

# NATIVE THREE-SPECIES RESEARCH

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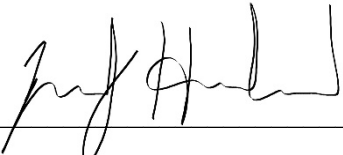
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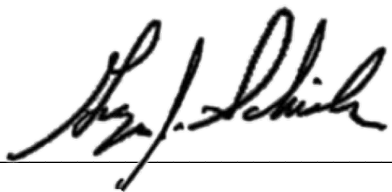
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Date: December 13, 2024

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

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## Three-species tributary use and spawning investigations

Period Covered: March 1, 2023 to December 30, 2023

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### **RESEARCH PRIORITY:**

Test a resistance board weir as a means of controlling the entire Roubideau Creek spawning run, allowing for the selective exclusion and removal of non-native and hybridized Catostomid suckers. *This priority is an ongoing project that has been covered in prior reports. In 2023, we collected the last set of larval sucker samples.*

### **AUTHORS:**

Zachary Hooley-Underwood, Jillian Campbell, and Dr. Elizabeth Mandeville.

### **OBJECTIVES:**

- I) Test the functionality and operability of a resistance board weir located near the mouth of Roubideau Creek.
- II) Evaluate the effect of the weir on the species composition of the larvae produced in the Roubideau drainage by sampling larvae and genetically assessing their species identity.
- III) Compare the extent of tributary use between native and non-native suckers via longitudinal larval sucker sampling and genetic identification.

### **INTRODUCTION**

In this study, we investigated the ability of a resistance board weir to restrict nonnative sucker access into native Bluehead (*Pantosteus discobolus*; BHS) and Flannelmouth (*Catostomus latipinnis*; FMS) sucker spawning habitat of Roubideau Creek, in the Gunnison River Basin (Colorado, USA). We investigated how well selective removal of non-native White (*C. commersonii*; WHS) and Longnose (*C. catostomus*; LGS) suckers and their hybrids at the weir translates to a decrease in non-native sucker ancestry within the sampled larval populations. If selective removal of non-native suckers was successful, we would expect lower proportional genetic contributions of WHS to larval fish cohorts in years with restricted access to Roubideau Creek. In addition, we evaluated the relative genetic contribution to the larval population of the three species of interest at different spawning locations upstream to understand the longitudinal ancestry trends within the spawning habitat. Based on prior knowledge of this system, native suckers were believed to use upstream reaches of the spawning habitat in Roubideau Creek, while WHS were expected to remain farther downstream, closer to the weir. We accomplished both objectives through genomic analysis of larval fish specimens. The majority of this project is complete and the full report is available in the Three-Species 2022 progress report. In addition, a publication on this research objective was submitted to *Evolutionary Applications* and was *in*

*review* at the time of writing. In 2023, we did not operate the weir, but collected an additional season's worth of larval catostomids. The purpose of these samples was to see if there appeared to be any sort of lag effect from the previous years of non-native exclusion.

## **METHODS**

We collected a full set of larvae as described in the 2022 progress report from Roubideau Creek. In summary, we used drift and dip nets to collect larvae (or eggs which were allowed to hatch in incubation trays) at three sites from the mouth of Roubideau Creek to approximately 25 miles upstream. A total of 80 larvae was the target sample size at each location, and at each site, collections were spread out temporally, between early May and early July to get a representative collection of all larvae produced.

Collected larvae were preserved individually in 90% undenatured ethanol, and were sent to University of Guelph for DNA extraction, library assembly, and were then sent to the Toronto Sick Kids Hospital for Illumina sequencing.

## **RESULTS AND DISCUSSION**

In total, we collected and prepared 240 larvae from the 2023 season. At the time of this report, these samples were still awaiting sequencing at the sequencing facility. Snow pack in the drainage in 2023 was the highest on record since 1991. We suspect that the resulting record-high runoff probably favored native sucker spawning and production, so it may be difficult to discern whether any lasting effect of the weir occurred.

## **ACKNOWLEDGMENTS**

Kevin Thompson conceptualized the project and set it in motion. We thank Tracy Kittell and Steve Patterson for assistance with capital development, and Cramer Fish Sciences for designing and fabricating the weir. We thank Jesse Anderson of Cramer Fish Sciences for assistance and training in installation and maintenance of the resistance board weir. Dan Cammack, Dan Kowalski, Eric Gardunio, Jenn Logan, Russ Japuntich, Cole Brittan, Rachel Jones, Mark Richman, Stuart Sinclair, Codi Inoles-Williams, Katie Birch, Sawyer Morain and many CPW technicians assisted with electrofishing, weir operation, and larval fish sampling.

