NATIVE THREE-SPECIES RESEARCH

Zachary Hooley-Underwood
Aquatic Research Scientist

Annual Report

Colorado Parks & Wildlife

Aquatic Wildlife Research Section

Fort Collins, Colorado

May 2022

STATE OF COLORADO

Jared Polis, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Dan Gibbs, Executive Director

COLORADO PARKS & WILDLIFE

Dan Prenzlow, Director

WILDLIFE COMMISSION

Carrie Besnetter Hauser, Chair
Charles Garcia, Vice Chair
Luke B. Schafer, Secretary
Taishya Adams
Karen Michelle Bailey
Betsy Blecha
Marie Haskett
Dallas May
Duke Phillips IV
James Jay Tutchton
Eden Vardy

Ex Officio/Non-Voting Members:
Kate Greenberg, Dan Gibbs, and Dan Prenzlow

AQUATIC RESEARCH STAFF

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

Alexandria Austermann, Librarian

Prepared by: _____

Zachary Hooley-Underwood, Aquatic Research Scientist

Approved by: _____

George J. Schisler, Aquatic Wildlife Research Chief

Date: ___May 2, 2022_____

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

TABLE OF CONTENTS

LIST OF FIGURES

Three-species tributary occupancy and spawning investigations

Period Covered: March 1, 2021 to November 31, 2021

PROJECT OBJECTIVE: To determine the current distribution of Bluehead *Catostomus discobolus* and Flannelmouth *C. latipinnis* sucker and Roundtail Chub *Gila robusta* (collectively referred to as the three-species) in tributary streams of Colorado's mainstem, western rivers (Green, Yampa, White, Colorado, Gunnison, Dolores, and San Juan) and to evaluate spawning ecology of the fishes and limit hybridization of the two sucker species with introduced nonnative suckers.

RESEARCH PRIORITY:
Identify the current distribution of three-species fishes in tributary streams of western Colorado Rivers.

OBJECTIVES:
Identify precise distribution of the three-species in several streams and drainages in the Colorado, Dolores, and Gunnison basins.

INTRODUCTION
Bluehead Sucker *Catostomus discobolus* (BHS), Flannelmouth Sucker *C. latipinnis* (FMS), and Roundtail Chub *Gila robusta* (RTC) are collectively known as the "three-species" under conservation regimes shared by numerous western states that contain habitats occupied by these fishes. All three are native to the Colorado River basin, and FMS and RTC are endemic to the basin while BHS also occupy portions of the Snake and Bear River drainages to the north of the Colorado River basin. All three species have experienced significant population reductions, and have been extirpated from many habitats.

Much of the available data and literature on the distribution of the three-species focuses on large rivers and major tributaries – habitat that is indeed important to a substantial component of these species' populations. However, small tributaries and even intermittent waters can be important for all three species annually, and some populations are even restricted to small tributaries. Therefore, to truly understand the three-species distribution in Colorado and to spatially and temporally implement conservation efforts with the most effect, it is necessary to identify the distribution and changes in the distribution of these fishes in these tributary habitats. Over the past decade, we have sampled extensively to model the fishes' distribution in Colorado, the

results of which are available in our 2019 Technical Report. In 2021, we continued to sample targeted locations to refine our knowledge of the specifics of the species' distribution. Our efforts were reduced in 2021 compared to previous years, as the list of waters to investigate has been reduced, and we shifted effort to more in-depth evaluations of several waters (see final priority in this report).

METHODS

We consulted the three-species database created in winter 2019/2020 for areas where data was missing or indeterminate. We conducted presence/absence surveys with backpack electrofishers. We did one or two pass surveys. If no three-species fishes were captured or observed on the first pass, we did not do a second pass. Occasionally, time limitations precluded a second pass even when presence was established. Results were incorporated into the range-wide three-species database.

RESULTS AND DISCUSSION

For our completed range-wide sampling and modeling results, please see the following technical report.

- Thompson, K. G., and Z. E. Hooley-Underwood. 2019. Present Distribution of Three Colorado River Basin Native Non-game Fishes, and Their Use of Tributary Streams. Colorado Parks and Wildlife Technical Publication 52.

In 2021, we sampled several waters with no sampling history, and sampled multiple locations on previously sampled intermittent waters where we wanted to confirm the continued presence of three-species fishes. The only newly discovered occupied site was on Disappointment Creek (Dolores River drainage) west of Lone Cone State Wildlife Area. We sampled two locations, and found BHS at one site (our upstream survey). However, we only discovered three individuals in a 900 foot reach. A local landowner/rancher indicated that our downstream site is intermittent, while the upstream site is perennial (outside of runoff, Disappointment Creek loses volume as it flows from ponderosa forest into dry pinon-juniper and sage desert). We found Speckled Dace (SPD) but no BHS at the downstream site. The presence of BHS at the upstream site is important, because the only other location any of the three-species have been found in the drainage is 20 miles (straight-line) downstream near the stream's confluence with the Dolores River. Mid-way between the stream's mouth and the occupied site, the stream flows over a large sandstone sill that forms an 8 to 12 foot waterfall. The presence of BHS at the one site we surveyed is evidence that a resident population exists above this natural barrier in Disappointment Creek. Further exploration should be conducted upstream from our occupied site to determine the extent of the occupied stream reach. Please see the final priority in this report for the results of the rest of our occupancy refinement sampling.

ACKNOWLEDGMENTS

CPW Aquatic Research Technician John Fesenmaier assisted with all of the sampling.

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:

Determine annual spawning tributary fidelity of PIT-tagged three-species fishes.

