

# NATIVE THREE-SPECIES RESEARCH

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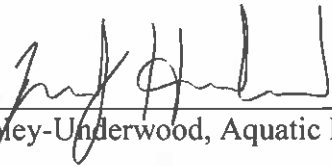
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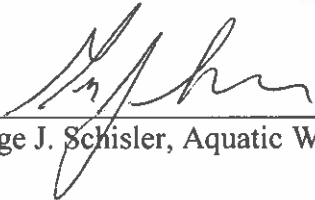
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*The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Colorado Parks & Wildlife policy by the Director or the Wildlife Commission.*

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## **Three-species tributary use and spawning investigations**

Period Covered: March 1, 2024 to November 1, 2025

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### **RESEARCH PRIORITY:**

Determine which tributaries to the Gunnison River support sucker (native and non-native) recruitment to Gunnison River populations across different hydrologic conditions, and identify effects of water quality on recruitment.

### **AUTHORS:**

Zachary Hooley-Underwood, Tawni Firestone, and Brian Avila

### **OBJECTIVES:**

- I) Rank tributaries by relative recruitment contribution for both native and non-native suckers.
- II) Monitor tributary associated recruitment trends over time, and correlate recruitment to hydrologic conditions.
- III) Associate tributary recruitment to water quality parameters.

### **INTRODUCTION**

Bluehead Sucker (BHS) and Flannemouth Sucker (FMS) are native to the Colorado River basin (CRB), where they presently occupy less than 50% of their historic ranges (Bezzerides and Bestgen 2002). Reasons behind these range reductions are varied, but one of the most widespread, ongoing risks is hybridization with non-native suckers. Nonnative White Suckers and, to a lesser degree, Longnose Suckers have invaded the majority of Bluehead and Flannemouth Sucker habitat in Colorado, are widespread throughout the CRB, and can hybridize with both native species without any apparent loss of viability (Bezzerides and Bestgen 2002; Mandeville et al. 2015). In many river systems, nonnatives and their hybrids have become as or more common than the natives, and extirpation or extinction by hybridization is a very possible future scenario. Options to reverse the spread of nonnatives and limit hybridization are scarce as the species are already widespread and inhabit large rivers and lakes where all forms of removal or suppression are cost- and resource-prohibitive.

Native sucker spawn in both perennial and intermittent tributaries (Levick et al. 2008; Laub et al. 2018; Hooley-Underwood et al. 2019). We may be able to prevent hybridization in a subset of river populations because tributaries are typically small enough that relatively low-cost exclusion or removal efforts can prevent comingling of native and non-native suckers during spawning. For example, we recently evaluated the feasibility of using a resistance board weir and fish trap to capture suckers immigrating into Roubideau Creek (a tributary to the Gunnison River near Delta, Colorado) and manually remove nonnative and hybrid suckers before allowing the remaining native suckers to migrate upstream and spawn. This allowed for tens of thousands of native suckers to spawn in the presence of fewer nonnative suckers, resulting in decreased hybridized larval production. This method is likely to become a long-term annual or semi-annual operation, requiring a significant resource commitment on the part of Colorado Parks and Wildlife and

collaborators. Before these resources are committed, it is necessary to ensure that a measurable benefit to the basin’s native sucker population will be realized. Thus, we aim to identify the relative recruitment provided by individual tributaries by identifying shallow genetic divergence among different tributary spawning populations in the basin, completed with a low-cost, next-generation, high-throughput genotyping method. We have identified a high degree of tributary fidelity in Gunnison River native sucker populations, so shallow genetic divergence is expected. We will evaluate the thermal landscape and water quality that out-migrating larvae experience to determine if there are recruitment bottlenecks associated with thermal transitions from warm tributary habitats to the colder, dam-manipulated mainstem Gunnison River, or if there are any water quality related factors that limit recruitment in certain tributaries. Lastly, we will use movement data ascertained through detections of PIT-tagged fish, to verify that adult fish participate in tributary spawning in accordance with genomics results.

