

Coldwater Stream Ecology Investigations

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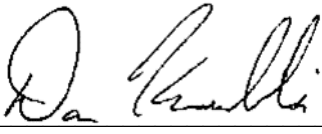
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COLDWATER STREAM ECOLOGY INVESTIGATIONS

PROJECT SUMMARY

Period Covered: July 1, 2023 to June 30, 2024

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 Firing 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 Mottled Sculpin *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 feet 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, 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 will 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 Colorado River Connectivity Channel (CRCC) 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.

PROGRESS

Construction activities began on the CRCC in 2022 and the official groundbreaking occurred on August 23, 2023 (Figures 5-6). After more than four decades of impeded fish passage and alteration of the natural flow and sediment regimes, water flowed into the bypass channel on October 25, 2023 and the upper Colorado River was re-connected. Northern Water Conservancy District anticipates completion of channel including revegetation work to conclude in 2024 and official post project invertebrate and sculpin sampling will begin in 2025. All pre-project invertebrate and sculpin sampling was completed in 2018-2021, earlier progress reports contain summary of pre-project invertebrate data (Kowalski 2019; Kowalski 2022).

METHODS

Pre-project aquatic invertebrate samples were taken at six sites on the Colorado River in 2018-2021 and fish sampling occurred at four sites (Table 1, Figures 1-2). 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 natural riffles at each site.

The USGS method involved taking five replicate macroinvertebrate samples at each site using a 0.086 m² Hess sampler with a 350 µm mesh net. Because a known and exact area of stream bottom is sampled by the Hess sampler, true density estimates can be made. Macroinvertebrate samples were sorted and sub-sampled in the laboratory using a standard USGS 300-count protocol, except that replicates were not composited (Moulton et al. 2000). Approximately 300 individual organisms were identified from each replicate and a 15-minute search for large or rare organisms was conducted on the entire sample. All organisms, except for chironomids and non-insects, were identified to genus or species. Chironomids were identified to family and non-insects were identified to class. Each replicate sample was processed separately so that more individual specimens were identified from each site to ensure rare organisms 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 an independent taxonomist (Moulton et al. 2000).

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). 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. Processing the MMI samples involves subsampling and identifying 300 individual organisms from the entire sample, including chironomids to species.

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).

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. Because a standardize area is sampled and specific time limits, relative densities of insects can be calculated.

Table 1. Aquatic invertebrate sampling sites on the Colorado River 2018-2021.

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.