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## **RESEARCH PRIORITY**

Identify tributary fidelity rates and spawning movement patterns in Three-Species fishes as well as non-native suckers in the Roubideau Creek drainage.

### **OBJECTIVES:**

- I. Determine annual spawning tributary fidelity of PIT-tagged Three-Species fishes.
- II. Identify which spawning tributaries Three-Species PIT tagged in the Gunnison River mainstem select for.

### **INTRODUCTION**

Research conducted by Colorado Parks and wildlife and others has established tributary spawning is a major life history component for the Three-Species (Bonjur et al. 2023; Fraser et al. 2017; Hooley-Underwood et al. 2019). Additionally, we have shown that in the Gunnison River drainage, individual fish generally return to the same tributaries to spawn annually (Thompson and Hooley-Underwood 2019). These findings strongly suggest that conservation actions may be the most economical in tributary habitats, as we can access large components of the population in relatively small waters. As discussed in the previous research priority, we have evaluated whether using mechanical exclusion of non-native suckers during spawning migrations into tributaries is an effective way to manipulate hybridization. Findings from those studies have suggested that exclusion can be effective, but only under favorable flow conditions. Moving forward, CPW aquatic managers are considering larger and more robust designs for exclusion devices. However, these devices are likely to be very costly to build and operate. Therefore, we need to continue to monitor tributary fidelity rates and identify tributaries that receive the greatest amount of spawning use to maximize the benefits of future projects. We are continuing to monitor fidelity rates of previously tagged fish, as described in (Thompson and Hooley-Underwood 2019), and are using a new PIT tagging approach to compare tributary use.

### **METHODS**

Since 2014, CPW and partners have been PIT-tagging Three-Species fishes in the Lower Gunnison basin. Many of those have been tagged in the Roubideau Creek drainage. In 2015, we installed a PIT-tag detecting, passive interrogation array (PIA) at the mouth of Roubideau Creek. The PIA has been operated continuously since 2015, and in 2016, we began deploying portable, submersible PIT-Tag readers (SPRs) in various locations in Roubideau Creek and its tributaries. We have used redetections of PIT-tagged fish on the PIA and SPRs to determine fidelity to the Roubideau drainage as a whole (via PIA detections), and to specific tributaries within the drainage (via SPR detections; Thompson and Hooley-Underwood 2019). We have estimated annual fidelity rates as the proportion of fish detected in a given year that return in the following year. More detailed methodology on this Research Priority (through 2018 sampling) can be found in the publication referenced in the results and discussion section below. In 2023 specifically, we tagged new individuals of all three species, and continued to monitor redetections with the Roubideau PIA, and SPRs. We also installed SPR's in new locations – the North Fork of the Gunnison River and the Uncompahgre River.

To begin to identify tributary selection, we began a new tagging program in the mainstem of the Gunnison River. The Three-Species migrate into spawning tributaries in March and April, spawn in May and June and then return to the mainstem by July. They remain in the river during the remainder of summer, fall, and winter. We began tagging fish during August, September, and October in 2023 in the mainstem of the Gunnison River when fish are expected to be behaving independently from any spawning related behavior. We captured fish via Raft electrofishing with the goal of deploying up to 2,000 tags in as much of the mainstem as possible from the confluence of the North Fork of the Gunnison to the Redlands Diversion (just upstream of the Gunnison’s confluence with the Colorado). We did not sample continuously while floating river sections, instead shocking relatively short reaches so that individual captures could be easily associated with geographic coordinates. All captured fish were weighed and measured, sexed if possible, scanned for an existing PIT tag, and if not previously tagged, implanted with a 12mm PIT tag. All fish were released within 1 km of their capture location.

## RESULTS AND DISCUSSION

Results from 2016 - 2022 are available in our previous technical and annual reports. During 2023, 2,191 additional PIT tags were deployed in the Gunnison Drainage. Detections of PIT tagged fish at the PIA give us the best indication of tributary fidelity as antenna efficiency is typically 100%. In previous years, we saw a high proportion of individuals of both BHS, FMS, and RTC returning to spawn from one year to the next, and this trend held true for fish detected in the system in 2022 returning in 2023 (Figure 1). In 2023, species-specific return rates

