Colorado Coldwater Stream Ecology Investigations

Project Summary

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PROJECT SUMMARY

Period Covered: July 1, 2019 to June 30, 2020

PROJECT OBJECTIVE

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

RESEARCH PRIORITY

Quantifying the Habitat Preferences of the Stonefly *Pteronarcys californica* in Colorado

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

OBJECTIVE

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

INTRODUCTION

The salmonfly (*Pteronarcys californica*) is the largest stonefly species in North America and can attain high densities in some western rivers. They play a critical ecological role as shredders in stream ecosystems (DeWalt and Kondratieff 2019), and can be extremely important to stream dwelling trout as a food resource. Nehring (1987) reported that *P. californica* was the most common food item of trout in the Colorado River, comprising 64-75% of the mean annual stomach contents. Because of their high biomass and hatching behavior, they also play an important role in supplementing terrestrial food webs and riparian communities with stream-derived nutrients (Baxter et al. 2005; Walters et al. 2018). Salmonflies have a 3-4 year life cycle in various parts of their range including Colorado (DeWalt and Stewart 1995; Nehring 1987). Therefore, as one of the longest-lived aquatic insects in the Nearctic, salmonflies are more susceptible to habitat alterations than other taxa (DeWalt and Stewart 1995).

Salmonflies are one of the most synchronously emerging aquatic invertebrates, with emergence at any one site only lasting from 5-13 days (DeWalt and Stewart 1995). They hatch at night by crawling from the water onto riparian vegetation and other vertical structures, such as rocks, cliff faces, and bridge abutments, where they emerge from the larvae's exoskeleton or exuvia that is left attached to the structure. The synchronous emergence and hatching behaviors allow *P. californica* to be sampled in unique ways, similar to techniques used to survey Odonata (dragonflies and damselflies) see Raebel et al. (2010), and DuBois (2015). Nehring, Heinold, and Pomeranz (2011) used multiple-pass removal density estimates of the shed exuvia as an index of salmonfly density in rivers in Colorado. This technique was validated and applied to other studies as a cost- and time-efficient index of salmonfly density (Walters et al. 2018; Heinold et al. 2020). Therefore, we applied this technique to index salmonfly density and explore its relationships with stream habitat variables.

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

The extirpation or decline in range of *P. californica* in several western rivers has led to several re-establishment attempts. Reintroduction by direct transfer of larval salmonflies into formerly occupied habitat has been attempted in at least three waters including the Logan River in Utah, two attempts in the Arkansas Rivers in Colorado, and two or more times in the upper Gunnison River in Colorado (Colburn 1986; Vinson 2011; Kowalski 2015; Benzel 2016). All of the attempts in Colorado have failed to establish *P. californica* populations. In the upper Colorado River near Granby, Colorado, *P. californica* has declined in range and numbers due to the downstream impacts of a mainstem reservoir (Nehring et al. 2011). As part of a mitigation and enhancement package for increased water diversions associated with that reservoir, a habitat improvement project, flow management program, and reservoir bypass channel has been proposed (Northern Water Conservancy District 2011). One of the explicit goals of that plan is to improve the stream habitat downstream of the reservoir for aquatic invertebrates including *P. californica.* These efforts reflect a desire by biologists and water managers to restore salmonflies to areas of its range where they have been extirpated. However, in rivers where water quality appears sufficient to support *P. californica*, little is known about the specific habitat requirements that may be deficient and could hamper or preclude the restoration efforts. The motivation for this study was to quantitatively define habitat preferences of the salmonfly with commonly used variables to guide restoration efforts and further the understanding about this ecologically important indicator species.

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

METHODS

Salmonfly density estimates and habitat variable measurements were made at 18 sites on the Colorado, Gunnison, and Rio Grande Rivers (Fig. 1). These rivers are 6th order streams with pool-riffle or pool-riffle/plane bed morphology in the Rocky Mountains of western Colorado, USA (Montgomery and Buffington 1997). A flood-frequency analysis was performed for each watershed to estimate the 1.5-year flood at each study site, which is considered an approximation of the bankfull flow (Dunne and Leopold 1978). Study sites within the Gunnison River in southwest Colorado have an average 1.5-year flow of 88 m^3/s, an average drainage basin area of 11,711 km^2, and range in elevation from 1,539-1,639 m. Ranging in elevation from 2,070-2,376 m, study sites within the Colorado River in west central Colorado have an average 1.5-year flow of 42 m^3/s, and an average drainage basin area of 3,691 km^2. In the Rio Grande River in south central Colorado, study sites have an average 1.5-year flow of 70 m^3/s, an average drainage basin area of 1,777 km^2, and range in elevation from 2,579-2,613 m.

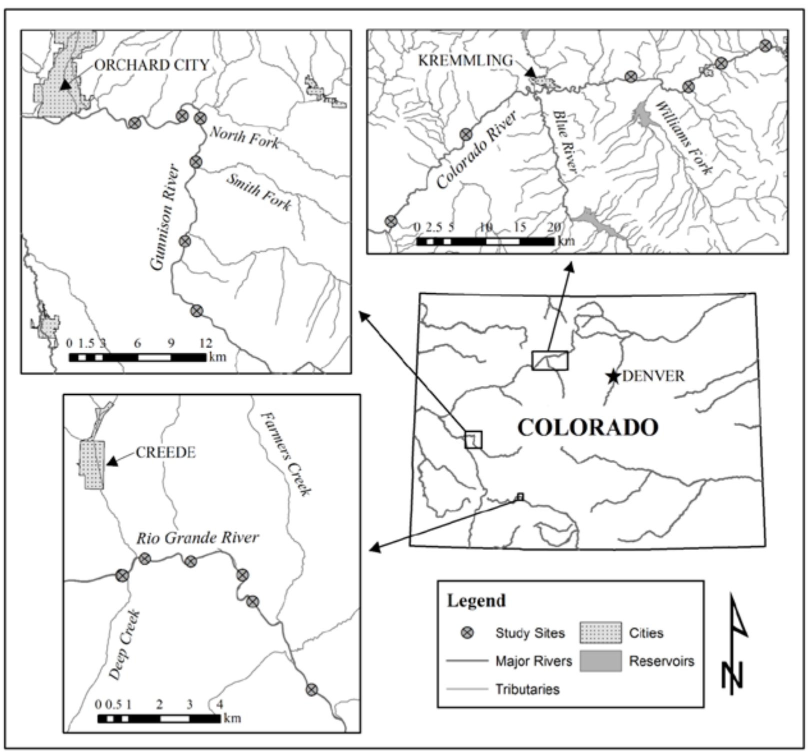

Figure 1. Salmonfly habitat study sites on the Gunnison, Colorado, and Rio Grande Rivers.