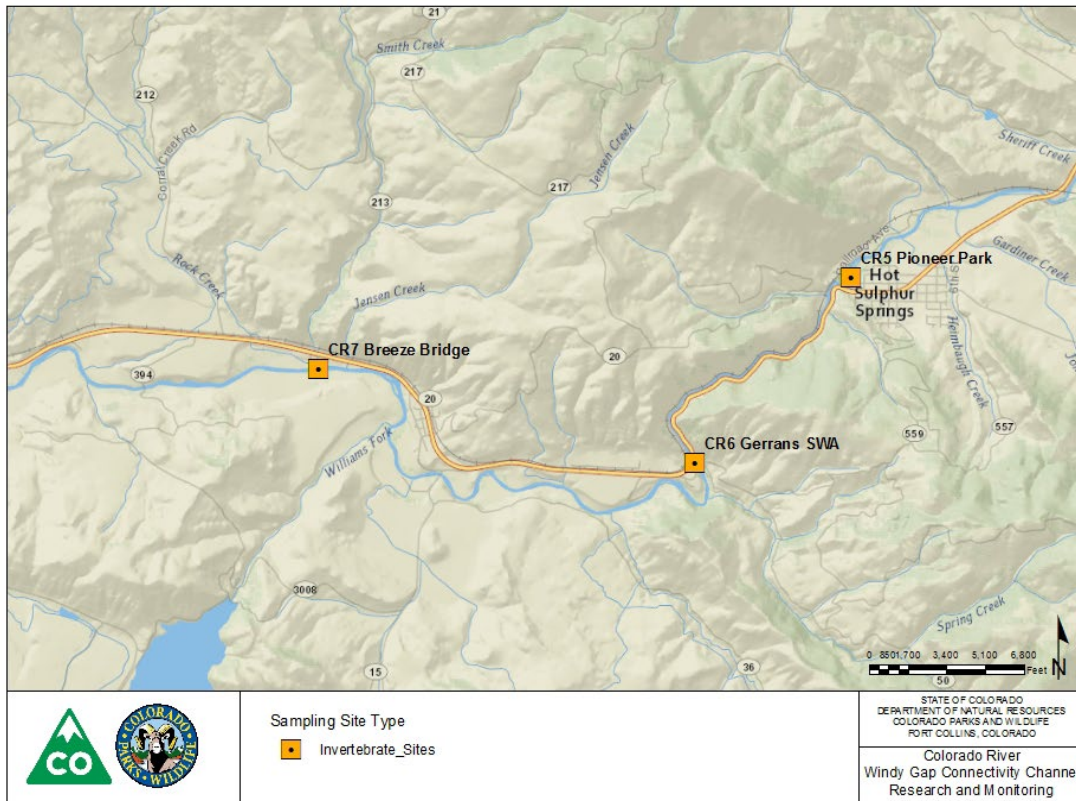


Figure 2. Map of the lower benthic macroinvertebrate sampling sites on the Colorado River in 2020.

RESULTS AND DISCUSSION

Fish and aquatic invertebrate sampling results from the upper Colorado River 2018-2021 reflect the patterns presented in previous work (Nehring et al. 2011; Kowalski 2019). Generally, while healthy and diverse invertebrate communities exist upstream of the reservoir, sites downstream of Windy Gap Reservoir are less diverse, have fewer sensitive species, and are lower in density and diversity of stonefly species (Fig 3). Several sites below Windy Gap Reservoir fall below the state standard for coldwater stream impairment on some years. Fish sampling results from 2018-2021 also reflect patterns previously observed in the upper Colorado River, native Mottled Sculpin continue to be absent from sites below Windy Gap Reservoir while they are common above the reservoir and in tributary streams

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.

Interestingly, there has been an improvement in invertebrate community diversity at the Hitching Post site immediately below WGR in 2020 and 2021. This improvement appears to be restricted to this site, as most of the other sites downstream have generally been stable or declining in community diversity indices (Figure 3). 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.

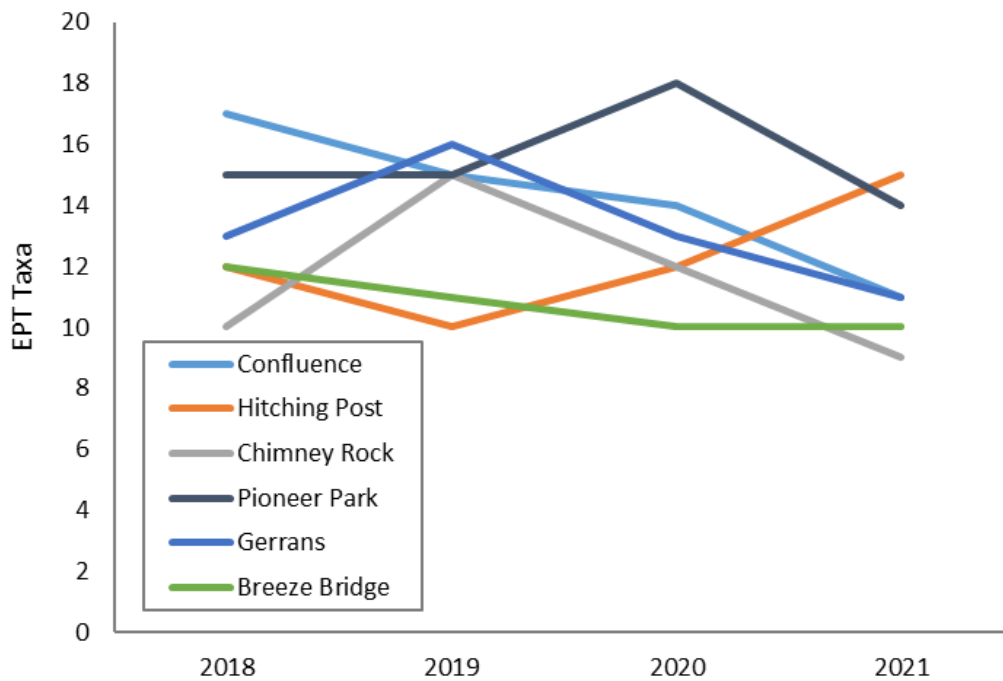


Figure 3. EPT taxa richness of invertebrate sampling sites on the Colorado River 2018-2021.



Figure 4. Windy Gap Reservoir in September 2020. The reservoir was drained in the fall of 2020



Figure 5. The Colorado Connectivity Channel looking upstream at the new diversion into Windy Gap in October 2023 re-connecting the upper Colorado River.



Figure 6. The Colorado Connectivity Channel looking downstream through the former bed of Windy Gap Reservoir.

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.

