the bacteria in wild trout populations.

The objectives of this study were to document the distribution and prevalence of *R. salmoninarum* in Colorado’s wild trout fisheries, investigate the influence of environmental variables and historic fish stocking practices on that distribution, and to evaluate common testing methods under conditions found in wild trout populations. We hypothesized that the bacterium would be detected frequently in wild trout fisheries and that bacteria levels would be higher in large, lower elevation rivers, where more fish from potentially positive hatcheries had been stocked historically.

METHODS

To investigate the prevalence of *R. salmoninarum* in wild trout streams, we used the Colorado Parks and Wildlife aquatic data management system to randomly select third to fifth order streams in management categories 302 (wild salmonid recreation streams) and 303 (wild salmonid recreation special regulations streams) from each major river basin. Colorado Parks and Wildlife fish biologists vetted the list of selected streams. Waters were removed for reasons such as intermittent stream flow and replaced by the next randomly selected water. Sixty-eight streams were sampled in all major river basins in Colorado from an elevation of 1,393 m to 3,078 m. A total 3,809 individual fish were sampled from June to October in 2016 and 2017.

Fish were examined for signs of clinical disease and tissue was collected for PCR and DFAT from the anterior, middle, and posterior regions of the kidney. Samples were stored in centrifuge tubes containing 70% EtOH. The remaining kidney tissue was placed in a Whirl-Pak-Bag for ELISA testing, stored on ice, and frozen as soon as practical. Individual fish tissue samples were combined into composite samples of five fish of single species. We summarized data by three metrics: samples, lots, and waters. A sample was a single five-fish composite group, a lot was all the samples from one species from one water, and waters were all the lots from a single stream. Because the focus of this study was screening wild trout populations in the context of fish health inspections, we focused on comparing testing results among waters and lots rather than individual fish samples.

Diagnostic Assays

Samples were tested with ELISA at the Colorado Parks and Wildlife Aquatic Animal Health Laboratory. Quantitative polymerase chain reaction (qPCR), nested polymerase chain reaction (nPCR), and DFAT screening occurred at the U.S. Fish and Wildlife Service Bozeman Fish Health Center. All assays followed standard operating procedures of American Fisheries Society Fish Health Section Blue Book (Elliott 2012, Elliott et al. 2014). All samples were screened with ELISA, DFAT, and qPCR but only positive results from qPCR tests were tested with nPCR. We compared fish lots (single species from a single water) to evaluate the various assays and considered an individual water “positive” by a specific assay if any lots from that water were positive.

Enzyme-Linked Immunosorbent Assay

Because of the unknown status of waters in this study for *R. salmoninarum*, we used a liberal testing threshold to reduce the chance of false-positive results. We adopted a negative-positive threshold for optical density values (OD) of 0.100 following Munson et al. (2010) and the considerations outlined in Elliott et al. (2013) and Meyers et al. (1993a). We used a tiered classification system to characterize antigen levels (Elliott et al. 2013). Optical density values between the negative-positive threshold (0.100) and 0.199 were 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.

A double-sandwich ELISA was used to detect soluble antigens of *R. salmoninarum* (Jansson et al. 1996, Elliott et al. 2014). Kidney tissues were prepared to a 1:4 (w/v) dilution in PBS-T20 (Phosphate-buffered saline pH 7.4, 0.05% (v/v) Tween-20, and 0.01% (w/v) Thimerosal), homogenized, heated at 100 degrees Celsius for 15 minutes, then stored at -20°C until we screened the sample. Affinity purified *R. salmoninarum*-goat antibodies (KPL) were used as the coating

antibody and horseradish-peroxidase labeled *R. salmoninarum*-goat antibodies were used as the antibody conjugate as previously described (Pascho et al. 1991). Colorimetric development in each sample was measured at 450 nm and positive samples were determined by dilutions of positive controls compared to negative controls for each set of samples.

Direct Fluorescent Antibody Test

Kidney tissues samples were homogenized, imprinted onto a 12-well Shandon glass slide, and fixed with methanol to reduce inhibitory lipid concentrations. Fluorescein isothiocyanate-conjugated (FITC) *R. salmoninarum*-goat antibody (KPL) was prepared at a working dilution of 1:40 (v/v) and clarified with a 0.45 µm filter. One drop of the FITC-antibody stain was added to each prepared well containing tissue and to a positive control slide. After staining, slides were immersed in 1X PBS pH 7.1 + Thimerosal and air-dried before microscopic examination. Stained slides were read with a Zeiss Axio Lab.A1 LED Epi-Fluorescence microscope at 100X and 50 fields of vision examined for the presence of *R. salmoninarum*.

Quantitative PCR

Extractions of *R. salmoninarum* DNA from fish kidney tissues were performed with the DNeasy Blood and Tissue Kit (Qiagen) following protocols from American Fisheries Society-Fish Health Section Blue Book with the addition of a 4X lysozyme lysis buffer to enhance break-down of the gram-positive cell wall (Elliott 2012, AFS-FHS 2014). Final DNA concentration and purity was determined with a NanoDrop spectrophotometer (ThermoFisher). When necessary, DNA concentration was diluted with Buffer AE for a final range of 100–300 ng/µl. All DNA was stored at -20°C until use.

Extracted DNA from each sample was used for the qPCR assay as described in Chase et al. (2006) with primers (RS 1238 F and RS 1307 R) and the probe (RS 1262 MGB) to target a 69 bp region of the *msa* gene. The master mix was modified from TaqMan Universal Master Mix for the substitution of PerfeCTa Multiplex 5X qPCR ToughMix with ROX. Each PCR mixture was then optimized to contain 5 µL of 1X PerfeCTa Multiplex qPCR ToughMix, 500 nM each *R. salmoninarum*-specific primer, 250 nM *R. salmoninarum*-specific probe, 2.5 µL of 1X TaqMan Exogenous internal positive control (IPC), and 0.5 µL of 1X TaqMan Exogenous IPC DNA, and 5 µL DNA. Negative template controls (PCR grade water) were added with each experiment. Standard amplification incubations were carried through each set of samples as follows: 95°C at 15 minutes for denaturing and 60°C at 60 seconds for anneal/extending. Cycle threshold values (Ct) values greater than 35 considered positive by qPCR and only positive samples were tested by nPCR to confirm the presence of *R. salmoninarum* DNA.

Nested PCR

For confirmation, samples positive by qPCR were analyzed for the presence of *R. salmoninarum* DNA by nPCR as described in Chase and Pascho (1998). Primers for p57 gene of *R. salmoninarum* used were Forward P3 (5' -AGCTTCGCAAGGTGAAGGG-3') and M21 (5' -GCAACAGGTTTATTTGCCGGG-3') for the first round and Forward P4 (5' -ATTCTTCCAATTCAACAGTACAAGG -3') and Reverse M38 (5' -CATTATCGTTACACCCGAAACC -3') for the second round (Pascho et al. 1998). A no-template

negative extraction control and a positive amplification control was included in each set of samples. Amplification rounds were slightly modified with a BioRad thermal cycler optimized at 30 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min for the first round. Thermo cycler programming was used for second round amplification except for only 20 cycles. PCR products were analyzed on 2% (w/v) agarose gel in an electrophoresis apparatus and was considered positive when 320-bp region bands were present.

Statistical Analysis

Fish lots (all samples from single species from a single water) were compared across waters and a lot or water was considered “positive” if the criteria for any individual samples were deemed positive by any of the assays. To report difference in ELISA OD values among species and between stocked and unstocked waters, we followed the recommendations of Burnham and Anderson (2002) and Wasserstein et al. (2019) and reported effect sizes (difference between means), standardized effect sizes (difference between means divided by the standard deviation), and 95% confidence intervals on the differences between means. We used multiple linear regression and AIC model selection to investigate how environmental variables and past stocking practices affect bacteria levels (Burnham and Anderson 2002). We identified five primary variables through literature review and professional judgment about factors that may influence bacteria levels in inland trout: elevation, stream order, drainage area, stream temperature, and the number of hatchery fish historically stocked in each stream. The variables represented specific hypotheses about how bacteria levels in fish may be influenced by geomorphic, environmental, and anthropomorphic characteristics of a stream.