Approach

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

Habitat variables

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

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

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

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

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

Salmonfly density

We estimated the density of salmonflies at our sites using the method described in Nehring et al. (2011) and evaluated by Heinold et al. (2020). This technique is an improvement on the exuvia collection methods of Richards et al. (2000) by applying a multiple-pass removal technique to account for imperfect detection probability. If sites are visited soon after emergence, the density of emerged salmonflies can be estimated by conducting multiple-pass removal sampling of exuvia left attached to riparian vegetation or structure. There is a high correlation ($R^2 = 0.90$) between post-emergence exuvia density estimates and more traditional pre-emergent quantitative benthic sampling and this method is more effective than traditional benthic surveys at detecting *P. californica* at low density sites. (Heinold et al. 2020).

We completed annual salmonfly density estimates in June 2013-2016 by searching 30 m sections of stream bank for *P. californica* exuvia adjacent to riffle habitat. If possible, each site was visited two to three times to encompass the entire emergence. If a site was visited only once, estimates were done as soon as possible after the emergence was complete (emergence lasted from 7-13 days at our sites during this study). Stream flow changes and weather conditions were also taken into account when planning surveys to best estimate the total emergence at each site. Riparian areas were intensively searched by three to seven people within a search area that extended 1-20 m from the water's edge. The search area varied by site and depended on the thickness and structure of riparian vegetation. The area was extended laterally from the water's edge until no exuvia were encountered, with the exuvia at most sites being found within the first 3 m from the water's edge. On a single sampling occasion, each site was searched completely with two to four passes with identical search areas, effort and personnel on each pass. The Huggins Closed Captures model in Program MARK was used to estimate the total density of exuvia at each site (Huggins 1989; White and Burnham 1999). All sites had at least three years of exuvia density estimates, with a minimum of two years of data collected under favorable flow and weather conditions (e.g. low winds and stable or declining stream flows) that did not compromise the estimates by possibly removing exuvia from streamside vegetation and structure.

Analysis

As an exploratory study, we focused on a basic analysis of a limited number of variables to produce simple descriptive model(s) and rank top variables. To evaluate associations between habitat variables and salmonfly density, a two-step modelling approach was used. The five habitat variables were first screened with Pearson's product moment correlation coefficient and then analyzed with multiple linear regression. Linear regression modelling was performed with the lm function in program R version 3.5.0 (R Foundation for Statistical Consulting, Vienna, Austria). Model assumptions of homogeneity of variance and normality were evaluated by examining residuals of the global model. The response variable, salmonfly exuvia per square meter, was analyzed with the Box Cox procedure due to patterns in the residuals (Box and Cox 1964). The lambda value of 0.295 had a 95% confidence interval that included 0.5, so a square root transformation was used on the response variable to better meet assumptions of the linear regression model.

Because of the sample size (n = 18), only a limited number of models could be considered without potentially identifying spurious effects and having problems estimating parameters from noisy data (Burnham and Anderson 2002). The three variables with the highest correlation coefficients were evaluated using the information theoretic approach (Burnham and Anderson 2002) to identify the top predictive model(s) using the small sample size version of Akaike's Information Criterion (AIC*c*). Single variable models with an intercept and an error term were considered as well as a global model of all three top variables. No other additive effect models or interaction models were considered due the sample size restrictions and our main objective of ranking the top variables. Model-averaged parameter estimates were based on model weights, and the sum of weights for each parameter was used to infer variable importance. The analysis was conducted with the AICcmodavg package (Mazerolle 2016) in program R version 3.5.0 (R Foundation for Statistical Consulting, Vienna, Austria).

RESULTS

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

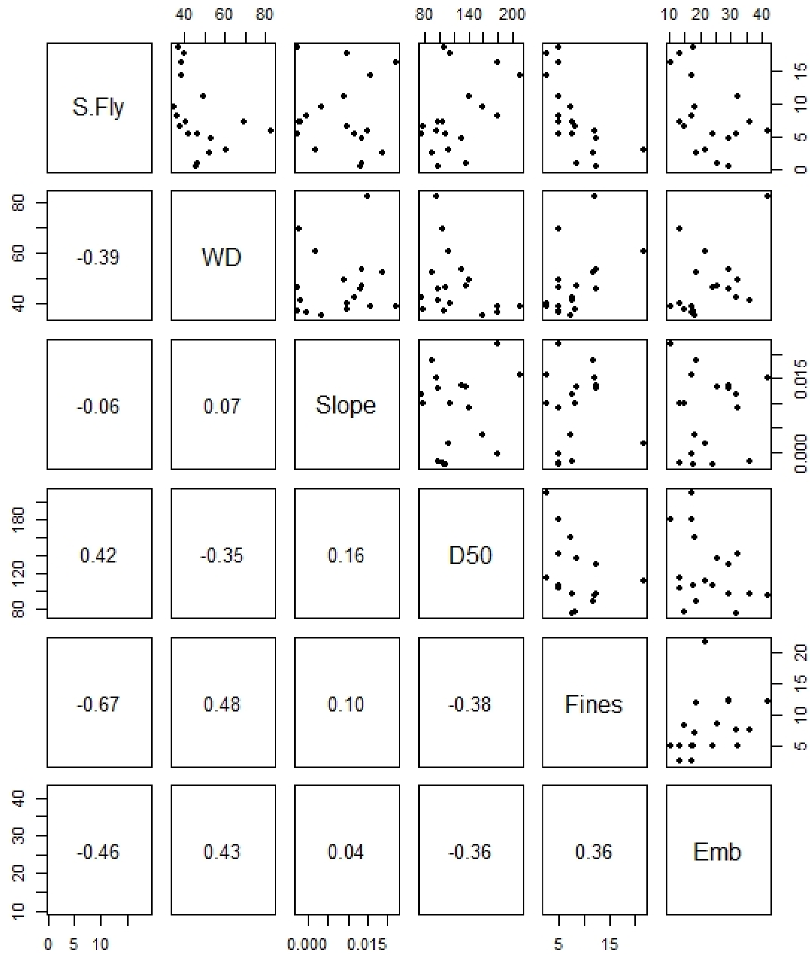

Figure 2. Pearson correlation matrix of habitat variables and Box Cox-transformed salmonfly exuvia density (S. Fly). WD is the width to depth ratio, slope is stream bed slope, D50 is the 50% cumulative particle size in mm, fines is percent of sand, silt and clay particles <2 mm, Emb is percent embeddedness.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