The improvement of the invertebrate community at the Hitching Post site is likely related to changes in reservoir operations at Windy Gap 2019-2023. In preparation for construction of the CRCC, Windy Gap was drained each fall for during preparation and construction 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.

Overall, the results of benthic sampling in the Upper Colorado River 2018-2021 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 upstream of 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.

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RESEARCH PRIORITY

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

Coauthor: Jackson Birrell, Graduate Research Associate, University of Montana, Missoula, Montana.

OBJECTIVE

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

INTRODUCTION

Giant Salmonflies, *Pteronarcys californica*, are among the largest of all stoneflies (Insecta: Plecoptera) and are endemic to Western North America. Salmonflies frequently occur in high densities ($> 400 \text{ m}^{-2}$) in mid-sized mountain streams and are often a major component of in-stream biomass (Nehring et al. 2011). They play a key role in nutrient cycling as shredders of leaf material (DeWalt and Kondratieff 2019; Vannote et al. 1980), provide an important food resource for trout populations (Nehring 1987), and transfer massive amounts of carbon to terrestrial systems during their large, synchronous emergences (Walters et al. 2014). Giant Salmonflies are sensitive to human disturbances and are used as bioindicators of river health (Barbour et al. 1999). They are also recreationally important to anglers because of the quality of the fishing during their emergence. Despite their ecological and cultural importance, reduction in the range and density of *P. californica* populations have been observed across the western United States.

Salmonfly declines have been reported in at least 10 rivers in the western U.S. They have been lost from $>550 \text{ km}$ of river in Montana, including reaches on the Madison, Smith, Big Hole and Clark Fork Rivers (Stagliano 2010). In Colorado, *P. californica* has been extirpated from the Arkansas River (Benzel 2016), and from several reaches of the Colorado (Nehring et al. 2011) and the upper Gunnison River (Elder and Gaufin 1973; Wiltzius 1976; Colborn 1985). In Utah, they have also been lost from the Logan River (Vinson 2011) and much of the Provo (Birrell et al. 2019) and Ogden Rivers. The factors influencing the declines of *P. californica* are not well understood. Changes in physical habitat, stream temperature, and oxygen levels may play a role (Anderson et al. 2019; Birrell et al. 2019; Kowalski and Richer 2020). In the Gunnison River, when temperature is not limiting, fine sediment deposition and cobble embeddedness may be driving Salmonfly range and density (Kowalski and Richer 2020). However, Giant Salmonfly disappearances in Utah do not appear to be correlated with high levels of fine sediments. Although abiotic factors, such as temperature, oxygen, and sedimentation, may play a role in *P. californica* declines, little work has been done to assess the importance of biotic interactions, such as diet and food availability. The interactions of abiotic and biotic factors likely influence

the range and distribution of this stonefly, and more work is needed to explore these factors in the Gunnison and other rivers.

In the Gunnison River specifically, Salmonflies have declined in range after the completion of the Aspinall project both above Blue Mesa Reservoir and immediately below Crystal Reservoir (Elder and Gaufin 1973; Wiltzius 1976). Currently, there is a thriving population of Salmonflies approximately five miles below the lowest of the three hydroelectric dams in the lower part of the Black Canyon National Park (BCGNP) and downstream throughout the Gunnison Gorge NCA. The density and distribution of larval Salmonflies declines closer to Crystal Dam, likely due to temperature and physical habitat limitations related to the large ecological impacts of regulated flow and altered temperature regime caused by the large bottom release impoundment.

The objective of this study is to explore the abiotic (temperature and physical habitat) and biotic (diet) factors that may be influencing Salmonfly density and range and to explain their disappearance from specific rivers, specifically below regulated impoundments.

PROGRESS

Sampling in 2023 and 2024 focused on monitoring the emergence time of Salmonflies at all 15 sites, monitoring temperature and dissolved oxygen at select sites and collecting Salmonflies for diet analysis. Where Salmonfly emergences occurred, the first and last day of emergence was observed and density estimates were made at a subset of the sites.

Habitat data is being currently being compiled and analyzed and will be completed in 2025. Analysis of the diet samples is ongoing and the project is expected to be complete, including draft manuscripts, in 2025

METHODS

Fifteen sites on the Gunnison River were sampled from Almont to Austin, Colorado in 2022 for Salmonfly density and abiotic habitat factors (Table 2). At eight sites that contained Salmonflies, 3-10 individual larvae were collected and frozen for diet analysis to be completed by collaborators and N.C. State University. Abiotic habitat sampling involved measuring dominant particle size (D50) of riffles with a pebbled count, fine sediment with a visual grid method, cobbled embeddedness with the Bain visual method as well as an estimate of force to move cobble particles. Temperature is being monitored with Hobo Pendant temperature logger and dissolved oxygen was monitored with a PME MiniDOT meter. Flow will be estimated using USGS gage data and discharge models. Three of these sites in BCNP were also sampled for aquatic invertebrate community structure because little invertebrate work has been done in this part of the river historically. Invertebrate sampling was done following the state of Colorado standard MMI method for invertebrate community health.

