Three-Species Investigations

Kevin Thompson
Aquatic Research Scientist

Job Progress Report

Colorado Parks & Wildlife

Aquatic Research Section

Fort Collins, Colorado

May 2017

STATE OF COLORADO

John W. Hickenlooper, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Bob Randall, Executive Director

COLORADO PARKS & WILDLIFE

Bob Broscheid, Director

WILDLIFE COMMISSION

Chris Castilian, Chair
Jeanne Horne, Vice-Chair
James C. Pribyl, Secretary
William G. Kane
Robert “Dean” Wingfield
Alexander Zipp
Robert William Bray
John V. Howard, Jr.
James Vigil
Dale E. Pizil
Michelle Zimmerman

Ex Officio/Non-Voting Members: Don Brown, Bob Randall and Bob Broscheid

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 Fitzpatrick, Aquatic Research Scientist, Eastern Plains Native Fishes
Eric E. Richer, Aquatic Research Scientist/Hydrologist, Stream Habitat Restoration
Matthew C. Kondratieff, Aquatic Research Scientist, Stream Habitat Restoration
Dan Kowalski, Aquatic Research Scientist, Stream & River Ecology
Adam Hansen, Aquatic Research Scientist, Coldwater Lakes and Reservoirs
Kevin B. Rogers, Aquatic Research Scientist, Colorado Cutthroat Studies
Kevin G. Thompson, Aquatic Research Scientist, 3-Species and Boreal Toad Studies
Andrew J. Treble, Aquatic Research Scientist, Aquatic Data Management and Analysis
Brad Neuschwanger, Hatchery Manager, Fish Research Hatchery
Tracy Davis, Hatchery Technician, Fish Research Hatchery
Christopher Praamsma, Hatchery Technician, Fish Research Hatchery

Kay Knudsen, Librarian

Prepared by: ____
Kevin Thompson, Aquatic Research Scientist

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

Date: ______________________________

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 Tables

List of Figures

Project Title:	Three-Species investigations
Project Number:	None
Project Objective:	To gather information that will allow Colorado to manage Bluehead Sucker, Flannelmouth Sucker and Roundtail Chub in a way that will enhance their current range and minimize the probability of listing under the Endangered Species Act.
Job No. 1	
Job Title:	Three-species genetics
Job Objective:	Characterization of genetic purity and relatedness/diversity among basins for the three-species
Period Covered:	January 1, 2014 to December 31, 2016

Introduction

The so-called three-species assemblage comprises Flannelmouth Sucker *Catostomus latipinnis*, Bluehead Sucker *C. discobolus,* and Roundtail Chub *Gila robusta*. Natives of the Colorado River basin, each species is estimated to occupy just 45 – 55% of its historic native range in the upper Colorado River basin (Bezzerides and Bestgen 2002; the upper basin includes the Colorado River and its tributaries from Glen Canyon Dam upstream). Of the three, Roundtail Chub is considered a species of special concern by Colorado, whereas the two sucker species hold no special status. For all three, there is concern that populations are exhibiting downward trends. Roundtail Chub is a candidate for Endangered Species Act listing as a “distinct population segment” across the southern portion of its native range (Federal Register 2012). Collectively the three-species are the subjects of a range-wide conservation agreement to which Colorado is signatory (UDWR 2006).

The strategy preferred by Colorado Parks and Wildlife (CPW) with regard to the three-species is to protect and enhance what remains as first priority. After these avenues are addressed adequately CPW could then pursue opportunities to expand the present distribution of three-species back into historic portions of their range not currently occupied. Both enhancement under the first priority and expansion or repatriation may necessitate the use of hatchery-produced offspring of captive broodstock. Prior to the initiation of this project the Native Aquatic Species Restoration Facility (NASRF) housed 27 Bluehead Suckers from the Yampa basin. There are also mature Roundtail Chubs at NASRF representing four different populations; production from the Roundtail broodstock is stocked in the San Juan basin inside and outside of Colorado.

Previous genetic analyses for the Bluehead Sucker suggested that populations within sub-basins in Colorado may be quite different, and that much genetic variation was evident at the population level for this species (Shiozawa et al. 2003, Colorado Parks and Wildlife Three species draft plan 2011). Genetic diversity in Bluehead Sucker populations has also been described as moderate to high in a study encompassing samples from five states (Hopken et al. 2013). This same study indicated that a smaller proportion of total genetic variability was detected from among basins compared to within populations. Since such a scenario allows, but not necessarily requires, the development of potentially several broodstocks with the concomitant space and manpower requirements, it would be prudent to confirm the results of the previous analysis. Moreover, additional genetic analyses are advisable to lessen the uncertainty associated with previously conducted studies.

Specific Objectives:

1. Assist in collection of genetic samples to ensure proper geographic representation of each species in an overall analysis designed to characterize Colorado-wide genetic diversity.
2. Evaluate within- and among-basin diversity of Bluehead and Flannelmouth Suckers in Colorado.
3. Evaluate purity of suckers deemed "pure" by visual inspection in the field, and the probability that CPW researchers and biologists are encountering hybrid suckers that appear pure.
4. Facilitate training of field personnel in sucker and hybrid sucker identification so that the integrity of native species data is ensured.
5. Evaluate need for further genetic sampling based upon initial results and questions thereby raised.
6. Collaborate with cutthroat trout researcher Kevin Rogers in the eventuality that three-species sucker genetic results can shed light on native cutthroat trout genetics.

Methods

Biologists and researchers collected samples opportunistically when sampling waters from which genetic specimens were desired. These waters were broadly representative of suitable habitats in both the Northwest and Southwest regions. A sample of tissue was removed from the top lobe of the caudal fin in most cases. However, if fish were sampled for age and growth analysis by the removal of the first pectoral fin ray, excess tissue from the distal end of the fin ray was sometimes collected. Tissue samples were preserved in 70% ethanol, labeled with unique identifying codes, and entered into a spreadsheet data repository and an in-house database prior to shipping to the analysis laboratory.

Genetic purity - Genetic analyses were conducted at Pisces Molecular LLC using microsatellite markers. Amplifications were conducted using forward and reverse polymerase chain reaction (PCR) primers for six loci previously developed for Catastomid suckers (Tranah et al. 2001), except that for each locus the published forward primers were modified to include a 23 base-pair M13 phage sequence on the 5' end. The PCR amplicons were labeled by the addition of a third fluorescently labeled M13 primer to the PCR reactions allowing for triplex (3 color) fragment analyses. After PCR amplification, the fluorescently labeled amplicon fragments were diluted into molecular biology grade H_2O to normalize the fluorescence signals across all three dyes. The dilutions were run in triplicate on an ABI3130 Genetic Analyzer. Fragment presence and size data were scored using GeneMapper® 4.0 and exported into an Excel spreadsheet for input into a population analysis. The laboratory transitioned from GeneMapper® to Geneious® in 2013 for DNA analysis. Additionally, in 2014 the program TANDEM was implemented to automate the bin-calling procedure. Nearly all previously analyzed specimens have been subjected to re-analysis using the TANDEM program along with Geneious®.

The genetic fingerprints of individuals were subjected to population analysis using program

STRUCTURE 2.3 (Falush et al. 2007) to determine the genetic similarity or dissimilarity among six sucker species encountered in western Colorado (Bluehead *Catostomus discobolus,* Flannelmouth *C. latipinnis,* Longnose *C. catostomus,* White *C. commersonii,* Mountain *C. platyrhynchus,* and Razorback *Xyrauchen texanus*). Initial analysis included 96 representatives of the six species and was intended to create a set of "reference" populations against which future samples could be tested. Each fish in this candidate set was selected based on biologist assessment of phenotype as a pure representative of the appropriate species. All samples were processed as "unknown" with regard to population, resulting in blind scoring of genetic identity to establish reference populations.

Following establishment of the reference populations, further samples of Bluehead (n = 137) and Flannelmouth (n = 139) suckers from across western Colorado were analyzed for purity and then diversity. Later, a mixed assemblage of purported pure and hybrid sucker specimens was analyzed for purity and the accuracy of field identifications. One set of 197 samples analyzed for purity were run through STRUCTURE with population information made available. A larger set of 412 samples that incorporated the first set also was analyzed with new binning software to automate the allele calls (TANDEM, Matschiner and Salzburger 2009) and was run through STRUCTURE without population information. Reference fish were included in this analysis, but STRUCTURE was not informed they were reference fish, resulting in a more "blind" run. Results of the latter analysis are reported herein.

A second genetic method is generally recommended as a means of confirming results. In addition to microsatellite analysis, we conducted analysis of the mitrochondrial ND2 gene from 463 fish representing pure specimens as well as specimens field- and microsatellite-identified as hybrids. As mitochondrial genes are inherited maternally, this method allows a check that will provide a definitive answer only if an unexpected species assignment arises from the mother.

Genetic diversity - The genetic fingerprints of Bluehead and Flannelmouth Sucker samples evaluated as "pure" in the genetic purity analysis were further analyzed using STRUCTURE to determine the genetic diversity in populations of those species from different river drainages across Colorado. Separate analyses were conducted for each species, and in each case included the reference population fish as well as pure samples from the purity analysis. STRUCTURE was set to conduct the analyses without any prior population information; hence the samples were analyzed as unknowns. The analysis for each species was run several times with increasing latitude for how many different populations to which the program was allowed to assign specimens (known as "K" values). A final analysis for each species was run after the specimens were labeled and sorted by geography, but the program was still required to treat the samples as unknowns.

Collaboration - University of Wyoming PhD candidate Liz Mandeville conducted a study on hybridization between native and non-native catostomid fishes in the Upper Colorado River basin, using single nucleotide polymorphisms (SNPs) and high throughput, next-generation sequencing techniques to generate large volumes of data. Objectives included examining differing rates and outcomes of hybridization among multiple hybridizing pairs and among rivers. Although the study commenced with a collection of fishes largely from Wyoming, this sort of analysis will be much more powerful if a greater portion of the upper Colorado River basin were to be represented. We collected and submitted for this project 639 sucker tissue samples representing four river basins: White River, Yampa River, Dolores / San Miguel rivers, and Gunnison River. This sampling scheme provides two rivers in each of CPW's western regions, and in each region one river is characterized by the presence of non-native and hybrid suckers whereas the other is not. Some of the samples submitted represented brood fish held at the Native Aquatic Species Restoration Facility, and therefore gave an additional analysis to evaluate their suitability as brood stock.

A second collaboration was initiated with members of the Turner Lab, University of New Mexico, to synthesize the genetic information collected since 2010 from 859 suckers in western Colorado, with additional specimens collected in eastern Colorado from the native range of White and Longnose suckers, as well as specimens of Desert and Utah suckers from outside of Colorado. This effort used microsatellite DNA markers to evaluate population structure and incidence of hybridization among native and non-native suckers. Additionally, we will generate an overall analysis of the nuclear and mitochondrial dna collected from 274 Bluehead Suckers and 241 Flannelmouth Suckers to characterize diversity within and among the tributary river basins to formulate broodstock recommendations.

Results and Discussion

Genetic purity – Progress in 2014 and 2015 included the addition of fish to the reference populations for Flannelmouth, White, Longnose, and Mountain suckers to achieve a better balance with the 60-fish reference population established for the Bluehead Sucker. More White and Longnose sucker specimens were obtained from eastern Colorado waters, which represent native range of these two species, reducing the likelihood that the reference populations could be skewed from limited introduction events on the Western Slope. These two actions should result in more robust results from purity analyses. In addition, we obtained and added 20 Desert Sucker *C. clarki* samples from the Virgin River as a new reference population to address the possibility of genetic material from this species possibly showing in the San Miguel Bluehead Sucker broodstock, as hypothesized in a previous progress report (Thompson 2014).

In conjunction with transition to using the TANDEM automated binning software that was occurring at the same time, these new reference populations and the addition of Desert Sucker alleviated the concern that the latter species was represented in the San Miguel River specimens. In fact, the TANDEM procedure alone seemed to clear up many of the uncertainties associated with those fish.