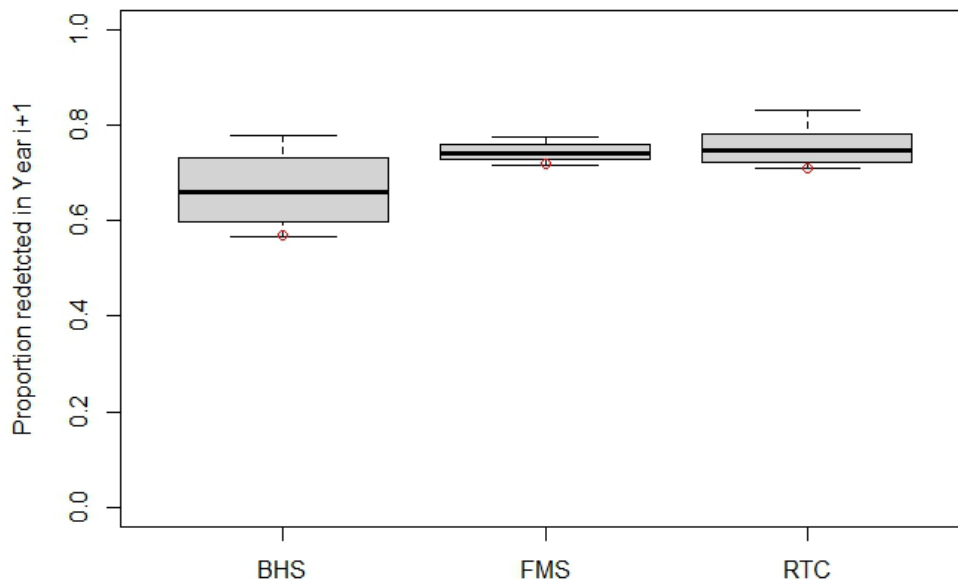


Figure 1. Annual fidelity rates for PIT-tagged Bluehead Sucker (BHS), Flannemouth Sucker (FMS) and Roundtail Chub (RTC) based on Roubideau Creek PIA detections between 2016 and 2023. Proportion redetected is the proportion of individual fish that were detected in year<sub>i</sub> redetected in year<sub>i+1</sub>. Red circles indicate 2023 proportions.

(proportion of fish detected in 2022 redetected in 2023) were 57.4, 72.5, and 71.1% for BHS, FMS, and RTC, respectively. These rates are similar to those observed annually since 2016, but were slightly lower for all three species. This is unsurprising given the high runoff volume which may have allowed some fish to bypass the PIA antennas laterally, or pass over them high in the water column outside of the read range. Despite this we still saw overall high fidelity rates, especially when considering that this estimation does not account for mortality.

In both 2022 and 2023, we struggled to maintain SPRs under the high flow conditions, and there were up to two months during which we were unable to collect data from SPRs. Therefore, we were unable to estimate tributary specific returns. However, we did detect PIT-tagged fish accessing Cottonwood, Buttermilk, Potter, and Upper Roubideau creeks in abundance. The SPRs deployed in the North Fork of the Gunnison and in the Uncompahgre both detected fish. In the North Fork, we detected 12 individual fish, and in the Uncompahgre we detected ten individual fish. Again, high flows made it impossible to change these antenna batteries for a period of up to two months.

We have now monitored fidelity rates and tributary usage during multiple years of extreme flows, extreme drought, as well as more average conditions. Across these years, Roubideau Creek fidelity rates have remained in the 60 to 90% range for all three species, and we have seen immigration into the smaller tributaries annually, as long as they actually flow.

In order to identify tributary selection of Gunnison River Three-Species, we tagged 1560 fish between August 8<sup>th</sup> and September 28<sup>th</sup> in the mainstem Gunnison River between the North Fork and Wells Gulch confluences. Additionally, we tagged 382 fish in the spring near the north Fork of the Gunnison confluence. In total, 962 BHS, 616 FMS, and 366 RTC were tagged. Detections of these fishes in starting in spring 2024 will be used to assess tributary selection.

## **ACKNOWLEDGMENTS**

Former Aquatic Researcher Kevin Thompson initiated and conducted the bulk of the work described here through 2020. Dozens of people over the years PIT-tagged the suckers and chub used and re-detected in this priority. In 2023, technicians Jackie Tauberman, Collin Thorburn, and Meredith Selhmeyer helped deploy and manage antennas, and capture and tag new fish.

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## **RESEARCH PRIORITY:**

Monitoring “perennial island” Three-Species populations in an intermittent stream-scape.

## **OBJECTIVES:**

- I) Identify perennial segments of intermittent streams that support the Three-Species.
- II) Monitor population demographics within the perennial segments across seasons and assess mobility of fish within these populations.