Elevation, stream order, and drainage area for each sampling location was obtained from the U.S. Geologic Survey NHDPlus hydrologic dataset (U.S. Geological Survey 2017). We estimated stream temperatures at our site with modelled temperatures from NorWeST, a western United States stream temperature model (Isaak et al. 2017). This model uses extensive thermograph data (>220,000,000 temperature recordings from >22,700 sites) to create a spatial statistical stream network model. It has a 1 km resolution and has been shown to give accurate and unbiased stream temperature predictions ($R^2 \sim 0.90$, $RMSE < 1.0$ °C). From the NorWeST model we used the output variable S1 (Mean August Stream Temperature 1993-2011) as an index of relative summer stream temperature for all the sites. Historical stocking records were obtained from Colorado Parks and Wildlife databases and the total number of fish stocked into each water from 1987-2008 was compiled for each water. We used records from this period because most waters are not currently stocked and have been managed solely for wild trout since the early 1990’s or earlier.

The five variables were evaluated with Pearson’s product moment correlation coefficient (R) and then analyzed with multiple linear regression and the information-theoretic approach to identify the best predictive models and most influential explanatory variables (Burnham and Anderson 2002). Linear regression modelling was performed with the `lm` function in R, version 3.5.2 (R Foundation for Statistical Consulting, Vienna, Austria). Model assumptions of homogeneity of variance and normality were evaluated by examining residuals of the global model (additive combination of all individual variables). The response variable, average OD values from ELISA assay, was transformed with the Box Cox procedure due to patterns observed in the residuals (Box and Cox 1964). The lambda value had a 95% C.I. that included -1 so an inverse transformation was used on the response variable. Ten linear regression models were built with additive combinations of

uncorrelated variables and interaction models were tested when they made biological sense. Model selection was completed with the small sample size version of Akaike's information criterion (AIC_c) following Burnham and Anderson (2002). The lm function in R and the statistical packages MASS and AICcmodav were used for data analysis, model fitting, and model selection (R Core Team 2018).

RESULTS

All waters (100%) had some fish that tested positive for *R. salmoninarum* by ELISA and 48.7% of all individual samples were positive with the 0.100 negative-positive threshold. Almost six percent (5.9%) of all waters had tissue samples test positive by DFAT, 23.5% tested positive by qPCR and 11.8% were confirmed positive by nPCR (Figure 1).

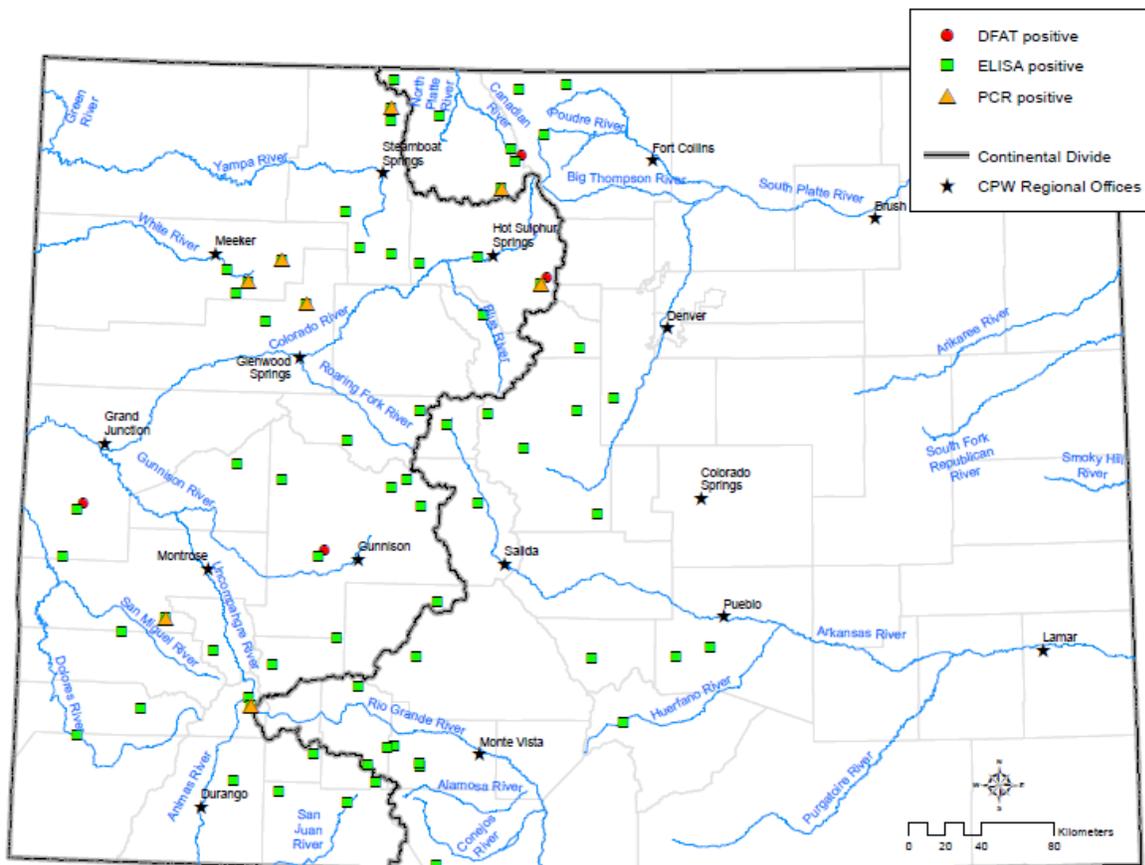


Figure 1. Wild trout sampling sites in Colorado 2016-2017 that tested positive for *R. salmoninarum* with DFAT, ELISA, and qPCR confirmed with nPCR.

ELISA Results

While prevalence of *R. salmoninarum* was high among wild trout waters, most of the samples had relatively low antigen levels. Of the 103 lots tested, 12.6% were negative, 48.5% had low antigen levels (OD < 0.199), 30.1% had moderate antigen levels (OD 0.200-0.999), and 8.7% had high antigen levels (OD > 1.000). Within a single water, prevalence was moderate. Less than half of samples from each water (48.7%) had OD values greater than 0.100. The negative-positive threshold of 0.100 in the ELISA assay accomplished the objective of being liberal and reducing the risk of false-positive results. Forty-two ELISA runs were completed to test all of the samples. If the negative-positive threshold was computed as twice the standard deviation of the negative controls of each run (the more conservative method), the threshold would have averaged 0.072 (SD = 0.011).

DFAT Results

Only five lots of fish from four different waters tested positive by DFAT, which is the screening assay generally used by Colorado Parks and Wildlife. The waters were all high elevation Brook Trout populations, one of which had sympatric Rainbow Trout test positive as well. One of the four lots, Brook Trout in the Fraser River, tested positive by qPCR and was confirmed by nPCR. Concordance between DFAT and ELISA lots was low (15.5%) with a negative-positive threshold of 0.100. However, using the tiered classification system of Elliott et al. (2013) produced good agreement (88.3%) in prevalence in lots by DFAT and ELISA High category (OD values > 1.000). While the agreement was good between DFAT and ELISA High category due to all of the negative lots, DFAT did a poor job of identifying “severe” cases with high or DNA antigen levels by other assays. Of the five lots that tested positive by DFAT, all of them were in the ELISA Low category (average ELISA OD value was 0.120, range 0.074-0.186) and four of the five lots positive by DFAT were negative by qPCR.

PCR Results

Sixteen waters (23.5%) tested positive by qPCR and eight of those waters were confirmed by nPCR. Of the 103 lots (single species, single water) tested, 15.5% were positive by qPCR with a Ct threshold of 35. Concordance between qPCR and ELISA results among lots was low (28.2%) with the negative-positive threshold of 0.100, but there was good agreement (82.5%) in prevalence in lots by qPCR and ELISA High category (OD values > 1.000). Likewise, concordance between nPCR and ELISA was low (20.4%), but there was good agreement (89.3%) in the prevalence of lots by nPCR and ELISA High category. There was an 81.6% agreement in prevalence of positive lots between qPCR and DFAT and an 89.3% agreement between nPCR and DFAT.

Historical Fish Stocking

Thirty-seven of the wild trout waters (54.4%) were stocked at some point historically, but the prevalence and average OD values for those waters were similar to wild trout waters with no stocking records. The difference between average ELISA OD values of historically stocked waters (0.134) and unstocked waters (0.130) was only 0.004 (95% C.I. -0.029–0.021), $p = 0.743$. The correlation between fish stocking and inverse transformed OD values was relatively weak ($R = 0.27$) and stocking was negatively correlated with OD values: antigen levels were higher in waters with lower levels of fish stocking.