A few of the San Miguel River Bluehead brood fish have died in captivity, and several others were recommended to be removed from consideration as brood fish because genetic analyses judge them to be less than 95% pure by current methods (even though the San Miguel and Dolores basin carry a well-deserved reputation as being only lightly invaded by non-native suckers). A number of these brood fish were also represented in the SNP analysis conducted at University of Wyoming (Table 1). That analysis primarily corroborates that the San Miguel Bluehead Suckers collected were genetically appropriate for brood fish. One surprising anomaly was a Bluehead Sucker that exhibits affinity with Utah Sucker *C. ardens*, a native of the Bonneville Basin and the Snake River above Shoshone Falls that was included in the overall University of Wyoming analysis although no specimens originated in Colorado. The presence of purported Utah Sucker in some Colorado specimens may argue for the need to acquire reference specimens of this species in order to protect Colorado's broodstock decisions.

The mitochondrial ND2 gene analysis showed that all 30 of the San Miguel River Bluehead Suckers collected as potential broodstock in 2012 grouped with Bluehead Sucker, indicating that the maternal line of each fish was Bluehead. A good result, but not definitive since there were just

Table 1. Genetic analysis results of San Miguel River Bluehead Sucker brood fish. Pisces results were based on the latest TANDEM – STRUCTURE analysis method using 6 microsatellite loci; Wyoming results (not all brood fish represented) were based on >11,000 single nucleotide polymorphisms. All fish exhibited BHS maternal identity through analysis of the mitochondrial ND2 gene. Fish highlighted with blue have died, and those highlighted with rose in the BHS Pisces column were recommended for removal from consideration as brood fish. In addition to the five primary species, Utah Sucker (UTS) were represented in the Wyoming analysis.

	Pisces – microsatellite results					University of Wyoming – SNP results					
Pisces #	BHS	FMS	LGS	MOS	WHS	BHS	FMS	LGS	MOS	WHS	UTS
116043	0.98	0.01	0	0	0	1	0	0	0	0	0
116044	0.98	0.01	0.01	0	0	0.99	0.01	0	0	0	0
116045	0.98	0.01	0	0	0	0.99	0	0.01	0	0	0
116046	0.95	0.03	0	0.01	0	0.99	0	0	0	0	0
116047	0.98	0.01	0	0	0	1	0	0	0	0	0
116048	0.98	0.01	0	0	0	0.98	0.01	0	0	0	0
116049	0.90	0.01	0.07	0.01	0.01	1	0	0	0	0	0
116050	0.96	0.02	0.01	0.01	0.01						
116051	0.98	0.01	0	0	0						
116052	0.97	0.01	0	0	0.02	0.95	0	0.05	0	0	0
116053	0.98	0.01	0	0	0.00						
116054	0.97	0.02	0.01	0	0.01	0.99	0	0	0	0	0.01
116055	0.96	0.01	0.01	0	0.02	0.96	0	0.04	0	0	0
116056	0.97	0.01	0.01	0	0.02						
116057	0.98	0.01	0.00	0	0.00	0.97	0	0.02	0.01	0	0
116058	0.97	0.01	0.01	0	0.01	0.99	0	0.01	0	0	0
116059	0.95	0.01	0.01	0.02	0.01	1	0	0	0	0	0
116060	0.98	0.01	0.01	0.01	0.00	0.98	0.01	0.01	0	0	0
116061	0.99	0.01	0	0	0.00						
116062	0.95	0.01	0	0	0.04						
116063	0.97	0.01	0.01	0	0.01						
116064	0.95	0.01	0.03	0	0.01						
116065	0.92	0.03	0.03	0.01	0.01	0.96	0	0.04	0	0	0
116066	0.96	0.03	0.01	0	0.01						
116067	0.97	0.01	0.00	0.01	0.00	0.99	0	0	0	0	0.01
116068	0.98	0.01	0	0	0.01	0.71	0	0	0	0	0.29
116069	0.97	0.01	0.01	0	0.01	0.99	0	0.01	0	0	0
116070	0.97	0.02	0.01	0	0.00	0.91	0.02	0.03	0.02	0	0.01
116071	0.98	0.01	0.00	0	0.01	1	0	0	0	0	0
116072	0.99	0.01	0.00	0	0.00	1	0	0	0	0	0

30 specimens. If introgression occurred at very low levels, say 5%, and it is assumed that half of

all hybrids have a non-Bluehead Sucker maternal line, 30 specimens would yield p ~ 0.5 of detecting hybridization. However, 20 Flannelmouth Suckers were also in the ND2 analysis pool, and all of them grouped with Flannelmouth Sucker, indicating Flannelmouth maternal heritage. Flannelmouth Suckers hybridize with White Suckers as readily as Bluehead Suckers, providing additional evidence that at least there is not widespread White Sucker hybridization in the San Miguel River. Using the same assumptions with a sample of 50 fish, the probability of detecting hybridization would be ~ 0.7. As it stands now, if the “admixed” fish from the San Miguel River are genuinely hybrid suckers, biologists have little hope of accurately discerning such hybrids in the field, since they were collected on the basis of exhibiting classic Bluehead Sucker morphological characteristics.

Genetic purity – Accuracy of field identifications

A TANDEM – STRUCTURE analysis of 412 varied sucker specimens revealed congruent field versus genetic identification results in 76.2% of specimens, with wide variation in the number of congruent identifications among species. This rate of accurate field identification was heavily influenced by hybrid suckers (see Tables 2 and 3 and associated discussion, Thompson 2014). In contrast, the SNP analysis of 601 specimens provided to the University of Wyoming revealed a higher rate of congruent field and genetic identifications (90.2 % overall, Table 2). This accuracy rate would be even slightly higher if the near-certain field recording errors were eliminated that resulted in Bluehead / Flannelmouth and Flannelmouth / Bluehead mis-matches. The SNP data set represents specimens collected more recently than most of those in the microsatellite data set. This suggests that collectors have made strides in accurate identifications, particularly of hybrids.

Table 2. Comparison of field identification of sucker specimens with SNP genetic assignment.

Field identification	SNP identification	N	% correct
BHS	BHS	150	96.8
	Hybrid	4	
	FMS	1	
FMS	FMS	195	94.7
	Hybrid	10	
	BHS	1	
LGS	LGS	23	82.1
	Hybrid	5	
MOS	MOS	0	0
	Hybrid	0	
	BHS	0	
WHS	WHS	35	94.6
	Hybrid	2	
Hybrid	Hybrid – correct	139	79.1
	Hybrid – incorrect	32	
	FMS	1	
	LGS	1	
	WHS	2	

As a final observation on the sucker purity topic, I note that genetic analysis has confirmed the presence of hybrid suckers in the San Miguel (one Flannelmouth x White Sucker), White (White Sucker and Longnose Sucker dna detected in some samples), and Dolores (one Flannelmouth x

White Sucker) rivers. Nevertheless, the presence of non-native and hybrid suckers in these streams remains less of a problem than in other streams.

Genetic diversity – Collaboration –

A contract was initiated with researchers at the University of New Mexico and the Southwestern Museum of Biology – Fishes to conduct an overall analysis backed with landscape genetics expertise and push the genetic data toward publication. A draft manuscript was submitted to CPW in June 2016, but afterward it became evident that ND2 analyses were necessary on additional fish for which msat data already existed. The additional ND2 analyses were completed in December 2016, bringing the total number of fish specimens in the dataset to 1140. This total includes Bluehead Sucker, Flannelmouth Sucker, White Sucker, Longnose Sucker, Mountain Sucker, Utah Sucker, and Desert Sucker. All samples have been provided to University of New Mexico collaborators, who are presently conducting additional analyses that will be described in the manuscript.

Conclusions and Recommendations

The genetic assignment test based on six microsatellite loci generally appears to perform well, with the ability to distinguish the five represented sucker species from one another. With updated reference populations in 2014 and the use of TANDEM to more objectively assess microsatellite alleles, its performance should improve further. Additional species additions may help as well. Desert Sucker specimens were added, although results since then suggest the Desert Sucker reference population will not be necessary to routinely include in analyses. It served its initial purpose well in clarifying that Desert Sucker genetics were not significantly present in the Dolores River basin.

Broodstocks held at NASRF for native suckers include Bluehead Suckers from both the Northwest and Southwest Regions, and Flannelmouth Suckers from only the Northwest Region. Presently, there is no holding room available at NASRF to support a Flannelmouth Sucker broodstock from the Southwest Region. However, it would be prudent to seek ways to accommodate additional Flannelmouth Sucker there, perhaps by holding the two regional broodstocks in the same raceways, to be distinguished by PIT tags prior to any breeding efforts.

Additionally, the size of founding populations is considered crucial to the long-term success of new populations stemming from those founders. For broodstocks, this principle dictates that losses from the various native sucker broodstocks at NASRF be frequently replenished, so that progeny originating at NASRF continue to represent the broadest possible genetic diversity given the space constraints there. Any such broodstock replacement fish should be genetically tested to ensure purity.

Accurate field identification of sucker hybrids will continue to be an issue, and especially in waters where hybrids as well as back-crossed individuals may be encountered. To alleviate this situation as best as is possible, it would be prudent to continue asking biologists to use the identification materials assembled as a result of the 2012 identification workshop and to train new temporary hires in the use of those materials if they are expected to work in waters where these fish will be encountered. Periodically, hands-on sucker identification workshops should be conducted for permanent personnel.

Job No. 2	Part A
Job Title:	Life History Investigations
Job Objective:	Investigate reproductive and fish community response to thermal and flow gradients in the upper White River drainage, Colorado.
Period Covered:	January 1, 2014 to December 31, 2016

Investigators

Gregory Fraser, M.S., Colorado State University
Kevin R. Bestgen, Ph.D., Senior Research Scientist and Assistant Professor, Larval Fish Laboratory, Colorado State University
Dana L. Winkelman, Ph.D., Unit Leader, Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University
Kevin Thompson, M.S., Aquatic Research Scientist, Colorado Parks and Wildlife

Introduction

This portion of Job 2 has been completed, and Mr. Fraser's thesis serves as a Final Report on this job segment. It has been disseminated appropriately, as well as posted on Colorado Parks and Wildlife's public website at the following url:
http://cpw.state.co.us/Documents/Research/Aquatic/pdf/Publications/Three-Species-Investigation-2015.pdf
Additionally, chapter one of the thesis was accepted by Transactions of the American Fisheries Society (Fraser et al. *in press*). This paper relates to spawning movement timing, temperatures at which these movements occur, and estimates of fidelity to Coal Creek.

However, there are many PIT-tagged Bluehead and Flannelmouth Suckers remaining in the study reach, and those fish continue to be monitored to assess spawning activity residence time, site fidelity, and survival rates.

Methods

Native suckers previously PIT-tagged during the course of Job 2A are monitored passively with a PIT tag antenna array in lower Coal Creek (the "Downstream Antenna Array" of Fraser 2015). Individual antennas consisted of a 50 cm wide loop formed of 10-gauge wire spanning the entire stream. The array comprised two antennas separated by about 1 m distance, allowing assessment of movement direction. In 2014 - 2016 the array was deployed in early to mid April before suckers begin to migrate into Coal Creek and removed in mid to late July after tagged fish were no longer being detected. The system was powered by two (2014) or three (2015-16) deep-cycle 12V batteries coupled with two small solar panels to charge the batteries. The reader was visited about bi-weekly to retrieve PIT tag data, and to deploy freshly charged batteries.

Estimates of residence times for individual fishes were constructed from PIT tag passive antenna array detections. A tagged fish was considered to have entered Coal Creek if its PIT tag was sequentially detected on the downstream and then the upstream antenna, and to have exited Coal Creek if detections were in the reverse order on the antennas. However, many detections exist in the data that do not follow this pattern. If an entry was uncertain due to detection on only one of the antennas, but a later definitive exit occurred, the uncertain entry was considered to have been a true entry. Uncertain exits were judged in the same way, considered true if preceded by a known entry and the fish was not detected upstream again. Fish for which we could not reasonably determine an entry as well as an exit were excluded from residence time analyses. In some cases, individual fish entered and exited Coal Creek more than once each year. In such instances, those fish contributed more than one data point to time of residence analysis. Lengths of stay equaling less than one day were excluded, assuming such explorations were not indicative of active spawning behavior. Only fish that were originally tagged in Coal Creek (Fraser 2015) were included because we were also examining the spawning site fidelity of these two sucker species (many additional native suckers were PIT tagged during electrofishing surveys in the White River).