INTRODUCTION

Information is relatively sparse on whether individual BHS and FMS suckers tend to select specific tributaries and locations for spawning repeatedly or if they stray among tributaries. If they do exhibit high rates of spawning tributary fidelity, efforts to limit hybridization in tributaries such as those described in the following research priority are more likely to result in decreased hybridization in the basin over the long term. In this scenario, a higher proportion of natives are likely to return to controlled tributaries as genetically pure fish recruit to the spawning population following control measures, even if hybridization continues to increase in uncontrolled portions of a basin. Alternatively, if fish stray from tributary to tributary among years, we would expect to see a long-term increase in hybridized fish in a controlled tributary, reflecting the basin wide continued increase in hybridization incidence. Therefore, in conjunction with testing the feasibility of spawning run control measures (see following Research Priority), we deemed it important to simultaneously evaluate tributary fidelity among the three-species fishes. In recent years we identified high tributary fidelity rates in Gunnison River tributaries. We are continuing to monitor these movement patterns to see if these patterns are affected by the highly variable climatic and hydrographic conditions typical of the Colorado River basin.

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). We have estimated short term fidelity rates as simply 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.

RESULTS AND DISCUSSION

This Research Priority is partially complete. Our 2019 technical report including this project can be referenced for detailed methodology and results through 2018.

- Thompson, K. G., and Z. E. Hooley-Underwood. 2019. Present Distribution of Three Colorado River Basin Native Non-game Fishes, and Their Use of Tributary Streams. Colorado Parks and Wildlife Technical Publication 52.

During 2021, additional PIT tag data was collected. The following is a brief summary of the published results of this Research Priority, as well as an update on the results of the 2021 data collection:

In 2016 -2020, we observed high rates of tributary fidelity to the Roubideau Creek drainage as reported in Thompson and Hooley-Underwood (2019), and more recent progress reports. Of the PIT-tagged native suckers detected entering Roubideau Creek during the spawning period in any given year, 63 - 78% of those fish (not adjusted for any annual mortality) were detected again the following year during the spawning period (Figure 1). For Roundtail Chub, rates ranged from 72 - 80%. Non-native suckers and hybrids were tagged in low numbers, and after 2016 recaptured tagged fish were culled, but fidelity rates were still observed as high as 72%. Additionally, fidelity to specific tributaries within the Roubideau Creek drainage appeared high, with the majority of the fish detected in different tributaries having been originally tagged in those same tributaries.

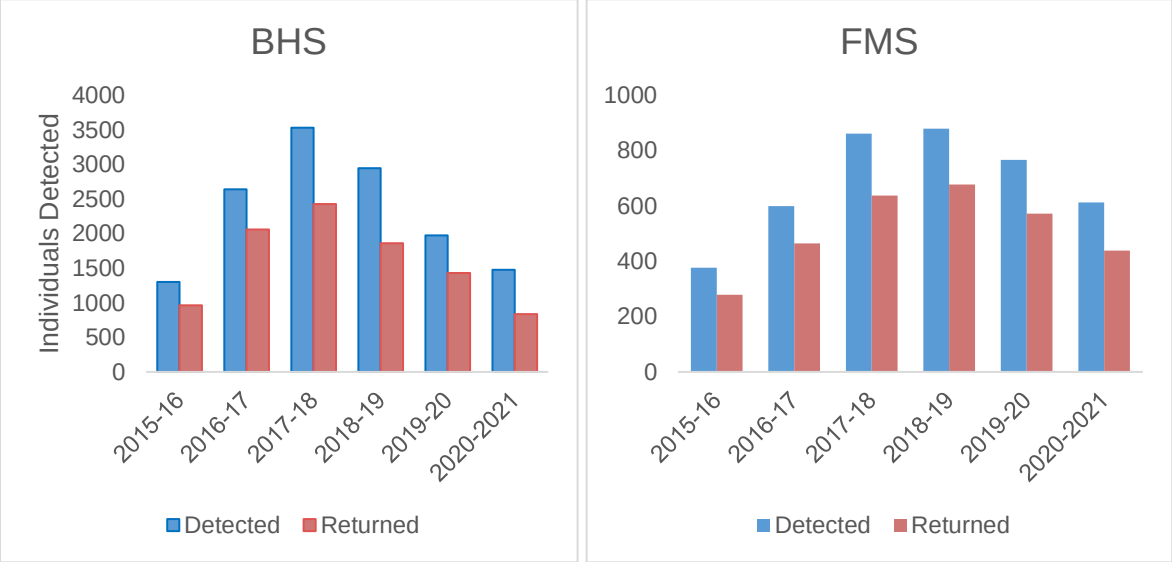

Figure 1. One-year fidelity rates for PIT-tagged BHS and FMS based on Roubideau Creek PIA detections. Bar-pairs represent the number of individual fish detected in $year_i$ (Blue) and redetected in $year_{i+1}$ (Red). The difference between annual pairs represents the number of individuals that did not return. Reduced tagging efforts starting after 2017 are responsible for the overall declines in detections in more recent years.

In 2021, runoff was poor despite near-average annual snowpack. Much of the accumulated snow did not materialize as runoff due to exceptionally dry soil conditions in summer and fall 2020. Of the PIT-tagged three-species fishes and non-native (and hybridized) suckers detected in 2020 on the PIA, redetection rates in 2021 were 56.6% for BHS, 71.6% for FMS, 76.3% for RTC, and 37.5% for other suckers. Specific tributary redetections via SPRs were collectively low due to poor water conditions and decreasing numbers of tagged fish.

