Coldwater Stream Ecology Investigations

Dan A. Kowalski
Aquatic Research Scientist

2022 Progress Report

Colorado Parks and Wildlife

Aquatic Research Section

Fort Collins, Colorado

August 2022

STATE OF COLORADO

Jared Polis, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Dan Gibbs, Executive Director

COLORADO PARKS & WILDLIFE

Heather Dugan, Acting Director

WILDLIFE COMMISSION

AQUATIC RESEARCH STAFF

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

Prepared by: ______________________________________
Dan Kowalski, Aquatic Research Scientist

Approved by: ______________________________________
George J. Schisler, Aquatic Wildlife Research Chief

Date: ___August 31, 2022___________________________

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.

Table of Contents

COLDWATER STREAM ECOLOGY INVESTIGATIONS
PROJECT SUMMARY

Period Covered: July 1, 2021 to June 30, 2022

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

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). On the Colorado River, not only have dams altered the temperature and flow regime of the river, but trans-basin water diversions remove approximately 67% of the annual flow of the 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 different species, the Colorado Sculpin *C. punctulatus* or Eagle River Sculpin *C. annae* (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 2019).

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

In 2021, aquatic invertebrate samples were collected at six standard monitoring sites on the Colorado River (Table 1, Figures 1-2). 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 500µ 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. But, because a standardize area is sampled and specific time limits, relative densities of insects can be calculated. Community indices were calculated according to methods outlined in CDPHE (2020) and Barbour et al. (1999). Six metrics were used to compare sites: total taxa richness, EPT taxa richness (number of Ephemeroptera, Plecoptera, and Trichoptera taxa), Plecoptera richness, relative Plecoptera density, Shannon Diversity Index (SDI), and the MMI.

Table 1. Aquatic invertebrate sampling sites on the Colorado River in 2021.

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

Figure 1. Map of the upper benthic macroinvertebrate sampling sites on the Colorado River in 2020.

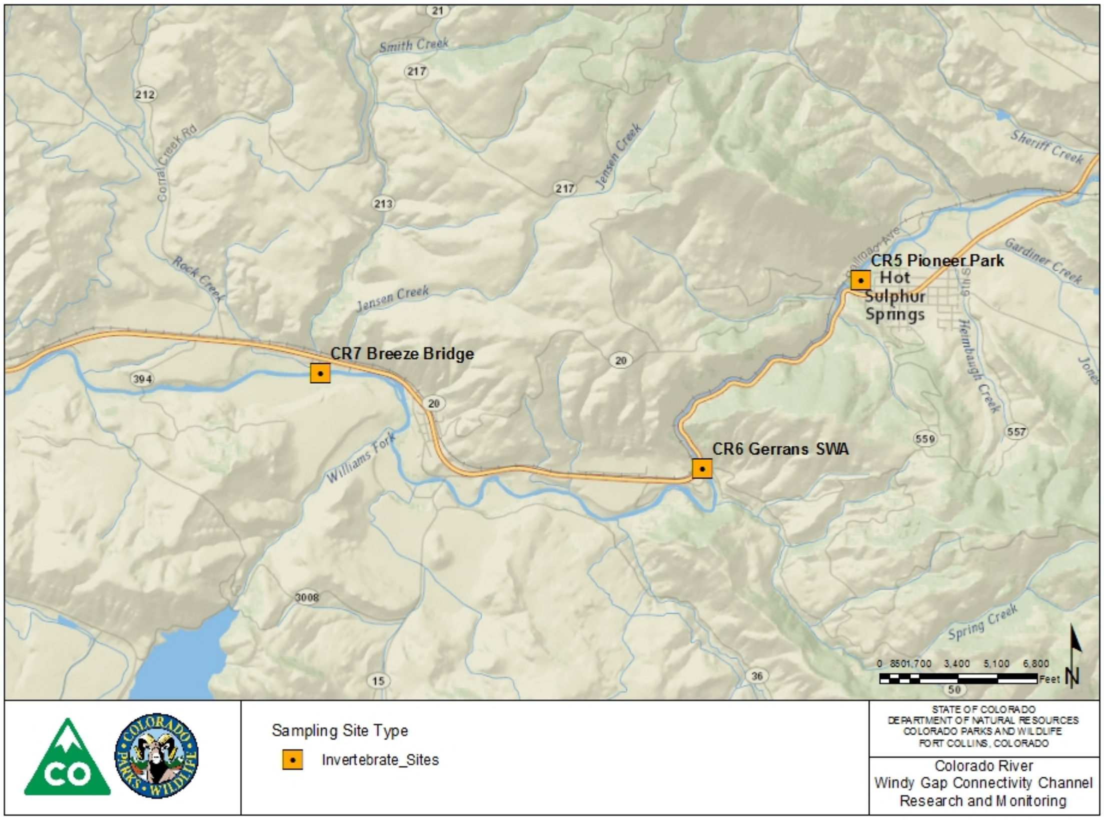

Figure 2. Map of the lower benthic macroinvertebrate sampling sites on the Colorado River in 2020.