Input data for survival analysis was also obtained from detections on the passive antenna array. A fish was considered to have survived the preceding year(s) and to have been re-encountered if there was at least one detection of that fish's tag on the reader during the spawning season. The accumulated detection data were formed into individual annual capture histories in a Cormack-Jolly-Seber (CJS) format and analyzed using Program MARK (White and Burnham 1999). Modeling started with a suite of pre-defined models available for CJS data in MARK including species and time differences in apparent survival and capture probability and interaction terms between them. However, one assumption of CJS models is that every marked animal in the population immediately after time (i) has the same probability of surviving to time (i+1). Examining the capture histories and the numbers of fish never seen again suggested that there may have been a tagging effect on apparent survival (or permanent emigration), which would result in a violation of the assumption. Therefore additional models were constructed that allowed for differing first-year apparent survival and capture probability among tagged cohorts. Model selection was conducted using QAICc (quasi-likelihood AIC, variance adjusted for over-dispersed data) in the context of the information-theoretic approach of Burnham and Anderson (2002).

Results

Tagged suckers of both species continue to return to Coal Creek during spawning season; in 2015 37.7% of all Flannelmouth Suckers originally tagged in Coal Creek during 2011 - 2013 visited Coal Creek, as did 36.8% of Bluehead Suckers (Table 3). In 2016, 30.6% of all Flannelmouth Suckers and 24.5% of all Bluehead Suckers visited Coal Creek. However, many suckers PIT-tagged in Coal Creek remain undetected since the tagging event. This is especially true of fish tagged in 2012 and 2013, with more than 50% of these fish not yet re-detected in Coal Creek. This result suggests there are differences among tagged cohorts in first-year apparent survival, which may be driven by true differences in survival (a tagging effect), permanent emigration, or tag loss. If the former, then estimated site fidelity among these two native sucker species, while moderate at best, could be adjusted upward from that reported by Fraser (2015). However, each year to date some fish have returned and been detected for the first time since the year they were tagged (Table 3).

Native suckers of both species that do return to Coal Creek during the spring spawning season may visit multiple times. Overall, average lengths of stay in Coal Creek tend to be a bit longer on the part of Bluehead Suckers compared to Flannelmouth Suckers (Table 4), but range from about 10-20 days depending on species and year. Lengths of stay appear somewhat longer during the last three years when no trapping or electrofishing activities were pursued in Coal

Table 3. Unique capture histories, numbers, and percentage of fish (for each cohort) exhibiting each history for three cohorts of Flannelmouth and Bluehead Suckers PIT-tagged in 2011, 2012, and 2013. A ‘1’ indicates capture or antenna detection for a given year and ‘0’ indicates no detection. The first digit of the capture history is 2011, the last 2016.

Capture History 2011-2015	FMS	%	BHS	%
Total 2011 tags	53		40	
111111	7	13.2	3	7.5
111110	0		1	2.5
111100	3	5.7	0	
111000	2	3.8	3	7.5
110101	0		1	2.5
110001	1	1.9	1	2.5
110000	3	5.7	3	7.5
101111	5	9.4	2	5.0
101110	0		3	7.5
101100	1	1.9	1	2.5
101011	1	1.9	0	
101000	2	3.8	4	10.0
100111	6	11.3	0	
100110	1	1.9	1	2.5
100100	2	3.8	2	5.0
100011	1	1.9	1	2.5
100010	3	5.7	1	2.5
100000	15	28.3	13	32.5
Total 2012 tags	224		40	
011111	26	11.6	4	10.0
011110	5	2.2	0	
011101	2	0.9	0	
011100	5	2.2	2	5.0
011011	2	0.9	0	
011010	6	2.7	0	
011001	1	0.5	0	
011000	4	1.8	0	
010111	13	5.8	5	12.5
010110	1	0.5	2	5.0
010101	2	0.9	0	
010100	3	1.3	1	2.5
010011	14	6.3	0	
010010	16	7.1	3	7.5
010001	10	4.5	0	
010000	114	50.9	23	57.5

Table 3 concluded. Unique capture histories, numbers, and percentage of fish (for each cohort) exhibiting each history for three cohorts of Flannelmouth and Bluehead Suckers PIT-tagged in 2011, 2012, and 2013. A '1' indicates capture or antenna detection for a given year and '0' indicates no detection. The first digit of the capture history is 2011, the last 2016.

Capture History 2011-2015	FMS	%	BHS	%
Total 2013 tags	158		26	
001111	27	17.1	8	30.8
001110	10	6.3	3	11.5
001101	1	0.6	0	
001100	6	3.8	1	3.8
001011	8	5.1	1	3.8
001010	12	7.6	1	3.8
001001	6	3.8	0	
001000	88	55.7	12	46.2
Total tagged fish	435		106	
2014 detections:	125	28.7	40	37.7
2015 detections:	164	37.7	39	36.8
2016 detections:	133	30.6	26	24.5

Table 4. Mean residence time (days; SE and range parenthetically) of Bluehead (BHS) and Flannelmouth (FMS) suckers initially PIT-tagged in Coal Creek and returning to Coal Creek one or more years later, calculated from tagged fish detections on passive antenna arrays. Fish were PIT-tagged in 2011 – 2013. Years 2012 and 2013 are from Fraser (2015); no SE or antenna deployment dates reported.

Year	BHS	FMS	Antenna deployment dates
2012	11.2 (1–27) n=6	10.7 (1–16) n=10	
2013	11.8 (1–30) n=20	10.5 (1–32) n=74	
2014	14.1 (1.5315; 1-38) n=42	13.6 (1.1318; 1-54) n=130	4/17/2014 – 7/11/2014
2015	20.0 (2.7152; 1-52) n=42	13.8 (0.8503; 1-51) n=215	4/02/2015 - 7/22/2015
2016	19.0 (3.0723; 1-56) n=29	15.7 (1.1030; 1-88) n=183	4/14/2016 - 7/21/2016

Creek. The lack of these disruptive activities may lead to longer periods of spawning residency, although potential (but unexamined) effects of differing flow conditions may also play a role.

Survival analyses based on the capture histories in Table 3 confirmed the suspicions that first year apparent survival was lower than apparent survival in later years for both species. The five top-ranked models indicate that only those with a term separating the first-year post-tagging apparent survival from later years for each cohort garner any support from the data (Table 5). By far, the most weight is given to the model showing a first-year effect on both survival and probability of capture, but no differences between the two species. Additionally, the parameter estimates suggest that the first-year effect on apparent survival was most pronounced for the 2012 and 2013 cohorts of fish (Figure 1). If fish survive the first year, apparent survival in later

Table 5. Modeling results for survival of native suckers PIT-tagged in Coal Creek from 2011-2013. These models represent only the five top-ranked models. Model symbols are: Φ = apparent survival, p = probability of capture, t1 = a first year post-tagging effect on survival or capture probability, and sp = species differences between Bluehead and Flannelmouth Suckers.

Model	QAICc	ΔQAICc	Model Wts	Model Likelihood	Parms	QDeviance
Φ(t1) p(t1)	1011.920	0.0000	0.8481	1.0000	8	106.3915
Φ(sp+t1) p(t1)	1016.511	4.5912	0.0854	0.1007	12	102.8174
Φ(sp*t1) p(t1)	1017.282	5.3621	0.0581	0.0685	11	105.6356
Φ(sp+t1) p(sp+t1)	1021.192	9.2725	0.0082	0.0097	16	99.2692
Φ(t) p(t)	1029.260	17.340	0.0002	0.0002	10	119.6571

Figure 1. Apparent survival (± SE) of three cohorts of Flannelmouth and Bluehead Suckers PIT-tagged in Coal Creek and followed over time with passive PIT-tag antenna arrays. Estimates charted here were produced by the top ranked model of Table 5.

years is high, as would be anticipated from long-lived fish such as these native catostomids. The second ranked model estimates a somewhat lower annual survival for Bluehead Suckers than for Flannelmouth Suckers once they have survived the initial year after being tagged.

There are two possible explanations for the differences in apparent survival among these cohorts over the first year. One, that there truly are differences in survival. If this were the true explanation it would suggest that those differences are driven by mortality caused by the tagging, a hypothesis that gains support from the substantial numbers of fish noted as "bleeding" after insertion of the PIT tag. Two, that short-term tag loss is the culprit. Since these fish were not double-marked, there is no way of evaluating this hypothesis, especially since the fish are no longer handled, otherwise tagging scars may serve as a fairly reliable second mark.

Conclusions and Recommendations

The continued deployment of the antenna array in Coal Creek is yielding valuable information on the level of spawning season use and site fidelity and should be continued. The growing data set on detection histories is also allowing insight into the survival of these fish, and the potential for tagging effects on survival.

Given the level of effect on first-year survival for these fish, perhaps the use of 32-mm PIT tags should be re-examined. However, the potential differences between the first year survival of the 2011 cohort versus the later cohorts may also suggest that the effects may be a combination of tags used, and the experience of taggers. These large tags are 3.8 mm diameter and require a syringe needle 4.45 mm diameter for insertion, and the physical data sheets from the years of tagging indicate that many of the fish bled upon insertion of the tag. Perhaps adjustments to tagging location or to tagging technique would be necessary if such large tags were to be used in the future. Other work involving PIT tags covered in this report involves 12.5 mm long x 2.1 mm diameter tags and produce much less tissue disruption.

Coal Creek appears to be an important spawning tributary for these native suckers in the White River Basin. Fraser (2015) did not find strong evidence of native suckers using the White River or its tributaries much above the confluence of Coal Creek. Evidently these areas are too cold to be attractive as spawning or rearing areas. Downstream of Coal Creek there are few tributaries that are sufficiently large to be attractive to large runs of fish. Those that are large enough – e.g. Flag Creek and Piceance Creek – suffer from issues that inhibit widespread use. Flag Creek is inaccessible due to an irrigation diversion and then a 30 meter culvert under County Road 13. Piceance Creek suffers from both irrigation withdrawals and numerous irrigation diversions such as tarp dams in the creek that inhibit fish access. Therefore, a tributary such as Coal Creek becomes even more important.

Access to Coal Creek is not what it could be. While it is known from Fraser (2015) that fish can pass the first three culverts in Coal Creek (one on the White River Ranch, one under County Road 8, and the lowermost one on the Strang Ranch), we never found evidence during Fraser's research project that they were able to ascend the second culvert on the Strang Ranch. Further upstream there are additional imposing culverts that would likely prevent upstream access, such as under County Road 6. Improved access to more of Coal Creek would very likely benefit the native suckers, and CPW would do well to seek ways to accomplish such improved access in collaboration with landowners, Rio Blanco County, NRCS, and perhaps conservation-minded NGOs such as The Nature Conservancy.

Job No. 2	Part B
Job Title:	Life History Investigations
Job Objective:	Pursue greater understanding of the life history requirements and preferences of the three-species to facilitate effective management decisions.
Period Covered:	January 1, 2012 to December 31, 2013. Note: Age and Growth studies were suspended due to funding cuts.

Introduction

The so-called three-species assemblage comprises Flannelmouth Sucker *Catostomus latipinnis*, Bluehead Sucker *C. discobolus*, and Roundtail Chub *Gila robusta*. Natives of the Colorado River basin, they each occupy an estimated 45 – 55% of their historic native range in the upper Colorado River basin (Bezzerides and Bestgen 2002; the upper basin includes the Colorado River and its tributaries from Glen Canyon Dam upstream). Of the three, Roundtail Chub is considered a species of special concern by Colorado, whereas the two sucker species hold no special status. For all three, there is concern that population trends are negative.

Basic life history information such as general habitat associations in larger streams, age at sexual maturity and general timing and water temperature at spawning has been summarized in several recent publications (Bezzerides and Bestgen 2002; Rees et al. 2005a, 2005b; Ptacek et al. 2005). However, many gaps exist in the accumulated life history knowledge. The importance of tributaries in the completion of life history on a large geographic scale is uncertain. It is known that tributaries are widely used, but are they critical? Do adult fish exhibit fidelity to spawning sites or spawning tributaries? Are populations in tributaries ever distinct from those in mainstems? In the case of Escalante Creek, there exists an apparently self-sustaining three-species assemblage above a barrier that prevents immigration from the Gunnison River or the lower three miles of Escalante Creek, but do fish from above the barrier contribute to the downstream populations?