In 2019 - 2021, we also estimated the mean fidelity rate for each species across all years of sampling. For this estimate, we plotted all individuals redetected in $year_{i+1}$ as a function of the number detected in $year_i$. To see if fish are likely to return multiple years in a row, we followed groups of fish across time. For example, we used all fish detected in 2015 ($year_i$) coupled with the number of those redetected in 2016 ($year_{i+1}$) as a data point, as we had in the previous analysis, but then also used those particular 2016 redetections of 2015 detections (i.e. $year_i$ & $year_{i+1}$) coupled with the number of those redetected again for the third time in 2017 ($year_{i+2}$) as a data point. We applied this scheme to all years of data for each species, and fit linear regressions. In the case of non-native suckers (including non-native hybrids), we plotted the data points that were affected by our culling efforts at the 2017 Cottonwood Creek weir (during which we culled previously tagged non-natives) separately from points unaffected by those removals, and fit regressions for both data sets. The slopes of these regressions approximate the average annual return rates, and deviations from the average 1-year rates presented above indicate that groups of fish that return in multiple consecutive years exhibit return rates that vary from the 1-year average. For BHS, FMS, and RTC, the regressions including all years fit the data very well (Figure 2). Analyzed this way with the inclusion of 2021 detections, annual fidelity rates remained surprisingly steady (as indicated by the large r^2 values) and are actually somewhat depressed compared to our single year rates for BHS, but slightly elevated for RTC and FMS. This may suggest that FMS and RTC that return at least once to the stream are more likely to return in subsequent years. For non-native suckers, when considering only the data unaffected by 2017 culling, a regression with a slope of 0.78 fit the data very well ($r^2 = 0.99$). This indicates that non-native suckers also have high rates of tributary fidelity. The annual rate estimate that includes our culling effort was 39.7% ($r^2 = 0.70$) indicating that even with non-native removal occurring only at Cottonwood Creek, we were still able to greatly decrease the number of non-native fish returning to the Roubideau drainage as a whole (it is important to note that we base this estimate off of only six data points however). These findings offer encouragement for the resistance board weir project because removal efforts at the weir are likely to have a reducing effect on non-native suckers in following years.

Considering specific tributary fidelity within the Roubideau Creek drainage in 2021, we redetected fewer fish than in previous years in general. Similar to 2020, this was largely due to the low water year which resulted in shorter (and later) windows of accessible flow in the smaller streams throughout the drainage. Even Roubideau Creek upstream of Buttermilk Creek (which is heavily supplemented by irrigation return flows) had flows low enough that access was hindered during a substantial portion of the spawning period. We deployed SPRs and detected fish at the

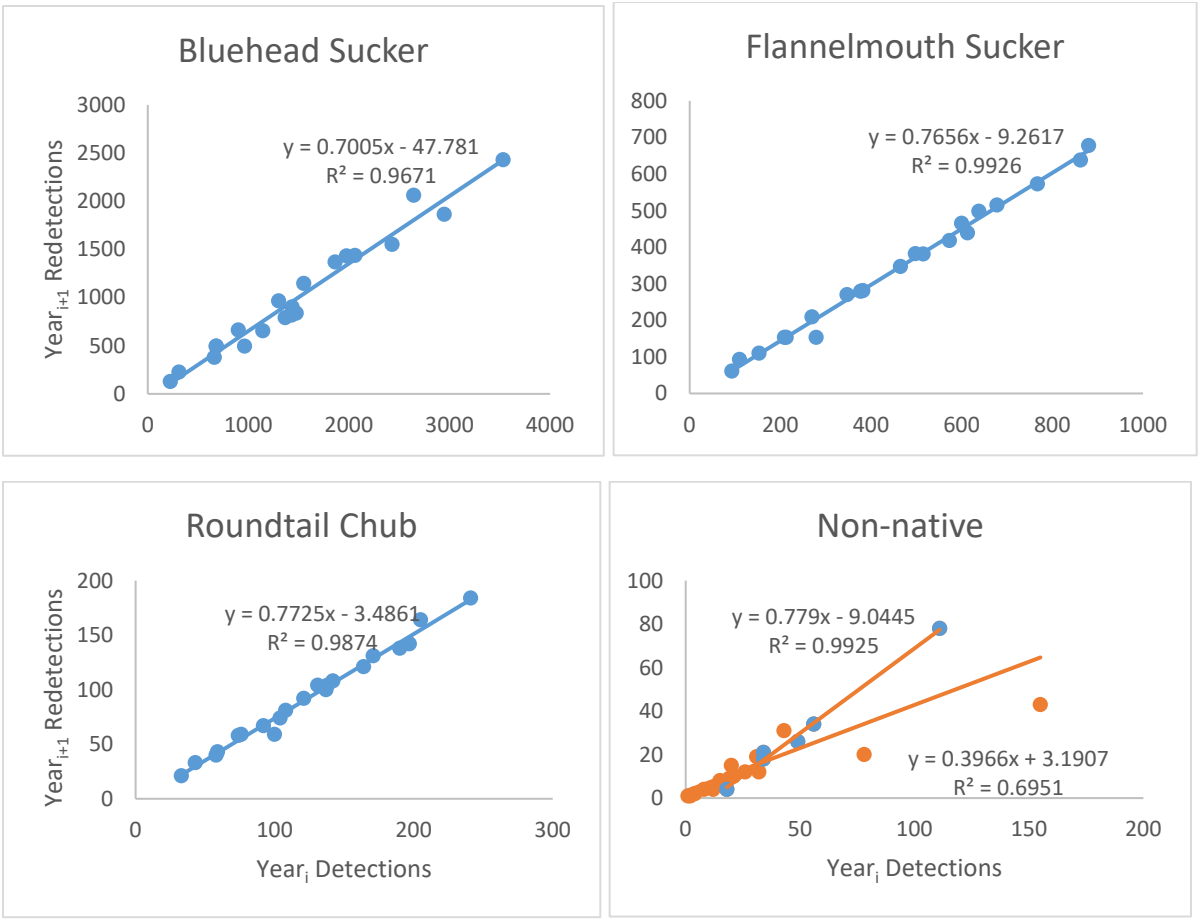

Figure 2. Redetections of PIT-tagged BHS, FMS, RTC, and Non-native suckers (WHS, LGS, and hybrids combined) as a function of the total number of individuals detected in the previous year for all years of data (2015 detections-2021 redetections). Included are multiple year redetections (i.e. number of redetections of year$_i$ fish in year$_{i+2}$ as a function of the total number of year$_i$ fish detected in year$_{i+1}$). Slopes approximate the overall annual return rate for each species. For non-natives, we plotted data that was affected by our culling efforts of PIT-tagged fish on the Cottonwood Creek Weir in 2017 (blue) independently of the entire data set (orange).