AICc model selection results indicate that the single variable model with percent fines was the top model with a model weight (*wi*) of 0.89 (Table 1). The global model with an additive combination of all 3 variables was 4.7 AICc units behind the top model, and explained 51.4% of the variation in salmonfly density. Akaike weights for each variable were summed across the model set to infer relative variable importance (Burnham and Anderson 2002). Percent fines was the most influential variable with a cumulative weight of 0.94, followed by embeddedness at 0.11 and D50 at 0.10. A null (intercept) model was also evaluated and was 7.6 ΔAICc units behind the top model and would be the lowest ranked model in the set if included.

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

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

Mean August water temperature (average = 16.3 C, SE = 0.46) varied little over our study sites (range = 13.8-19.7 C). Water temperature exhibited low correlation to salmonfly density (Pearson r = -0.18) and was not significant at an α level of 0.05 ($p = 0.472$). The amount of variation in transformed stonefly density that mean August temperature explained was low (R2 = 0.03). Mean August temperatures would be 10.0 ΔAICc units behind the top model (percent fine sediment) if it was included as a single variable model in our set of physical habitat models.

Salmonfly density increased at sites with low amounts of fine sediment, low embeddedness, and larger median substrate size. We made model predictions to summarize the values of the stream habitat variables associated with the range of salmonfly densities encountered at our sites (Table 2). An average salmonfly density site (median of the observed values) could be expected with 6% fine sediment, while high densities (75% quartile of observed values) would be expected only at sites with low amounts of fine sediment (< 3%). Width to depth ratio had the fourth highest correlation coefficient and was left out of the model selection exercise but was still marginally related to salmonfly density ($R^2 = 0.16$, $p = 0.11$). Salmonfly density increased with lower width to depth ratios, an average density site could be expected with a width to depth ratio of 38, while high densities would be expected only at sites with width to depth ratio less than 24.

DISCUSSION

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

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

Relative density	Exuvia/m²	% Fines	D50 (mm)	% Embeddedness
Moderate (25% quartile)	20	13	64	36
Median	48	10	104	27
Average	96	6	150	17
High (75% quartile)	147	3	187	9
Maximum observed	353	0	295	0

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

Salmonfly distribution and abundance are likely driven by many factors, and may be limited by different environmental factors at different scales and locations. While temperature may be driving salmonfly density in the Madison River, it appears that habitat factors like fine sediment are more highly associated with salmonfly density in these three rivers in Colorado. Our objective was to describe relationships of physical habitat variables to salmonfly density at the reach scale (Frissell et al. 1986). Within the range of sites we studied, aspects of substrate composition like percent fine sediment and cobble embeddedness, and the geomorphic characteristic median sediment size were related to salmonfly density.

Salmonflies are an indicator species for quality coldwater stream habitat in the western U.S. They are also a large, easily identifiable invertebrate species that are conspicuous and can be sampled in novel ways (Heinold et al. 2020). On some rivers, like the upper Colorado, there have been changes in the invertebrate community and reduced species diversity related to impacts of a mainstem impoundments (Nehring et al. 2011). After a shallow surface-release reservoir impounded the Colorado River, species diversity of invertebrates and native fish declined and many sensitive species of Ephemeroptera and Plecoptera were reduced in number or extirpated immediately below the dam (Nehring et al. 2011; Kowalski 2019). If impaired aquatic habitat can be restored with the explicit objective of improving populations of an indicator species like *P. californica*, then the entire aquatic community should benefit.

While different abiotic factors influence invertebrate distribution at different scales, many are likely to be difficult for land managers to influence at the river reach level. River geomorphology and sedimentation in rivers, however, can be influenced by land use practices, alterations to stream flows and sediment supplies, and even direct physical river restoration (Leopold et al. 1964; Rosgen and Silvey 1996; Wood and Armitage 1997). If conservation of salmonfly habitat is a goal of resource managers, then flow management, land-use decisions, and habitat restoration activities should focus on reducing the input of fine sediment in rivers and encouraging flow regimes and channel morphology that maintain low cobble embeddedness and larger median substrate size in riffles. These recommendations could expand the range and population of an indicator species like the salmonfly and also support the broader invertebrate and fish community.

This research priority is complete. Two manuscripts related to this work have been published in the scientific peer reviewed literature (River Research and Applications and PlosOne) within this reporting period.

Kowalski, D. A. and E. E. Richer. *In Press*. Quantifying the habitat preferences of *Pteronarcys californica* in Colorado rivers. River Research and Applications.

Heinold, B. D., D. A. Kowalski, and R. B. Nehring. 2020. Estimating densities of larval Salmonflies (*Pteronarcys californica*) through multiple pass removal of post-emergent exuvia in Colorado rivers. PLOSONE 15(4): e0227088.

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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 impacts on fish and aquatic invertebrates (Ward and Stanford 1979). Trans-basin water diversions remove approximately 67% of the annual flow of the upper Colorado River and future projects will deplete flows further. Previous work by CPW researchers identified ecological impacts of streamflow reductions and a mainstem reservoir on the invertebrates and fish of the 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). The health of the invertebrate community also 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 stonefly *Pteronarcys californica* was common in the upper Colorado River but have become rare immediately below Windy Gap Reservoir (USFWS 1951; Nehring et al. 2011).