Correlation, Linear Regression Modeling, and Model Selection

The correlation analysis indicated that the variables explained the most variability in transformed OD values were Drainage Area ($R = 0.35$, $p = 0.004$), Total Trout Stocked ($R = 0.27$, $p = 0.027$), and Stream Order ($R = 0.26$, $p = 0.030$) (Figure 2). August stream temperature had low correlation with transformed ELISA OD levels ($R = 0.078$, $p = 0.526$). All explanatory variables were negatively correlated with untransformed ELISA OD values and, contrary to our hypotheses, average OD values decreased with total trout stocked. In Colorado, the antigen of the *R. salmoninarum* is highest in trout from smaller (low order) streams with a small drainage area that were less likely to be historically stocked. Average OD values decreased with increasing August temperature, further suggesting that the bacterium is a cold-water pathogen (Delghandi et al. 2020). Of the explanatory variables, Total Stocked Trout and Drainage Area were highly correlated ($R = 0.79$) as well as August Stream Temperature and Elevation ($R = -0.82$). The collinear pairs of variables were not included in the same model to avoid problems with parameter estimation due to multicollinearity (Dormann et al. 2013).

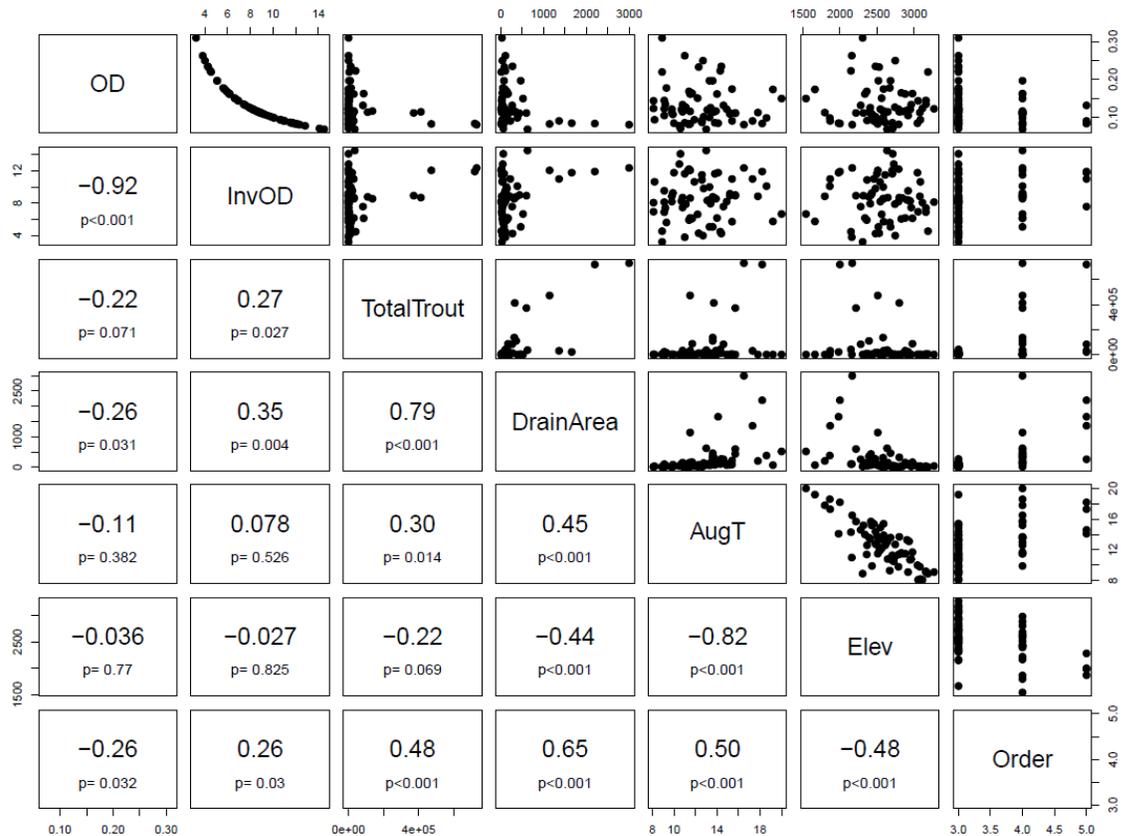


Figure 2. Pearson correlation matrix of explanatory variables and untransformed (OD) or inverse transformed ELISA optical density values (InvOD) from wild trout populations in Colorado 2016-2017.

AICc model selection results indicate that the single variable model with Drainage Area was the top

model with a model weight of 0.45 (Table 1). An additive combination of Drainage Area and August temperature was 1.65 AIC_c units behind the top model and explained 12% of the variation in OD values. Overall, the stocking and environmental variables that we explored in this study explained relatively little variation in OD values (0-13%) so more work is needed to investigate factors that are related to *R. salmoninarum* antigen levels in trout in Colorado.

Table 1. Model selection results of linear regression models of environmental and fish stocking variables. Included are the number of model parameters (K), Akaike’s information criterion corrected for small sample size (AIC_c), the difference in AIC_c values (Δ AIC_c), AIC_c model weight (w_i), and multiple R².

Model	K	AIC _c	Δ AIC _c	w_i	R ²
Drainage Area	3	321.79	0	0.45	0.12
Drainage Area + August Temp	4	323.44	1.65	0.20	0.13
Drainage Area + August Temp + Order	5	325.28	3.50	0.08	0.13
Drainage Area * August Temp	5	325.35	3.56	0.08	0.13
Total Stocked	3	325.40	3.61	0.07	0.07
Order	3	325.60	3.81	0.07	0.07
Total Stocked + August Temp + Order	5	327.81	6.02	0.02	0.10
Total Stocked * Order	5	328.24	6.45	0.02	0.10
August Temp	3	330.06	8.27	0.01	0.01
Elevation	3	330.42	8.64	0.01	0.00

Species Trends

We encountered (in decreasing frequency): Brown Trout *Salmo trutta*, Brook Trout *Salvelinus fontinalis*, Rainbow Trout *Oncorhynchus mykiss*, Mountain Whitefish *Prosopium williamsoni*, and Cutthroat trout *Oncorhynchus clarkii*. Brook Trout occupied the smallest, highest elevation streams, with the coldest mean August temperature (Table 2). Streams that Brook Trout were the dominant species also had the lowest historical stocking rates. Brook Trout lots had the highest average ELISA OD values followed by Brown Trout and Rainbow Trout (Figure 3). Brook Trout lots had the highest average ELISA OD values followed by Brown Trout and Rainbow Trout (Fig. 3). Brook Trout had average ELISA OD values 0.032 (0.4 Std. Dev.) higher than Rainbow Trout (95% C.I. 0.000–0.065), $p = 0.051$. Brook Trout had average ELISA OD values 0.016 (0.2 Std. Dev.) higher than Brown Trout (-0.019–0.051), $p = 0.354$, and Brown Trout had average ELISA OD values 0.016 (0.3 Std. Dev.) higher than Rainbow Trout (-0.003–0.036), $p = 0.101$. Not enough waters contained Cutthroat Trout and Mountain Whitefish to calculate confidence intervals or compare statistically to lots of other species. Brook trout also had the highest prevalence by qPCR: 26.5% of Brook Trout lots were positive, 12.0% of Rainbow Trout lots, 9.5% of Brown Trout lots, and no lots of Mountain Whitefish or Cutthroat Trout.