same locations as in previous years (see 2019 Technical Report), except that we did deploy an SPR both at the county-line site on Roubideau Creek and in Roubideau Creek above Potter Creek (at nearly the same location as in 2018; see the technical report). As in 2018 and 2020 when flows were also exceptionally low, the tributary that resulted in the most individual fish detected this year was Buttermilk Creek, which corroborates our past conclusion that Buttermilk Creek is only used by substantial numbers of fish when alternative tributaries are unusable. The fish detected in Buttermilk Creek were mostly BHS, with only a handful of FMS and RTC. As in 2020, nearly 70% of all individuals were originally tagged in Cottonwood Creek, which is unsurprising considering Buttermilk Creek is the next tributary that fish encounter when

Cottonwood Creek is low or dry. Cottonwood Creek did not flow in 2021. In Potter Creek and in Roubideau Creek above Potter Creek, few fish were detected (n=6 in Potter, n=42 in Roubideau), and all but one (which was originally tagged in the Gunnison River) were originally tagged in either Roubideau or Potter creeks. Due to the limited volume of data this year, it is difficult to draw strong fidelity conclusions based on SPR detections, but there is nothing that counters our conclusion from past years that fish tend to return to the stream in which they were originally tagged when they can.

ACKNOWLEDGMENTS
Former Aquatic Researcher Kevin Thompson initiated and conducted the bulk of the work described here. Dozens of people over the years PIT-tagged the suckers and chub used and re-detected in this priority. Aquatic technicians Chase Garvey, John Fesenmaier, Gwen Harris, Jackie Tauberman, Sam Redmond, and Kelsey Marshall helped with SPR deployment and maintenance in 2021.

RESEARCH PRIORITY:
Test a resistance board weir as means of controlling the entire Roubideau Creek spawning run, allowing for the selective exclusion and removal of non-native and hybridized suckers.

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

Options for mitigating the threat of hybridization to BHS and FMS are increasingly limited due to the overall range-wide abundance of non-native and hybrid suckers. Protection from hybridization has been attempted and sometimes achieved for other freshwater species utilizing isolation of key habitats, mechanical removal, chemical removal, or a combination. These methods are largely impractical for BHS and FMS because both species are typically associated with large rivers where financial, resource, social, and political limitations are insurmountable. One conservation action that remains feasible and has the potential to preserve the genetic integrity of large population components involves the mechanical control of spawning runs into tributary habitats that lack resident non-native or non-native hybrid adult suckers. Having determined that maintaining a picket weir in a small intermittent tributary through typical spring runoff conditions was very difficult, we searched for alternative control devices. A search for a better weir design led to consideration of resistance board weirs (Figure 3). This design was

originally conceived for use in Alaskan salmon runs, and is amenable to streams much deeper and wider than the intermittent Cottonwood Creek. It allows accumulating debris to depress the weir panels so that debris can flow over the weir, while still constraining upstream fish movement. Thus, it is a promising design for the mainstem Roubideau Creek.

Our objective here is to test the feasibility and efficacy of mechanically excluding the vast majority of non-native and hybrid suckers from the Roubideau drainage during the spawning season using a resistance board weir (RBW). If effective, a decrease in the proportion of larval suckers in the drainage with non-native genetic influence is expected compared to sampling in previous years. Our ultimate goal is to develop a conservation strategy that can be applied to other tributaries throughout the range of BHS and FMS.

The Roubideau Creek drainage is a prime candidate for a manipulation of this nature. Roubideau Creek lacks significant resident populations of adult suckers, especially above the confluence of Buttermilk Creek, and offers more than 25 stream-miles of spawning habitat, with more available in tributaries of Roubideau Creek. In 2018, approximately 12% of all fish implanted in 2014-2015 with passive integrated transponder (PIT) tags in the Gunnison River from the Colorado Highway 65 Bridge, east of the city of Delta, to the confluence of Roubideau Creek were detected in the Roubideau Creek drainage. Considering this is a conservative estimate because not all PIT-tagged fish were still alive, it highlights the importance of the drainage to the Gunnison River population as a whole. Additionally, high rates (70-80%) of tagged suckers return from one year to the next, suggesting that spawning tributary fidelity is high (as described in the previous Research Priority). Excluding non-natives and hybrids from the drainage would create a hybridization-free spawning opportunity for a significant portion of the Gunnison River native sucker population, and annual fidelity would allow for the perpetual protection of the genetic purity of the Roubideau Creek-spawning population component, even if hybrid and non-native abundance continues to grow elsewhere in the overall Gunnison River population.

Additionally, in light of the differences in both adult and larval species composition observed between Cottonwood and Potter creeks discussed in Chapter 2 of the above mentioned Technical Report (i.e. a much higher proportion of White Sucker and their hybrids in Cottonwood Creek than in Potter Creek), we hypothesized that there may be differences in the overall mileage of spawning tributaries that native and non-native suckers use. If this is so, then there may be a natural level of protection against hybridization in some tributaries, wherein pure native sucker recruits are produced in tributary reaches farther from mainstem rivers. We seek to continue evaluating this hypothesis by examining the species composition of larval sucker collected at discrete locations between the mouth and headwaters of Roubideau Creek.

METHODS

RBW operation – In 2019, we secured funding and began the planning process for this Research Priority. We developed a contract with a knowledgeable and experienced firm to fabricate a RBW and fish trap suitably sized for Roubideau Creek. The RBW consists of PVC picket panels that are anchored on the upstream end to the substrate along a rail upon which they pivot, and are held above the water's surface downstream by hydraulic lift generated by large boards attached

to the underside of the panels (Figure 3). One panel has an “entry chute,” essentially a tunnel of PVC pickets that allows fish to pass through the weir and a fyke into a large (6 x 10 x 5 foot) cage. We planned to deploy the RBW near the mouth of Roubideau Creek from early March through the middle of May in 2020 and 2021, capturing what detections on the PIA have indicated is the entire immigration period. The developing COVID-19 pandemic in 2020 forced us to cease operations on April 1. Therefore, we adjusted the project timeline to include 2021 and 2022 as manipulation years. In 2021, we continually staffed the weir, in an attempt to minimize the amount of time fish were held in the cage. All fish were identified to species, and those that were deemed pure native suckers (and RTC) were passed upstream to continue their spawning migration, while non-native and hybrid suckers were removed from the population. Additionally, length and weight data were taken on a daily subset of each species, and a subset of native suckers and RTC were PIT tagged. The number of PIT tags implanted per species per week was based on the number of PIA detections per species per week averaged over all years since 2015.