Salmonfly Density Estimates

Salmonfly density estimates varied by site, but generally followed expected patterns previously observed (Figure 1). There were no Salmonflies observed upstream of Blue Mesa Reservoir or immediately downstream of Crystal Reservoir. Approximately five miles below Crystal Reservoir, Salmonflies were found at Gunnison Point. Densities increased downstream with the highest densities observed in Ute Park in the Gunnison Gorge NCA. Salmonfly densities generally decline below Ute Park and they were present in low densities at Smiths Mountain and absent from Drysdales site near Austin, Colorado, approximately eight miles below the confluence with the North Fork of the Gunnison.

Aquatic Invertebrate Community

Preliminary analysis of the MMI sampling revealed interesting, but not unexpected results. Downstream of Crystal Reservoir the invertebrate community has low diversity but high density of select taxa (Tables 3-4). All three sites were dominated by tolerant, coldwater species like midges, blackflies, and scuds. This pattern has been reported before and is typical of invertebrate communities downstream of large bottom-release reservoirs (Ward and Stanford 1979; Vinson 2001).

The East Portal site was dominated by *Gammarus lacustris* (30.6%), and 33.4% *Baetis tricaudatus*, with only one stonefly species present *Hesperoperla pacifica*. The Gunnison Point site was dominated by oligochaete worms in the genus *Nais* (38.0%) and Chironomidae (37.1%), mostly genus *Tvetenia*. Two stonefly species were also present (*H. pacifica* and *P. californica*). This is farthest upstream (closest to Crystal Dam) that Salmonflies have been documented as larvae. The Red Rocks site was dominated by Simuliidae (67.9%) and also had two stonefly species *H. pacifica* and *P. californica*. The densities of Salmonflies were higher at the Red Rocks site than Gunnison Point. The diversity of the invertebrate communities and the MMI scores was low at all sites but increased going downstream (Table 3).

Table 2. Gunnison River Salmonfly sampling sites.

Site Code	Site	UTM (NAD83, Z13)	Sampling Completed
GR1	Almont Campground	338493, 4280034	Benthic, Temp, DO, Habitat
GR2	Garlic Mikes	332499, 4271989	Benthic, Habitat
GR3	Gunnison Whitewater	329747, 4266462	Benthic, Temp, Habitat
GR4	East Portal	269116, 4267719	Benthic, Temp, DO, Habitat, MMI
GR5	Gunnison Point	266346, 4271222	Benthic, Diet, Temp, DO, Habitat, MMI
GR6	Red Rocks	257277, 4275405	Benthic, Diet, Temp, Habitat, MMI
GR7	Chukar Trail	253421, 4278775	Benthic, Habitat
GR8	Bobcat	251353, 4280344	Benthic, Diet, Temp, Habitat
GR9	Ute Park	252376, 4284894	Benthic, Diet, Temp, Habitat
GR10	Smith Fork	253338, 4291889	Benthic, Temp, DO, Habitat
GR11	Goldmine	253728, 4295747	Benthic, Diet, Temp, Habitat
GR12	Cottonwood	252129, 4295940	Benthic, Diet, Temp, Habitat
GR13	Orchard Boat Ramp	247947, 4295297	Benthic, Diet, Temp, DO, Habitat
GR14	Smith's Mountain	246534, 4295614	Benthic, Diet, Temp, Habitat
GR15	Drysdales	245053, 4296502	Benthic, Temp, DO, Habitat