The MMI is the multimetric index that is the standard regulatory method used by the state of Colorado to determine stream impairment under 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. When compared with more intensive and fully quantitative approaches, the MMI was found to be efficient, cost-effective, and superior method 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 (Kowalski 2019). However, more intensive fully quantitative methods were superior for detecting rare species, fully characterizing the diversity at each site, and giving true density estimates.

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).

RESULTS

Results of the invertebrate sampling in 2021 produced some similar trends as previous years' results as well as some new spatial patterns (Table 2). The invertebrate community is generally less healthy and diverse as moving downstream from the confluence of the Fraser and Colorado River. The mean MMI score below Windy Gap Reservoir is about the same as above (62.5 vs. 62.1) and sites below WGR had on average slightly more EPT taxa. In 2021, the Hitching Post site CR2 had the highest total taxa richness, EPT taxa richness, and highest MMI score of all the sites sampled. This site previously was one of the least diverse sites on the river and consistently has had the lowest MMI scores as well as all other community health indices. Plecoptera relative density and Shannon Diversity were both lower at this site than above, but there still appears to be a positive trend in some aspects of the invertebrate community in recent sampling.

Table 2. Community metrics and index scores for invertebrate sampling sites on the Colorado and Gunnison Rivers in 2021.

Community Metrics	CR1	CR2	CR3	CR5	CR6	CR7	Ave. Below WGR
Total Taxa Richness	28	29	23	21	21	20	22.8
EPT Taxa Richness	11	15	9	14	11	10	11.8
Plecoptera Richness	4	4	1	1	1	1	1.6
Relative Plecoptera Density	300	195	90	15	15	15	66.0
SDI	3.6	3.0	2.7	3.1	2.4	2.2	2.7
MMI	62.1	71.6	58.5	63.9	58.2	60.2	62.5

The positive community diversity trends at the Hitching Post Bridge site were a result of an increase in EPT taxa richness driven by Plecoptera species. Four stonefly species (Chloroperlidae, *Isoperla fulva*, *Claassenia sabulosa*, and *Skwala americana*) were found where previously only one or two species were present 2018-2020. No *P. californica* or *Pteronarcella badia* have been sampled at this site since 2018 despite being present before Windy Gap Reservoir. Other sampling methods, such as exuvia surveys during emergence, designed to detect rare invertebrates, have confirmed that *P. californica* at this site in very low densities, whereas they are rarely found using the standard MMI sampling protocol.

Interestingly, this improvement in invertebrate community diversity appears restricted to this site as most of the other sites downstream have generally been stable or declining in community diversity indices (Figure 3). This is likely due to changes in reservoir operations at Windy Gap in

2020 and 2021. In preparation for construction of the bypass channel, Windy Gap has been drained each fall for preconstruction work (Figure 4). This has likely had some major ecological effects on the river below the reservoir. The drawdown created a more natural stream channel through the bed of the reservoir and reconnected the river above and below Windy Gap. Evidence for the temporary reconnection of the river includes documented fish movement both upstream and down through the reservoir channel during the drawdown and when the dam's auxiliary gate was open. Downstream dispersal of aquatic invertebrates was also likely during this time and may explain the increase species richness at the Hitching Post site in 2020 and 2021.

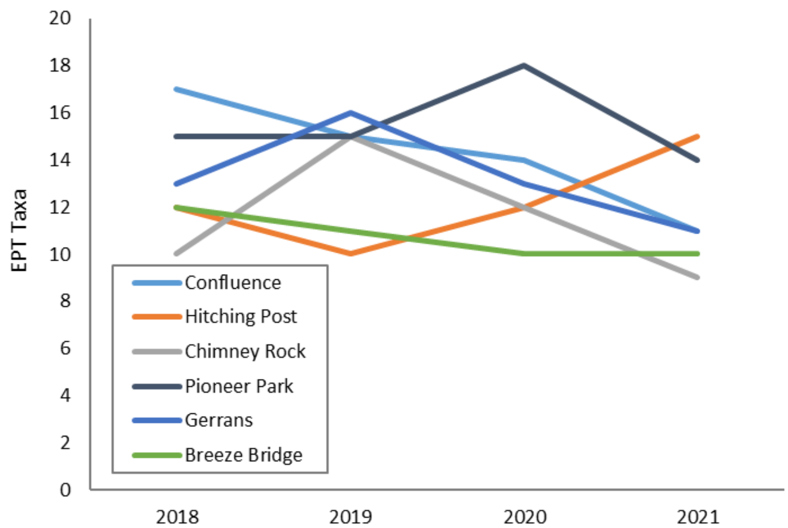

Figure 3. EPT taxa richness of invertebrate sampling sites on the Colorado River 2018-2021.