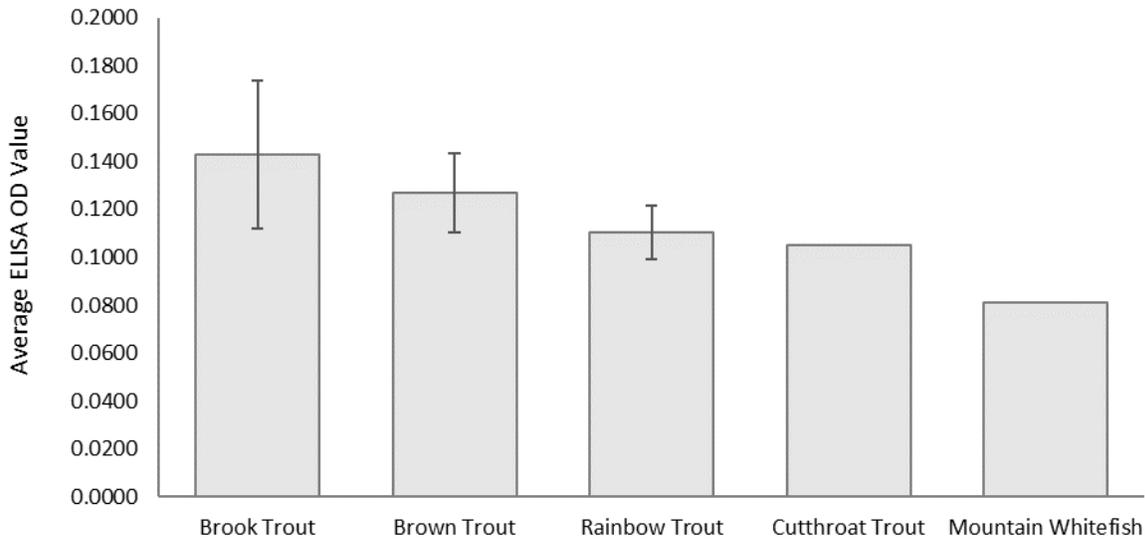


Figure 3. Average ELISA OD values and 95% confidence intervals of fish lots sampled from wild trout populations in Colorado 2016–2017.

Table 2. Summary of environmental and stocking variables of waters sampled in Colorado 2016–2017 by the dominant species present at each site.

Dominant Species	Elevation (m)	Drainage Area (km ²)	Mean August Temp (C)	Stream Order	Total Fish Stocked 1987-2008
Brook Trout	2,758	60	11.2	3.1	4,130
Brown Trout	2,509	384	13.3	3.6	103,850
Rainbow Trout	2,236	299	15.3	3.4	5,149
Mountain Whitefish	1,980	1,656	14.1	5.0	20,266

DISCUSSION

The causative agent of bacterial kidney disease, *R. salmoninarum*, is widespread in Colorado’s wild trout fisheries. While common, bacteria levels are generally low, and clinical disease is rare. After sampling 3,809 individual fish from 68 waters from all major river basins (all of which showed evidence of the presence of soluble antigen of the bacteria), no cases of clinical BKD were observed. A larger surveillance effort of statewide waters (not randomly selected or restricted to wild trout streams) came to the same conclusion; that clinical BKD was rare in feral trout populations in Colorado (Kowalski 2019). In that effort, over 12,800 individual fish from 194 statewide waters were sampled and only two clinical cases of BKD were observed, both in Brook Trout in small high altitude streams.

Our results support the hypothesis that *R. salmoninarum* is common in inland trout populations, generally occurs at low levels, and that resident trout are somewhat resistant to the bacteria and are generally refractory to clinical BKD (Meyers 1993b, Kingswood 1996). High prevalence of *R. salmoninarum* in wild, non-anadromous trout and char has been reported in Iceland (Jónsdóttir 1998,

Guðmundsdóttir et al. 2017), Alaska (Meyers et al. 1993b), Michigan (Eissa et al. 2007), and Wyoming (Mitchum et al. 1979). The bacterium was also common in wild trout in Rocky Mountain National Park in Colorado (Kingswood 1996). All of the streams sampled in the park had fish that tested positive for the antigen of *R. salmoninarum* by ELISA. Even though 82% of all individual fish from those streams tested positive by ELISA, all of the streams contained robust, self-sustaining fish populations with no clinical signs of disease (Kingswood 1996).

Environmental and fish stocking variables that we hypothesized to influence *R. salmoninarum* antigen levels in wild trout did not explain much variability (0-13%) in ELISA OD values. While the effect sizes were not large, there were influential correlations between antigen levels and stream order ($p = 0.030$), drainage area ($p = 0.004$), and historical stocking rates ($p = 0.027$). Antigen levels were highest in small streams (low stream order and small drainage area) that had low historical stocking rates. Differences in species distribution and the infection intensity of the trout species could explain these patterns. Brook Trout are more likely to occupy smaller high elevation streams in Colorado due to colder temperature preferences than Brown or Rainbow Trout (Behnke 2002, Table 2). Brook Trout had the highest average ELISA OD levels as well as the highest prevalence by DFAT and qPCR of the fish species we tested. Therefore, the trend of higher OD values in smaller streams could be an artifact of those streams being dominated by Brook Trout. Brook Trout are known to have higher prevalence of *R. salmoninarum* and have higher antigen levels than other resident trout species (Snieszko and Griffin 1955, Mitchum et al. 1979, Faisal and Eissa 2009). The smaller, Brook Trout dominated streams in our study were also less likely to be stocked, so the negative correlation between ELISA OD values and fish stocking could be an artifact of the species distribution on the landscape and unrelated to fish stocking. More work is necessary to explore species-related differences in bacterial levels as well as the environmental factors that may influence antigen and DNA levels of *R. salmoninarum* in trout fisheries in Colorado.

We expected the low sensitivity and lack of concordance between DFAT results and the results from other assays. The fluorescent antibody test is inconsistent at detecting low antigen levels and, under those conditions, DFAT results do not correlate well with other assays, especially in non-anadromous salmonids (Pascho et al. 1987, Pascho et al. 1991, Meyers et al. 1993b, Elliott et al. 2013). In one of the larger studies of *R. salmoninarum* in resident trout, the DFAT assay would not detect soluble antigen in samples with ELISA OD values less than 0.17 and inconsistently detected the antigen at OD values less than 0.98 (Meyers et al. 1993b). Eighty-seven percent of our samples had ELISA OD values less than 0.17 and 99.6% were less than 0.98. The vast majority of fish samples in our study would be unlikely to test positive by DFAT but actually have low levels *R. salmoninarum* antigen. Using DFAT as a liberal screening method to identify only severe cases does not appear to be an effective strategy; it did a poor job of discerning high antigen or DNA cases identified by ELISA or qPCR. The results of DFAT testing were somewhat stochastic in what samples tested positive in comparison to other assays. Our results, as well as previous work, suggest that DFAT is not the optimal screening assay for the *R. salmoninarum* levels commonly observed in inland wild trout (Meyers et al. 1993b).

There may not be exact agreement between ELISA and PCR because they measure different bacterial macromolecules (antigen vs. DNA). These differences should not be interpreted as conflicting results but as useful information indicating different states of infection (Nance et al.

2010). Fish with various stages of infection would be expected to be present concurrently in wild salmonid populations (Faisal and Eissa 2009, Nance et al. 2010, Elliott et al. 2013). The antigen of *R. salmoninarum* can be present in kidney tissue in fish recovering from infection and can be detected by ELISA up to three months after viable bacteria have been cleared from the fish (Pascho et al. 1997, Sami et al. 1992). Many lots and samples in our study were positive by ELISA but negative by qPCR, a pattern that reflects naturally resolving infections (Nance et al. 2010). Because *R. salmoninarum* is common in Colorado trout fisheries and resident trout are more resistant to the bacteria, it is likely that many trout in wild populations would have low level, resolving infections. Our results emphasize the importance of using both a molecular (qPCR) and immunological assay (ELISA) to reveal different stages of infection especially in non-anadromous trout (Nance et al. 2010, Elliott 2012, Elliott et al. 2013).

The strengths of using ELISA to test samples from wild trout populations were twofold: it had the ability to detect low levels of antigen present in mild or resolving infections and it was able to estimate antigen levels rather than giving binary negative/positive results. This allowed the ELISA scores to produce a continuous response variable for the linear regression exercise and was useful in differentiating lots with low levels of antigen that other assays did not detect. One limitation of our study was that we did not take advantage of the same quantitative feature of the PCR assay. With the proper methods targeting specific primers, qPCR can effectively be used to quantify the number of viable bacteria cells present (Suzuki and Sakai 2007, Nance et al. 2010, Elliott et al. 2013). A weakness of the qPCR assay is the small volume of the kidney tissue that is tested. The heterogeneous distribution of bacterial DNA in the kidney can lead to decreased analytical sensitivity and stochastic results from sampling variation with a small tissue sample (Bruno et al. 2007, Elliott et al. 2013). We think that, properly applied, qPCR is an effective assay for testing inland trout populations for *R. salmoninarum* under the conditions that are commonly encountered. More work is necessary to explore the quantitative features of the qPCR assay, apply them to testing trout in Colorado, and to pair it with a serological assay like ELISA to give a more complete picture of bacterial levels in wild trout.