## **INTRODUCTION**

The biological and physical benefits of intermittent and ephemeral streams are not given nearly the consideration of perennial streams due to their short wetted periods. In fact, these streams are often not even afforded the protections of regulatory laws such as the Clean Water Act in the United States. Intermittent streams are those that flow continuously only at certain times of year, while ephemeral streams are those that flow only briefly in direct response to local precipitation. Both are often overlooked with respect to aquatic organisms. In the arid, lower elevations of the Colorado River basin, the majority of waterways are intermittent or ephemeral. Recently, researchers and biologists have gathered a wealth of data showing that intermittent streams are important for Three-Species fishes when it comes to fulfilling certain life-history components. In prior research priorities, we have identified heavy use of intermittent tributaries by the Three-Species for spawning and early larval rearing. Many of the streams we have studied closely, including Cottonwood Creek, Roubideau Creek, and other streams draining the Uncompahgre Plateau, flow during April and May, when spawning, hatch-out, and larval drift occur, and then dry up in June. Therefore, we’ve viewed the streams as only important seasonally. However in March 2020, CPW aquatic technician Chase Garvey observed small suckers in pools in a short flowing segment of the Dry Fork of Escalante Creek prior to runoff. The observation was significant, as the stream flows less regularly than Cottonwood Creek, and was partially isolated from the mainstem of Escalante Creek by a derelict irrigation diversion. The fact that fish were present in these pools following a record dry summer and fall was surprising, suggesting that these fish had survived at the location through at least one significant drought year. More so, the presence of the barrier downstream likely meant that these fish survived in isolation in the short perennial reach for many years. We sampled the segment and another that we subsequently identified, and confirmed the suckers were BHS, and that Speckled Dace (*Rinichthys osculus*; SPD) were also present. Following this discovery, we prioritized identifying other perennial islands of occupied habitat among the largely intermittent streams of the area. Our goal was to identify locations that may have perennial flow due to groundwater input, and then sample those areas to determine if they were occupied. For a subset of occupied habitats, we will monitor population dynamics over several seasons, and look at movement between populations. Additionally, the isolating barrier was removed during summer 2021, so monitoring these reaches will determine whether movement occurs between the Dry Fork and Mainstem Escalante creeks. Generally, documenting the presence of these populations increases the amount of

occupied stream length in the state. Studying these habitats will better our understanding of the diversity of habitats that Three-Species fishes use, will allow us to increase the precision of the range-wide database, and will further inform management and conservation practices for intermittent desert streams.

## METHODS

We initiated this priority in summer 2021 and continued to monitor fish populations and stream conditions in 2022 and 2023. First, we used Google Earth aerial imagery to search drainages on the east slope of the Uncompahgre Plateau for stream segments that were potentially perennially wet (Figure 2). We compared imagery from different dates to assess wet and dry conditions through time. Imagery captured on August 8<sup>th</sup>, 2019 was particularly helpful, as the quality was good, and summer of 2019 was very dry, so visible water was scarce. What was visible in the images was likely perennial, and reliably so even under moderately to extremely dry years. We selected a subset of reaches to confirm whether water was present, and if so, sample for fish. Fish sampling was conducted with one LR24 backpack electrofisher (Smith-Root®), as all segments



Figure 2. Aerial imagery (A) of Cottonwood Creek captured August 8<sup>th</sup>, 2019 used to identify a potential perennial reach. The reach begins at the marked “spring” and flows north for approximately 800 feet. Downstream of the indicated spring (B) water was present and fish were abundant in October 2021. Upstream (C) the stream bed was dry, and abundant plant growth indicated substantial flow has been absent for a large amount of time. Below the spring, there was active flow (D). In the center of image D, our temperature logger is visible, anchored to a boulder with a rock-climbing bolt and chain.

were very narrow and relatively shallow. If fish were present on the first pass, additional passes were completed to achieve adequate depletion. All fish were counted, identified to species, weighed, and measured. Any native suckers and chub captured over 120 mm TL were implanted with a 12mm PIT tag. At a subset of occupied sites, we established a “wet” sampling reach and upstream and downstream “dry” sampling reaches, based on water and fish presence at the time of the survey. Features such as short drops or shallow riffles (expected under runoff conditions) were used to demarcate reach termini, and lengths were held between 300 and 500 stream-feet. Within each reach, we placed an Onset MX2203 temperature logger to record temperature throughout the season. Additionally, these loggers have the ability to log whether they are in water or air, so they will be able to collect data on whether reaches remain wet or dry throughout seasons. We attempted to place loggers in locations that would best indicate whether water was flowing if they were wet (we avoided deep pools that would remain wet well after flow stopped, and shallow riffles that may register as dry if only a rivulet of flow existed. Loggers were downloaded annually.