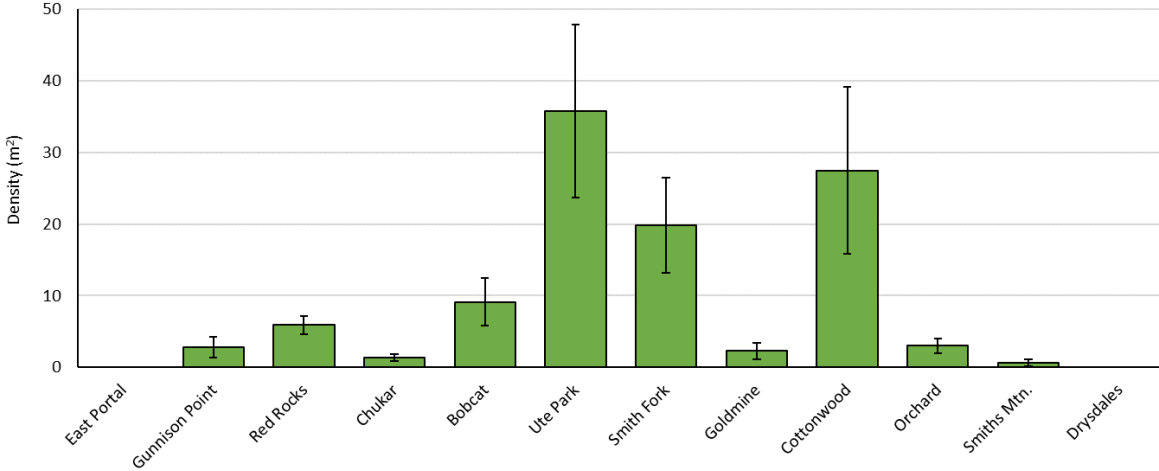


Figure 7. Salmonfly larvae density and 95% confidence intervals at Gunnison River sites below the Aspinall Unit reservoirs in 2022. No Salmonflies were sampled at Gunnison Whitewater, Garlic Mikes, Almont Campground above the dams or East Portal, or Drysdale sites below the dams.

Table 3. Community metrics and index scores for invertebrate sampling in Black Canyon of the Gunnison National Park in 2022.

Community Metrics	East Portal	Gunnison Point	Red Rocks
Total Taxa Richness	20	26	29
EPT Taxa Richness	3	6	8
Plecoptera Richness	1	2	2
SDI	2.62	2.55	1.65
MMI	12.9	15.9	37.0

Table 4. Relative abundance invertebrate orders in Black Canyon of the Gunnison National Park in 2022.

Order	East Portal	Gunnison Point	Red Rocks
Nematoda	0	0	0.1
Oligochaeta	22.7	41.4	2.8
Amphipoda	30.6	0.4	0.2
Ephemeroptera	33.6	17.5	17.7
Plecoptera	0.1	0.1	0.4
Trichoptera	0.0	0.1	0.7
Coleoptera	1.4	0.9	2.9
Diptera	11.6	39.4	74.6
Gastropoda	0	0.1	0.1
Bivalvia	0	0	0.4

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RESEARCH PRIORITY

Sculpin Phylogeny, Diversity, and Morphology in Colorado

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

Sculpin are members of a diverse family fish (Cottidae) that occur in a variety of marine and freshwater habitats throughout the northern hemisphere. In western North America, sculpin commonly inhabit coldwater streams with good quality habitat that is not excessively impacted by sedimentation or pollution (Adams and Schmetterling 2007). They are important ecologically in stream communities and can be better bioindicators of coldwater stream habitat than the salmonid species that they usually co-occur with (Adams and Schmetterling 2007).

There has long been taxonomic uncertainty about the identity of lineages of sculpins in Colorado (Woodling 1985; Adams and Schmetterling 2007). 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 two species do not differentiate them in Colorado 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. Those conclusions were based on both molecular and morphological data, he found difference in mitochondrial DNA and morphological characteristics, including higher lateral line pore counts among western forms. 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, 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 began in 2022 and will continue through 2025. Phase two of the project is a cooperative study with Colorado State University and will involve exploring the morphological differences between our two sculpin species.

In the first phase of the project, Colorado Parks and Wildlife biologists and researchers 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). The results here are summarized from Young et al. (2020).

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 *C. annae* in Colorado. 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, the results from the first phase of the study demonstrate that there are two distinct lineages of sculpin in Colorado and that they are different species from their current classification. This important conclusion indicates that two of the 13 fish species native to the upper Colorado River basin in Colorado may be misclassified. These results also emphasize the need for future work to properly describe the fish and will inform decisions on properly naming the fish, potentially resurrecting the name Eagle River Sculpin *C. annae*.

The second phase of this project began in July of 2023 and field collections continued through November 2024. This phase is a collaboration between CPW and Kevin Bestgen of the Larval Fish Laboratory at Colorado State University. The objective is to provide a morphological description of two provisional sculpin species in Colorado. A primary outcome of the study will be an improved understanding of morphological differences between the two taxa, and whether they are useful to differentiate taxa, is required to aid effective management and conservation efforts. The project is expected to be complete by July 2026.