Using DFAT to screen inland trout populations for *R. salmoninarum* is not recommended. Due to the lower bacteria levels of wild trout and the unreliable nature of the DFAT assay at these levels, it is an inappropriate screening assay at worst and uninformative at best. A quantitative tool that more reliably detects the DNA or antigen of *R. salmoninarum* at lower levels like qPCR or ELISA would be more useful. Using these tools to estimate bacteria levels and adopting a liberal threshold for “positive” waters such as ELISA OD values of 1.0 to 2.0 would be a logical strategy if waters must be classified for management or regulatory reasons. Managers should instead focus on using multiple assays (molecular and serological) to quantify bacterial levels and interpret results with nuance. The overly simplistic paradigm of considering populations “positive” or “negative” is not an informative way of thinking about *R. salmoninarum* in resident trout.

The bacteria *R. salmoninarum* is common and widespread throughout Colorado’s wild trout fisheries but bacterial levels (indicated by antigen and DNA) are generally low and clinical disease is uncommon. Active infections (indicated by the detection of DNA) are rare but the presence of lower levels of the bacteria’s antigen is common and widespread, supporting the paradigm that resident trout are commonly resistant carriers of the bacteria. Clinical BKD is occasionally observed in

spawning Brook Trout in small high elevation streams in Colorado, but we have not documented any population level effects. More work is necessary to investigate disease dynamics in wild trout populations. Bacterial kidney disease appears rare in wild trout populations in Colorado despite being an ongoing and potentially increasing problem in trout hatcheries.

ACKNOWLEDGEMENTS

Colorado Parks and Wildlife and the Federal Aid in Sportfish Restoration Program Grant F-237 provided funding for this work. The Colorado Parks and Wildlife Aquatic Animal Health Lab completed some of the sample collection and all of the ELISA testing. Vicki Milano was integral in project planning and completion. April Kraft, Victoria Vincent, Weston Niep, and Cody Minor were invaluable by assisting with sample collection and processing. This work was a close collaboration with U.S. Fish and Wildlife Service Bozeman Fish Health Center and the National Wild Fish Health Survey. Lacey Hopper and Molly Bensley completed all of the PCR and DFAT testing.

LITERATURE CITED

- AFS-FHS (American Fisheries Society-Fish Health Section). 2014. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2020 edition. Accessible at: <https://units.fisheries.org/fhs/fish-health-section-blue-book-2020/>
- Austin, B., and D. A. Austin. 2016. Bacterial fish pathogens: disease of farmed and wild fish. Springer, Switzerland.
- Behnke, R. 2002. Trout and salmon of North America. Simon and Schuster, New York.
- Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. J R Stat Soc, B 26:211–243.
- Bruno, D., B. Collet, A. Turnbull, R. Kilburn, A. Walker, D. Pendrey, A. McIntosh, K. Urquhart, and G. Taylor. 2007. Evaluation and development of diagnostic methods for *Renibacterium salmoninarum* causing bacterial kidney disease (BKD) in the UK. Aquaculture 269:114–22.
- Burnham, K., and D. Anderson. 2002. Model selection and multi-model inference Second Edition. Springer-Verlag, New York.
- Chase, D. M., D. G. Elliott, and R. J. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. J Vet Diagn Invest 18:375–380.
- Chase, D. M., and R. J. Pascho. 1998. Development of a nested polymerase chain reaction for amplification of a sequence of the p57 gene of *Renibacterium salmoninarum* that provides a highly sensitive method of detection of the bacterium in salmonid kidney. Dis Aquat Org 34:223–229.

- Delghandi, M. R., M. El-Matbouli, and S. Menanteau-Ledouble. 2020. *Renibacterium salmoninarum*- The causative agent of bacterial kidney disease in salmonid fish. *Pathogens* 9:845.
- Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. Marquéz, B. Gruber, B. Lafourcade, P.J. Leitão, and T. Münkemüller. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36:27–46.
- Eissa, A. E., E. E. Elsayed, and M. Faisal. 2007. Prevalence and shedding of *Renibacterium salmoninarum* in Brook trout (*Salvelinus fontinalis*) in Michigan. *Nature and Science* 5:8–17.
- Elliott, D. G. 2012. 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, 2020 edition. Accessible at: <https://units.fisheries.org/fhs/fish-health-section-blue-book-2020/>
- 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. *J Fish Dis* 36: 779–809.
- Elliott, D. G., D. M. Chase, and M.K. Purcell. 2014. 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, 2020 edition. Accessible at: <https://units.fisheries.org/fhs/fish-health-section-blue-book-2020/>
- Faisal M., and A. E. Eissa. 2009. Diagnostic testing patterns of *Renibacterium salmoninarum* in spawning salmonid stocks in Michigan. *Journal of wildlife diseases* 45:447–456.
- Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. 2020. Efficacy of Erymicin 200 injections for reducing *Renibacterium salmoninarum* and controlling vertical transmission in an inland rainbow trout brood stock. *Pathogens* 9:547.
- Fryer, J. L., and C. N. Lannan. 1993. The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in Pacific salmon. *Fisheries Research* 17:15–33.
- Guðmundsdóttir, S., L.J. Applegate, Í. Ö. Árnason, Á. Kristmundsson, M. K. Purcell, and D. G. Elliott. 2017. Detecting *Renibacterium salmoninarum* in wild brown trout by use of multiple organ samples and diagnostic methods. *Bull Eur Assoc Fish Pathol* 37:31–40.

- Isaak, D. J., S. J. Wenger, E. E. Peterson, J. M. Ver Hoef, D. E. Nagel, C. H. Luce, . . . and S. L. Parkes-Payne. 2017. The NorWeST summer stream temperature model and scenarios for the western U.S.: A crowd-sourced database and new geospatial tools foster a user community and predict broad climate warming of rivers and streams. *Water Resources Research*, 53(11), 9181–9205. doi:10.1002/2017WR020969.
- Jónsdóttir H., H. J. Malmquist, S. S. Snorrason, G. Gudbergsson, S. Gudmundsdottir. 1998. Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland. *Journal of Fish Biology* 53(2):322–339.
- Kingswood, R. W. 1996. The range and effect of *Renibacterium salmoninarum* on trout in Colorado. MS thesis. Colorado State University, Fort Collins, CO.
- Kowalski, D. A. 2019. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237-R26, Progress Report, Fort Collins.
- Meyers, T. R., S. Short, C. Farrington, K. Lipson, and H. J. Geiger. 1993a. Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaskan Pacific salmon. *Dis Aquat Org* 16:191–197.
- Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger, and R. Gates. 1993b. 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. *Dis Aquat Org* 16:181–189.
- Mitchum, D. L., L. E. Sherman, and G. T. Baxter. 1979. Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Board of Canada* 36:1370–1376.
- Munson, D.A., D. G. Elliott, 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. *N Am J Fish Manage* 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. *Dis Aquat Org* 91:113–119.
- Pascho, R.J., and D. G. Elliott. 1991. Brood stock segregation of spring chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. *Dis Aquat Org* 12:24–40.
- Pascho, R. J., D. G. Elliott, R. W. Mallett, and D. Mulcahy. 1987. Comparison of five techniques

- for the detection of *Renibacterium salmoninarum* in adult coho salmon. *Trans Am Fish* 116:882–890.
- Pascho, R. J., D. Chase, and C. L. McKibben. 1998. Comparison of the membrane-filtration fluorescent antibody test, the enzyme-linked immunosorbent assay, and the polymerase chain reaction to detect *Renibacterium salmoninarum* in salmonid ovarian fluid. *J Vet Diagn Invest* 10:60–66.
- Pascho, R. J., T. D. Goodrich, and C. L. McKibben. 1997. Evaluation by enzyme-linked immunosorbent assay (ELISA) of *Renibacterium salmoninarum* bacteria affected by persistence of bacterial antigens. *J Aquat Anim Health* 9:99–107.
- R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria <http://www.R-project.org/>
- Sami, S., T. Fischer-Scherl, R. W. Hoffmann, and C. Pfeil-Putzien. 1992. Immune complex-mediated glomerulonephritis associated with bacterial kidney disease in the rainbow trout (*Oncorhynchus mykiss*). *Veterinary Pathology* 29:169–174.
- Snieszko, S. F., and P. J. Griffin. 1955. Kidney disease in brook trout and its treatment. *Prog Fish-Cult* 17:3-13.
- Suzuki, K., and D. K. Sakai. 2007. Real-time PCR for quantification of viable *Renibacterium salmoninarum* in chum salmon *Oncorhynchus keta*. *Dis Aquat Org* 74:209–223.
- U.S. Geological Survey. 2017. National Hydrography Dataset (NHD), accessed December 2017 at www.usgs.gov/core-science-systems/ngp/national-hydrography/access-national-hydrography-products.
- Wasserstein, R. L., and A. L. Schirm, N. A. Lazar. 2019. Editorial: moving to a world beyond $p < 0.05$. *Am Stat* 73:1–19.