	Reach	Baseflow condition	Pre-Runoff (March/April)		Post-Runoff (June)		Fall (September/October)	
			Condition	SPP present	Condition	SPP present	Condition	SPP present
Dry Fork Escalante	DED1	Dry	Wet	None	Wet	BHS, SPD	Dry	None
	DEW1	Wet	Wet	SPD	Wet	BHS, SPD	Wet	BHS, FMS, SPD
	DEW2	Wet	Wet	None	Wet	BHS, SPD	Wet	BHS, FMS, SPD
	DED2	Dry	Wet	None	Wet	SPD	Dry	-
	DEW3	Wet	Wet	BHS, SPD	Wet	BHS, FMS, SPD	Wet	BHS, SPD
	DED3	Dry	Wet	SPD	Wet	SPD	Dry	-
Cottonwood	CCD1	Dry	n/a	-	n/a	-	Dry	-
	CCW1	Wet	n/a	-	n/a	-	Wet	BHS, FMS, SPD
	CCD2	Dry	n/a	-	n/a	-	Wet	SPD

Table 1: Sampling sites in Dry Fork Escalante and Cottonwood creeks. Both creeks are primarily intermittent but have short reaches of perennial flow. Baseflow conditions indicate whether the site has flowing water during typical summer baseflow conditions. In 2023, sites were sampled one to three times. For each sampling occurrence, condition indicates whether flowing water was present, and detected species are listed under SPP present (BHS = Bluehead Sucker; FMS = Flannelmouth Sucker; SPD = Speckled Dace.

A survey of each reach (assuming they are wet) was completed prior to runoff, on the descending limb of runoff, and in fall. We will conduct depletion sampling using multiple passes (at least two) to achieve adequate depletion to estimate abundance. We will track population size and demographics over time ending in spring 2025. Additionally, we will scan all fish captured for a PIT tag, and use recapture data to determine whether there is movement among reaches and analyze annual survival.

## **RESULTS AND DISCUSSION**

From satellite imagery, we identified over 13 candidate locations for further investigation in 2021. Findings from 2021 and 2022 are available in those respective annual reports. In 2023, we visited all 6 Dry Fork Escalante sites three times (pre and post runoff, fall) but were only able to access Cottonwood sites in the fall due to snow pack in the March and heavy rains in June (Table 1). Wet conditions in 2023 resulted in the entire stream being wetted pre and post runoff, but by fall, the normally dry reaches had ceased to flow. The most notable findings were that all reaches were occupied by fish post- runoff, and one FMS was caught representing the first record of this species in these reaches. During the fall sampling, YOY FMS were abundant in the lower wet reaches indicating successful reproduction by the species. The removal of the diversion barrier prior to 2022 likely allowed for the immigration and spawning of FMS from Escalante Creek, which we believe is the source of these FMS. Similar to 2022, DEW2 had few fish compared to 2021. The pools that were previously heavily occupied in the reach still had sediment deposits in the spring. Some scouring occurred during runoff, but previous pool depth wasn't fully restored. Some recolonization of those pools did occur during runoff, and was the reach with the highest density of FMS when sampled in the fall. In Cottonwood Creek, BHS, FMS and SPD were present as is typical. In both creeks, recaptures were limited, and no movements outside of the original tagging reaches were identified through re-detection elsewhere.

## **ACKNOWLEDGMENTS**

CPW aquatic technicians Jackie Tauberman, Colin Thorburn, and Meredith Selhmeyer assisted with sampling, and logger deployment.

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## **RESEARCH PRIORITY:**

Comparing larval sucker hatch timing and growth rates among intermittent and perennial tributary, and mainstem habitats.

## **OBJECTIVES:**

- I) Identify differences in larval sucker hatch timing among three habitat types.
- II) Identify differences in larval sucker growth rates among three habitat types.
- III) Identify thermal regime differences among three habitat types during the larval sucker development period.