While previous work identified declines in the range of some species of aquatic invertebrates, the 2021 sampling did document the presence of several species of interest at sites below Windy Gap Reservoir. Salmonflies were sampled at site CR6 but 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 CR7. 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 2021. Mayflies in the genus *Rhithrogena* were present in the river before reservoir construction and were documented in 2020 at sites several sites, but were not found at any sites in 2021. Mayflies in the genus *Heptagenia* were reported at multiple sites in the early 1980s, but were absent at all sites in 2010-2021. 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 and are occasionally sampled above the reservoir.

Site CR6, the Gerrans Unit of the Hot Sulphur Springs SWA, was the only site sampled in 2021 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 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 related to decreased fine sediment, larger median cobble size, lower cobble embeddedness, and lower width to depth ratio (Kowalski and Richer 2020).

Figure 4. Windy Gap Reservoir in September 2020. The reservoir was drained in the fall of 2020 and 2021 leaving a remnant river channel passing through the bed of the reservoir potentially reconnecting the river for some time and allowing passage of fish and invertebrates.

Overall, the results of the 2021 benthic sampling reflect the patterns in invertebrate community of the Colorado River presented in previous work (Nehring et al. 2011; Kowalski 2019) but with some interesting new patterns. Generally, while healthy and diverse invertebrate communities exist above the reservoir and at some sites downstream, most sites below Windy Gap Reservoir are less diverse, have lower numbers of sensitive species, and are lower in the density and diversity of stonefly species. 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 (Nehring et al. 2011; Kowalski and Richer 2020). Recent changes in reservoir operations show some promising trends and bode well for an improvement in the invertebrate community after the river is reconnect with a bypass channel.

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. 2019. 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

Habitat Preferences of the Stonefly *Pteronarcys californica* and Factors Related to Declines in Range

OBJECTIVE

Investigate the habitat use of the salmonfly *Pteronarcys californica* in Colorado rivers and explore the factors related to their decline.

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 (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). 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 by wildlife agencies and non-governmental conservation groups. 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 is to document the density and rage of *P. californica*, measure physical habitat variables related to their distribution in rivers in Colorado and Utah, and explore factors related to declines in rage and number in the large rivers of the Rocky Mountains.

PROGRESS

The first phase of this project was completed in 2020 (Richer and Kowalski 2020). The publication of that paper led to a collaboration with researchers at the University of Montana in Missoula investigating the disappearance of salmonflies from rivers in Utah, Montana, and Colorado. The second phase of this project began in 2021 where a larger scale project that intends to build upon what was learned earlier on the Colorado, Rio Grande, and Gunnison Rivers. The objective was to more directly investigate sites where salmonflies have been extirpated and explore reasons for the declines in range. The Gunnison and Colorado Rivers will be sampled to compare salmonfly densities, habitat features, stonefly diet preferences, water temperature, and flow regime of sites where salmonflies have been extirpated as well as sites that support healthy densities of salmonflies. The study will address two of the largest documented range declines on salmonflies in the state; the Gunnison River above the Black Canyon and Blue Mesa Reservoir and the upper Colorado River below Windy Gap Reservoir. Fieldwork for this phase began in 2022 and will continue through 2023.

REFERENCES

Anderson, H. E., Albertson, L. K., and Walters, D. M. 2019. Water temperature drives variability in salmonfly abundance, emergence timing, and body size. River Research and Applications, 35(1), 1–2.

Barbour, M. T., Gerritsen, J., Snyder, B. D., and Stribling, J. B. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish (second edn.). EPA 841– (pp. D–97–D–002). Washington, DC: United States Environmental Protection Agency, Office of Water.

Baxter, C. V., Fausch, K. D., and Carl Saunders, W. C. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology, 50(2), 201–220.

Benzel, L. 2016. Arkansas River: Restoration of Giant salmonflies (*Pteronarcys californica*) hasn't had the hoped for results - yet. Coyote Gulch. Retrieved from https://coyotegulch.blog. Aug 22, 2016.

Birrell, J. H., Meek, J.B., and Nelson, C.R. 2019. Decline of the giant salmonfly *Pteronarcys californica* Newport, 1848 (Plecoptera: Pteronarcyidae) in the Provo River, Utah, USA. Illiesia, 15, 83–97.