In 2023, 10 sampling sites were visited and 100 individual sculpin specimens were taken and preserved in 10% formalin solution (Figure 8). Paired genetic samples were taken from each individual specimen, preserved on chromatography paper, and sent to the U.S. Forest Service National Genomics Center for Wildlife and Fish Conservation. In 2024, 25 sites were sampled and 250 individual sculpin specimens were taken and preserved. Field collections are now complete, analysis of the samples is ongoing and expected to be complete in 2025 with final reports and publications submitted by July 2026.

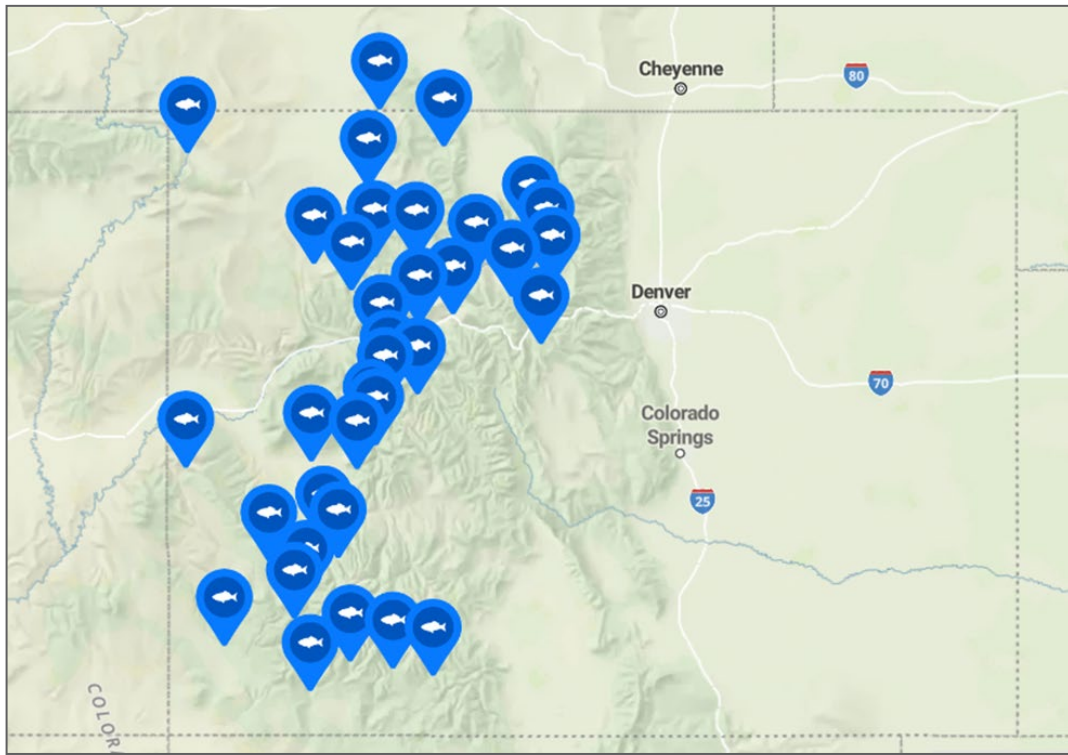


Figure 8. Sculpin specimen sampling locations 2023-2024. Thirty five sites in all major river basins on the western slope of Colorado were sampled.

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RESEARCH PRIORITY

Investigate the influence of streamflow patterns, reservoir operations, and fish stocking on the population dynamics of the trout fishery of the Uncompahgre River tailwater in Southwest Colorado.

OBJECTIVE

The objective of this project is to investigate the biotic and abiotic factors affecting the sport and native fisheries of the Uncompahgre River and Cow Creek and explore how fish populations are effected by stream flows, reservoir operations, and fish management activities.

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). Dams affect river ecosystems in a variety of ways including altering the natural pattern of water flow, transforming the biological and physical characteristics of river channels and floodplains, and fragmenting the continuity of rivers (Poff et al. 1997). How reservoirs are operated directly influences the flow and temperature of rivers and can dictate the diversity of aquatic invertebrate communities in rivers as well as the quantity and quality of fish habitat (Vinson 2001; Olden and Naiman 2010). Despite the many negative ecological effects, dams often benefit a subset of species. For instance, modified tailwater sections of river immediately downstream of dams can provide ideal conditions to support economically and recreationally important salmonid fisheries (Dodrill et al. 2016). Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*), two popular sport fish, thrive in tailwaters rivers throughout the Western U.S. because of the cool and clear water, stable flows, optimal water temperatures, and high production of algal and invertebrate resources.