**METHODS**

In 2024 and 2025, we attempted to collect larval suckers from each of nine tributaries suspected to contribute to recruitment (Table 1). Collections were primarily made with fine meshed dip-nets during May, June and July. To increase capture numbers, we also used drift nets. We hoped to sample at least 30 individual larvae/juveniles of each species (BHS, FMS, WHS) from each of the nine tributaries for genetic analysis, but field identification of larvae to species is functionally difficult, thus we collected excess larvae (goal of n = 200 per tributary per year) to identify to species in the laboratory. Within each tributary, we collected larvae from disparate dates and locations (at least spread over a minimum of 0.5 km) as possible to ensure that attained a randomized collection of larvae. Generally, this should not be a problem as larvae mix randomly as they undergo a passive drift-phase after hatch-out. Larvae and early juveniles were stored in 90% EtOH. The Larval Fish Laboratory (LFL) at Colorado State University (Fort Collins, Colorado) conducted all larval sucker identifications and will eventually select larvae that appear morphologically pure for inclusion in the genomic data set. We plan to use genotyping-by-thousands panel development (GTseq; Campbell et al. 2014) to identify tributary specific differentiation within larvae groups (spp. x tributary; Figure 1). This development will be completed in 2026 by the Eagle Fish Genetics Lab (Idaho Department of Fish and Game, Eagle, ID).

Table 1. The nine tributaries to the Gunnison River that likely support spawning and recruitment of Flannelmouth, Bluehead, and White suckers.

<b>Water Code</b>	<b>Tributary</b>	<b>Water Code</b>	<b>Tributary</b>
40458	Gunnison River #3	49343	North Fork of the Gunnison
43656	Tongue Creek	46905	Uncompahgre River
42717	Roubideau Creek	40054	Escalante Creek
20800	Kannah Creek	46498	East Creek
22765	Whitewater Creek		

Adult suckers (BHS, FMS, WHS and hybridized suckers) were collected from their home-range habitat in the Gunnison River via raft or boat electrofishing during the months of August-October in 2024 and 2025. These collections will continue for at least three more years. All adult fish were morphologically identified to species, weighed, measured, and tagged using an abdominally implanted 12.5 mm, 134.2 kHz, full duplex PIT-tag. Caudal fin clips were collected from adults and preserved on Whatman chromatography paper for genetic analysis. We also collected pectoral fin rays from each sampled sucker which will be used to age individuals so recruitment trends can be compared across time. We divided the Gunnison River from the Redlands Diversion near its mouth in Grand Junction to the confluence with the North Fork of the Gunnison River into five zones of equal length (roughly 14 miles each) to obtain a relatively uniform longitudinal sample. We collected genetic samples from equal numbers of BHS, FMS, and WHS or WHS hybrids in combination ( $n =$  at least 40 samples of each species per zone, which is roughly equal to three fish per mile per species). We also recorded the specific location of each sampled fish to conduct distance-based analyses of origin. DNA from adult fish will be extracted and run through the GTseq panels. Each individual will be assigned back to its tributary of origin, producing estimates of overall specific tributary recruitment by river segment.