Box, G. E. P., and Cox, D. R. 1964. An analysis of transformations. Journal of the Royal Statistical Society: Series B, 26(2), 211–243.

Bryce, S. A., Lomnicky, G. A., and Kaufmann, P. R. 2010. Protecting sediment-sensitive aquatic species in mountain streams through the application of biologically based streambed sediment criteria. Journal of the North American Benthological Society, 29(2), 657–672.

Colborn T. 1986. The use of the stonefly, *Pteronarcys californica* Newport, as a measure of bioavailable cadmium in a high-altitude river system, Gunnison County, Colorado (Doctoral dissertation, University of Wisconsin, Madison). Retrieved from https://search.proquest.com/docview/16358129?accountid=150414

DeWalt, R. E., and Kondratieff, B. C. 2019. Chapter 16- Plecoptera. In R. Merritt, K. Cummins, and M. Berg (Eds.), An introduction to the aquatic insects of North America (pp. 429-520). Dubuque, IA: Kendall Hunt Publishing, Co.

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

DuBois, R. B. 2015: Detection probabilities and sampling rates for Anisoptera exuviae along river banks: influences of bank vegetation type, prior precipitation, and exuviae size, International Journal of Odonatology, 18(3), 205-215.

Elder, J. A., and Gaufin, A. R. 1973. Notes on the occurrence and distribution of *Pteronarcys californica* Newport (Plecoptera) within streams. Great Basin Naturalist, 33, 218–220. Retrieved from https://scholarsarchive.byu.edu/gbn/vol33/iss4/2

Freilich, J. E. 1991. Movement patterns and ecology of Pteronarcys nymphs (Plecoptera): observations of marked individuals in a Rocky Mountain stream. Freshwater Biology, 25(2), 379–394.

Heinold, B. D., Kowalski, D. A., and Nehring, R. B. 2020) Estimating densities of larval Salmonflies (*Pteronarcys californica*) through multiple pass removal of post-emergent exuvia in Colorado rivers. PLOSONE, 15, 1-10.

Kowalski, D. A. 2015. Colorado River aquatic resources investigations (Federal Aid in Sportfish Restoration Project F-237R-22). Fort Collins, CO: Colorado Parks and Wildlife.

Kowalski, D. A. 2019. Colorado River aquatic resources investigations (Federal Aid in Sportfish Restoration Project F-237R-26). Fort Collins, CO: Colorado Parks and Wildlife.

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. 1987. Stream fisheries investigations (Federal Aid in Sportfish Restoration Project F-237R-18). Fort Collins, CO: Colorado Division of Wildlife.

Nehring, R. B., Heinold, B. D., and Pomeranz, J. 2011. Colorado River aquatic resources investigations (Federal Aid in Sportfish Restoration Project F-237R-18). Fort Collins, CO: Colorado Division of Wildlife.

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

Stagliano, D. M. 2010. Evaluation of salmonflies in Montana's rivers: are statewide populations really declining? Retrieved from http://mtnhp.org/reports/ZOO_MT_Salmonfly_2010.pdf.

Vinson, M. R. 2011. Returning salmonflies to the Logan River. In J. L. Li and M. T. Barbour (Eds.), Wading for Bugs: exploring streams with the experts (pp. 29–33). Corvallis, OR: Oregon State University Press.

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

RESEARCH PRIORITY

Sculpin Phylogeny, Diversity, and Morphology in Colorado

Coauthored by Michael K. Young, National Genomics Center for Wildlife and Fish Conservation, Missoula, MT. Results are summarized from Young et al. (2020) and Young et al. (2022).

OBJECTIVE

Use molecular techniques to identify sculpin from Colorado to evaluate diversity within and between species, document their distribution, and to assess their phylogenetic relatedness to other lineages of sculpin. Compare morphological and meristic characters of sculpin in Colorado to identify distinctive characters and evaluate the physical differences among sculpin in Colorado.

INTRODUCTION

There has long been taxonomic uncertainty about the 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. Colorado Parks and Wildlife biologists and researchers have long suspected that sculpin in Colorado do not morphologically align with the described type specimens of Mottled Sculpin and Paiute Sculpin and recent publications have supported that hypothesis.

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, Young et al. 2022).

The second species of sculpin recognized from Colorado, *C. annae*, was originally described from individuals collected from the Eagle River near Gypsum, Colorado (Jordan 1896). With little justification, Bailey and Bond (1963) synonymized this species with the Paiute Sculpin *C.*

beldingii which was originally described from Lake Tahoe, Nevada (Eigenmann and Eigenmann 1891).