Our knowledge of specific spawning and rearing sites in Colorado is quite incomplete. We do not know to what extent the three-species spawn or spend early life in tributary streams compared to mainstems, or to what extent fish reared in tributaries eventually become members of the mainstem population. We do not know if there are smaller tributaries that host self-sustaining populations without influence from mainstems.

Numerous studies in recent years have sought to describe the effects on fish communities resulting from the placement of dams and the resulting altered flow regime. Osmundson et al. (2002) described such altered flow regimes and effects on the riverine food web supporting the endangered Colorado Pikeminnow *Ptychocheilus lucius* in the Colorado River. Although migrations of this fish were impaired by low-head irrigation diversion dams, substantial effects were attributed to dams much further upstream in the system that actually altered flow regime, and thus sediment and nutrient transport. Since the Colorado Pikeminnow is a top predator in this system, it follows that the food web effects would impact lower trophic level fishes as well.

The serial discontinuity concept (Ward and Stanford 1983, Stanford and Ward 2001) holds that dams and resulting regulated flows perturb river ecosystems for some distance downstream, with increasing distance resulting in a return to more normal conditions. McPhee Dam represents such a perturbation in the Dolores River basin. The three-species fishes in the Dolores River below McPhee have experienced declines, and it has been hypothesized that they no longer successfully spawn and recruit in the 31 miles of the Dolores River between McPhee Dam and the Dove Creek pumps. Instead, those fish that remain are thought to be remnants of once robust populations and that the populations will likely be extirpated in that area (Bestgen et al. 2011). Having worked on this stream during 1992 – 1994 as a graduate student, I can attest that Flannelmouth and Bluehead Suckers were commonly encountered in electrofishing sampling during spring or on the descending limb of the runoff hydrograph, especially in 1993 when miles 13 – 31were electrofished (this section is difficult to float and water releases from the dam are rarely conducive).

One method to ascertain if the native suckers are still spawning or recruiting in this reach is to attempt age and growth analyses from fishes collected in this reach. Moreover, it would be advantageous to compare this system with other, less impaired, river reaches. Several other rivers in western Colorado would be suitable candidates: the White River, the San Miguel, and the lower Gunnison. The native suckers are still present in good numbers in all these streams, and to a lesser extent Roundtail Chubs may be found as well. Age analysis of fish from the Dolores River ought to allow identification of flow conditions that resulted in successful spawning attempts and subsequent recruitment.

Pectoral fin rays are proposed as the method of aging these long-lived fish. Scales are unreliable in such fishes, and Quist et al. (2007) demonstrated that there is good agreement between fin ray sections and otoliths in the three-species. Since otolith sampling is lethal, it is preferable for these sensitive species to use fin rays.

Specific Objectives:

1. Intensively explore issues of tributary use, tributary fidelity, and spawning/rearing locations in the White River drainage. This objective will be primarily accomplished through a graduate student (see part A of Job 2).
2. Study age and growth of the three-species with particular attention to examining the influence of dam discharge and water temperature on spawning and recruitment success.

Methods

Age and growth – Streams selected for age and growth studies are the Dolores River (to encompass both the impaired section downstream of McPhee and the healthier reach below the San Miguel confluence and including the lower San Miguel River), the Gunnison River, and the White River.

Fin rays were encased in epoxy and sectioned according to the protocol of Koch and Quist (2007). Resulting sections were then polished with three progressively finer grit sandpapers to remove saw marks and clarify annuli. After polishing they were photographed under microscope and each photographic file saved with a unique name identifying fish origin, date of collection, and specimen number. Aging was mostly conducted by examining these photographs, although in some instances the examination of sections under microscope provided better clarity. Each section was aged independently by two individuals, and without knowledge of species or fish length initially. After aging, the two ages were compared. If the ages were in disagreement, the two agers consulted together to resolve discrepancies and assign a final age.

The ages so obtained were back-calculated to year of origin and plotted in order to visualize particularly strong or weak recruitment years within the adult population. I hypothesize that, at least in the Dolores River below McPhee, recruitment will be sporadic. Evaluating stream flow, temperature and other abiotic conditions in the various rivers during years when good recruitment was realized will allow the formulation of management recommendations.

Marking trials – Escalante Creek upstream of a barrier hosts a robust population of young Roundtail Chubs ranging from 45 to 80 mm during fall surveys. This raised the question of whether this population contributes members to the downstream Escalante Creek population or the Gunnison River population. Fish of this size are difficult to mark individually except perhaps with coded wire tags. For individual identification coded wire tags require lethal sampling. Batch marks could be applied to small fish such as these with either coded wire or visible implant elastomer (VIE). To evaluate whether VIE would be an option for marking young Escalante Creek Roundtail Chubs for later detection lower in the system, we tested red VIE in captive Roundtail Chubs at CPW's Native Aquatic Species Restoration Facility (NASRF).

Eighty-one Roundtail Chub averaging about 53 mm total length were used for the trial. Fish were anesthetized in small groups, and red fluorescent VIE was applied at the base of the right pectoral fin. The first fish marked was randomly assigned to one of two recovery buckets, and thereafter fish were alternated between the two buckets and eventual rearing tanks to create two groups. Fish were marked on November 20, 2012 and evaluations were conducted on January 8, April 19 and September 25 of 2013.

Evaluations included length, weight, and as assessment of mark visibility. Each mark was assigned to one of four numeric categories: 0 = not detectable; 1 = detectable with blue light and amber glasses; 2 = detectable with blue light; and 3 = detectable without any aid.

Results and Discussion

Age and growth – Pectoral fin rays were collected in 2012 and 2013 from the lower San Miguel, the White, and from the Gunnison River tributaries Escalante Creek, Roubideau Creek, and Potter Creek. The latter are spawning tributaries of the Gunnison River. It was not possible to collect large numbers of fin rays in the Dolores River study section in 2012 or 2013 because adequate electrofishing flows were unavailable, a result of poor snowpack and resulting strictures on McPhee Dam operations. However, 66 fin rays were obtained from Dolores River fish in late summer and early fall 2013, and 31 more from the San Miguel tributary Tabeguache Creek.

Close to 1,200 fin ray specimens were sectioned, polished, and aged in 2013. Discrepancies in age were common, and we discovered that it is often very difficult to accurately identify the first annulus. We initially considered an often solidly white interior region as the first year's growth, but upon back-calculating lengths at age one for these locations found that such lengths were often between 20 – 35 mm, too small to be the typical length of these fishes at age one. Adjustments to the ages had to be made for each case individually. Consequently the ages presented in this progress report should be considered provisional data.

Dolores River Basin

The few fin rays obtained from the Dolores River were actually picked up off the surface as mortalities following a severe storm event in late August 2013 that killed many fish in the Big Gypsum reach of the river. All the fish were relatively small, supporting the hypothesis that the adult population does not make use of this section during low flow periods. On a positive note, young age classes of Flannelmouth Sucker were collected during the event, indicating that juvenile life stages make use of the habitat in the upper Dolores River. Most of the fish collected were Roundtail Chubs, and of interest are the small sizes of these fish considering the age estimates (Figure 2). It has been thought that the Roundtail Chubs of the upper Dolores River are stunted compared to other populations and these data support that concept. The reasons for this small size remain uninvestigated, but perhaps would include habitat limitations brought about by low flows through this reach compared to the size of the channel.

Elsewhere in the Dolores River basin we aged fish from the San Miguel River and Tabeguache Creek, tributary to the San Miguel (Figures 8 and 9). The San Miguel confluence is the point at which the Dolores regains a more normal hydrograph. In these streams both native suckers were collected, and multiple age classes were present indicating a population that continues to recruit members to the adult population. Bluehead Suckers up to age 10 and Flannelmouth Suckers up to age 15 were represented.

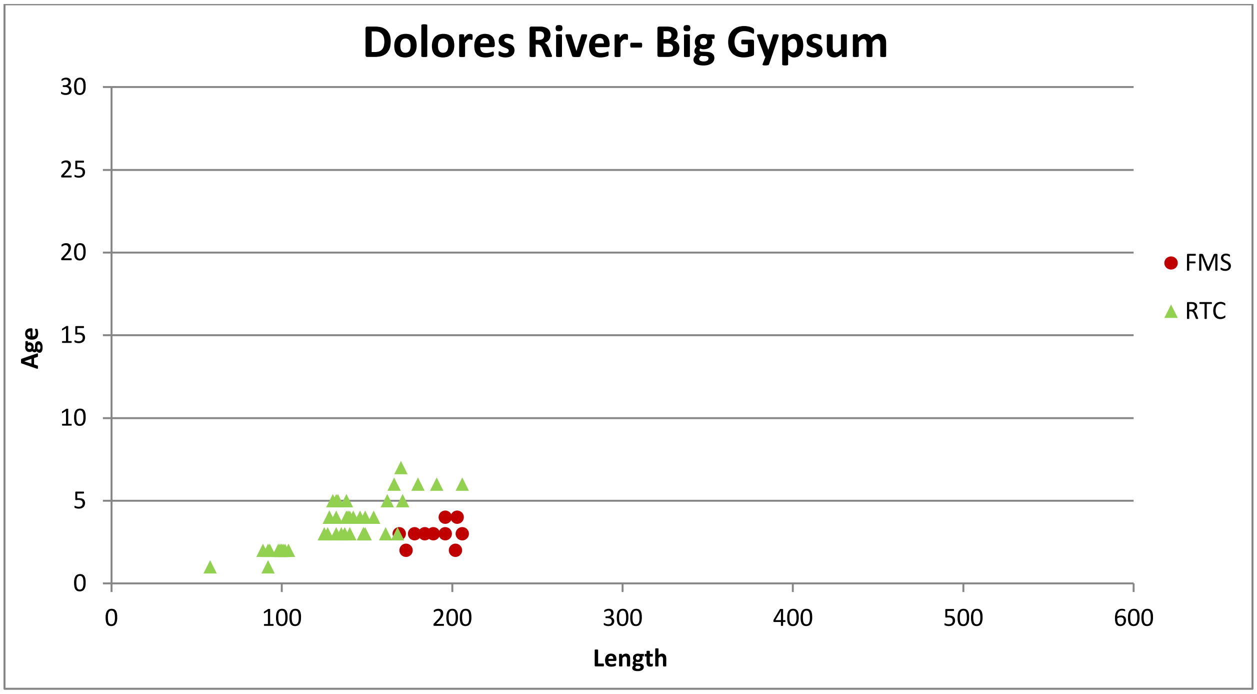

Figure 2. Age estimates of fish from the Dolores River, by length. Collected August 2013.

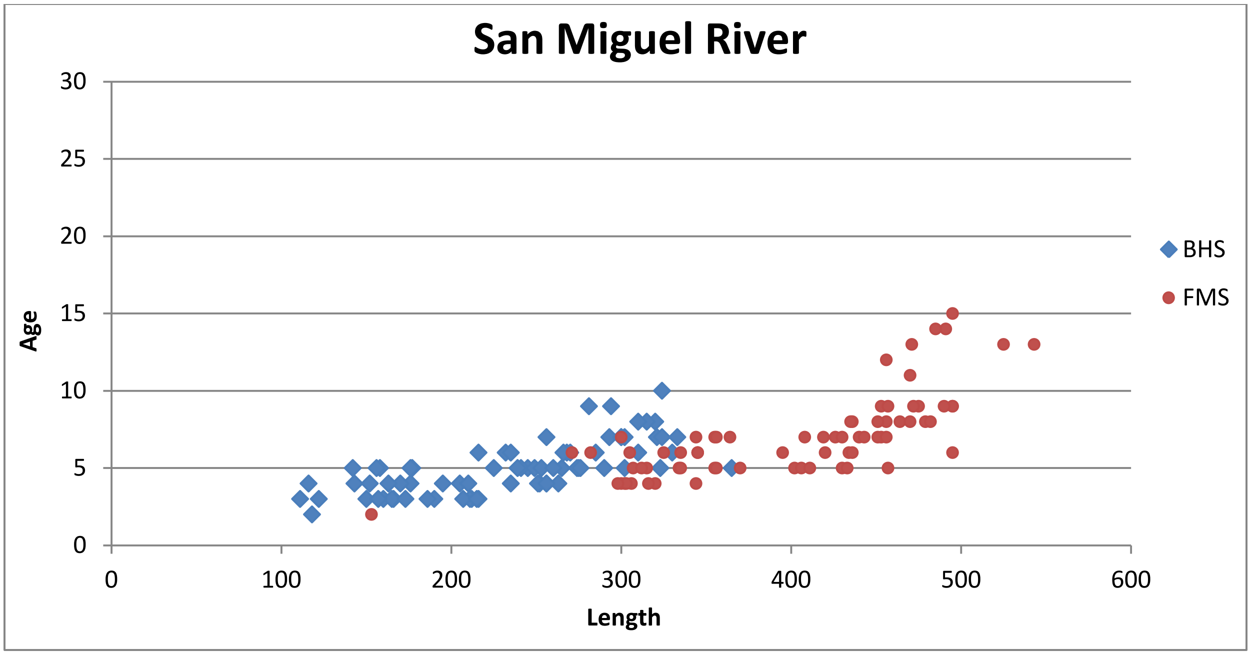

Figure 3. Age estimates of fish from the San Miguel River, by length. Collected May 2012.