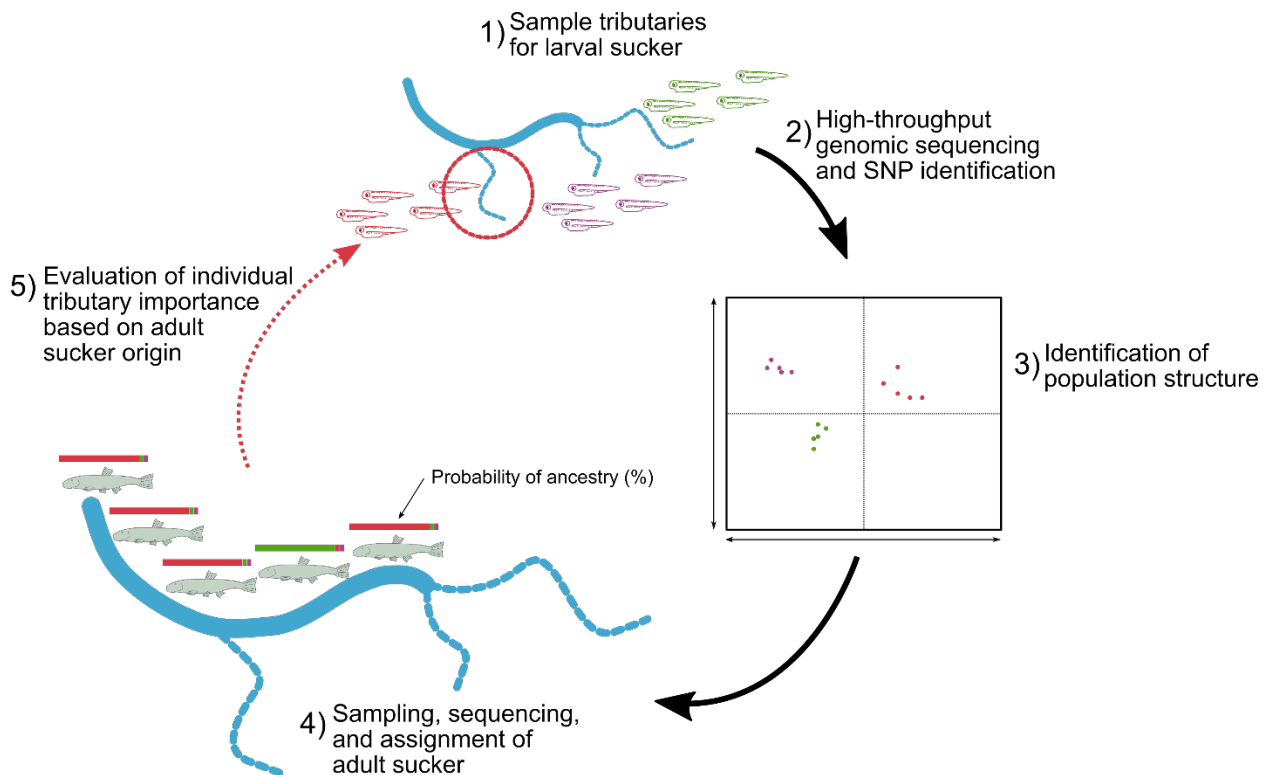


Figure 1. Conceptual diagram of the methods used for evaluating individual tributary importance in relation to native sucker production in the Gunnison River Basin.

## RESULTS AND DISCUSSION

Larval and adult fish collections were completed as planned in 2024 and 2025. In 2024, we collected 1,429 larvae from 11 tributaries. We collected larvae from Alkali, Currant, and Seep Creeks, not originally included in our list of nine primary tributaries, to identify any potential unexpected sources of recruitment. After sampling these added tributaries, we determined that in 2024, access was limited and few if any larvae were produced in any of those tributaries. Additionally, we determined that Whitewater Creek was not accessible to spawning Three-Species and was removed from our tributary list. In the remaining eight tributaries, we were able to collect 180-250 larvae per tributary, except for Kannah Creek in which we only collected four sucker larvae across four sampling attempts. These larvae were identified to species by the LFL (some additional identification work will be completed on questionable individuals during the 2025 sample identification work; Table 2).

Table 2. Preliminary species composition (%) by tributary estimates for 2024 larval sucker collections. The total number of larvae collected per tributary is indicated under “n,” while all other numbers represent the indicated species’ percentage of the total per-tributary. The “Hybridized WHS” includes all larvae that were deemed to have some degree of WHS ancestry, but that weren’t thought to be pure WHS. The “Unidentified or non-sucker” column includes larvae that were badly damaged or were of the genus Cyprinidae (and included in our collection by mistake). Green shading indicates populations where > 50% of individuals are pure, native ancestry, while orange shading indicates populations where >50% are of non-native ancestry.

Water	n	BHS	FMS	WHS	Hybridized WHS	Unidentified or non-sucker
East Creek	201	12.9	10.0	23.4	42.8	9.0
Escalante Creek	249	8.4	30.1	38.6	20.5	2.0
Gunnison River #3	201	8.5	2.5	66.7	20.4	2.0
Kannah Creek	4	25.0	0.0	75.0	0.0	0.0
North Fork Gunnison River	200	4.0	6.0	79.5	10.5	0.0
Roubideau Creek	201	19.4	61.2	8.0	6.0	3.0
Tongue Creek	179	8.4	3.4	20.7	65.9	1.7
Uncompahgre River	14	0.0	7.1	7.1	71.4	7.1

While larval identifications will likely change to a small degree after final revision by the LFL, early results show that WHS and WHS hybrids made up greater than 50% of the total species composition in all tributaries besides Roubideau Creek. Roubideau Creek samples were dominated by FMS larvae.