Many of Colorado's best trout fisheries are in tailwater rivers so dam operations have a direct and strong influence on many the state's best and most highly used fisheries. Wild trout populations in Colorado are frequently driven by the strength of annual recruitment because spawning and adult habitat are generally abundant in most rivers and streams (Nehring and Anderson 1993; Latterell et al. 1998). Young of the year trout recruitment is heavily influenced by the rivers' hydrograph pattern with the timing of spring runoff as a major influence on trout recruitment success and the subsequent year class strength (Nehring and Anderson 1993). Therefore, the flow and temperature patterns below dams are driving both the biotic and abiotic factors that dictate the character and quality of many important trout streams in Colorado.

The Uncompahgre River is a tailwater trout fishery in Ridgway State Park between south of Montrose in southwest Colorado. The river is the outflow from Ridgway Reservoir, a 1,030-acre (84,591 AF) flood control and water supply reservoir that is a "participating project" of the Colorado River Storage Project. The Dallas Creek Project was authorized by the Colorado River Basin Project Act of 1968 was constructed on the Uncompahgre River in 1987 to increase water

supplies for irrigation, provide water for municipal and industrial purposes, and to provide flood control. Recreational fishing was not a primary purpose of the project but is an ancillary benefit from it. The Uncompahgre tailwater supports a highly used coldwater sport fishery that, while it is well publicized and popular, suffers from habitat and flow limitations that may limit the quality of the fishery available to anglers (Kowalski 2009; Dillingham 2018). The river is heavily used by anglers and is a major draw for Ridgway State Park (CPW Creel data 2007, 2022).

The river is managed by CPW as a category 507 coldwater mixed stocking stream with intensive sportfish management strategies, meaning it can receive supplemental stocking of both subcatchable and catchable sized trout to provide angling recreation in lower productivity waters. It has special fishing regulations that mandate the use artificial flies and lures only and all trout must be returned to water immediately (catch and release, flies and lures only) and is recognized on CPW's Quality Waters list. At the USGS gage below the dam, the river has a drainage area of 265 square miles, an average daily flow of 195 cfs, a minimum daily flow 28 cfs, and a maximum daily flow 1,400 cfs.

The specific factors that most influence the fishery are dynamic and have changed over time, but fish habitat limitations, gas super saturation, and low wintertime stream flows have been identified as factors affecting the fishery at various times in recent years (Kowalski 2009). Fish populations in the river were studied intensively in the late 1990's through middle 2000's by CPW biologists, researchers, instream flow coordinators, as well as scientists from the Bureau of Reclamation. More recently, two proposed water projects could substantially alter the flow regime of the river and have emphasized the need for more updated information. The first proposed project is Rams Horn Reservoir, a new reservoir project with a conditional water right first proposed in the 1950's as part of the original Dallas Creek Project. It would create a new 235 surface acre 25,000 acre-foot reservoir on Cow Creek near the U.S.F.S. Uncompahgre Wilderness Area. 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. The Rams Horn project would drastically alter the flow and sediment regime of Cow Creek and could influence how water is released from Ridgway Reservoir due to water exchanges and a proposed pipeline to the reservoir (Sackett 2021).

The second proposed project that could change flow patterns on the Uncompahgre River below Ridgway Reservoir is the Project 7 Regional Water Resiliency Program Water Supply Project. This is a plan for municipal suppliers to use water out of Ridgway Reservoir to diversify its water supply for the communities of Montrose, Olathe, and Delta Colorado. Together with Ramshorn Revoir, the future flow patterns in the Uncompahgre River could change dramatically and more information was needed on how the flow patterns and temperature regimes in the Uncompahgre River below Ridgway affect trout populations in the river.

The specific research objectives of this project are to explore how temperature, flow, and hydrograph pattern affect trout populations of the Uncompahgre River tailwater and how fish

management activities such as stocking and habitat improvement projects can be used to improve the fishery. Specific goals to accomplish this objective are to:

1. Estimate growth, survival, diet composition, immigration, and emigration of trout in the Uncompahgre River to evaluate biological and environmental factors that limit the trout population.
2. Investigate instream flow needs of sport and native fish in the Uncompahgre River and Cow Creek.
3. Suggest mitigation efforts, management strategies, and reservoir operations to improve the sport fishery in the Uncompahgre River.
4. Document the biological and environmental baselines of Cow Creek fish populations and habitat to inform mitigation efforts of the planned future water project.

Management implications of this study will be an improved insight into the biology and ecology of the native and sport fisheries of Cow Creek and the Uncompahgre River to better inform fish management strategies and comments on upcoming large-scale water projects. This project was proposed through CPW's research project selection process in 2023 by Eric Gardunio with the support of CPW staff Katie Birch, Ryan Unterreiner, Kelly Crane, Rachel Sralla, and John Freeborn.