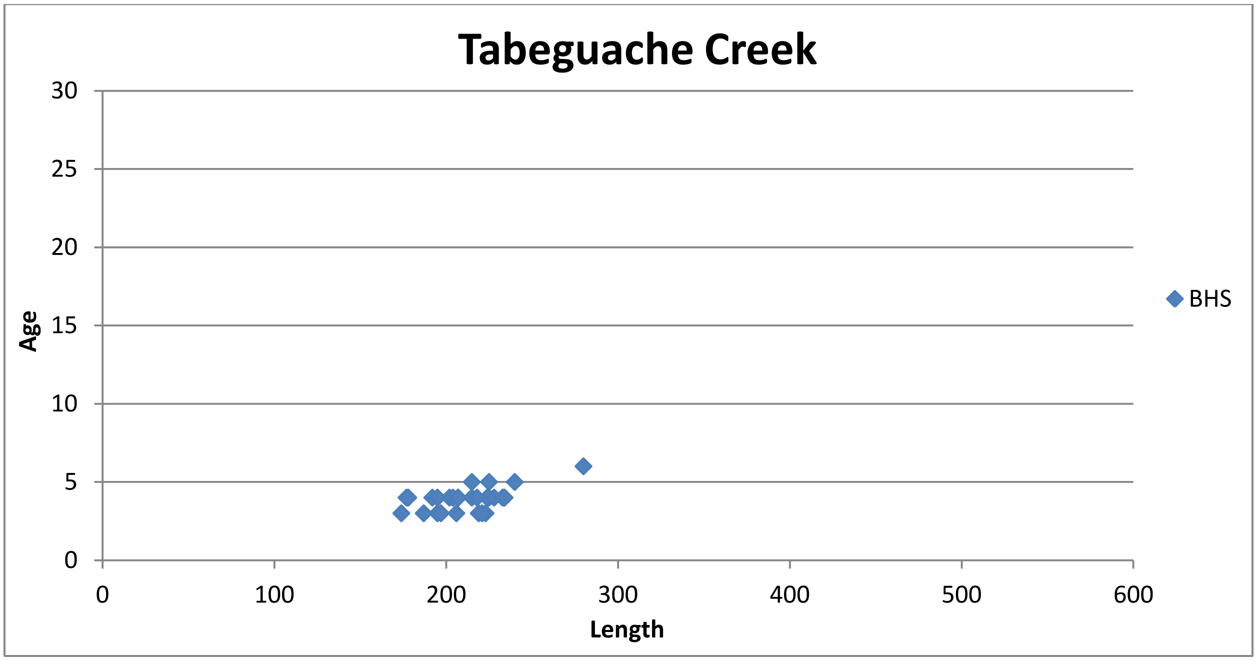

Figure 4. Age estimates of fish from Tabeguache Creek, by length. Collected May 2013.

Gunnison River Basin

Fin ray collections were obtained from three Gunnison River tributaries during spring spawning runs, Escalante Creek (Figure 5) and Roubideau and Potter creeks (Figure 6, presented together). Most suckers collected from these tributaries were aged between 4 and 13 years during the spawning runs; a view of the figures suggests that Bluehead Sucker may

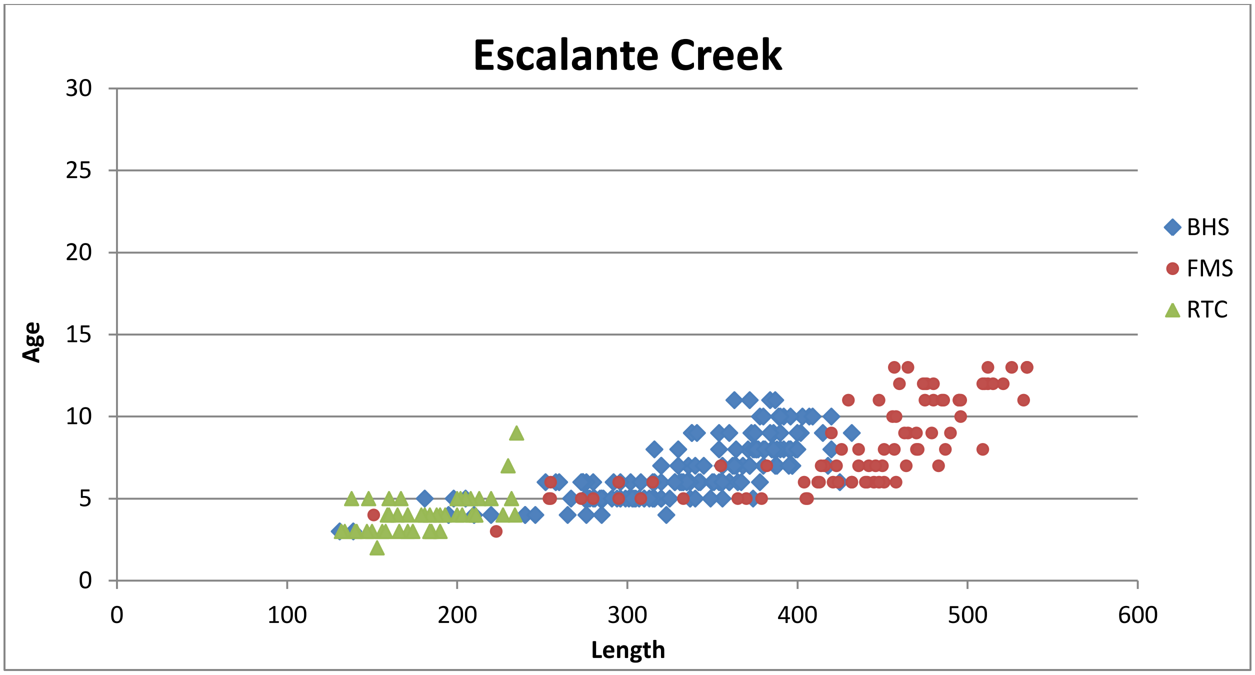

Figure 5. Age estimates of fish from Escalante Creek, by length. Collected May, 2012 and 2013.

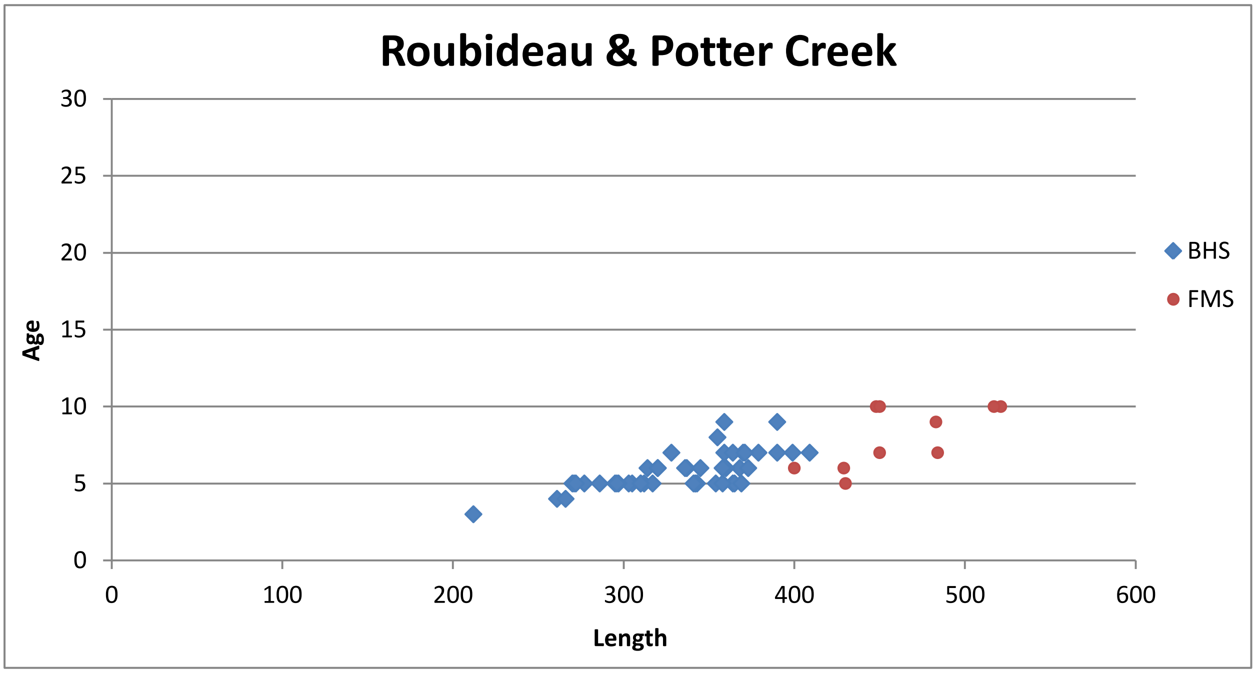

Figure 6. Age estimates of fish from Potter and Roubideau creeks, by length. Collected May 2013 at the confluence of the two streams.

enter the spawning population at age 4 or 5, and Flannelmouth Sucker at age 5 or 6. More Bluehead Suckers were encountered than Flannelmouth Suckers, as was the case in the San Miguel River.

The Roundtail Chub captured in Escalante Creek were about 20 days later than the sucker collections, at which time the suckers were mostly gone. This comports with the review of Bezzerides and Bestgen (2002) which indicates the Roundtail Chub are the latest spawners of the three-species. Although the Roundtail Chub were all less than 250 mm total length, many were ripe males. The absence of larger Roundtail Chub was a curiosity, unless the larger fish stay in the Gunnison River to spawn or exhibit different timing. Sampling by CPW demonstrates that Roundtail Chub much larger than those captured in Escalante Creek live in the Gunnison River.

White River Basin

Fish were aged from two locations in the White River, a section in Meeker (Figure 7) and another about 5 miles above Kenney Reservoir (Figure 8). In contrast to the other drainages, older fish were among those aged, with Bluehead Suckers up to age 18, Flannelmouth up to age 28, and Roundtail up to age 19. Also in contrast, more Flannelmouth were collected than Bluehead. The oldest suckers were found in the higher section of the river at Meeker, but all the younger suckers were found in the downriver section. Roundtail Chub were only captured in the downriver section, a result corroborated by Roundtail Chub larvae collections no higher than Piceance Creek (Fraser 2015).