In 2025, we experienced greater difficulty collecting larval suckers. We increased our overall effort compared to 2024 but collected only 646 sucker larvae total. We suspect that decreased snowpack at the beginning of 2025 and the resulting diminished runoff may have led to poor spawning conditions in many tributaries. Only Roubideau and Escalante creeks had abundant larvae. In other tributaries, we collected between 40 and 90 presumed sucker larvae across

multiple sampling efforts. These larvae will be sent to the LFL and identification work will begin in late 2025.

### **KEY MANAGEMENT IMPLICATIONS**

- From data thus far generated under this project, Roubideau Creek in 2024 appeared to support much more native sucker recruitment compared to all other tributaries from which larvae were sampled.

### **ACKNOWLEDGMENTS**

Audry Harris (Eagle Fish Genetics Lab), Dr. Elizabeth Mandeville (Northern Michigan University), and Dr. Catherine Wagner (University of Wyoming) provided input on genomics methods. Numerous Colorado Parks and Wildlife and BLM permanent and seasonal employees helped with the extensive larval and adult fish sampling. Several municipal and private partners graciously provided access to key sampling locations.

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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**

Tributary spawning is a major life history component for the Three-Species (Bonjour 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. We have previously 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 2024 and 2025 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 in 2025 – Tongue Creek, Kannah Creek, and East Creek.

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 reside in the river during the remainder of summer, fall, and winter. We tagged Three-Species fishes during August, September, and October in 2023-2025 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 4 km of their capture location.

## **RESULTS AND DISCUSSION**

Results from 2016 - 2023 are available in our previous technical and annual reports. During 2024, we implanted 853 PIT tags into the three-species in the Gunnison River. We implanted a similar number in 2025, but data entry was ongoing at the time of writing. Detections of PIT tagged fish at the PIA give us the best indication of tributary fidelity as antenna efficiency is typically 100%. In Tongue, Escalante and Kannah creeks, we deployed paired SPRs (side by side) to improve channel coverage and allow for detection efficiency estimation. In future years we will be able to estimate fidelity rates for those locations as well. For the Roubideau PIA specifically, we have consistently recorded a high proportion of individuals of 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 2023 and 2024 returning in 2024 and 2025, respectively (Figure 1). In 2024, species-specific return rates (proportion of fish detected in 2023 redetected in 2024) were 82.5, 86.7, and

80.1% for BHS, FMS, and RTC, respectively. In 2025, species-specific return rates (proportion of fish detected in 2024 redetected in 2025) were 73.7, 85.0, and 72.7% for BHS, FMS, and RTC, respectively. These rates are similar to those observed annually since 2016, but were on the higher end of the typical ranges for all three species both years, except for RTC in 2025. Runoff in 2024 and 2025 was lower than in previous years during which fidelity was on the low end of the range for all three species. Differences in observed fidelity rates could be explained by either a negative correlation between runoff intensity and true fidelity, or by lower detection efficiency in high flow events. We have confirmed that high detection efficiency persists under high flow events to a degree, but 2022 and 2023 (both lower fidelity years) had extreme runoff levels beyond what we have been able to evaluate for array efficiency in the past. 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 65 to 90% range for all three species.