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, and to assess their phylogenetic relatedness to other lineages of sculpin, especially *C. beldingii* and *C. bairdii* from near their type locations. The secondary objective was to compare lineages of sculpin in Colorado to explore any morphological or meristic difference between them.

PROGRESS

The first phase of this project was completed in 2020 (Young et al. 2020; Young et al. 2022). The second phase of this project will begin in 2022.

In the first phase of the project, Colorado Parks and Wildlife biologists and researches sampled 262 specimens from 93 waters around the western slope of Colorado. These specimens were sent to the U.S. Forest Service National Genomics Center for Wildlife and Fish Conservation as part of a larger study of *Cottus* species across the west (Young et al. 2022).

Phylogenetic analyses based on DNA barcoding placed the Colorado specimens in two primary lineages. One lineage (referred to here as *C. punctulatus*) is currently called Mottled Sculpin *C. bairdii* but is notably divergent from that taxon. Mottled Sculpin from eastern North America was a highly supported lineage that differed substantially (mean pairwise distance, 2.1%) from a primarily western group found in the Great Basin, Colorado, and Columbia River. Pairwise distances of this size are generally indicative of differences between full species (Ward 2009). The second lineage in Colorado (referred to here as *C. annae*) was unambiguously affiliated with the *C. beldingii* species complex, particularly those in Nevada, Idaho, Utah, and Wyoming, but was divergent from *C. beldingii*. The Colorado member of the Paiute Sculpin group was found to be geographically discrete, genetically divergent, and monophyletic and is likely and unique species endemic to Colorado.

Specimens of *C. punctulatus* were more widely distributed than those from *C. annae* in Colorado (Figures 5 and 6). The fish previously referred to as Mottled Sculpin, now thought to be *C. punctulatus*, were found in every river basin in western Colorado that was a tributary to the Colorado River. In contrast, *C. annae* was not found in samples from the San Juan and Green River basins in Northern Colorado, implying that the extent of its range was the Colorado River basin above the mouth of the Dolores River. It is currently unknown if the range extends to parts of the Dolores River basin in Utah on the eastern side of the La Sal Mountains, but *C. punctulatus* was present in La Sal Creek near Paradox, Colorado.

The two sculpin lineages were found to be sympatric in the main-stem Dolores River, Dallas Creek (Gunnison River basin), the Eagle River, and the Crystal River. The co-occurrence of these taxa has been reported before; Jordan (1896) noted that *C. bairdii punctulatus* was abundant at the type location for *C. annae*. More recently, Shiozawa et al. (2010) detected both groups in samples from the Frying Pan River.

Interestingly, the distribution of *C. annae* is equivalent to that of the “green” lineage of Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus* and the range *C. punctulatus* is the same as “blue” lineage of Colorado River cutthroat trout (Bestgen et al. 2019). Because these species complexes share similar ranges, their distribution implies that *C. annae* and “green” lineage cutthroats may have established in Colorado at a similar place and time, in a way that differed from *C. punctulatus* and “blue” lineage cutthroats.

Overall, these results demonstrate that there are two distinct lineages of sculpins in Colorado and they are different from what they are currently identified as. We conclude that these findings can form a basis for resurrecting the names Eagle River Sculpin *C. annae* and Colorado Sculpin *C. punctulatus* for the sculpins of Colorado, and for adding to the recognized diversity of aquatic species in the West.