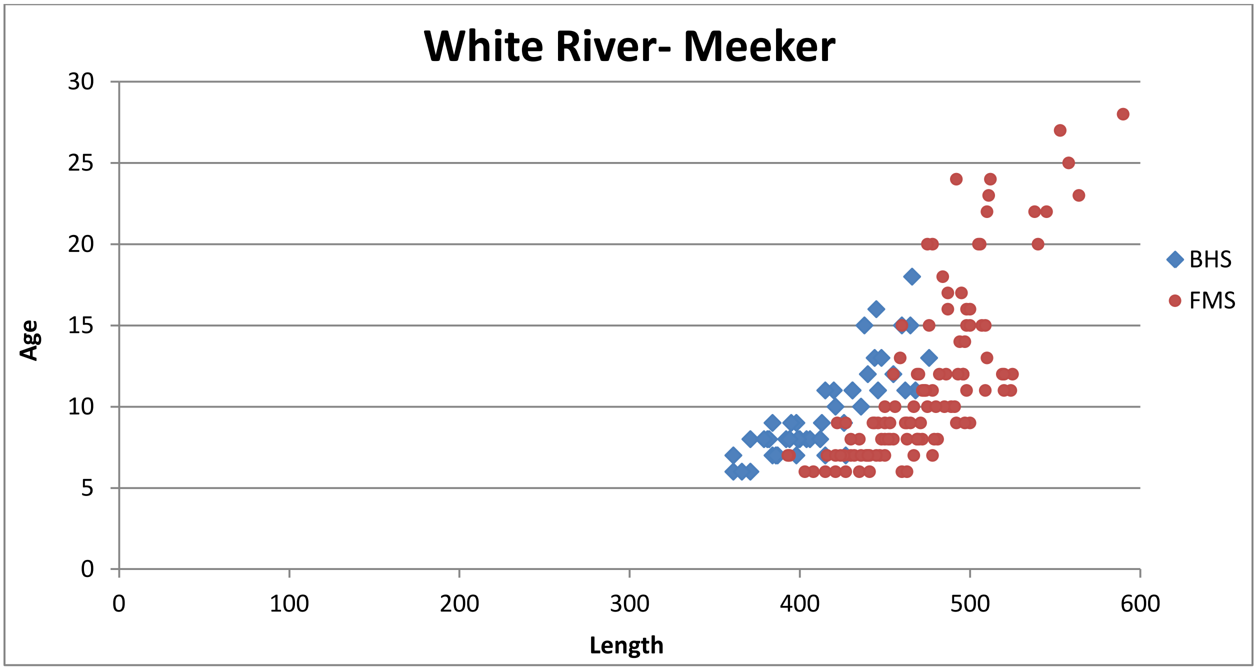

Figure 7. Age estimates of fish from the White River at Meeker by length. Collected June 2012 and June 2013.

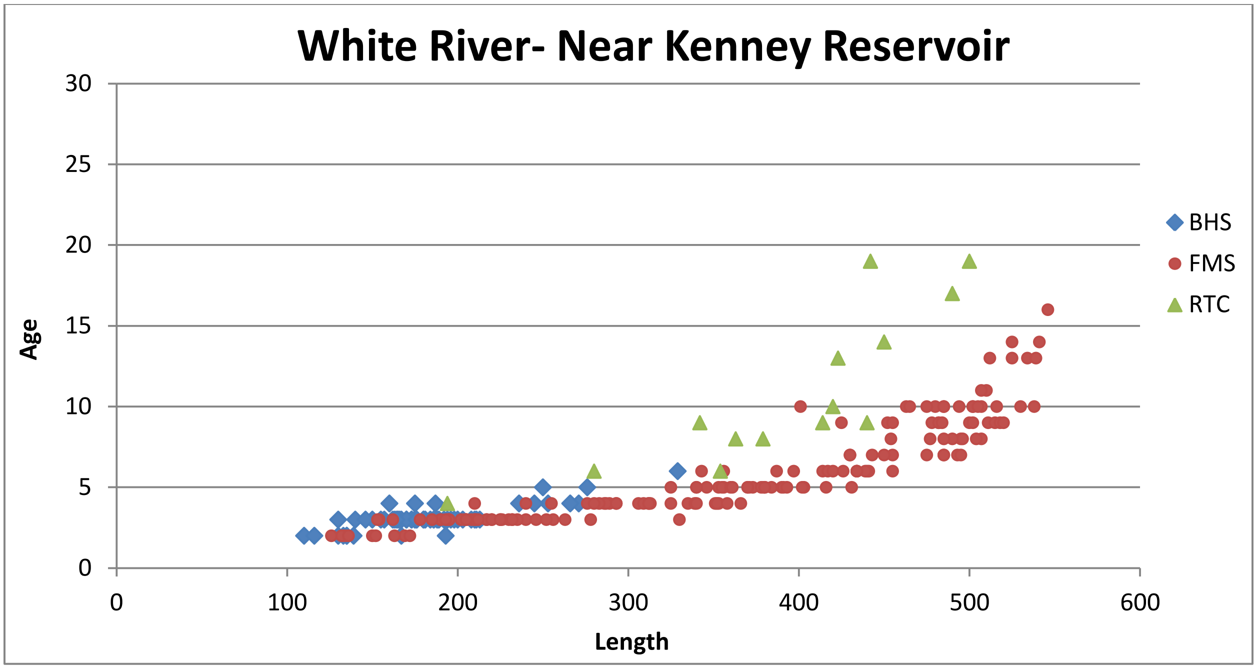

Figure 8. Age estimates of fish from the White River above Kenney Reservoir by length. Collected June 2012 and June 2013.

Unfortunately, the goal of comparing age structure and strength of age classes between the Dolores River and the other rivers did not materialize because Dolores River flows over the last two summers did not permit the widespread intensive boat sampling required to access the fish. Now, as a result of budget cuts in CPW's fiscal year 2014-15, this portion of the project was eliminated or at least suspended until such time as funding is restored.

Marking trials - There were 41 fish in group one averaging 52.9 mm (se = 0.9) and 40 fish in group two averaging 53 mm (se = 0.7). All marks were visible with the naked eye without aid upon completion of the marking November 19, 2012 and there were no associated mortalities. Weights were not obtained on the marking occasion.

On January 8, 2013 measured lengths averaged smaller for each group than in November. This may have been the result of differing measuring boards and for the remaining evaluations the same board was used as in January. Group one average 51.9 mm (se = 0.9) and group two averaged 51.9 (se = 0.7), and both groups averaged 1.3 gm weight. All marks in both groups were visible without aid or with only blue light in indoor conditions. Average mark score was 2.85 (se = 0.06) in group one and 2.9 in group 2 (se = 0.05).

On April 19, 2013 it was discovered that the two groups had been combined into one rearing tank due to a misunderstanding of the length of the trial. There had been one mortality, and the remaining 80 fish averaged 55.2 mm and 1.54 gm. Average mark score was 2.69, but all marks were still visible without aid or with the aid of only blue light in indoor conditions, 5 months after marking. By September 9, 2013 the fish had grown to 83.6 mm average length (se = 1.05) and 4.4 gm (se = 0.17). However, mark integrity had diminished greatly, with an average score on this final occasion of 1.9 (se = 0.13). Only 7 fish had marks still visible without aid, and 12 fish had marks that required blue light and amber glasses to see, and 2 marks could not be seen at all.

These results indicate that VIE batch marks applied to the base of pectoral fins are not suitable for determining over a period of at least more than one year whether age 0 or age 1 Roundtail Chubs emigrate from the section of Escalante Creek above the barrier to points downstream.

Job No. 3
Job Title: <u>Current distribution of the three-species</u>

Job Objective: Ascertain the proportion of native range in Colorado currently occupied by each of the three-species.

Period Covered: January 1, 2012 to December 31, 2016

Introduction

The best evidence currently available suggests that the three-species fish currently occupy only 45 – 55% of their historic native range in the upper Colorado River basin (Bezzerides and Bestgen 2002). They estimated historic range from extensive searches of the historical literature, giving greater weight to collection records supported by voucher specimens. Percentages of native range still occupied were derived by comparing pre-1979 data and post-1979 data. The post-1979 era was chosen because these species overlap considerably with the habitat of the four Colorado River basin endangered fishes, the subjects of intensive field research from 1980 to the present. Therefore a fair amount of ancillary information on the three-species was available for the post-1980 timeframe.

Despite extensive sampling in the upper Colorado River basin driven by work on the four endangered species, recent information on the three-species is not extensive in smaller streams. The work on the endangered species largely occurs in mainstem rivers, and CPW staff have determined that many HUC-12 basins have not been sampled since 1980.

An effort to rigorously determine the present extent of three-species range in Colorado will require sampling in areas other than mainstem channels. One way to accomplish such sampling in a scientifically defensible way is to pursue a form of “dual frame” sampling. This strategy couples visits to historic sites (a “list” frame) with visits to randomly selected sites where it is possible the species may occur (a “random” frame). Such a sampling strategy allows inference to the entire range within Colorado, as opposed to a strategy in which previously unvisited sites are selected non-randomly (perhaps based on convenient access).

Methods

Random Perennial or Intermittent sites – Random sampling locations were selected using the Reversed Randomized Quadrant-Recursive Raster (RRQRR) algorithm (Theobald et al. 2007). The algorithm permits the selection within a GIS framework of random sites that are spatially balanced with respect to availability across the landscape of interest. The result of the exercise was a list of UTM coordinates on streams in western Colorado. Separate lists of random sites were selected for perennial and intermittent waters. Filters were implemented to limit site selection as follows:

- An upper elevation limit of 8500 feet.
- No first order streams.
- No lentic waters.
- No random sites in the mainstems of the Yampa River below Stagecoach Reservoir, White River, Colorado River, Gunnison River, Uncompahgre River, Dolores River below McPhee Reservoir, San Juan River, Animas River, and La Plata River.
- No sites in any stream above Blue Mesa Reservoir, Ridgway Reservoir, Vallecito Reservoir, and Lemon Reservoir.

- Stream sites were selected with varying inclusion probability according to stream order (Table 6).
- For sampling year 2013 and later, random sites were excluded if stream gradient at the site, as measured on topographic maps, exceeded 4.0%.

Table 6. Inclusion probability for any site from a given stream order for perennial and intermittent streams.

	Inclusion probability	
Strahler Stream order	Perennial	Intermittent
2	0.1	0.1
3	0.1	0.1
4	0.1	0.2
5	0.1	0.4
6	0.2	1
7	0.5	---
8	1	---

A restriction placed upon such sampling schemes is that the selected random sites are to be visited in the order they appear on the list. This restriction was relaxed somewhat to make travel and sampling more efficient. We held to the restriction in the sense that, at the end of the field season, all sites on the list up to the highest-numbered visited site had actually been visited as well, or eliminated for reasons other than mere convenience (e.g., de-watered, permission denied, excessively steep gradient).

Prior to planning field sampling events, random sites were scouted in the office via topographic maps and Google Earth. Sites situated on stream sections exceeding 4.0% stream gradient were excluded from consideration. This additional filter criterion was applied following the 2012 field season when several random sites were sampled that clearly had no chance of hosting the target species. Examination of historic data from CPW's ADAMAS database revealed that nearly all three-species detections in the past have come from stream sections with gradient less than 2.0%. We chose the conservative cutoff of 4.0% or greater because there were a very few historic detections listed in the CPW ADAMAS database that occurred in gradients of up to 4.0%.

Upon visiting a random site, the actual sampling station was selected. We attempted to keep the random site UTM point near the midpoint of the sampling station while ensuring that a proper length of stream was sampled and appropriate start and stop points were selected. Site photographs for future reference were taken at the midpoint and at the upper and lower station termini. An image of a small whiteboard with UTM coordinates, photo point location, and orientation on the stream was captured with each stream photograph.

We sampled a minimum of 500 feet of stream, or 20 times the average stream width for streams greater than 25 feet average width. Fish sampling was conducted primarily with electrofishing equipment, usually backpack electrofishers. On rare occasions a bank electrofisher with multiple electrodes, or raft- or boat-mounted electrofishers were necessary. Two passes were conducted at each sampling station (with rare exceptions). All fish from each pass were identified, however since presence or absence was our primary objective, if the catch was large only a portion of each species catch may have been measured and weighed.

At some sites a seine was also deployed as a second capture technique in 2012. This secondary method was used extensively with the dual frame sampling effort on the eastern plains because of conductivity levels that may compromise electrofishing effectiveness, as well as the species richness encountered there with the accompanying habitat segregation. The use of a secondary method was important in that context to help break covariance between species detection and sampling gear (Ryan Fitzpatrick, CPW, personal communication). Seining was removed from the three-species sampling protocol after 2012 because the target fish are all suitably vulnerable to capture by electrofishing and in only one stream in 2012 did the seine capture a species that was not captured with electrofishing.