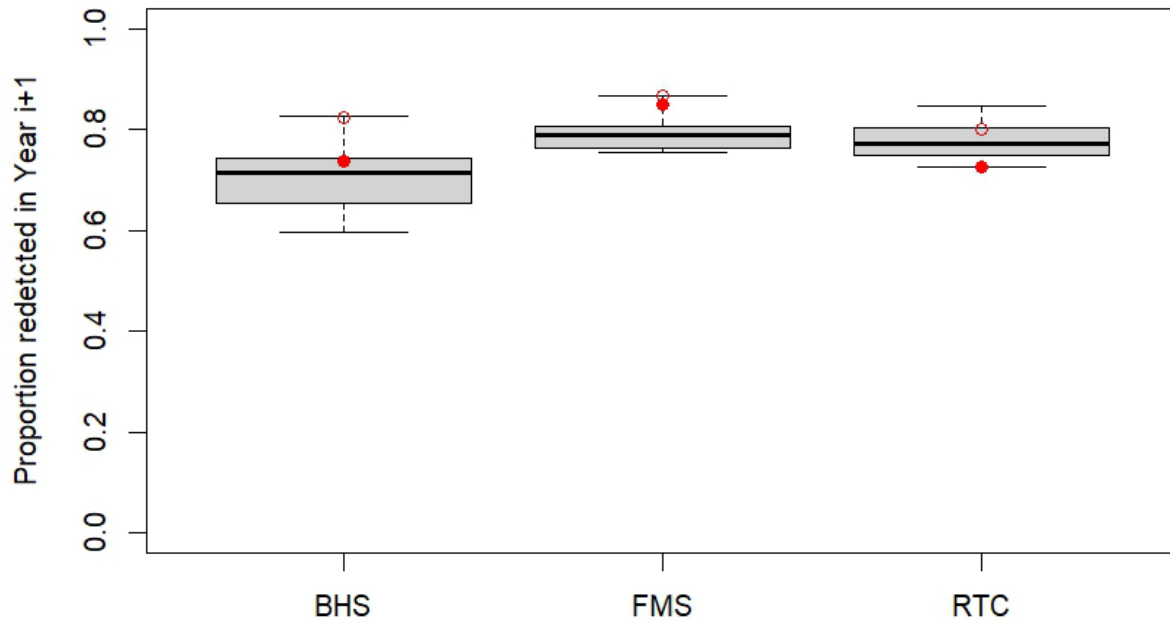


Figure 2. Annual fidelity rates for PIT-tagged Bluehead Sucker (BHS), Flannemouth Sucker (FMS) and Roundtail Chub (RTC) based on Roubideau Creek PIA detections between 2016 and 2025. Proportion redetected is the proportion of individual fish that were detected in year<sub>i</sub> redetected in year<sub>i+1</sub>. Box plots represent means and quartiles for fidelity rates across all years, while solid red circles indicate 2024 proportions, and open red circles represent 2025 proportions.

In order to identify tributary selection of Gunnison River Three-Species, in 2024 and 2025 we PIT tagged over 1600 fish between mid August and the end of October in the mainstem Gunnison River between the North Fork confluence and the Redlands Diversion. In 2026, redetection analyses of these fish will be compared across the North Fork of the Gunnison and Uncompahgre rivers, and the Tongue, Escalante, Kannah, and East creek SPRs to determine which tributaries support migrating fish.

## **KEY MANAGEMENT IMPLICATIONS**

- The Three-Species exhibit very strong annual fidelity to Roubideau Creek regardless of annual environmental conditions.
- Access to Roubideau Creek for the Three-Species is necessary for life-history completion and potentially the persistence of Gunnison River populations.

## **ACKNOWLEDGMENTS**

Former Aquatic Researcher Kevin Thompson initiated and conducted the bulk of the work described here through 2020. Dozens of people from multiple agencies over the years PIT-tagged the suckers and chub used and re-detected in this priority. In 2024, Colorado Parks and Wildlife technicians Giulio Del Piccolo and Meredith Selhmeyer, and in 2025 technicians Aarohi Barman, Cameron Brown, and Chris Craft 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 important, 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 major 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 through 2025. 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 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.



Figure 3. 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

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 -2023 are available in those respective annual reports. Reasonably wet conditions in 2024 contrasted to fairly dry conditions in 2025, which likely resulted in the presence of more species in 2024 in general. We had intended to end this study after pre-runoff sampling in 2025, but the dry conditions warranted sampling in the fall of 2025 to capture the effect of another season of drought. Indeed, we saw overall low catch rates and species diversity

in the fall of 2025. In 2023, we noted the first occurrence of FMS in Dry Fork Escalante above a barrier that was removed in 2021, and in 2024 we documented the first presence of RTC as well. The removal of the diversion barrier prior to 2022 likely allowed for the immigration and spawning of FMS and RTC from Escalante Creek, which we believe is the source of these fishes.

Table 3. Sampling sites in Dry Fork Escalante and Cottonwood creeks and results of sampling occasions. 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 2024 and 2025, 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; RTC= Roundtail Chub; SPD = Speckled Dace).