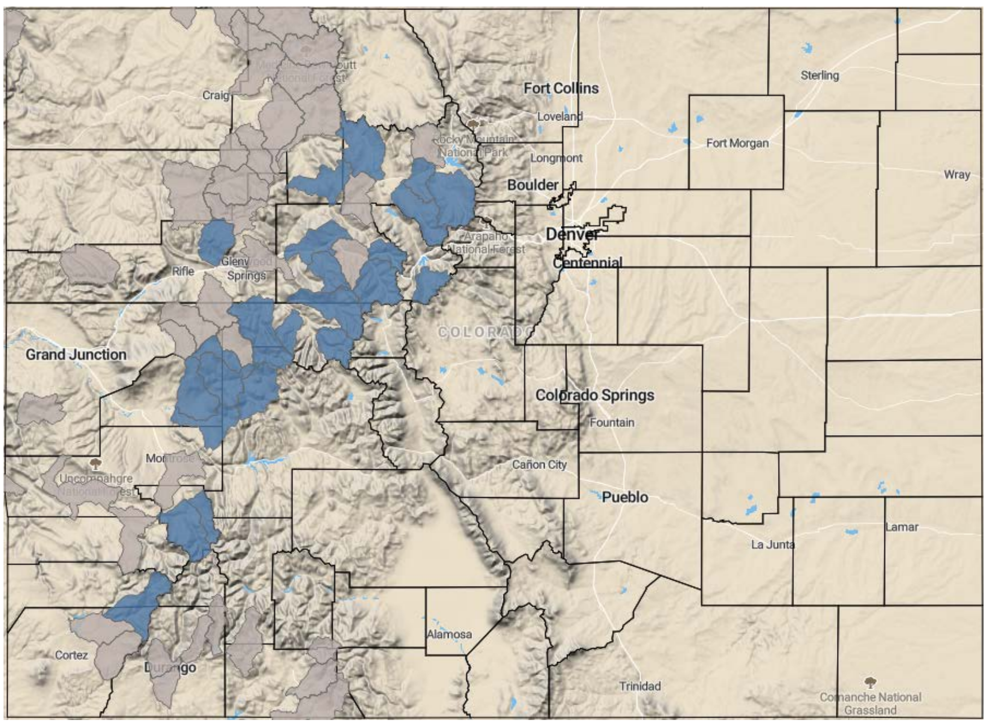

Figure 5. Distribution of *C. annae* (formerly thought to be Paiute Sculpin) in Colorado. Map created by A. Treble, CPW.

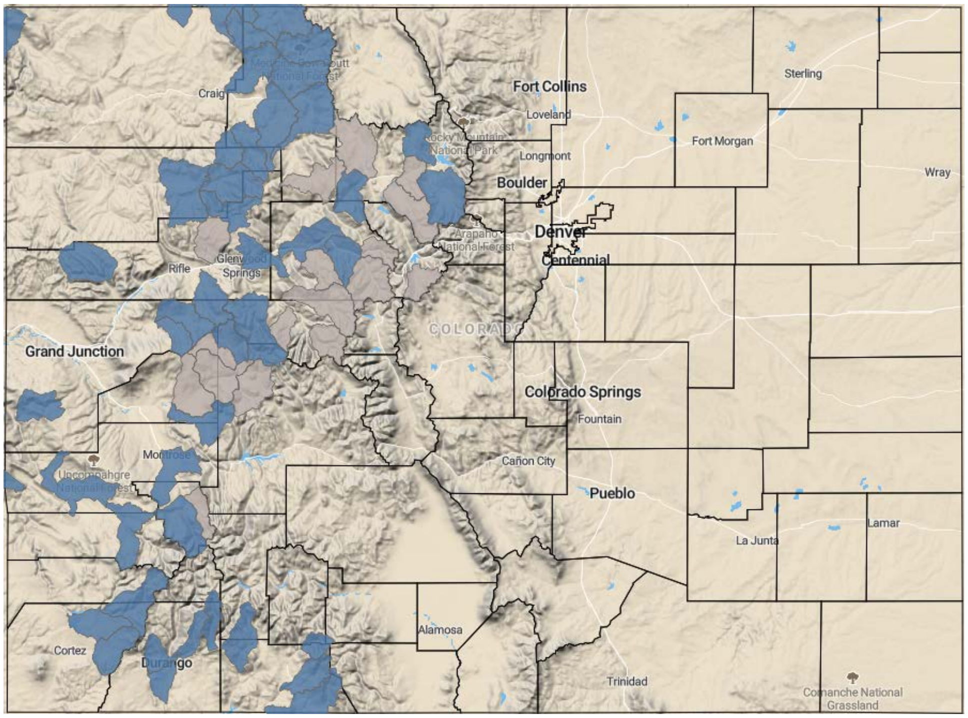

Figure 6. Distribution of *C. punctulatus* (formerly thought to be Mottled Sculpin) in Colorado. Map created by A. Treble, CPW.

REFERENCES

Bailey, R. M. and C. E. Bond. 1963. Four new species of freshwater sculpins, genus *Cottus*, from western North America. Occasional Papers of the Museum of Zoology University of Michigan, 634.

Bestgen, K. R., Rogers, K. B., and Granger, R. 2019. Distinct phenotypes of native cutthroat trout emerge under a molecular model of lineage distributions. Transactions of the American Fisheries Society 148:442-463.

Gill, T. 1862. Observations on the genus *Cottus*, and descriptions of two new species (abridged from the forthcoming report of Capt. J. H. Simpson). Proceedings of the Boston Society of Natural History, 8:40-42.

Hebert P. D., Ratnasingham S., De Waard J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270:S96-S99.

Jenkins R. E., Burkhead N. M. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland. 1080 p.

Jordan, D. S. 1896. Notes on fishes little known or new to science. Proceedings of the California

Academy of Sciences, 6:201-244.

Kinziger, A. P., R. M. Wood, and D. A. Neely. 2005. Molecular systematics of the genus *Cottus* (Scorpaeniformes: Cottidae). Copeia 2005(2): 303-311.