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

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

METHODS

Aquatic invertebrate samples were taken at seven sites on the Colorado River in 2019 and fish sampling occurred at four sites (Table 3, Figures 3-4). Invertebrate samples were collected by two different protocols commonly used in Colorado, the standard USGS method used by the National Water Quality Monitoring Laboratory (Moulton et al. 2000) and the MMI method used by Water Quality Control Division of the Colorado Department of Public Health and Environment (CDPHE). Samples were taken by both methods from the same riffle at each site.

The USGS method involved taking five replicate samples at each site using a 0.086 m^2 Hess sampler with a 350 µm mesh net. Because a defined and exact area of stream bottom is sampled by the Hess sampler, true density estimates can be made. Samples were collected September 18-20, 2019. All replicates were collected from the same riffle with predominantly cobble substrate by disturbing the streambed to a depth of approximately 10 cm. Field samples were washed through a 350-µm sieve and preserved in 80% ethanol. Samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol (Moulton et al. 2000). Approximately 300 individual organisms were identified from each replicate and a 15-minute search for large or rare organisms was conducted on the entire sample. All organisms were identified to genus or species except chironomids were identified to family and non-insects were identified to class. Each replicate was processed separately so that more individual specimens were identified from each site to ensure rare organism were identified and to increase the power of the comparisons between riffle sites in close proximity (Vinson and Hawkins 1996). All taxonomic identifications followed recommendations by Moulton et al. (2000) and were completed by a single CPW invertebrate taxonomist. Recommended quality control and quality assurance procedures were followed and at least 10% of all individual identifications were verified by Dr. Boris Kondratieff at Colorado State University (Moulton et al. 2000). All invertebrates and material remaining after the subsampling procedure was also checked for the presence of non-represented species.

The MMI is a multimetric index that is that standard regulatory method used by the state of Colorado to determine stream impairment under the Colorado Water Quality Control Act and the Federal Clean Water Act (CDPHE 2010a). Multimetric indices combine invertebrate community information with expected species composition and community metrics from reference sites. They have been shown to be an effective and cost-efficient method for invertebrate bioassessment (Hughes and Noss 1992; Barbour et al. 1995; Karr 1998). The Colorado MMI is made up of metrics that represent various aspects of the community structure and function and are grouped into five categories: taxa richness, composition, pollution tolerance, functional feeding groups, and habit. Combining metrics from these categories into a multi-metric index transforms invertebrate sampling data into a unit-less score that ranges from 0-100 that indicates the community health and stream condition (CDPHE 2010a). Sampling protocols followed standard methods and involved collecting a semi-quantitative kick net sample from each site (CDPHE 2010b). Approximately one square meter of stream bottom was disturbed for a timed one minute and all organisms were preserved in 80% ethanol. Sampling occurred on the same day and from the same riffles as the USGS method. Samples were sent to the Colorado Department of Public Health and Environment, Denver, CO and processed using the same methods, taxonomists, and facilities as CDPHE-collected samples. Processing the MMI samples involves subsampling and identifying a fixed count of 300 individual organisms from the entire sample, including chironomids, to species. Because the area of stream bottom sampled is approximated and sampling time is restricted, the MMI method cannot provide true density estimates but instead is a community index of invertebrate quality collected by standardized methods where sites can be compared to each other as well as to reference sites of similar stream types.

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

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

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

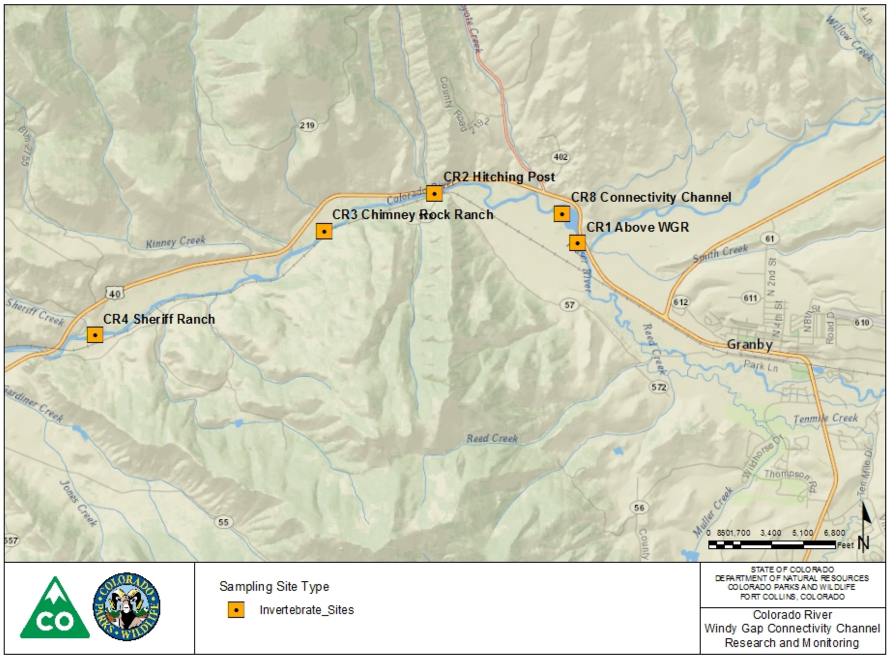

Figure 3. Map of the upper benthic macroinvertebrate sampling sites on the Colorado River. Site CR8 will be sampled in the future after construction of the connectivity channel. Fish sampling occurred at CR1 and CR2.