RESEARCH PRIORITY

Colorado River Ecology and Water Project Mitigation Investigations

OBJECTIVE

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

INTRODUCTION

Dams are known to drastically alter the habitat of rivers and have a multitude of effects on fish and aquatic invertebrates (Ward and Stanford 1979). Trans-basin water diversions remove approximately 67% of the annual flow of the upper Colorado River and future projects will deplete flows further. Previous work by CPW researchers identified ecological impacts of streamflow reductions and a mainstem reservoir on the invertebrates and fish of the river (Nehring et al. 2011). The health of the invertebrate community has declined after the construction of Windy Gap Reservoir, with a 38% reduction in the diversity of aquatic invertebrates from 1980 to 2011. A total of 19 species of mayflies, four species of stoneflies, and eight species of caddisflies have been extirpated from the sampling site below Windy Gap Reservoir (Erickson 1983; Nehring et al. 2011). Historically, the Salmonfly (*Pteronarcys californica*) was common in the upper Colorado River but has become rare below Windy Gap Reservoir (USFWS 1951; Nehring et al. 2011).

In addition to impacts on the aquatic invertebrate community, Windy Gap Reservoir has altered the fish community of the upper Colorado River. Native sculpin, once common, are now rare or extirpated immediately below Windy Gap Reservoir (Dames and Moore 1977; Nehring et al. 2011). These fish currently recognized as *Cottus bairdii* are likely a different species, the Colorado Sculpin *C. punctulatus* (Young et al. 2020). Stream reaches below several dams and water projects in Middle Park have reduced density and range of sculpin (Nehring et al. 2011). The decline in sculpin distribution appears both temporally and spatially related to impoundments (Kowalski 2014). A survey in 1975-1976, before Windy Gap Reservoir construction, documented sculpin at all sampling sites (Dames and Moore 1977). In 2010, a project investigating the distribution of sculpin in the upper Colorado River revealed that their density was 15 times higher in sites above impoundments compared to downstream sites (Nehring et al. 2011). In the main stem Colorado River between Windy Gap Reservoir and the Williams Fork, a single fish was sampled in 3,200 ft of river sampled by electrofishing. This study attributed the decline of sculpin in the upper Colorado River to habitat changes related to flow alterations, changes in sediment dynamics, and water depletions below the reservoir. Surveys in 2013, 2018, and 2019 confirmed these patterns finding sculpin common above impoundments on the upper Colorado River but rare or absent downstream (Kowalski 2014, Kowalski 2020).

The planned Windy Gap Firming Project will increase trans-basin water diversions from the

upper Colorado River. There are ongoing efforts to implement mitigation measures to reduce the impact of the new projects (Northern Water Conservancy District 2011). A large component of the mitigation plan is the construction of a bypass channel around the reservoir. This would reconnect the Colorado River and address various effects of a large, main-stem impoundment but overall the firming project will exacerbate flow depletions from the system. The planned bypass channel offers a unique opportunity to evaluate the effects of reconnecting the river and investigate if mitigation measures can offset the impacts of large flow depletions on the ecology of the river.

METHODS

Aquatic invertebrate samples were collected in 2020 at seven sites on the Colorado River and two sites on the Gunnison River for comparison (Table 3, Figures 3-5). Fewer sites were sampled in 2020 than in years past due to budgetary restrictions and the sampling plan developed around construction of the Windy Gap bypass channel. In addition, two new sites were sampled on the Kemp Breeze State Wildlife Area to monitor and evaluate the planned physical habitat improvement project there.

Invertebrate samples were collected by the standard method used by Water Quality Control Division of the Colorado Department of Public Health and Environment (CDPHE 2020). A semi-quantitative sample was collected with an 18" x 8" rectangular frame kick net with 500 μ mesh using CDPHE protocols. Approximately one square meter of stream bottom was disturbed above the kick net for one minute and all organisms were collected, elutriated, sorted, and preserved in 80% ethanol. Samples were sent to Aquatic Associates Inc. in Fort Collins Colorado where all processing and identification took place. Processing samples with the CDPHE method involves subsampling and identifying a fixed count of 300 individual organisms (including chironomids) to species. The method generates a standardized multimetric index score specifically developed for Colorado streams, the MMI. Because the area of stream bottom sampled is approximated and sampling time is restricted, the CDPHE method cannot provide true density estimates. Instead, it is an index of invertebrate community health collected by standardized methods where sites can be compared to each other as well as to reference sites of similar stream types. Community indices were calculated according to methods outlined in CDPHE (2020) and Barbour et al. (1999). Five metrics were used to compare sites: the MMI, Shannon Diversity Index (SDI), EPT taxa richness (number of Ephemeroptera, Plecoptera, and Trichoptera taxa), Plecoptera taxa richness, and total taxa richness.

The MMI is a multimetric index that is that standard regulatory method used by the state of Colorado to determine stream impairment under the Colorado Water Quality Control Act and the Federal Clean Water Act (CDPHE 2020). Multimetric indices combine invertebrate community information with expected species composition and community metrics from reference sites. They have been shown to be an effective and cost-efficient method for invertebrate bioassessment (Hughes and Noss 1992; Barbour et al. 1995; Karr 1998). The Colorado MMI is made up of metrics that represent various aspects of the community structure and function and are grouped into five categories: taxa richness, composition, pollution tolerance, functional feeding groups, and habit. Combining metrics from these categories into a multi-metric index

transforms invertebrate sampling data into a unit-less score that ranges from 0-100 that indicates the community health and stream condition (CDPHE 2020). The higher the MMI value, the healthier the invertebrate community is relative to its biotype in Colorado. The Shannon Diversity Index (SDI) is a metric commonly used to estimate the species diversity and evenness of an invertebrate community (Shannon 1948, Barbour et al. 1999). The SDI (also referred to as Shannon-Wiener Index) is a function of both the number of species in a sample and the distribution of individuals among those species, and the higher the value of the index, the greater the diversity of species in a particular community (Barbour et al. 1999).

Table 3. Aquatic invertebrate sampling sites on the Colorado River and Gunnison Rivers in 2020.

Site #	Site Name	UTM East	UTM North
CR1	Fraser Confluence	416914	4439457
CR2	Hitching Post	414652	4440330
CR3	Chimney Rock, Red Barn	412703	4439648
CR6	Hot Sulphur SWA, Gerrans Unit	403440	4434141
CR7	Breeze Bridge	398319	4435421
CR8	Kemp Breeze #2	397783	4435451
CR9	Kemp Breeze #3	397287	4435379
GR3	Smith Fork	253338	4291889
GR5	Cottonwood Rec Site	252094	4295941



Figure 4. Map of the upper benthic macroinvertebrate sampling sites on the Colorado River in 2020.