Random Historic sites – Emphasis shifted in 2014 from random site sampling to historic site sampling. All historic sites (including random sites from the previous two years' work where three-species fishes were captured) with sampling records after 1979 were placed in the candidate pool and selected similarly to the random sites, using the RRQRR algorithm, to ensure spatial balance. The previous filters were applied with regard to large streams, lentic waters, and upstream limits only (one Bluehead Sucker historic site had an estimated site gradient of 6.0% and was thus the only site in the data set with site gradient > 4.0%). Other sampling protocols remained the same as for random waters. However, since the database-listed coordinates of the aquatic station number for each historic site is reported as the downstream terminus, we made every effort to use those points as our re-sampling downstream terminus rather than the middle of the station. Also, we frequently sampled more stream length than was listed for a site in Adamas, in order to meet minimum length standards for this project.

In 2015, based upon consultation with CPW Aquatic Researcher Ryan Fitzpatrick and post-doctoral researcher Kristin Broms, emphasis shifted once again with respect to historic sites. In 2015 and 2016, we attempted to re-visit some sites across years and to re-visit some sites within years, and introduced fewer "new" sites to the sampling frame. The thrust of these adjustments was twofold – to better our understanding of year-to-year and seasonal variation in occupancy of these sites.

As a result of these sampling protocol adjustments, occupancy analyses of the dual-frame data set will be initially limited to the 2012-2014 time frame. These analyses were conducted in Program MARK as a suite of single-species analyses under a "single season" occupancy models (MacKenzie et al. 2002, 2006). However, for the purpose of this report all sampling conducted over the three years of dual frame sampling was considered as a season of sampling for each species. Therefore, they represent a "snapshot" of species occupancy over the three year period. For these analyses, I also divided historic sites into two groups – those that were historic for the species for which occupancy was being modeled, versus those that were historic for members of the three species other than the species being modeled.

Results and Discussion

Since 2012, 71 random and 84 historic sites were sampled over a total of 72 and 134 occasions, respectively (Table 7). All waters sampled under this research project from 2011 through 2016, including those sampled apart from formal three-species distribution assessment, are listed in Appendices 1 and 2.

Random Perennial, Intermittent and Historic sites, 2012 - 2014 – A total of 71 randomly selected sites on perennial and intermittent streams and 56 randomly selected three-species historic sites were sampled from 2102 to 2014 (Table 7). Those sites sampled in 2012 that exceeded 4.0%

stream gradient (n = 6) were excluded from occupancy analysis so that the gradient protocol was consistent among years, leaving 121 sampled sites in the 3-year analysis.

Table 7. Sites visited each year from 2012 – 2016, and number of total occasions represented.

Year	Random sites	Occasions	Historic sites	Occasions
2012	29	29		
2013	42	42		
2014			56	56
2015			25	40
2016	1	1	29	38

Bluehead suckers were physically captured at 26 of 45 species-specific historic sites and one of 11 historic sites where they had not been previously documented. The top 15 models for Bluehead sucker are listed in Table 8. The most-supported model estimated Bluehead Sucker occupancy at 0.625 (se 0.106) for species-specific historic sites, and at 0.230 (se 0.075) for the other three groups combined. The probability of detection estimated in the top model was 0.877 (se 0.090) on the first pass of a sampling effort and 0.590 (se 0.122) on the second pass. It decreased on second pass because the sampling efforts were almost always depletion sampling, so fewer specimens were available for detection on second passes. Using the second-ranked model, which separated all groups, estimated occupancy was 0.090 (se 0.119) in non species-specific historic waters, 0.132 (se 0.167) in intermittent waters, and 0.268 (se 0.088) in perennial waters. The estimate given by this model for species-specific historic sites was substantially the same as that given by the top model.

The top models all indicate that site conductivity was influential on the probability of capture, which is reasonable given that electrofishing was the method of survey used. Site gradient was an important factor predicting site occupancy, and the likelihood of Bluehead Sucker occupancy diminished with increasing gradient (Figure 9).

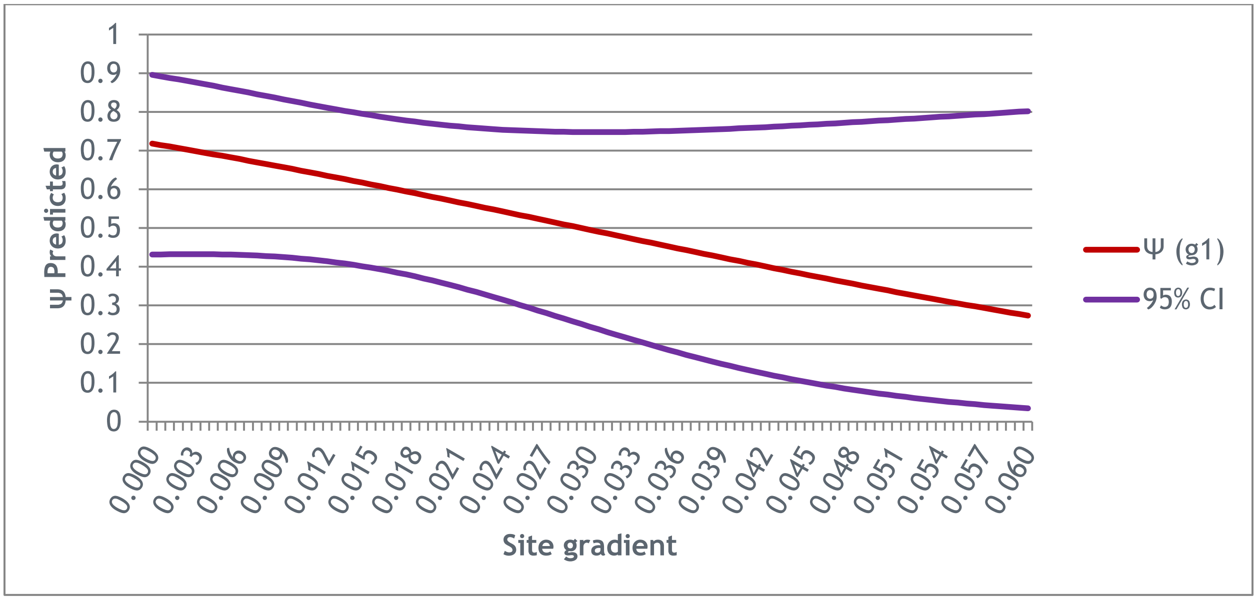

Figure 9. Predicted occupancy produced by the top-ranked Bluehead Sucker model for species-specific historic sites over the range of site gradients.

Table 8. Bluehead Sucker occupancy models for 2012-2014 sampling, ranked by order of support within the data. In model descriptions, p = detection probability (given presence), ψ = occupancy, t = time, g = group (of which there were four relating to the type of site: species-specific historic (denoted as 'g1' in the top-ranked model, in which the remaining three groups are modeled together), non species specific historic, intermittent, and perennial), cond = specific conductivity, grad = site gradient, and jday = Julian calendar day. Covariate abbreviations followed by a '2' indicate a squared term. A '+' indicates an additive effect, and a '*' indicates an interactive effect.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Parms
{p(t)+cond ψ(g1)+grad}	123.16	0.00	0.523	1.000	6
{p(t)+cond ψ(g)+grad}	126.49	3.33	0.099	0.189	8
{p(t) Ψ(g)}	127.07	3.91	0.074	0.142	6
{p(t)+cond ψ(g)+jday}	128.37	5.22	0.039	0.074	8
{p(t)+cond ψ(g)+cond}	128.37	5.22	0.039	0.074	8
{p(t)+cond ψ(g)+cond+grad}	128.51	5.36	0.036	0.069	9
{p(t)+cond ψ(g)+grad+jday}	128.66	5.51	0.033	0.064	9
{p(t)+cond+cond2 ψ(g)+grad}	128.69	5.53	0.033	0.063	9
{p(t)+cond ψ(g)+grad+grad2}	128.80	5.64	0.031	0.060	9
{p(.) ψ(g)}	129.29	6.14	0.024	0.047	5
{p(t) ψ(g_Historic groups together)}	130.36	7.21	0.014	0.027	5
{p(t)+cond ψ(g)+grad+jday+jday2}	130.46	7.30	0.014	0.026	10
{p(t)+cond ψ(g)+cond+jday}	130.69	7.53	0.012	0.023	9
{p(t)+cond ψ(g)+cond+grad+jday}	130.81	7.65	0.011	0.022	10
{p(t) ψ(.)	132.01	8.86	0.006	0.012	3

Flannelmouth suckers were physically captured at 11 of 30 species-specific historic sites. They were not captured at non species-specific historic sites, nor intermittent sites, but were captured at four of 62 randomly chosen perennial sites. Once again, the top models (Table 9) indicate that site gradient was an important determinant of site occupancy (Figure 10). The top models for this species generally estimated occupancy at considerably less than the naïve estimate (11/30 = 0.367), but these estimates were modeled on mean values for the gradient covariate. Covariate plots generated occupancy estimates of ~0.75 for stream gradient on the low end of the range sampled (~0.0015) and ~0.23 for stream gradient of ~0.01. Model 4 in Table 9, which was not modeled with covariates, generated a Flannelmouth Sucker occupancy estimate of 0.371 (se 0.089). The probability of detection estimated in the top model was 0.858 (se 0.149) on the first pass of a sampling effort and 0.738 (se 0.195) on the second pass.

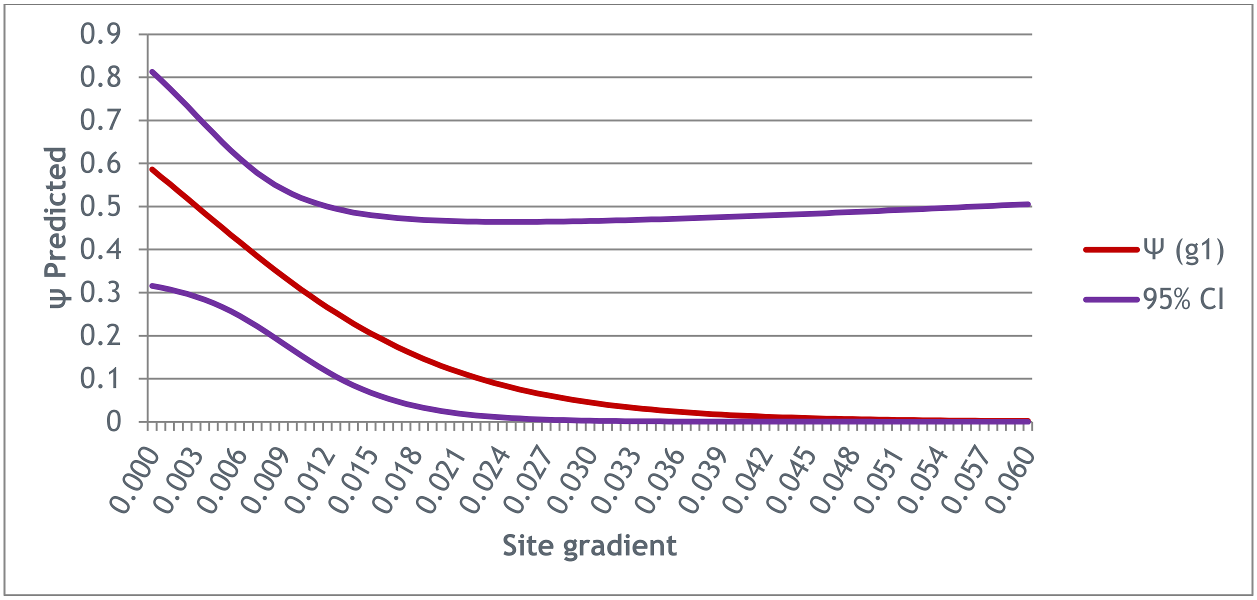

Figure 10. Predicted occupancy produced by the Flannelmouth Sucker model {p(t)+ cond+ cond2 Psi(g)+ grad} for species-specific historic sites over the range of site gradients.