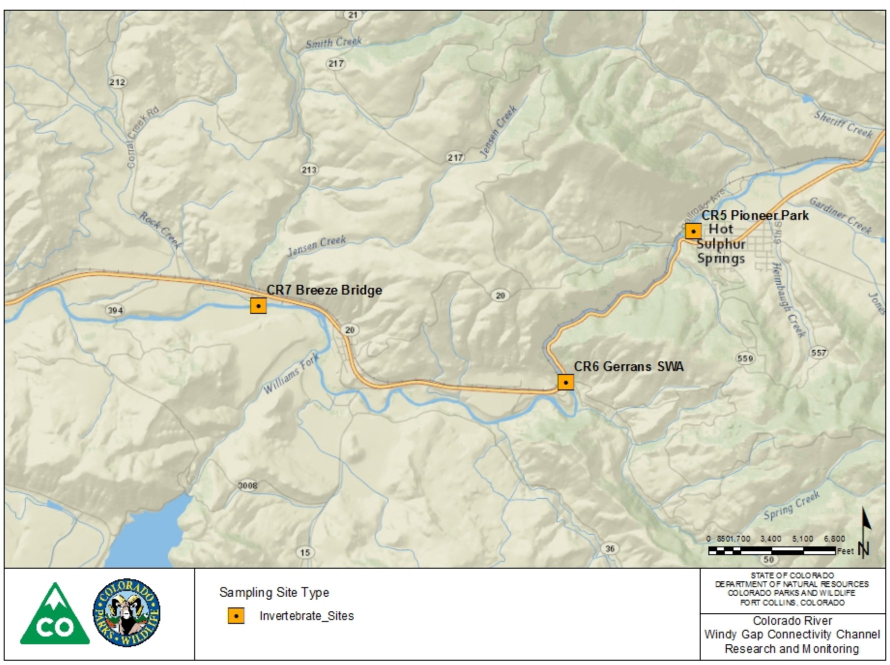

Figure 4. Map of the lower benthic macroinvertebrate sampling sites on the Colorado River. Fish sampling occurred at CR5 and CR6.

RESULTS

Invertebrate Sampling

Results of the 2019 invertebrate sampling are generally similar to previous years and reflect some of the historical patterns; the invertebrate community is generally less healthy and diverse immediately below Windy Gap Reservoir. Diversity and community health indices increased at all sites in 2019 compared to 2018 (Table 4). All sites attained the State of Colorado's MMI aquatic life standard of 48, although site CR2 only barely (48.6). Site CR6 had the highest MMI score (76.6).

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

	CR1	CR2	CR3	CR4	CR5	CR6	CR7
2018 MMI Score	67.6	57.8	38.1	37.9	59.0	62.3	35.7
2019 MMI Score	60.1	48.6	60.5	68.3	70.9	76.6	54.2

Generally, species diversity and community health metrics were lowest at site CR2 (below the dam) and CR7 (Breeze Bridge) and highest at sites CR1 (above the reservoir) and CR6 (Gerrans SWA) (Figures 5-8). Plecoptera density was much lower at all sites below Windy Gap Reservoir (Figure 9). Density was estimated at 1,018 per m² (SE 91) at site CR1 while the average of all the sites below Windy Gap Reservoir was 348 per m² (SE 39).

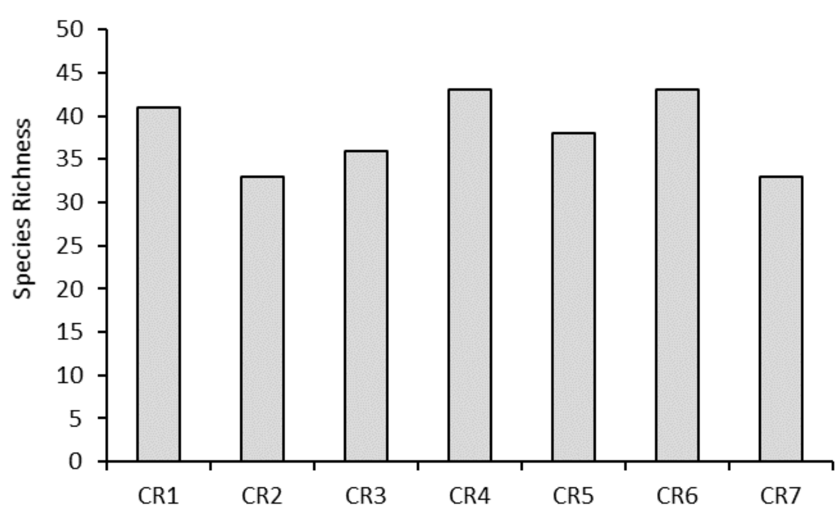

Figure 5. Total species richness from Colorado River invertebrate sampling with the USGS method in 2019.

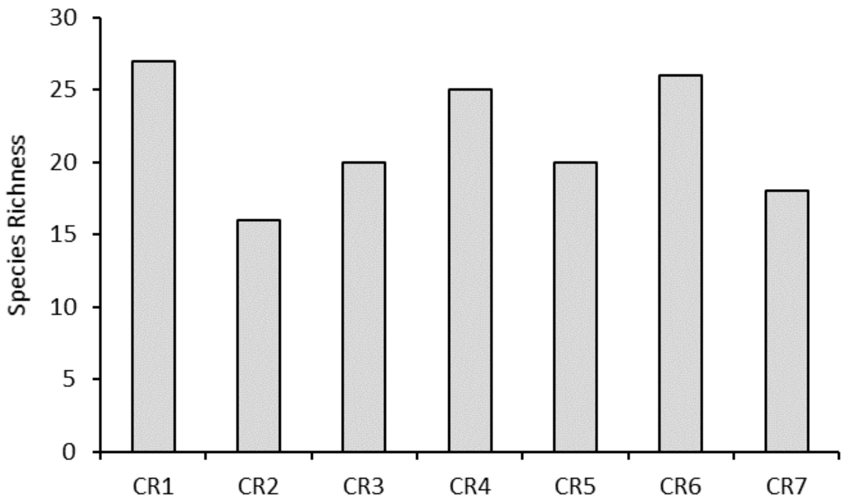

Figure 6. Ephemeroptera, Plecoptera, and Trichoptera species richness from Colorado River invertebrate sampling with the USGS method in 2019.

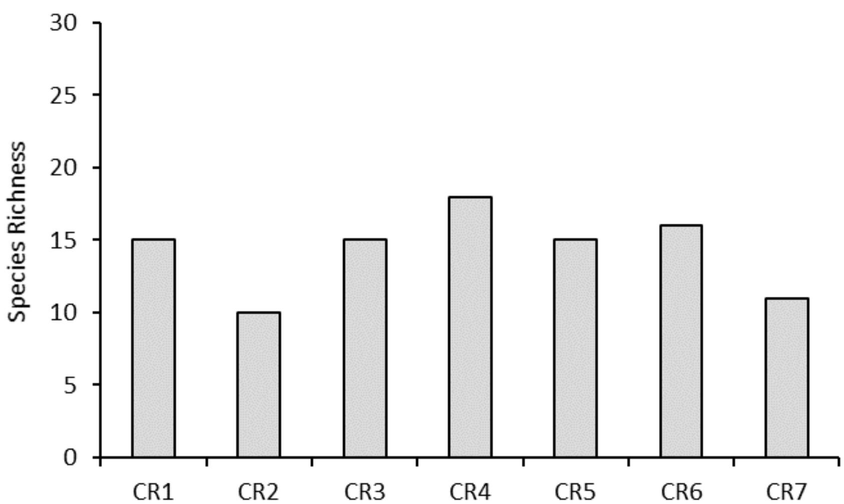

Figure 7. Ephemeroptera, Plecoptera, and Trichoptera species richness from Colorado River invertebrate sampling with the CDPHE method in 2019.