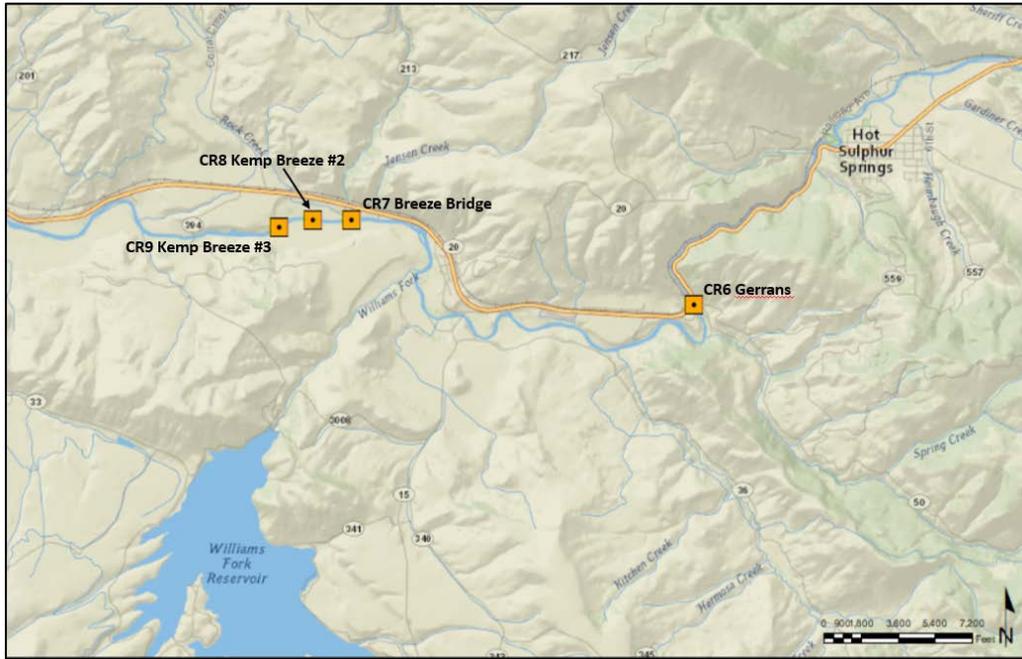


Figure 5. Map of the lower benthic macroinvertebrate sampling sites on the Colorado River in 2020.

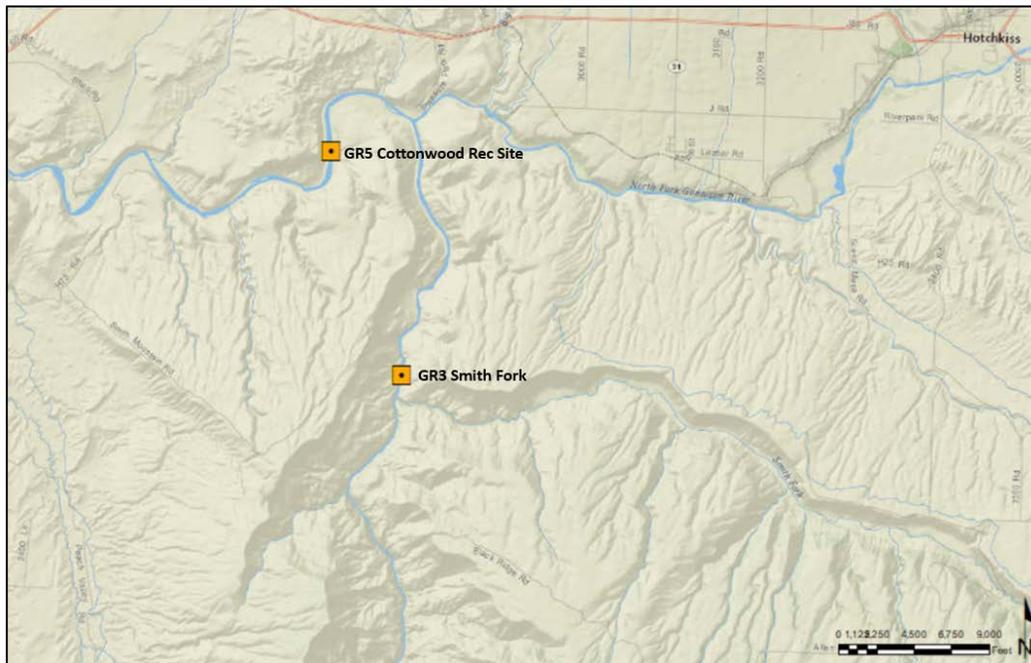


Figure 6. Map of benthic macroinvertebrate sampling sites on the Gunnison River in 2020.

RESULTS AND CONCLUSIONS

Results of the invertebrate sampling in 2020 produced similar trends as previous years' results; the invertebrate community is generally less healthy and diverse immediately below Windy Gap Reservoir (Table 5). Sites CR1 (Fraser Confluence) and CR8 (Kemp Breeze #2) had the highest MMI score at 68.8 and 68.3. Generally, species diversity and community health metrics were high above Windy Gap, lowest at site immediately below the dam, and increased in a downstream trend to the Kemp Breeze sites (Figure 7). As in previous years sampling, Plecoptera (stonefly) species richness was highest at site CR1 above Windy Gap Reservoir than in sites below (Figure 8). One species of Plecoptera, *Pteronarcella badia*, was only found above Windy Gap Reservoir at site CR1.

Table 5. Community metrics and index scores for invertebrate sampling sites on the Colorado and Gunnison Rivers in 2020.

Community Metrics	CR1	CR2	CR3	CR6	CR7	CR8	CR9	GR3	GR5
Total Taxa Diversity	29	23	28	24	18	32	28	37	36
EPT Taxa Diversity	14	12	12	13	10	17	14	15	18
SDI	2.6	1.8	2.3	2.2	1.6	2.3	2.4	2.8	2.9
MMI	68.5	60.1	63.4	64.5	60.3	65.9	68.3	76.8	80.3

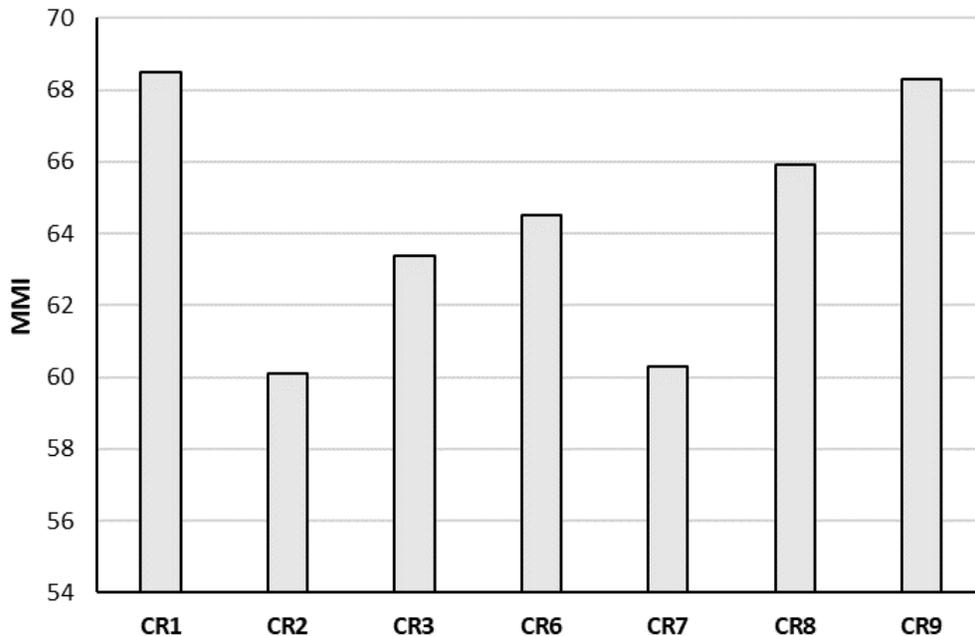


Figure 7. Trends in MMI scores of the Colorado River invertebrate sampling sites in 2020 from upstream (CR1) to downstream (CR9). Windy Gap Reservoir is between sites CR1 and CR2.

While previous work identified declines in the range of some species of aquatic invertebrates, the 2020 sampling did document the presence of several species of interest at sites below Windy Gap Reservoir. Salmonflies were sampled CR3 and CR6 but densities were low except at site CR6. While it was encouraging to document their presence at two of the seven sites, they remain rare or absent immediately below Windy Gap Reservoir. The mayfly *Drunella grandis*, which has declined in range in the Colorado River, was documented at sites CR2, CR7, CR8, and CR9. This species was rare or absent at sites immediately below Windy Gap Reservoir in 2010 and it continues to be rare but present there today. Several other disturbance-sensitive species present before Windy Gap Dam was constructed continue to be absent from sites below the reservoir in 2020. Mayflies in the genus *Rhithrogena* were present in the river before reservoir construction and were documented in 2020 at sites CR7 and CR8. Mayflies in the genus *Heptagenia* were reported at multiple sites in the early 1980s but were absent at all sites in 2010, 2018, 2019, and 2020. Stoneflies in the genus *Isogenoides* and *Pteronarcella* are also no longer found at sites below Windy Gap Reservoir where they had been documented before construction.