## **INTRODUCTION**

Bluehead and Flannelmouth sucker, commonly spawn in intermittent tributaries. We have documented spawning use by large numbers of adults, as well as substantial larval production in such waters (Hooley-Underwood et al. 2019). We presume there is a benefit bestowed by spawning in these waters due to the numbers of fish observed, and the high annual fidelity of those fish to those waters; however, there is also a hefty cost in that large numbers of larvae are stranded and perish as these streams dry during summer months. We suspect that this mortality is offset by early hatch dates and improved growth compared to larvae produced in the mainstem or in perennial tributaries. In intermittent waters we have studied, peak runoff occurs between mid-April and early May, compared to permanent tributaries or the mainstem where peak runoff occurs a full month later. These fishes typically spawn on the receding runoff limb, spawning can occur a full month earlier in intermittent streams. Additionally, intermittent streams often warm more rapidly as flows drop. We have regularly observed 5-7°C warmer mean daily water temperatures in Cottonwood Creek (an intermittent spawning tributary in the Gunnison basin), compared to the mainstem Gunnison River near, and maximum daily differences can be as high as 12-13°C. Warmer water temperatures result in shorter incubation periods and faster growth for larval suckers (Riepe et al. 2023; Riepe et al 2024). Therefore, larvae produced in intermittent streams that out-migrate before drying may have had an extra month of growth at more optimal temperatures compared to perennial tributary or mainstem larvae. This extra growth likely gives intermittent stream produced larvae both a survival advantage (through increased swimming performance, predator avoidance, and earlier dietary resource transition) as they mature, as well as a competitive edge over their smaller, mainstem produced cohorts. This theory has not been tested for BHS and FMS, so we are attempting to do this in the Gunnison River drainage. This project combines larval presence sampling, and larval growth estimation to assess whether this advantage exists, and then compares water temperature among habitats. Agencies like CPW are beginning to target intermittent waters more frequently for conservation actions, and understanding how these waters benefit native suckers will improve the design of those actions. Also, there are many highly altered intermittent streams on the landscape as they are often not considered important for fishes, and understanding how these habitats support native fishes may direct restoration efforts.

## **METHODS**

This project was conceptualized and initiated during the 2023 sucker spawning season, and methods developed over the course of the 2023 collection. Reported here are both the methods used in 2023, as well as methods to be used in future years.

*Sampling sites*-We established seven sampling locations. We selected Roubideau Creek and the Uncompahgre River as tributary locations, and then sampled in the Gunnison River both above and below each of their confluences (>250m, <1 km). We sampled one location in the Uncompahgre River (1.5 km above mouth) and two locations in Roubideau Creek. Roubideau Creek is mostly intermittent except for the lower 12.3 km which is perennially augmented by irrigation related flows, so we sampled both 0.7 km and 13.2 km upstream of the mouth.

*Fish collection* – Sucker eggs and larvae were caught with drift nets (eggs and larvae) and dip nets (larvae only). Drift netting began near peak runoff with the goal of determining the beginning of the spawn. While catostomid eggs are demersal and adhesive, we have observed a substantial number of viable eggs drifting post fertilization, and use the presence of these drifting eggs as evidence of active spawning. Larval suckers begin active nighttime drifting behavior when they become mobile. Both eggs and drifting larvae are susceptible to night-time driftnet capture. As flows drop and clear, and larvae become more mobile, they can be capture visually or blindly with dip netting in still or low velocity areas. In 2023, we attempted to collect eggs and larvae weekly from each site. Drift nets were typically set in moderate current for the ½ hour before first light, though high water and debris due to the large runoff made full sets difficult on occasion. In future years, we will set drift nets once per week at each site for 1 hour before first light when possible, and will measure water velocity at the beginning and end of each net set so that drift density can be estimated. Nets are set either fully submerged, but near the water surface, or if there isn't enough depth to fully submerge, submerged depth is measured at the beginning and end of each set. Once larvae became susceptible to dip netting, we collected around 40 per occasion once per week per site. This will remain the same in the future, though we will extend drift netting later into the season so we can continue to compare density.

*Sample preservation and processing* – After each drift net set, all material in the net was rinsed into a 5 gallon bucket with extra stream water. Samples were brought to the lab, and were picked through by transferring small amounts of sample and water to shallow trays. Larvae and eggs were set aside as the samples were picked. Eggs were sorted based on size, and larger eggs (~2.5mm vs 1.5-2mm) were counted as catostomid eggs (the smaller eggs are of cyprinidae). Larvae were kept alive in shallow dishes until processing. Each larvae was euthanized with dilute ethanol (small amount of 90% mixed with sample water), which promoted a straight, extended body position post death (full strength ethanol causes larvae to contort). Each larvae was confirmed as belonging to Catostomidae and measured (TL) under a dissecting microscope. Larvae were individually preserved with a unique id number. At a future date, all or some of the preserved larvae will be sent to the Larval Fish Laboratory at CSU for identification species and otolith aging, which will allow for back-estimation of hatch date.

*Temperature* – Hobo temperature loggers (MX tidbit 400 or U22-001; Onset) were placed at all sampling locations in 2023 except for the Gunnison River above the Uncompahgre River, as there is a USGS gauge there that records temperature. Unfortunately, due to the flooding and widespread erosion that occurred during the 2023 runoff, all loggers except for one in Roubideau were lost. Loggers were replaced for future study years.