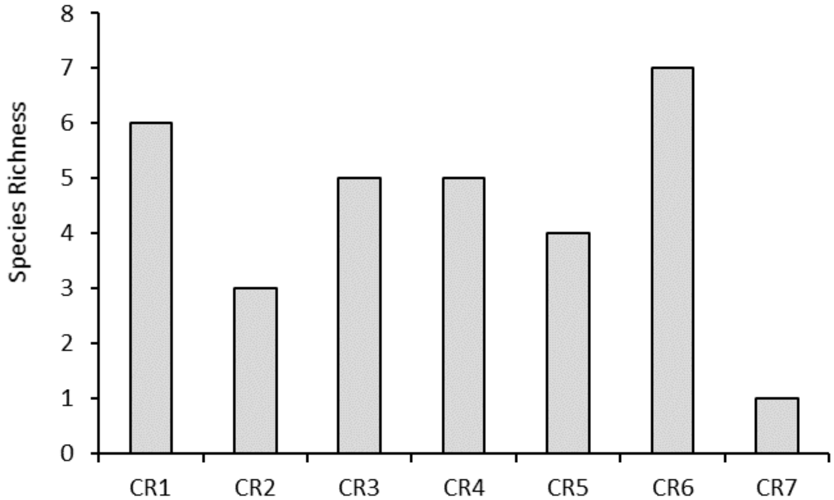

Figure 8. Plecoptera species richness from Colorado River invertebrate sampling with the USGS method 2019.

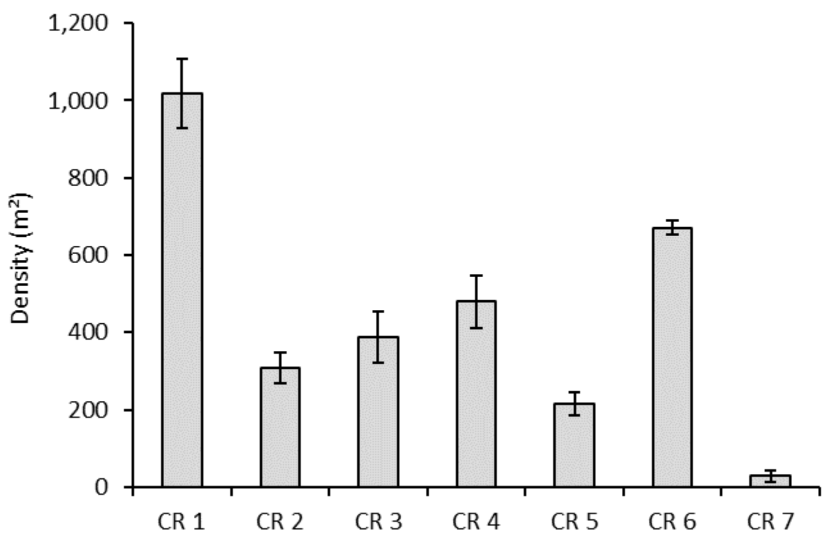

Figure 9. Plecoptera density and standard error bars from Colorado River invertebrate sampling 2019.

Site CR6, Gerrans Unit of the Hot Sulphur Springs SWA, was the most diverse and had the highest community health indices of all the sites. This site is below Byers canyon, a narrow higher gradient reach of Colorado River that has been identified as having the largest population of salmonflies of sites below the reservoir (but above Gore Canyon) (Nehring et al. 2011; Kowalski 2019). It appears that the increased velocity and gradient of the river in the confined reach in Byers Canyon leads to improved invertebrate community below, potentially due to decreased fine sediment, lower cobble embeddedness and lower width to depth ratio (Kowalski and Richer, *in press*).

While previous work identified declines in the range of some species of aquatic invertebrates, the 2018 and 2019 sampling did document the presence of several species of interest at some sites below Windy Gap Reservoir. Salmonflies were sampled with the USGS method at sites CR3, CR4, and CR6 and by the MMI method at sites CR3 and CR6. Densities of salmonflies were low at all sites except CR6. While it was encouraging to document their presence at three of the seven sites, they remain rare or absent immediately below Windy Gap Reservoir. The mayfly *Drunella grandis*, which has declined in range in the Colorado River, was documented at sites CR1, CR4, CR6, and CR7 by the MMI method and CR1, CR3, CR4, CR5, and CR7 by the USGS method. This species was rare or absent at sites immediately below Windy Gap Reservoir in 2010 and it continues to be absent there today. Several other sensitive invertebrate species that were present in the river in the early 1980's before Windy Gap Dam was constructed continue to be absent from sites below the reservoir. Mayflies in the genus *Rhithrogena* were reported pre-construction and are found at downstream sites (CR4-CR7) but not found in 2018 or 2019 at sites immediately below the reservoir. Mayflies in the genus *Heptagenia* were reported at multiple sites in the early 1980s but were absent in 2010, 2018, and 2019. Stoneflies in the genus *Isogenoides* and *Pteronarcella* are also no longer found at sites below Windy Gap Reservoir though they were documented there before construction.

The two sampling methods generally showed similar trends between the sites, but the USGS method always detected more species of invertebrates at each site (Table 5). The CDPHE method samples approximately $1m^2$ while the USGS method samples a total of $0.43\ m^2$. Despite sampling less than half of the streambed area of the CDPHE method, the USGS method identifies more individual insects from each site and those individuals come from a broader spatial area due to the replicate samples. A subsample of 300 individual invertebrates were identified per sample with the CDPHE methods while an average of 1,661 were identified with the USGS method and each replicate was entirely searched for large and rare organisms. Because of the larger number of identified invertebrates, the replicate samples, and the large/rare search of the entire sample, the USGS method appears to do a better job of representing more of the species present at each site. However, considering that the CDPHE method identifies less than 20% of the individual insects per site, it still detects on average 80.9% of the total number of species and 66.1% of the EPT species of the USGS method. Within each method, the same trends were shown between sites and generally the methods produced similar conclusions about aquatic invertebrates at the community level.