Figure 3. The fully assembled resistance board weir (RBW) in Roubideau Creek. The photo faces upstream. The PVC pickets in foreground are anchored on the upstream end to a substrate rail, but can pivot freely with water level. The only passage through which fish can pass is the PVC chute leading into the aluminum cage. The wall-tent in the back ground houses the fish-working station.

Pre-trapping removal - After we discovered adult non-native suckers overwintering in Roubideau Creek in March 2020, we attempted a removal effort over the entire 5.8 miles of Roubideau Creek between the weir and the Buttermilk Creek confluence each year. We were concerned that numerous resident WHS could be present, and that those fish could partake in the spawning event with migrant native suckers. We wanted to remove as many of those resident

WHS as possible so that the proportion of native to non-native suckers spawning in Roubideau Creek would be as high as possible. Removals were conducted over three days in early March, after we installed the RBW but before fish began migrating into Roubideau Creek. We used push barges with GPP 2.5 Smith-Root® electrofishers to capture fish. The 5.8 mile reach was divided into four sections. One section (2,000 feet in length) began at the RBW and extended upstream serving as a mark-recapture abundance estimation reach (closure was maintained with a blocknet upstream and the RBW downstream). The second section extended from the blocknet upstream approximately 1.2 miles and served as a two-pass depletion abundance estimation reach. We chose a long reach for the multiple-pass depletion to minimize the effects of out/inmigration, and to incorporate a wide range of habitat into the estimate. The third and fourth sections covered the rest of the stream length extending upstream to the Buttermilk Creek confluence, and were sampled with one electrofishing pass with the sole intent of removing as many adult WHS as possible in the reach. On day one of three, section 1 was electrofished and all adult (>280 mm) WHS were marked with a hole-punch in the upper caudal lobe and released. On day two, one electrofishing crew started at the RBW and shocked upstream through both the mark-recapture section (section 1), and the two-pass depletion section (section 2). A second crew started at the downstream end of section four (at the Cottonwood Creek confluence) and shocked upstream as far as day-length allowed. On day-3 one electrofishing crew completed the second pass of the depletion section (section 2) and then completed section 3, ending at the Cottonwood Creek confluence. The other crew completed the remainder of section 4. Habitat in all segments was fairly similar, with numerous deep pools that serve as good wintering habitat. Therefore, we calculated WHS density (by stream length) from our abundance estimates and applied to the entire reach to estimate the proportion of the total population removed.

Genetic evaluation - To assess our effect on the genetic composition of the larval production in Roubideau Creek, we collected larvae in May and June 2021 at three sites along a longitudinal gradient within Roubideau Creek. These larvae, and more collected in 2022, will determine how successful the RBW was at limiting hybridization in the drainage. We collected larvae in 2019 and 2020 (since the weir was not in use for the majority of the immigration) from the same sites to serve as baseline data. We plan to test the larvae genetically using a genotyping by sequencing (GBS) approach to determine species or species admixture of individual larvae. Additionally, we are testing a subset of adult fish caught at the RBW to confirm the accuracy of our visual identification. The genetic assessment will be conducted by Dr. Elizabeth Mandeville and graduate research assistant Jillian Campbell at the University of Guelph, and results will allow us to gauge the success of the weir. The presence and relative abundance of pure native sucker larvae will reveal if our effort to preclude the participation of non-native and hybrid suckers was successful.

Longitudinal larval sampling - To determine differences in the upstream extent of tributary use between native and non-native sucker, we continue to collect larval fish from three locations differing in distance from Roubideau Creek's confluence with the Gunnison River, adding to data collected in 2017-2020 under prior research efforts. Larvae identified as Catostomus were sent to the University of Guelph to determine species. Baseline data, in particular will shed light on longitudinal differences in the genetic composition of larval samples and help us to

understand differing life history behavior among native and non-native or hybrid sucker populations accessing Roubideau Creek for spawning purposes.

Size-retention study – During the first weeks of RBW operations in 2021, we discovered that smaller adult WHS were able to force through the cage bars (see RBW operation narrative below). We fixed the problem as described below, but did not think the solution was suitable for higher flows than those encountered in 2021. Therefore, in summer 2021, we set up the cage in Roubideau Creek, closed the fyke, and populated the cage with suckers and RTC. We captured fish with backpack electrofishing and trammel nets in Roubideau Creek, and by jet-boat electrofishing in the Gunnison River near the Roubideau Creek confluence. Our goal was to populate the cage with at least 80 fish from 200 to 400mm TL to fully bookend the transitional size range where the original observed escapement occurred. We measured (TL, weight, and maximum body width) and PIT or Floy® tagged all fish placed in the cage. We examined all fish in the cage twice per week for approximately four weeks and recorded which individuals were retained over time. We used logistic regression to model retention based on TL, and used the logit function to model probability of retention at size.

RESULTS AND DISCUSSION

RBW operation - We deployed the weir and trap, under the direction of Cramer Fish Sciences biologist Jesse Anderson, on March 2-3, 2021 in the same location as 2020, as the substrate rail that anchors the weir is a semi-permanent installation. Closure of Roubideau Creek was established the afternoon of March 2, and maintained through May 21. The first fish were captured on March 6. Eleven small (<300mm) WHS were present, but five were able to force their way through the cage bars. This was concerning as we had intended for the cage to hold all suckers over 280 mm. To rectify the issue, we created panels out of ridged hog-wire fence and flexible 1-inch poultry netting to line the cage and the sections of the weir panels along the substrate rail. We believe this alleviated escapement issues, but also made keeping the weir clean much more difficult. Flows were low due to drought, but eventually higher flows on May 3 displaced the mesh panels on the weir pickets and we removed them to prevent damage to the weir.