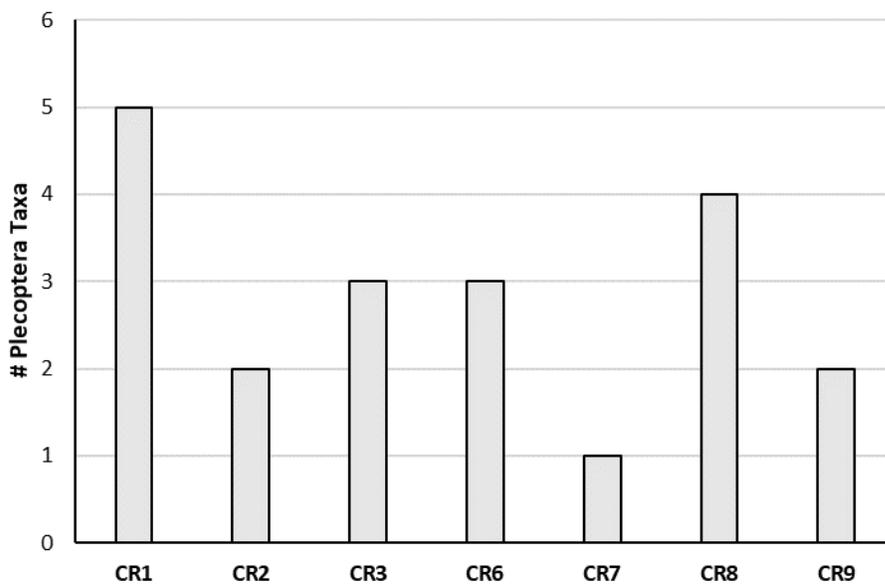


Figure 8. Plecoptera species richness of the Colorado River invertebrate sampling sites in 2020.

Site CR6, Gerrans Unit of the Hot Sulphur Springs SWA, was the only site sampled in 2020 that supported high densities of Salmonflies. This site is just below Byers Canyon, a narrow high gradient reach of Colorado River, and has been identified as having the largest population of Salmonflies of sites below Windy Gap but above Gore Canyon (Nehring et al. 2011; Kowalski 2020). It appears that the increased velocity and gradient of the river in the confined reach in Byers Canyon leads to improved invertebrate community below, potentially related to decreased fine sediment, larger median cobble size, lower cobble embeddedness, and lower width to depth ratio (Kowalski and Richer 2020).

All sites on the Colorado River had lower species diversity, richness, and lower community index scores than sites on the Gunnison River (Table 5). This reflects previous work that has documented healthier invertebrate communities on the Gunnison compared to the Colorado River (Nehring et al. 2011). Overall, MMI scores on the Colorado River were uniformly higher in 2020 than in previous years but the trends among sites were the same (Kowalski 2020). Some small differences in processing techniques individual laboratories may explain this, but more work is necessary to explore the trend. The MMI, in addition to having the weight of regulatory standards behind it, has been found to be an effective a cost efficient tool to monitor invertebrate communities on the Colorado River (Kowalski 2020).

Overall, the results of the 2020 benthic sampling reflect the patterns in invertebrate community of the Colorado River presented in previous work (Nehring et al. 2011, Kowalski 2020). Generally, while healthy and diverse invertebrate communities exist above the reservoir and at some sites downstream, sites immediately below Windy Gap Reservoir are less diverse, have lower numbers of sensitive species, and are lower in the density and diversity of stonefly species. The impaired invertebrate community below Windy Gap is likely due to habitat changes in the river associated with the shallow main stem impoundment and its associated water depletions.

ACKNOWLEDGMENTS

This work was supported in part by the Colorado Department of Public Health and Environment Water Quality Control Division. Technical assistance as well as direct support of the project was provided by processing MMI samples and we thank CDPHE and Chris Theel, Monitoring and Data Work Group Leader of the Water Quality Control Division, for the support.

REFERENCES

- Barbour, M. T., J. B. Stribling, and J. R. Karr. 1995. The multimetric approach for establishing biocriteria and measuring biological condition. Pp. 63-76 in W. S. Davis and T. P. Simon, editors. *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making*. Lewis Publishers, Ann Arbor, Michigan.
- 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-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- CDPHE. 2020. *Aquatic life use attainment, methodology to determine use attainment for rivers and streams policy statement 10-1*. Colorado Department of Public Health and Environment Water Quality Control Commission. Denver, CO. Available at: <https://cdphe.colorado.gov/wqcc-policies>.
- Dames and Moore. 1977. *Environmental assessment report Windy Gap project Grand County*,

- Colorado for municipal subdistrict, Northern Water Conservancy District. Denver, CO.
- 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.
- Hughes, R. M. and R. F. Noss. 1992. Biological diversity and biological integrity: current concerns for lakes and streams. *Fisheries* 17:11–19.
- Karr, J., 1998. Rivers as sentinels: using the biology of rivers to guide landscape management. In: Naiman, R., Bilby, R. (Eds.), *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. Springer-Verlag, New York, pp. 502–528.
- 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.
- Kowalski, D. A. 2020. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237-R26, Progress Report, Fort Collins.
- Kowalski, D. A. and E. E. Richer. 2020. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. *River Research and Applications* 36:2043– 2050.
- Nehring, R. B., B. D. Heinold, and J. Pomeranz. 2011. Colorado River aquatic resources investigations. Colorado Division of Wildlife, Federal Aid in Sportfish Restoration, Project F-237R-18, Final Report, Fort Collins.
- Northern Water Conservancy District. 2011. Windy gap firming project fish and wildlife enhancement plan. Retrieved from https://www.northernwater.org/sf/docs/default-source/wgfp-docs/document-library/wgfpenhanceplanjune2011.pdf?sfvrsn=a1c8894d_5
- Shannon, C. E. 1948. A mathematical theory of communication. *The Bell System Technical Journal* 27:379–423.
- 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.
- Young, M. K., R. Smith, and K. Pilgrim. 2020. A molecular taxonomy of *Cottus* from the Colorado River basin in Colorado and adjacent regions. National Genomics Center

for Wildlife and Fish Conservation, Missoula, MT.

RESEARCH PRIORITY

Technical Assistance

OBJECTIVE

Provide technical assistance to biologists, managers, researchers, and other internal and external stakeholders as needed in a variety of coldwater ecology applications.

INTRODUCTION

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

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

ACCOMPLISHMENTS

One paper was published in a peer-reviewed journal to summarize and disseminate information from stream ecology research projects:

Kowalski, D. A. and E. E. Richer. 2020. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. *River Research and Applications* 36: 2043– 2050. doi: 10.1002/rra.3733.

One paper was submitted to a peer-reviewed journal and is currently in review:

Kowalski, D. A., E. I. Gardunio, and C. A. Garvey. *In Review*. Evaluating the effects of an electric barrier on fish entrainment in an irrigation canal in Colorado. *River Research and Applications*.

Two reports were produced to summarize and disseminate information from the coldwater stream ecology research projects;

Kowalski, D. A. 2020. Colorado coldwater stream ecology investigations progress report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Kowalski, D. A. 2021. Trends in Fishing License Sales in Colorado 2018-2020. Colorado Parks and Wildlife, March 23, 2021.

One external press release was written disseminate results of aquatic ecology projects on the CPW website and external news outlets:

Kowalski, D. A. and R. Hampton. 2020. CPW and partners are studying important trout disease. Colorado Parks and Wildlife Press Release. December 14, 2020.
<https://cpw.state.co.us/aboutus/Pages/News-Release-Details.aspx?NewsID=7673>.

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

Kowalski, D. A. 2020. Bacterial kidney disease and *R. salmoninarum* aquatic research updates. Colorado Parks and Wildlife, December 17, 2020.

Kowalski, D. A., M. K. Young, and J. Logan. 2021. Muddled Sculpins: Diversity and Phylogeny of Colorado's Native Cottids. Colorado Parks and Wildlife Aquatic Biologist Meeting, January 20, 2021.

One external presentation was given to disseminate results of aquatic ecology projects to colleagues and other fishery professionals:

Kowalski, D. A. and E. E. Richer. 2021. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. Colorado-Wyoming American Fisheries Society Meeting, February 23, 2021.