*Analysis* – For each site, spawning onset (via egg presence), mean hatch timing (back-calculated from otolith ages), weekly drift density (from drift net catch and velocity measurement), and growth rate (from length and age data) will be estimated for each location. All sites will be compared and temperature metrics will be evaluated for correlation to timing and growth.

## RESULTS AND DISCUSSION

In 2023, we captured a total of 355 eggs and 1,767 larvae among all sites and occasions. Most of our analyses will occur after species ID and otolith aging occurs, but we did observe several trends. We sampled from May 10 through August 23<sup>rd</sup> (though weekly sampling ended July 17<sup>th</sup>), and captured the first eggs in Roubideau Creek on May 24<sup>th</sup>. Larvae and egg catch was generally the highest weekly in the Roubideau Creek sites. Gunnison and Uncompahgre river samples generally had few eggs or larvae, but the Gunnison site below the Roubideau confluence

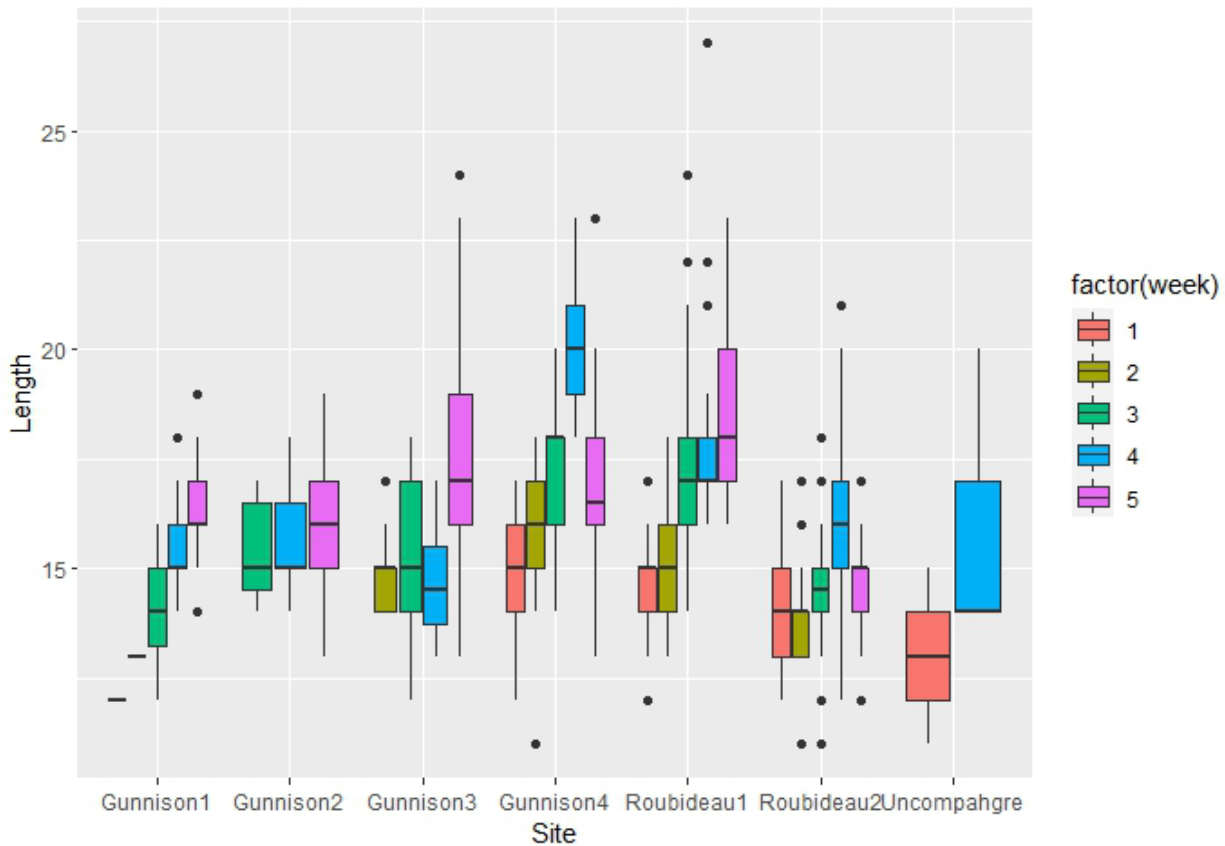


Figure 3. A comparison of larval sucker lengths at seven sites across five weeks. Larvae were collected using drift or dip nets weekly, and were measured to total length. Boxes represent mean and quantile lengths for each site and week. Gunnison 1 and 2 were above and below the Uncompahgre River confluence, respectively, Gunnison 3 and 4 were above and below the Roubideau Creek confluence, respectively, Roubideau 1 was near the mouth of Roubideau Creek, Roubideau Creek 2 was 13 km upstream in Roubideau Creek, and Uncompahgre was 1.5 km upstream of the mouth of the Uncompahgre River.