Due to the exceptionally dry soil conditions that were established during summer 2019 and throughout 2020, runoff in 2021 was late and diminished. As a result, we saw a delay in the spawning run from what we had expected. Aside from the first 11 WHS on March 6, we didn't trap fish until March 23. March 23 coincided with the first pulse of irrigation return water coming down Buttermilk Creek, which increased flows and turbidity in Roubideau Creek slightly (Figure 4). We believe this triggered the first immigrants, and in fact believe this anthropogenic input of water may be responsible for initiating immigration in most years. During March and most of April, flows, turbidity and fish immigration were generally low from what we had expected or experienced in the drainage in previous years, with only dozens to hundreds of fish migrating daily. During this period, we noted that Roubideau Creek above the irrigation inputs of Buttermilk Creek was not flowing until April 7, and flow was low (too low for fish to utilize) and clarity was high until sometime between April 22 and April 26. On April 25, flows and turbidity at the RBW increased slightly, and immigration increased sharply, with over 1,600 fish captured. On April 26 we noted that Roubideau Creek above Buttermilk Creek was flowing enough to allow fish movement and was very turbid. Roubideau flows and fish captures

remained high through April 4, and then both tapered off sharply over the following days, and remained low the rest of the season. The correlation we observed between Roubideau Creek flow above Buttermilk and the immigration numbers suggests that perhaps there was an olfactory cue associated with water from the Roubideau Creek drainage proper that triggered movement.

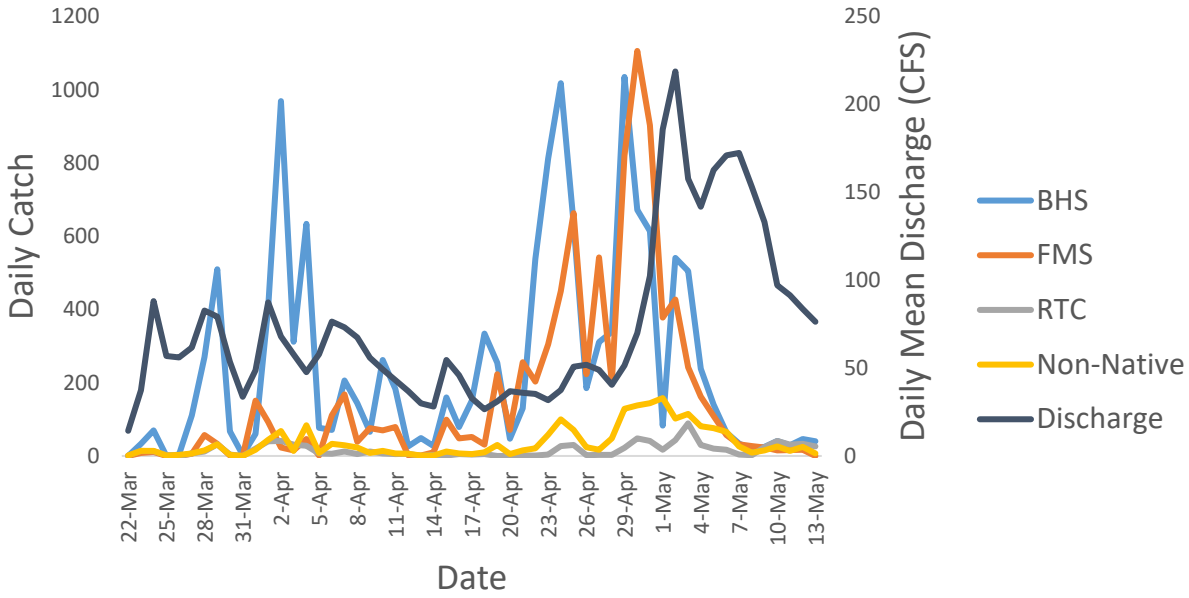

Figure 4. Comparison of daily catch (number per species) at the Roubideau Creek resistance board weir, and stream discharge (CFS) during the 2021 sucker immigration period. BHS = Bluehead Sucker; FMS = Flannelmouth Sucker; RTC = Roundtail Chub; Non-Native = any sucker with any suspected White or Longnose sucker genetics.

Overall, we captured 25,876 fish. Native suckers made up the vast majority, at 22,489 total. Of those, 13,768 were BHS and 8,721 were FMS. We removed 2,064 non-native and hybrid suckers, which made up 8.3% of the total suckers captured. We captured 989 RTC, but suspect many more passed the weir as the spacing of pickets was too wide to capture most RTC. The remainder of the catch was made up of additional non-catostomid, non-native fishes.

The majority of catch (immigration) at the RBW occurred in late April and early May, though there were several days at the beginning of April when we captured large numbers of BHS (Figure 4). We were expecting heavier movement in late March and early April which is usually the heaviest movement period based on PIA detections. Again, we suspect fish were waiting for an olfactory cue associated with snow-melt driven Roubideau Creek runoff (non-irrigation return water) that was late to occur in 2021. In Figure 4, note that immigration increased heavily around April 26th, which is when we observed that Roubideau Creek above Buttermilk Creek had begun running at a level that would allow for fish to use it. We suspect that the delayed immigration season, due to the late runoff, likely decreased the numbers of fish that participated in the spawning migration. We expect higher numbers of fish in years with a more typical (for the drainage; earlier and larger magnitude) hydrograph.