The CDPHE method is a superior method for information on Chironomidae and non-insect taxa like Oligochaeta due to the higher level of taxonomic identification for those families. Chironomidae can be useful for evaluating the presence of pollution-tolerant midge species and species level identification of Oligochaetes has utility if concern exists about the secondary host of Salmonid whirling disease *Tubifex tubifex*. The CDPHE method took less time to collect at each site and the sample processing costs were considerably less. The main benefit of the CDPHE method is that it is specifically calibrated to the native invertebrate communities in

Colorado and stratified by stream type. If the sampling objective is to generally characterize invertebrate community health, then the multimetric index of the CDPHE method (MMI) is a cost-efficient tool that also has the weight of regulatory authority behind it. However, the more time and labor intensive USGS method is superior for detecting rare species and giving quantitative density estimates.

Overall, the results of the 2019 benthic sampling reflect the patterns in invertebrate community of the Colorado River presented in previous work (Nehring et al. 2011). Generally, while healthy and diverse invertebrate communities exist above the reservoir 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 surface release main stem impoundment.

Table 5. The number of species of invertebrates collected by the two sampling methods 2019.

	Ephemeroptera		Plecoptera		Trichoptera		Total Taxa	
Site	CDPHE	USGS	CDPHE	USGS	CDPHE	USGS	CDPHE	USGS
CR1	5	11	4	6	6	10	32	41
CR2	4	4	2	3	4	9	23	33
CR3	5	5	3	5	7	10	33	36
CR4	6	10	4	5	8	10	42	43
CR5	3	7	3	4	9	9	33	38
CR6	6	7	4	7	6	12	26	43
CR7	7	8	0	1	4	9	27	33

Fish Sampling

Sculpin were found at a single site on the Colorado River (CR1) above Windy Gap Reservoir (Table 6). At that site there was an estimated density of sculpin of 0.12 per m^2 (95% confidence interval 0.11-0.13). The estimated capture probability of sculpin was 0.769 for the first pass and 0.768 for passes two and three. This density is similar to what has been observed in the Colorado River above the Fraser confluence but lower than the Fraser River itself (J. Ewert, Colorado Parks and Wildlife unpublished data). No sculpin were observed at the three sampling sites below Windy Gap Reservoir despite the sampling of 2,022 m^2 of stream and the capture of 573 other individual small-bodied fish. Extensive sampling near our study sites on the Colorado River for trout fry (multiple pass electrofishing at five sites sampled four times annually) also failed to find sculpin in 2019 below Windy Gap Reservoir (E. Fetherman, Colorado Parks and Wildlife personal communication). These results reflect the pattern of sculpin distribution reported in previous work; this native fish species continues to be absent in formerly occupied habitat in the Colorado River below Windy Gap Reservoir (Erickson 1983; Nehring et al. 2011; Kowalski 2014). Age-0 trout fry were noted to have clinical signs of Whirling Disease at sites CR2 (5.6% of brown trout and 40% of rainbows) and CR3 (100% of rainbows). No clinical signs were observed above Windy Gap or at the most downstream site, the Gerrans SWA.

Table 6. Summary of fish sampled at four monitoring sites on the upper Colorado River in 2019. MTS is sculpin, LOC is Brown Trout, RBT is Rainbow Trout, WHS is White Sucker, LGS is Longnose Sucker, LND is longnose Dace, FMW is Fathead Minnow, and IOD is Iowa Darter.

Site	CR1 Below Fraser	CR2 Hitching Post	CR5 Pioneer Park	CR6 Gerrans SWA
Stream Area Sampled	186.7 m²	528.4 m²	711.8 m²	595.0 m²
Species (# sampled)	MTS (22) LOC (7) RBT (7) IOD (6) WHS (1) LGS (1) FMW (1)	LND (100) LOC (18) RBT (5) WHS (1) LGS (1)	LND (84) LOC (40) LGS(15) WHS(1) RBT (1)	LND (218) LOC (52) LGS(13) WHS (1)

CONCLUSIONS

Fish and aquatic invertebrate sampling results from the upper Colorado River in 2019 reflect the patterns presented in previous work (Nehring et al. 2011; Kowalski 2019). Generally, while healthy and diverse invertebrate communities exist above the reservoir, sites below Windy Gap Reservoir are less diverse, have fewer sensitive species, and are lower in density and diversity of stonefly species. Several sites below Windy Gap Reservoir fall below the state standard for coldwater stream impairment on some years. Several species of disturbance-sensitive aquatic invertebrates that were rare or absent below Windy Gap Reservoir in 2010 were confirmed to be present, although in low numbers and not at all sites. Fish sampling results from 2019 also reflect patterns previously observed in the upper Colorado River, native sculpin continue to be absent from sites below Windy Gap Reservoir while they are common above the reservoir and in tributaries.

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

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RESEARCH PRIORITY

Sculpin Phylogeny and Diversity in Colorado

Coauthored by Michael K. Young, National Genomics Center for Wildlife and Fish Conservation, Missoula, MT.

OBJECTIVE

Use DNA barcoding and other molecular techniques to identify sculpin from Colorado to evaluate divergence within and among lineages, gauge their distribution, and to assess their phylogenetic relatedness to other lineages of sculpin.

INTRODUCTION

Sculpin have long been a taxonomic challenge and uncertainty exists about identity of lineages of sculpins in Colorado (Woodling 1985; Moyle 2002; Kinziger et al. 2005). Sculpin are among the most difficult freshwater fishes to identify based on morphological characteristics (Jenkins and Burkhead 1994), a difficulty compounded by geographic variation in phenotypically diagnostic characters within individual species (Maughan 1978; McPhail 2007). Currently there are two recognized species of sculpin in Colorado, the Mottled Sculpin *Cottus bairdii* and the Paiute Sculpin *C. beldingii*, but the morphological characteristics of those species do not differentiate them and are not diagnostic for identification.