	Reach	Baseflow condition	Pre-Runoff (March/April)		Post-Runoff (May/June)		Fall (September/October)	
			Condition	SPP present	Condition	SPP present	Condition	SPP present
2024								
Dry Fork Escalante	DED1	Dry	Wet	FMS*	Wet	BHS, FMS, RTC, SPD	Wet	None
	DEW1	Wet	Wet	BHS, SPD	Wet	BHS, FMS, RTC, SPD	Wet	BHS, FMS, SPD
	DEW2	Wet	Wet	BHS, FMS, SPD	Wet	BHS, FMS, SPD	Wet	BHS, FMS, SPD
	DED2	Dry	Wet	None	Wet	BHS, SPD	Wet	None
	DEW3	Wet	Wet	BHS, SPD	Wet	BHS, FMS, SPD	Wet	BHS, SPD
	DED3	Dry	Wet	BHS, SPD	Wet	BHS, SPD	Dry	-
Cottonwood	CCD1	Dry	n/a	-	Wet	None	n/a	-
	CCW1	Wet	n/a	-	Wet	BHS, FMS, SPD	n/a	-
	CCD2	Dry	n/a	-	Wet	BHS, SPD	n/a	-
2025								
Dry Fork Escalante	DED1	Dry	Wet	FMS, SPD	n/a	-	Dry	-
	DEW1	Wet	Wet	FMS	n/a	-	Wet	FMS, SPD
	DEW2	Wet	Wet	SPD	n/a	-	Wet	SPD
	DED2	Dry	Wet	None*	n/a	-	Dry	-
	DEW3	Wet	Wet	BHS	n/a	-	Wet	BHS, SPD
	DED3	Dry	Wet	None	n/a	-	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	-	Dry	-

\*One small fish observed but not captured.

The fact that we continued to capture juvenile FMS throughout 2024 and 2025 suggests that this species is taking up residency in this previously unavailable habitat. In contrast, no RTC were captured in the fall of 2024 or at any point in 2025, suggesting that in these years, RTC may only use Dry Fork Escalante during spring runoff (likely for spawning) and may do so only under higher water conditions. 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. More detailed analyses of population dynamics and migration will be conducted in 2026.

### **KEY MANAGEMENT IMPLICATIONS**

- Self-sustaining populations of Three-Species fishes can exist in small perennial habitats within intermittent stream networks. Likely intermittent waters should be thoroughly surveyed for perennial segments that may support such populations.
- When possible, removing derelict barriers or facilitating fish passage may result in rapid recolonization by the Three-Species.

### **ACKNOWLEDGMENTS**

In 2024 and 2025, Bureau of Land Management biologist Colin Brady assisted with fish sampling. CPW aquatic technicians Aarohi Barman, Cameron Brown, Christopher Craft, and Giulio Del Piccolo, Meredith Selhmeyer, and Kevin Thompson assisted with fish 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 were 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 the Gunnison River both above and below each tributary confluence (>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. 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 attempted to

collect around 40 larvae 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 to 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 prior to runoff in 2024, and remained active through 2025.

*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**

Most of our analyses will occur after species identification and otolith aging occurs, but we did observe several trends. For a summary of 2023 catch and growth data, please see our 2023 annual report. We captured a total of 102 and 159 eggs, and 689 and 1059 larvae in 2024 and 2025, respectively among all sites and occasions. We sampled weekly from May 7<sup>th</sup> through June 19<sup>th</sup> in 2024, from April 24<sup>th</sup> through June 25<sup>th</sup> in 2025. In 2024 the first eggs were captured May 7<sup>th</sup> in the lower Roubideau Creek site, and in 2025 the first eggs were captured May 6<sup>th</sup> at the upper Roubideau Creek site. The first larvae were captured May 28<sup>th</sup> both years, at the lower Roubideau Creek site in 2024 and the upper Roubideau Creek site in 2025. This aligns with observations from 2023 as well, when the earliest indication of spawning and hatch-out also occurred in Roubideau Creek. Overall, we had a much more difficult time capturing larvae in 2024 and 2025, than in 2023. Drift net catches were generally low and dip netting was consistently unproductive in all sites except for Roubideau Creek. This decreased catch compared to 2023 likely represents decreased abundance of larvae, as methods did not change but effort increased from 22.8 total drift net-hours in 2023, to 48.7 in 2024, to 66.4 in 2025. Larvae and egg catch was the highest weekly in the Roubideau Creek sites, similar to 2023.