Maughan, O. E. 1978. Morphometry of sculpins (*Cottus*) in the Clearwater drainage, Idaho. Great
Basin Naturalist, 38:115-122.

McPhail, J. D. 2007. The freshwater fishes of British Columbia. University of Alberta Press, Edmonton.

Moyle, P. B. 2002. Inland fishes of California: revised and expanded. University of California Press, Berkeley, CA.

Neely, D. A. 2001. A systematic and taxonomic reassessment of the mottled sculpin species complex, *Cottus bairdii* Girard (Teleostei: Cottidae). Doctoral dissertation, University of Alabama, Tuscaloosa.

Shiozawa, D. K., P. Unmack, and R. P. Evans. 2010. The separation of *Cottus bairdii* and *Cottus beldingii* from 20 locations in the Colorado River Basin in western Colorado. Report to Jenn Logan, Colorado Division of Wildlife.

Ward, R. D. 2009. DNA barcode divergence among species and genera of birds and fishes. Molecular Ecology Resources 9:1077-1085.

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

Young, M. K., R. Smith, K. L. Pilgrim, D. J. Isaak, K. S. McKelvey, S. Parkes, J. Egge, and M. K. Schwartz. 2022. A molecular taxonomy of *Cottus* in western North America. Western North American Naturalist, 82:307-345.

Young, M. K., R. Smith, and K. L. Pilgrim. 2020. A molecular taxonomy of *Cottus* from the Colorado River basin in Colorado and adjacent regions. Special Report to Colorado Parks and Wildlife. National Genomics Center for Wildlife and Fish Conservation Missoula, MT.

Young, M. K., K. S. McKelvey, K. L. Pilgrim, and M. K. Schwartz. 2013. DNA barcoding at riverscape scales: assessing biodiversity among fishes of the genus *Cottus* (Teleostei) in northern Rocky Mountain streams. Molecular Ecology Resources 13:583-595.

RESEARCH PRIORITY

Cow Creek Pre-Impoundment Studies (Technical Assistance)

OBJECTIVE

Collect baseline data on the aquatic invertebrate community of Cow Creek and the Uncompahgre River

INTRODUCTION

Cow Creek is a small mountain stream in the Uncompahgre River drainage in southwest Colorado. It has a drainage area of 46 square miles and a mean annual discharge of 62 cfs. Cow Creek is a high elevation drainage (mean 10,721 ft.) in the San Juan Mountains and is generally undeveloped with much of the watershed protected as federally designated wilderness areas managed by the U.S. Forest Service. In addition to its wilderness designation, the upper end of Cow Creek and its tributaries including Wetterhorn and Wildhorse Creek were found to contain "outstandingly remarkable values" by the U.S. Forest Service and to be eligible for designation under the Wild and Scenic Rivers Act.

Immediately downstream from the Uncompahgre Wilderness boundary there is a conditional water right for a 235 surface acre reservoir near Ramshorn Ridge. Recent water rights filings indicate the proponents of the reservoir may intend to pursue plans for the reservoir in the future. Cow Creek is known to support native Colorado River Cutthroat Trout (*Oncorhynchus clarki pleuriticus*) as well as a robust population of native Eagle River Sculpin (*Cottus annae*) and low to moderate numbers of native Bluehead Suckers (*Catostomus discobolus*) in its lower end. But, very little sampling has occurred of the aquatic invertebrate community and the fish sampling has been sporadic and not widespread in the drainage due to the extreme remoteness of this wilderness stream.

The objective of this project is to collect baseline data on the aquatic invertebrate and fish community of Cow Creek and the Uncompahgre River.

METHODS

Aquatic invertebrate samples were collected at three sites on Cow Creek and two sites on the Uncompahgre River in 2021 (Table 3). 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 500µ 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.

At the most downstream site on Billy Creek State Wildlife area, three replicate samples were taken from the same riffle to evaluate the spatial variability of the invertebrate community and the MMI subsampling procedures and to inform best practices for future sampling.

Table 3. Aquatic invertebrate sampling sites on Cow Creek and the Uncompahgre River in 2021.

Site #	Site Name	UTM East	UTM North
CC#1	Beckett SWA	259883	4236265
CC#2	Ramshorn	268788	4224643
CC#3	Courthouse	268971	4224382
Unc#1	Pacochupuk	258128	4236539
Unc#2	Billy Creek 1	258325	4240646
Unc#3	Billy Creek 2	258325	4240646
Unc#4	Billy Creek 3	258325	4240646

RESULTS

Cow Creek supports a relative healthy and diverse aquatic invertebrate community that is generally healthier than the Uncompahgre River below it based on the MMI scores (Table 4). One of the replicate sites on the Uncompahgre River on Billy Creek State Wildlife Area also shows a high number of taxa, good diversity of EPT taxa and a high MMI score.