Pre-trapping removal – On March 8, 2021, we captured, marked, and released 33 adult WHS (>280mm) in section 1, in preparation for the mark-recapture estimate. The marked fish were released in several locations within the reach to aid in redistribution. During this shocking event, we also culled 55 WHS and non-native hybrids under 280mm. On March 9, one crew resampled section 1, and completed the first pass of section 2 (the multiple pass depletion section). A second crew conducted a single pass removal on the downstream portion of section 4. On March 10, the same crew from March 9 resampled section 2, and then removed fish in section 3. The second crew finished the removal of section 4.

Unfortunately, the block net at the upstream end of section 1 collapsed over-night on March 8. We originally suspected that closure was still likely as riffles between pools were very shallow throughout the section, water temps were cold, and we don't see any sucker movement based on tag detections on the PIA in winter months. However, eight marked WHS were recaptured throughout section 2 over the two days of sampling. Because of a lack of closure, we expect that this estimate was flawed due to unequal escapement from section 1 among marked and unmarked fish. However it was encouraging that we recaptured 20 out of the 33 marked fish (60.6% recapture rate). We believe the multiple pass depletion estimate did not violate any assumptions (excluding the marked fish from section 1). We captured 36 and 8 adult WHS on passes 1 and 2, respectively. Using the Zippin method, capture probability was 77.8% and abundance ± 95% CI was 46 ± 5 fish. Density was 36 to 44 adult fish per mile. We captured numerous juvenile WHS as well, and estimated a capture probability of 52.3%, and a density of 49 to 97 fish per mile (95% CI; mean = 73). Assuming similar habitat and fish density throughout the entire 5.8 mile reach, we estimated there were 208 to 255 WHS > 280mm within the reach. In total, 342 adult WHS were removed. We clearly underestimated density. We suspect that either there was greater habitat and fish density variability between reaches, or that emigration from section 2 exceeded immigration between passes. Perhaps fish left the reach after the disturbance of our first pass, exhibiting a similar behavior as marked fish in section 1. We do suspect that capture probability was similar if not higher over the entire reach, as section 4 has similar or slightly shallower water than that encountered in section 2. Therefore, we are still confident we removed around 78% of the population. Overall, even 342 WHS adults is minuscule in comparison to the number of native fish that passed the weir, so hopefully the "resident" WHS population would have had little effect on the genetic composition of larvae with or without the removal effort.

Genetic evaluation – Larval samples from 2019, 2020, and 2021 were collected in full, and were sent to the University of Guelph. The full GBS method and analysis was completed for a small test set of larvae, and showed that species composition was clearly identifiable. As of December 2021, DNA extraction was complete for the majority of samples so-far collected, and sequencing was expected to occur in spring 2022.

Longitudinal larval sampling - Prior longitudinal larval sucker sampling in 2018 indicated that larval species composition ranged from 100% non-native or hybridized near the mouth to 70% and 100% native at 8.6 and 23.9 miles upstream from the mouth. Results from 2019 – 2021 are still being analyzed as stated above.

Size-retention study – We constructed the cage on June 15, 2021, and began collecting fish and populating the cage on June 16. We collected fish in Roubideau Creek one June 16, 17, 22, and 23, but struggled to obtain the numbers and the size range of fish we wanted. Therefore, on July 5, we collected fish from the Gunnison River via jet-boat electrofishing. In early collections, we retained all suckers and RTC for the study, but after short periods in the cage, many RTC exhibited mouth, belly, and caudal fin abrasion wounds, so we released them and did not conduct analyses for those fish. We ended the study on July 13. As we collected data near the end of the study, it became apparent that some of the larger fish that we fully expected to be retained were not present. At the PIA downstream, a number of smaller PIT-tagged native and non-native fish were accounted for but none of the larger missing fish were detected. Hypothesizing that in-cage mortality had occurred, we scanned the substrate under the cage floor and around the cage perimeter with a hand-held scanner (Biomark HPR) and detected eight tags from fish 321 to 386 mm TL. We suspect that fish succumbed to wounds and stress, and were quickly consumed by crayfish. We never observed signs of fish carcasses. Because many of our non-native fish were Floy® tagged and therefore not detectable on the PIA or by handheld scanner, we decided to model short term (any fish retained for at least two days) retention only. We justified this because, during trapping season, fish are held in the cage for hours at most, never days. We also removed the eight "recovered" PIT tags from analyses as they were known mortalities. With these corrections, we included 89 native, WHS, and hybrid suckers in analyses. Of those, 19 were Floy® tagged and 70 were PIT tagged. The logistic regression model indicated that at 304mm TL, probability of retention was 0.95 (Figure 5). No fish greater than 301 mm escaped the cage. In 2021, we collected length data on 632 non-native suckers captured with the poultry wire modification. Only 1.7% of those were under 304mm TL. Assuming that the size distribution of fish we measured was representative of the 2,064 non-natives captured, we estimate that we would have missed 35 captures had we not used the poultry wire modification. We therefore concluded the benefit of capturing the smallest non-native suckers in the spawning run was not great enough to outweigh the operational cost of the poultry wire modification.