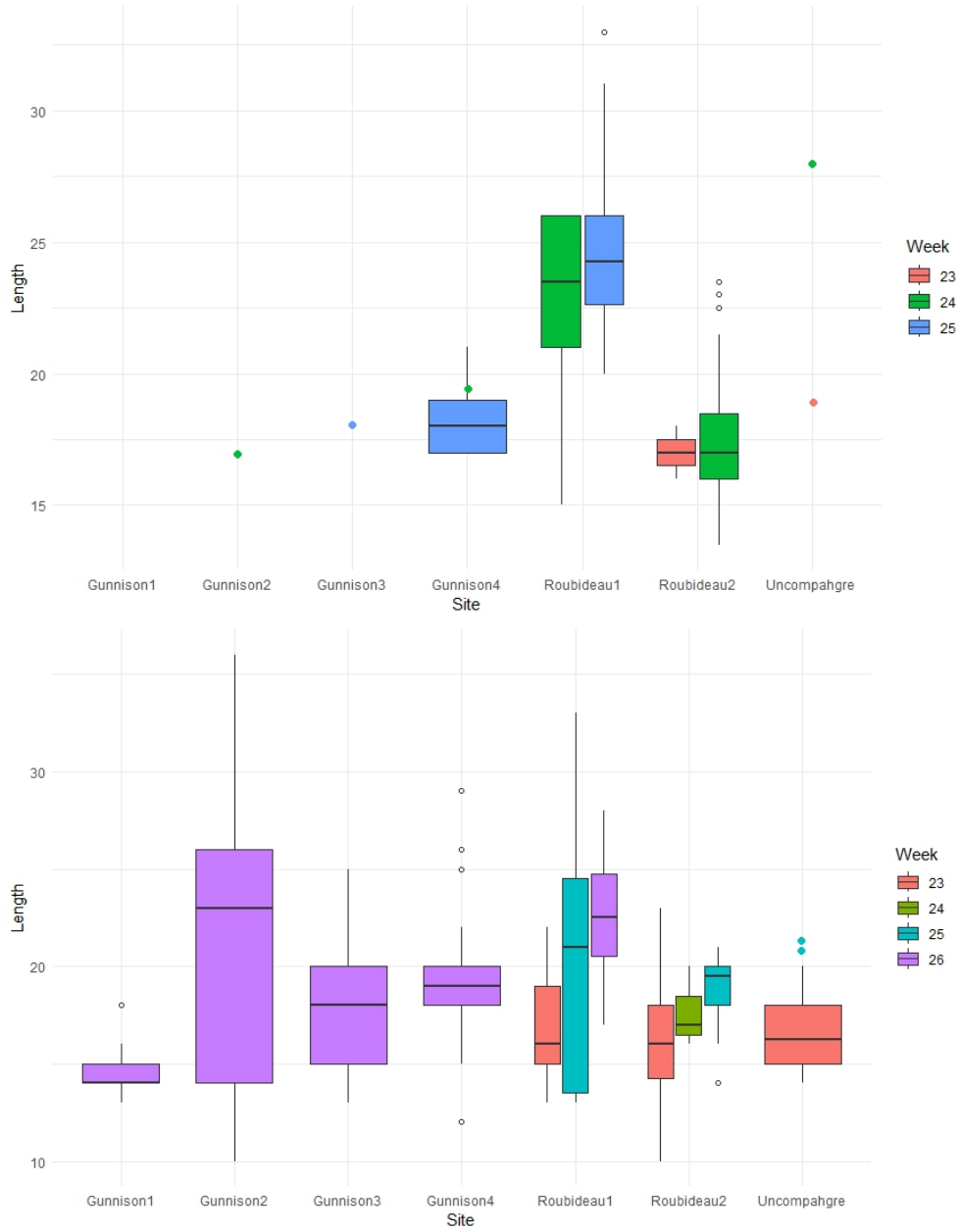


Figure 4. A comparison of larval sucker average lengths by calendar week at seven sites from 2024 (A) and 2025 (B). Larvae were collected using drift or dip nets weekly, and were measured to total length. Boxes represent mean and quantile lengths for each site and week. Colored circles represent weeks when less than three individuals were sampled at a site. 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 2 was 13 km upstream in Roubideau Creek, and Uncompahgre was 1.5 km upstream of the mouth of the Uncompahgre River.