consistently produced the highest egg and larvae catches in the Gunnison suggesting we were seeing the result of Roubideau Creek drift. Catches were so low in the Gunnison and Uncompahgre rivers that it is difficult to tell when spawning began, but it was fairly clear that spawning began in Roubideau Creek May 24<sup>th</sup>. We did not have a successful collection from the Uncompahgre River until June 9<sup>th</sup>, when one egg was collected, and all egg collections in the Gunnison River occurred below Roubideau Creek suggesting we were seeing the result of Roubideau Spawning. Eggs were present in every drift netting collection on Roubideau Creek from May 24<sup>th</sup> through June 14<sup>th</sup>.

We don't have hatch-date or growth data from 2023 yet, as we will have otoliths extracted all at once in the final year of study (this project will include 2024 and 2025 samples). In 2023 we were able to measure and preserve 110 larvae between the 4 Gunnison sites, 40 between the two Roubideau sites, and 20 from the Uncompahgre site. We were able to compare collective larval lengths across sites and weeks in 2023, and did see that the lower Roubideau Creek site as well as the Gunnison River below Roubideau Creek consistently had the largest larvae on average (Figure 3). Length differences from 2023 are only loosely indicative of growth differences as the samples likely include uneven mixes of BHS, FMS, WHS, and hybrids. However, it did appear that the largest average larvae were being produced in Roubideau Creek and in the Gunnison downstream.

#### **ACKNOWLEDGMENTS**

Dr. Kevin Bestgen at Colorado State University was consulted on this project and provided many useful suggestions. Technicians Jackie Tauberman, Colin Thorburn, Meredith Selhmeyer, and Chase Nicholson assisted with larval collections and/or sample picking and larval measurements.

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## **TECHNICAL ASSISTANCE AND COLLABORATIONS**

- Collaborated with CPW Research Scientist/Toxicologist Tawni Riepe with the development, planning, and grant acquisition for a project looking at sucker recruitment contribution by tributary in the Gunnison River.
- Collaborated with CPW Research Scientist/Toxicologist Tawni Riepe on the design and implementation of a thermal tolerance study on larval FMS. Collected wild spawn for this project, and participated in manuscript preparation.
- Collected streamflow data in the Roubideau Creek drainage and shared with Colorado Water Conservation Board and CPW's Water Section to aid in instream flow studies, and document out-of-bank flow dynamics. Shared local knowledge of flow timing, biological connections, and field work travel routes.
- Participated in the planning process for the eventual construction of a permanent Fish sorting structure on Roubideau Creek based on information learned during the RBW study.
- Dolores River investigations:
  - Assisted Dan Cammack (CPW Aquatic Conservation Biologist) with PIT tagging Three-Species in the Dolores and San Miguel rivers for ongoing movement studies.
- Reviewed CPW water management units designated as native waters with no native records within the Three-Species range, and determined whether occupancy by native fishes was likely or not.
- Assisted Tyler Swarr and Kristina Morben with the Grand Valley canal salvage projects, invasive mussel detection, and Boneytail Chub stocking.
- Maintained stream temperature loggers at tributary and mainstem sites in the Dolores, Gunnison, and White river basins to continue the long-term dataset that has been collected at those sites. Data from these loggers is used by CDPHE to support updates to the 303(d) Impaired Waters List.
- Provided technical expertise, wrote fish related informational sections for a grant application, and arranged CPW support for projects on the North Fork of the Gunnison aimed at modernizing diversion structure to increase flow and allow for fish passage.
- Discussed North Fork Gunnison River fish passage projects aimed at improving the Three-Species fishery. Provided data, and developed a movement monitoring plan.
- Wrote a letter of support for Dr. Derek Houston at Western University who was seeking a NSF grant looking at genetic history of western suckers.
- Collected sucker body depth data for Katie Birch with the CPW Water Unit to help inform instream flow needs for small sucker spawning tributaries.
- Worked with Trout Unlimited (TU) to monitor the fishery and physical conditions of the warm water section of Escalante Creek. Continued to develop a plan to assess effects of the improvements.
- Provided updates on CPW's Three-Species research progress to the Desert Fishes Council and Three-Species working group.
- Helped host, and presented in a symposium on non-perinneal streams at the Western Division AFS conference.
- Assisted Dr. Kevin Rogers with Lahonton Cutthroat Trout genetic material collections.

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