Gill (1862) first described a sculpin from the Colorado River basin as *Potamocottus punctulatus*, which was collected between Bridger Pass and Fort Bridger, Wyoming, likely from the Little Snake or Green River basins. Subsequently, sculpins of this lineage from the Colorado River basin were assigned a variety of generic, species, and subspecies names, and are presently recognized as Mottled Sculpin *C. bairdii*. Neely (2001) argued that *C. bairdii* should be restricted to sculpins from a portion of the Ohio River basin, and that the former members of this taxon in western North America constituted a mixed of named and unrecognized species. He proposed that those from the Colorado River basin be recognized as *C. punctulatus*, the Colorado Sculpin. Other researches have come to the same conclusions that the fish recognized as the Mottled Sculpin in Colorado (and throughout the basin) are not *C. bairdii* (McPhail 2007; Young et al. 2013).

The second species of sculpin recognized from Colorado, *Cottus annae*, was originally described from individuals collected from the Eagle River near Gypsum, Colorado (Jordan 1896). Bailey and Bond (1963) synonymized this species with the Paiute Sculpin *C. beldingii*–originally described from Lake Tahoe, Nevada in the Lahontan Basin (Eigenmann and Eigenmann 1891) but offered no corroborating evidence for this demotion.

The objective of this study was to use DNA barcoding and other molecular techniques to identify specimens of *Cottus* from Colorado, to evaluate divergence within and among lineages, to gauge their distribution, and to assess their phylogenetic relatedness to other lineages of sculpin, especially *C. beldingii* and *C. bairdii* from near their type locations.

METHODS

Aquatic researchers and biologists with Colorado Parks and Wildlife collected 262 specimens from waters across Colorado's western slope from all major river basins. A portion of the upper caudal fin removed from each specimen and stored in alcohol (earlier collections) or affixed to chromatography paper (later collections). Samples were sent to the USFS Rocky Mountain Research Station National Genomics Center for Wildlife and Fish Conservation for genetic analysis. DNA barcoding, the identification of species based on sequences of a portion of a mitochondrial gene, was used to explore the diversity and species identification of Sculpin in Colorado (Hebert et al. 2003, Young et al. 2013). All of the genetic analyses and interpretation was completed by M. K. Young and the USFS National Genomics Center for Wildlife and Fish Conservation, any use or citation of this work should recognize that as the primary source.

RESULTS

A brief summary of the results is presented below but the final report should be consulted for a more detailed presentation of results and discussion (Young et al. 2020).

Overall, the results indicate that there are two lineages of sculpins in Colorado but they are different than the currently recognized species. Rather than Paiute Sculpin and Mottled Sculpin, the fish in Colorado are more appropriately recognized as the Eagle River Sculpin (*C. annae* and the Colorado Sculpin (*C. punctulatus*).

The Eagle River Sculpin is the Colorado member of the *C. beldingii* species complex. It has a more limited distribution in river basins west of the Continental Divide and to date has only been sampled in Colorado. Relative to *C. beldingii*, this lineage was geographically discrete, genetically divergent, and monophyletic, and resurrecting the name *C. annae* for these specimens and rejecting synonymy with *C. beldingii* (Bailey and Bond 1963) appears warranted.

The fish currently referred to as Mottled Sculpin in Colorado is not a true *C. bairdii*, they are members of the *C. hubbsi* species complex are likely *C.* punctulatus, the Colorado Sculpin. These fish are widely distributed in all western Colorado basins as well as in other tributaries to the Colorado River in Utah and Wyoming. The Colorado Sculpin are found in every river basin in western Colorado that was a tributary to the Colorado River. In contrast, *C. annae* did not appear in samples from the San Juan and Green River basins, implying that the extent of its range was the Colorado River basin above the mouth of the Dolores River.

Members of both lineages were sympatric at five sites: the mainstem Dolores River, Dallas Creek (Gunnison River basin), the Eagle River, and two sites in the Crystal River (Colorado River basin). Co-occurrence between these taxa is not recent; Jordan (1896) noted that *C. bairdii punctulatus* was abundant at the type location for *C. annae*, and Shiozawa et al. (2010) detected both groups in samples from the Frying Pan River. Both species spanned a relatively wide elevation range, from 1,555 m in the Gunnison River near the confluence of the North Fork Gunnison River to 2,916 m in the Swan River near Breckenridge.

These conclusions support results of other studies that the most common sculpin in the state is not the Mottled Sculpin, but a currently unrecognized species previously called the Colorado Sculpin. We conclude that the results from this study support resurrecting the names *C. annae* and *C. punctulatus* for the sculpins of Colorado. More work is necessary to explore morphological differences between the two species under this new paradigm to properly describe and identify these species.

This research priority is complete. One summary report related to this work has been produced within this reporting period.

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.

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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 more effectively and efficiently 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

Two papers were published in peer reviewed scientific journals to summarize and disseminate information from the coldwater stream ecology research projects;

Kowalski, D. A. and E. E. Richer. *In Press*. Quantifying the habitat preferences of *Pteronarcys californica* in Colorado rivers. River Research and Applications.

Heinold, B. D., D. A. Kowalski, and R. B. Nehring. 2020. Estimating densities of larval Salmonflies (*Pteronarcys californica*) through multiple pass removal of post-emergent exuvia in Colorado rivers. PLOSONE 15(4): e0227088.

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

Kowalski, D. A. 2019. Colorado River Aquatic Resource Investigations. Federal Aid Project F-237-R26. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Kowalski, D. A. and B. D. Heinold. 2019. Windy Gap invertebrate and sculpin monitoring progress report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Kowalski, D. A., A. Treble, J. Drennan, V. M. Milano, L. Hopper, R. Cordes. 2019. Prevalence and distribution of *R. salmoninarum*, the causative agent of bacterial kidney disease, in Colorado's wild trout and stocked sport fisheries. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

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

Fetherman, E. R., E. Gardunio, D. A. Kowalski, and G. J. Schisler. 2020. Whirling disease resistance in the Gunnison River Rainbow Trout. CPW Conservation Over Virtual Interface Days 2020. Virtual. April 28, 2020.

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