Gunnison and Uncompahgre river samples generally had few eggs or larvae, but the Gunnison site below the Roubideau confluence consistently produced the highest egg and larvae catches in the Gunnison, suggesting we were seeing the result of Roubideau Creek out-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 during the first week in May in both 2024, and 2025. This was several weeks earlier than in 2023. Runoff conditions were below average in 2024 and 2025, compared to 2023, which saw significantly above average runoff. Despite higher water conditions (which could have a diluting effect on larvae abundance in the drift) and lower effort, we captured more sucker larvae at all sites in 2023 compared to 2024 and 2025. This suggests that 2023 conditions were more favorable for larval production. However, we did observe a delay in spawning and hatch-out in 2023.

Thus far, results are combined for all species, as larvae have not been identified to species, and we don't have hatch-date or growth data, as we will have larvae identified, and otoliths extracted and aged all at once in the final year of study. However, we were able to compare collective larval lengths across sites and weeks in 2023 - 2025, 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 thus far 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.

#### **KEY MANAGEMENT IMPLICATIONS**

- Spawning and hatch-out (based on egg and larval presence) consistently occur earlier in Roubideau Creek than in the mainstem Gunnison River, or the Uncompahgre River.
- Early evidence suggests that higher larval sucker growth rates may occur in intermittent Roubideau creek compared to the Perennial Uncompahgre River and the mainstem Gunnison River, but analyses are incomplete.

#### **ACKNOWLEDGMENTS**

Dr. Kevin Bestgen at Colorado State University was consulted on this project and provided many useful suggestions. Colorado Parks and Wildlife Aquatic technicians Aarohi Barman, Cameron Brown, Christopher Craft, Giulio Del Piccolo, Kellie Newcomer, Meredith Selhmeyer, and Kevin Thompson assisted with larval collections and/or sample picking and larval measurements.

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## **TECHNICAL ASSISTANCE AND COLLABORATIONS**

- Worked with Colorado Parks and Wildlife’s Water Program using multiple SPRs in Cottonwood Creek to try and assess minimum passage depth of riffles for Bluehead and Flannelmouth suckers.
- Participated in an ongoing movement study using SPRs and PIT-tagged fish in Escalante Creek.
- Provided data and support letters to the Western Slope Conservation Center for multiple grant pursuit phases for a rebuild of a diversion on the North Fork of the Gunnison, and worked with Jenn Logan and CWCB to secure match funding for installing a PIA with the diversion rebuild (Fall 2024/Spring & Summer 2025).
- Consulted with BLM and Nature Conservancy on aquatic Goals for the Escalante Ranch habitat reclamation project.
- Compiled Research data on BHS distribution and population size for GMUG Forest Service effort to add BHS to forest sensitive species list (June 2025).
- Sampled East Muddy Creek for BLM to assist with in-stream flow goals (July 2025).
- Assisted Billy Atkinson and Tyler Swarr with a White Sucker removal and Mountain Sucker translocation/Genetic ID project in Circle Creek, California Park (September/October 2024).
- Assisted with Grand Junction Canal Salvage and Salt Creek Sampling (November 2024).
- Assisted with BHS PIT tagging at NASRF (March 2025)
- Provided, modified, manufactured, and installed a picket weir on Great Sand Dunes NP to prevent entrainment of Rio Grande Sucker and Chub in an irrigation diversion. Worked with Dan Cammack (CPW) and NPS staff. (March/April 2025).
- Participated in sampling and removal efforts of non-native chub from Yellow Jacket Creek (April 2025).
- Led the installation of a resistance board weir in the East River on the Roaring Judy Hatchery property to direct Kokanee Salmon into the raceway outlet channel (August 2024).
- Collected Roundtail Chub fin clips for a Colorado Parks and Wildlife range-wide genomic evaluation
- Participated in the McIntire Spring reclamation project specifically conducted for the benefit of Rio Grande Sucker and Rio Grande Chub (October 2025).

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