METHODS

Fish populations will be sampled in the Uncompahgre River two times a year (November and April) with mark recapture electrofishing. All fish will be weighed to nearest gram, measured to the nearest millimeter, and individually tagged with 12.5 mm full duplex Biomark GPT12 passive integrated transponder (PIT) tags. A combination of Huggins Closed Capture models and Cormack-Jolly-Seber models in Program Mark will be used to estimate population size, capture probabilities, and survival of different size classes and types of fish for both winter and summer time periods (White and Burnham 1999). Instream flow modeling as well as foraging-bioenergetics food web modeling similar to methods in Dodrill et al. (2016) will be used to investigate the influence of stream flows and available prey resources on the trout population.

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Sackett, H. 2021. Ouray County water project faces opposition from state, others. *The Aspen Times*, Aug 29 2021.

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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

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

Technical assistance was provided to R. B. Nehring for whirling disease sampling efforts. A statewide sampling effort is being done to compare whirling disease in trout to historical collects from 20-30 years ago. Waters that were sampled in 2024 included the Lake Fork of the Conejos River, Los Pinos River, Gunnison River, and Middle Fork of the South Platte River.

A new collaborative effort with Dr. Yoichiro Kanno and Helen Acosta, fish ecology researchers from Colorado State University, was started on sculpin populations of the Eagle, Blue, and Colorado rivers. This project aims to compare growth, diet, reproduction, and ecology of sculpin in regulated and freestone rivers. Each river system was sampled in the summer to collect population density estimates and 50 sculpin specimens were taken in spring, summer, and fall for age and growth analysis (150 fish total for each river). Aquatic invertebrate samples were taken at each site and temperature was monitored throughout the ice-free season.

Technical assistance was provided to B. W. Avila on a Brown Trout (*Salmo trutta*) morphology and life history study on the South Platte River. A manuscript is in preparation, "The influence of population structure and life history on spotting pattern of brown trout in the South Platte River, Colorado" to be submitted in 2025.

A technical assistance project is ongoing with USGS scientists from Arizona and Colorado. A large data request was received from USGS scientists in 2022 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 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. Data collection is complete for this project and analysis and manuscript preparation is ongoing with the goal of three manuscripts being submitted in 2025.

COMMUNICATION AND INFORMATION TRANSFER

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

Kowalski, D. A. 2023. Coldwater stream ecology investigations progress report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.

One paper is in preparation to be submitted in 2025;

Avila, B. W., E. R. Fetherman, M. Kondratieff, E. Richer, D. Kowalski, M. Baerwald, A. Goodbla, and J. Spohn. *In preparation*. The influence of population structure and life history on spotting pattern of Brown Trout in the South Platte River, Colorado.

Three book chapters were completed for the Fishes of Colorado Book;

Kowalski, D. A. *In Review*. Brown Trout. In Treble, A. J., G. J. Schisler, J. Woodling, editors. Fishes of Colorado. Fort Collins, CO.

Woodling, J., D. A. Kowalski, A. J. Treble. *In Review*. Eagle River Sculpin. In Treble, A. J., G. J. Schisler, J. Woodling, editors. Fishes of Colorado. Fort Collins, CO.

Woodling, J., Kowalski, D. A., A. J. Treble. *In Review*. Colorado Sculpin. In Treble, A. J., G. J. Schisler, J. Woodling, editors. Fishes of Colorado. Fort Collins, CO.

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

Kowalski and Clements. 2024. The effects of mosquito control insecticides on aquatic invertebrates in Colorado streams. Rocky Mountain SETAC April 17, 2024 Fort Collins, CO.

Kowalski and Clements. 2024. The effects of mosquito control insecticides on aquatic

invertebrates in Colorado streams. Denver Ecotoxicologist/Risk Assessors Meeting, August 9, 2024.

Two posters were contributed to;

Smith-Nez, G., E. A. Scholl, M. A. Ford, T. A. Kennedy, C. B Yackulic, D. A. Kowalski, R.E . Zuellig, D. M. Carlisle. 2024. Dams, diets, and diversity: food webs in tailwater fisheries. Society for Freshwater Science Philadelphia, PA.

Avila, B. W., E. R. Fetherman, M. Kondratieff, E. Richer, D. Kowalski, J. Spohn. 2024. Red spots, black spots, Brown Trout. Colorado/Wyoming American Fisheries Society Annual Meeting 2024. Laramie, WY. February 28, 2024