The variability of the replicate samples on Billy Creek was generally moderate or low depending on the metric. Depending on the resolution of the data needed or the importance of community diversity indices, doing replicate samples could be advantageous. Previous work has found that while the MMI sampling protocol is and efficient and useful technique that generally does a good job of characterizing the invertebrate community, spatial replication of samples and a fully quantitative methods can be superior depending on the objectives of the sampling (Kowalski 2020).

Table 4. Aquatic invertebrate sampling sites on Cow Creek and the Uncompahgre River in 2021. The final column is the coefficient of variation of the three replicate samples from the same riffle on Billy Creek SWA.

Community Metrics	CC#1	CC#2	CC#3	Unc#1	Unc#2	Unc#3	Unc#4	Unc#2-4 C.V.
Total Taxa Richness	26	21	18	20	20	24	27	0.06
EPT Taxa Richness	11	14	12	12	11	13	14	0.04
Plecoptera Richness	2	4	3	1	3	5	5	0.07
SDI	3.4	2.9	2.6	2.4	2.1	1.8	2.9	0.19
MMI	62.4	67.0	60.2	57.5	51.5	55.5	61.4	0.05

REFERENCES

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.

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.

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

Two new technical assistance projects were started in 2021-2022; the Cow Creek Pre-impoundment Studies and the Tailwater Trout Diet Study. The first technical assistance project was to collect baseline aquatic invertebrate data on Cow Creek in southwestern Colorado. A new mainstream dam is being proposed this small tributary to the Uncompahgre River between Ridgway and Montrose, Colorado. Three sites on Cow Creek were sampled above and below the proposed reservoir site as well as two sites on the Uncompahgre River above and below the Cow Creek confluence.

The second new technical assistance project was a cooperative project with USGS scientists from Arizona and Colorado. A large data request was received from USGS scientists for fish population data on many of Colorado’s premier tailwater trout fisheries. Responding to that request began a collaboration to improve the project, aid in selection of sampling sites, and to improve the understanding of CPW fish data. This project will benefit the state of Colorado with an improved understanding of how flow and temperature influence tailwater trout fisheries, give specific information on the diet habitats of trout in the tailwater fisheries and how it relates to available drifting invertebrates, and how future climate change may affect the temperature regime of these fisheries.

The objective of this study is to investigate the diet habits of Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*) in tailwater fisheries and predict how they will respond to climate change and future water storage decisions. By providing insights into how flow and temperatures influence trout populations, our approach will be useful in evaluating current dam operations, ongoing drought impacts, and mitigating the future impact of climate change on important tailwater fisheries in Colorado.

COMMUNICATION AND INFORMATION TRANSFER

Two papers were published in a peer-reviewed journal to summarize and disseminate information from stream ecology research projects:

Kowalski, D. A., R. J. Cordes, T. B. Riepe, J. D. Drennan, A. J. Treble. 2022. Prevalence and distribution of *Renibacterium salmoninarum*, causative agent of bacterial kidney disease, in wild trout fisheries in Colorado. Diseases of Aquatic Organisms 149:109-120.

Kowalski, D. A., E. I. Gardunio, and C.A. Garvey. 2022. Evaluating the effects of an electric barrier on fish entrainment in an irrigation canal in Colorado. River Research and Applications 38: 539–547.

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

Kowalski, D. A. 2021. Colorado coldwater stream ecology investigations progress report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

Kowalski, D. A., R. J. Cordes, T. B. Riepe, J. D. Drennan, A. J. Treble. 2022. Prevalence and Distribution of *Renibacterium salmoninarum*, the Causative Agent of Bacterial Kidney Disease, in Wild Trout Fisheries in Colorado. Proceedings of the Wild Trout Symposium. West Yellowstone, MT.

Two external presentations were contributed to for dissemination of results of aquatic ecology projects to colleagues and other fishery professionals:

Fetherman, E. R., E. E. Richer, M. C. Kondratieff, J. Ewert, and D. A Kowalski. 2022. Update on Fraser River Ranch salmonid population and the Windy Gap fish movement study. Fraser River Ranch Fishing Club Party. Lakewood, Colorado. March 30, 2022.

Kondratieff, M. C., K. R. Bakich, E. E. Richer, D. A. Kowalski, B. F. Atkinson. 2021. A guidance document for reviewing whitewater park projects. 151st Annual Meeting of the American Fisheries Society. Baltimore, MD. November 10, 2021.