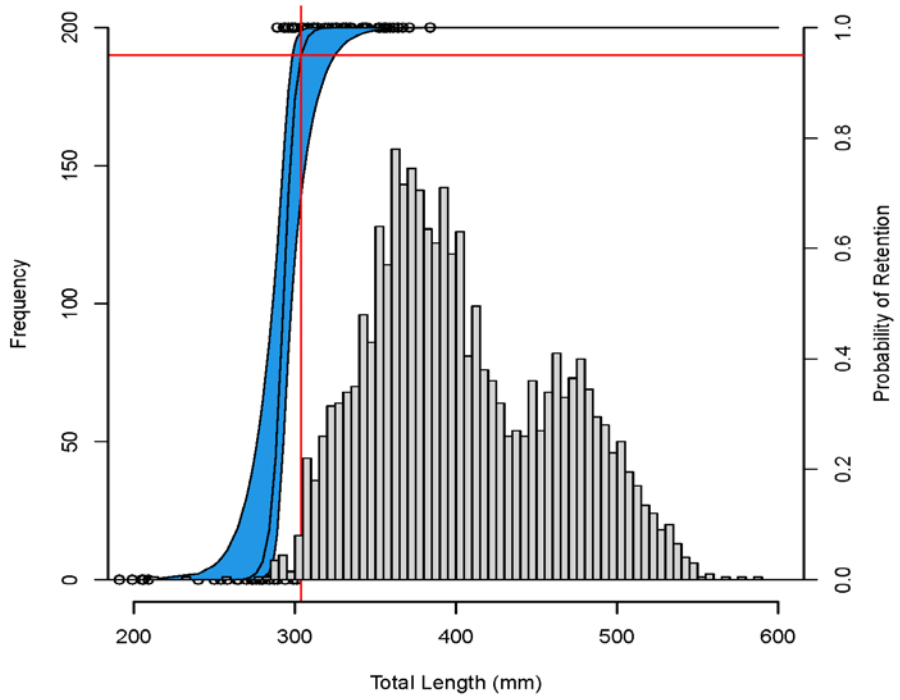

Figure 5. Comparison of a cage retention study (July 2021) and length-frequency data collected from the RBW in 2021. Gray bars indicate frequency of non-native suckers captured at the RBW within each 5mm total length (TL) bin. Open circles represent individual fish of different TL from the retention study that were either retained (probability of retention; POR = 1) or that escaped (POR = 0). The black curve is the logistic regression model (logit function applied) of the retention data, and the blue region is the 95% confidence interval. The red crosshairs show the TL (304mm) at which the model predicts POR = 0.95. Fish represented by the length-frequency bars were captured with poultry wire applied to the cage and weir which prevented escapement of all adult suckers, while the retention data was collected with no poultry wire. Fish represented by the length-frequency bars smaller than the vertical red line may have escaped the cage or passed the weir. These individuals represent only 1.7% of all non-natives measured.

ACKNOWLEDGMENTS

Kevin Thompson conceptualized the project and set it in motion. We thank Dr. Elizabeth Mandeville (University of Guelph) who assisted with planning this project and will be involved heavily with the genetic assessment portion of this research as the major advisor of the graduate research assistant. 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, Russ Japuntich, Cole Brittan, Rachel Jones, Mark Richman, Katie Birch, and CPW technicians listed in above priorities assisted with electrofishing, weir operation, and larval fish sampling.

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 while on a recreational hike. The observation was significant, as the stream flows less regularly than Cottonwood Creek, and is 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 probably means 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 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. 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. 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 6). We compared imagery from different dates to assess wet and dry conditions through time. Imagery captured on August 8, 2019 was particularly helpful, as the quality was good, and summer of 2019 was very dry, so visible water was scarce. What

Figure 6. Aerial imagery (A) of Cottonwood Creek captured August 25, 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.

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, a second pass was completed. 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 will be downloaded annually.

Moving forward, we will survey each reach (assuming they are wet) 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 or time for two more years. 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 September – November 2021, we visited seven suspected perennial reaches in the Roubideau and Escalante creek drainages. Above average precipitation occurred during summer 2021, but all intermittent streams we visited were in fact completely dry for most of their lengths. Six of the seven specific sites visited were wet and active flow was observed. We established one wet and two dry sampling reaches on Cottonwood Creek, and three wet and three dry reaches on the Dry Fork of Escalante Creek. All wet reaches had BHS and SPD, and the Cottonwood Creek reach also had FMS. Additionally, we identified occupied wet reaches in Potter and Monitor Creeks. We did not establish sampling reaches for the study at these sites due to limited logger funds, and access challenges. We do plan to revisit these sites occasionally in the future, and to visit additional sites identified from imagery. Data collection for this priority will occur throughout 2022 and 2023.

ACKNOWLEDGMENTS

CPW aquatic technicians John Fesenmaier and Gwen Harris assisted with sampling, exploration, and logger deployment.

TECHNICAL ASSISTANCE AND COLLABORATIONS

- Led the organization a symposium for the Western Division of the American Fisheries Society with co-organizers Dan Brauch (CPW) and Peter MacKinnon (Biomark) titled "Assessing fish movements across habitat networks using PIT technology."
- Helped author "Addendum to 'Biological Importance of Ephemeral and Intermittent Streams and Non-Adjacent Wetlands in Colorado." This document was an addendum to the State Of Colorado's Governor's and Attorney General's offices recommendations in favor of a scientifically based definition of the "Waters of the United States," addressed to the U.S. EPA and Department of the Army.
- Collected fish tissue samples from Roubideau Creek for the Colorado Department of Public Health (CDPHE), and shared local geographic knowledge. Collected data in incidence of spinal deformities in native fishes possibly linked to selenium exposure.
- 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. Shared local knowledge of flow timing, biological connections, and field work travel routes.
- 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.
    - Assisted Jim White, Dan Cammack, Eric Gardunio, and BLM biologist Russ Japuntich with multiple trials looking at Smallmouth Bass removal options given the low (no flow) water conditions in the Dolores River in summer 2021. Attempted to estimate abundance and conduct localized removals with electrofishing, netting, trapping, and angling.
- 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. Shared Dolores and San Miguel data with CDPHE to support updates to the 303(d) Impaired Waters List.
- Provided CPW biologists, and CDPHE with a literature summary of BHS, FMS, and RTC thermal biology, to inform impairment standards for western slope streams.
- Participated in Rio Grande sucker and chub surveys in the San Luis Valley with CPW and the US Fish and Wildlife Service.
- Provided a report on CPW sampling records, and stream temperature data to Trout Unlimited on the fishery and physical conditions of the warm water section of Escalante Creek. This data has been used to apply for grants to fund riparian and stream habitat restoration targeted at improving the three-species populations specifically. Wrote a letter of support for one grant proposal. Developed a plan to assess effects of the improvements.
- Proposed a barrier removal on Dry Fork Escalante Creek to BLM that was completed in 2021. Will study the results of the removal in future years with PIT technology.
- Contributed to a CPW letter of support for a property acquisition by BLM on Yellow Jacket Creek.