Table 9. Flannelmouth Sucker occupancy models for 2012-2014 sampling, ranked by order of support within the data. In model descriptions, p = detection probability (given presence), ψ = occupancy, t = time, g = group (of which there were four relating to the type of site: species-specific historic (denoted as 'g1' in the top-ranked model, in which the remaining three groups are modeled together), non species specific historic, intermittent, and perennial), cond = specific conductivity, grad = site gradient, and jday = Julian calendar day. Covariate abbreviations followed by a '2' indicate a squared term. A '+' indicates an additive effect.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Parms
{p(t)+cond ψ(g1)+grad+grad2}	95.10	0.00	0.327	1.000	6
{p(t)+cond ψ(g)+grad+grad2}	97.14	2.04	0.118	0.361	9
{p(t)+ cond+cond2 ψ(g)+grad}	97.59	2.48	0.094	0.289	9
{p(.) ψ(g)}	97.90	2.79	0.081	0.248	5
{p(t)+cond ψ(g)+grad}	98.25	3.15	0.068	0.207	8
{p(t)+cond ψ(g)+grad+jday}	98.26	3.16	0.067	0.206	9
{p(t)+cond+cond2 ψ(g)+grad+grad2+jday+ jday2}	98.61	3.51	0.056	0.173	12
{p(t)+cond ψ(g)+grad+grad2+jday+jday2}	99.39	4.29	0.038	0.117	11
{p(t) ψ(g)}	99.79	4.68	0.031	0.096	6
{p(t)+cond ψ(g)+cond+grad}	100.23	5.13	0.025	0.077	9
{p(t)+cond ψ(g)+cond+grad+jday}	100.29	5.18	0.024	0.075	10
{p(t)+cond ψ(g)+jday}	100.48	5.38	0.022	0.068	8
{p(t)+cond ψ(g)+grad+jday+jday2}	100.59	5.49	0.021	0.064	10
{p(t)+cond ψ(g)+cond+cond2+grad+grad2+ jday+jday2}	100.95	5.84	0.018	0.054	13

Roundtail Chub were physically captured at 11 of 15 species-specific historic sites. In addition, they were found at two of 41 non-species specific historic sites and four of 56 random perennial sites, but not at any random intermittent sites. Site gradient and Julian day of sampling occasions were important covariates determining site occupancy (Table 10). The most-supported model, which evaluated occupancy for species-specific historic sites against the other three site types combined, yielded an estimate of $\Psi = 0.536$ (se 0.2004) using the mean covariate value. However, plotting predicted occupancy versus site gradient shows that low gradient sites were more likely to be occupied (Figure 11). Time of year (Julian day of sampling) was also an important predictive covariate for Roundtail Chub, with later sampling dates more likely to reveal occupied sites (Figure 12).

Table 10. Roundtail Chub occupancy models for 2012-2014 sampling, ranked by order of support within the data. In model descriptions, p = detection probability (given presence), ψ = occupancy, t = time, g = group (of which there were four relating to the type of site: species-specific historic (denoted as ‘g1’ in the top-ranked model, in which the remaining three groups are modeled together), non species specific historic, intermittent, and perennial), cond = specific conductivity, grad = site gradient, and jday = Julian calendar day. Covariate abbreviations followed by a ‘2’ indicate a squared term. A ‘+’ indicates an additive effect.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Parms
{p(t)+cond ψ(g1)+grad+jday}	91.79	0.00	0.577	1.000	7
{p(t)+cond ψ(g)+grad+jday}	95.12	3.33	0.109	0.189	9
{p(t)+cond ψ(g)+jday}	95.61	3.82	0.085	0.148	8
{p(t)+cond ψ(g)+cond+grad+jday}	97.33	5.54	0.036	0.063	10
{p(t)+cond ψ(g)+grad+jday+jday2}	97.48	5.69	0.034	0.058	10
{p(t)+cond ψ(g)+cond+jday}	97.61	5.82	0.031	0.055	9
{p(t)+cond ψ(g)+grad+grad2+jday+jday2}	98.46	6.67	0.021	0.036	11
{p(t)+cond ψ(g)+grad}	99.11	7.32	0.015	0.026	8
{p(g*t)+cond ψ(g)+jday}	99.69	7.91	0.011	0.019	14
{p(g*t)+cond ψ(g)+grad+jday}	99.85	8.06	0.010	0.018	15
{p(t)+cond ψ(g)+cond+grad}	99.98	8.19	0.010	0.017	9
{p(t)+cond ψ(g)+cond+cond2+grad+grad2+ jday+jday2}	100.13	8.34	0.009	0.016	13
{p(t)+cond ψ(g)+grad+grad2}	100.34	8.55	0.008	0.014	9
{p(t)+cond ψ(g)+cond}	100.41	8.62	0.008	0.013	8

Figure 11. Predicted occupancy produced by the top-ranked Roundtail Chub model for species-specific historic sites over the range of site gradients.

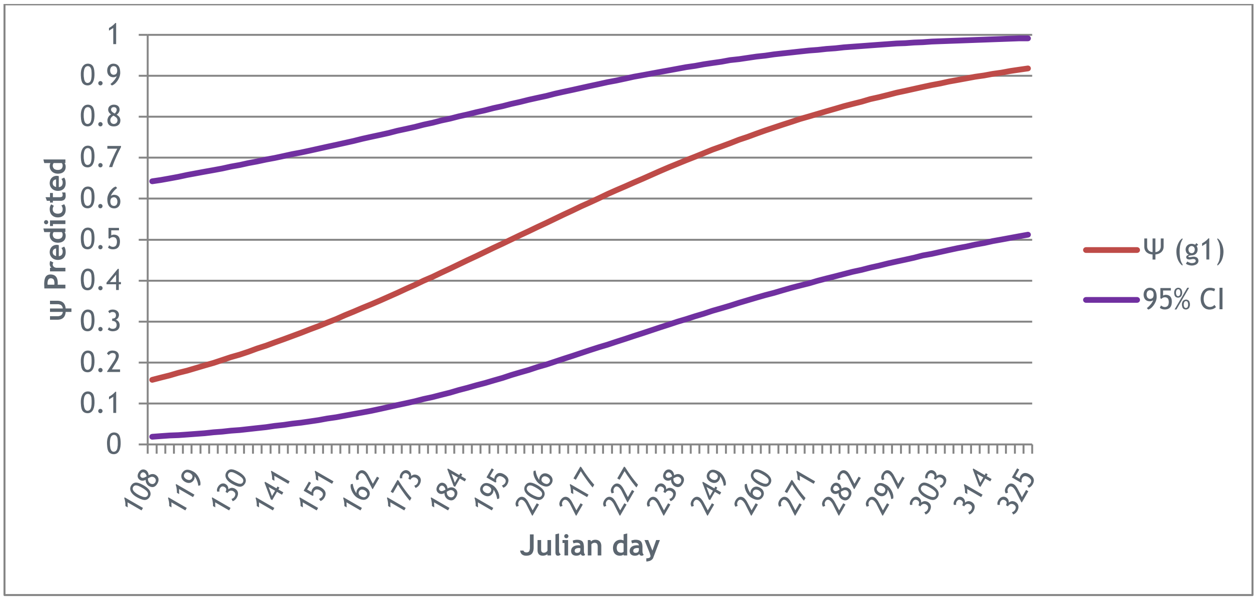

Figure 12. Predicted occupancy over the range of sampling dates produced by the top-ranked Roundtail Chub model.

The application of a stream gradient filter in 2013 removed many sites from consideration, but did not greatly increase the rate at which random sites were found to be occupied. Considering perennial and intermittent sites, three-species fishes were found at 24% of randomly selected sites sampled in 2012, and 26% in 2013. Considering only perennial sites, three-species fishes were found at 28% of sites in 2012 and 31% in 2013. In contrast, three-species fishes were found at 55% of randomly chosen historic sites sampled in 2014 (Table 11).

The historic site frame used for this sampling was limited to 1979 and later (with the exception of La Sal Creek, sampled at three sites in 2016 for the first time since 1974). Considering that Bezzerides and Bestgen (2002) used this same year to distinguish historic (for them, pre-1979) from recent data, the fact that we caught three-species fishes at just 55% of the sites that would have been considered "still occupied" by those authors may suggest that these fish species are still losing ground. However, a caution to accompany this viewpoint is that the sampling reported here was focused on tributary streams and hence on waters that are more prone to seasonal occupancy than the totality of the three-species range under consideration in Bezzerides and Bestgen (2002).

Historic sites, 2015 - 2016 – The number of sites visited and the total number of occasions for the sampling efforts in 2015 and 2016 are summarized in Table 7, and results by occasion are listed in Table 11. Although not evident in these tables, adult fish seasonal occupancy has been evident in the sampling. Locations such as Escalante, Roubideau, Piceance, Potter, Tabeguache and Cottonwood creeks are heavily used by spawning adult fish in the spring, but most of these locations are abandoned by adult fish the remainder of the year. Detections of PIT tags in Coal Creek (White River) reveal this phenomenon as well (Fraser 2015, Fraser et al. *in press*). Likewise, in Roubideau Creek, a channel-spanning passive interrogation array (PIA) installed to detect passing PIT tags reveals heavy use by adult native suckers from mid-March through early June, after which detections diminish greatly through the remainder of the summer and fall. In two winters of operation, the Roubideau PIA has not registered a single tag detection between mid-November and mid-March. Moreover, mobile antennas deployed for about 2 weeks in likely winter holding habitat in Roubideau Creek have also yielded no detections of tagged fish. These results indicate that adult fish do not use tributary habitats at all for winter habitat, and only lightly for summer and fall habitat.

In such tributaries, spring occupancy most often includes or is predominated by adult fish, whereas summer and fall occasions often reveal occupancy only by larval and juvenile fish. These results point to the importance of these tributary habitats to the life history of the three-species fishes. Although larvae and juveniles can be found in mainstem habitats (e.g., Fraser 2015), many resort to suitable tributary habitats for significant portions of the year. Additionally, it is evident from the work being conducted in Cottonwood Creek (see Job 4) that some important tributaries are ephemeral or intermittent. Although Cottonwood Creek only runs reliably during snowmelt, many hundreds to thousands of spawning adult three-species fishes have been found using that tributary during runoff periods in 2014 – 2016.

No formal occupancy analyses of the 2015 – 2016 data have yet been conducted, but occupancy by the three-species fishes was high over the occasions represented, which included multiple visits at some sites. Historic sites were found to be occupied by one or more of the three-species on 95% of sampling occasions in 2015 and 89.5% of occasions in 2016.

Table 11. Summary of the three-species, White Suckers, and select sucker hybrids detected at sites sampled from 2012 through 2016. Sites were spatially balanced from 2012 – 2014, but selected with investigator input from 2015 - 2016. SITE codes describe site type: “I” = intermittent, “P” = perennial, and “H” = historic. A “+” indicates that species or hybrid was detected at the site and a “-” indicates it was not. Area is the CPW Field Operations area.

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
I002	-	-	+	-	-	-	-	7	4/18/12	Kannah Creek
I005	-	+	-	-	-	-	-	6	5/7/12	Douglas Creek
I007	-	-	-	-	-	-	-	7	5/31/12	Dry Hollow Creek
I011	-	-	-	-	-	-	-	6	5/8/12	Cottonwood Creek
P001	-	-	-	-	+	-	-	15	9/26/12	Piedra River #1
P002	-	-	-	-	-	-	-	18	4/17/12	Spring Creek E Fork
P004	-	-	+	-	-	-	-	15	7/23/12	Cherry Creek
P005	-	-	-	-	-	-	-	6	5/9/12	Slater Creek #2
P006	-	-	-	-	-	-	-	18	5/10/12	La Fair Creek
P009	-	-	+	-	+	-	-	8	6/20/12	Roaring Fork #1
P010	-	-	-	-	-	-	-	18	6/15/12	Escalante Creek
P012	-	-	-	-	-	-	-	6	6/25/12	Spring Creek W Fork
P014	-	-	-	-	-	-	-	18	6/18/12	Big Bear Creek
P015	-	-	-	-	+	+	-	10	6/27/12	Trout Creek #1
P018	-	-	-	-	-	-	-	10	6/27/12	Mill Creek
P020	-	-	-	-	+	-	-	10	9/13/12	Elk River #1
P022	-	+	+	-	+	+	+	16	7/17/12	Muddy Creek
P025	-	-	-	-	-	-	-	6	6/28/12	Vermillion Creek
P026	-	-	-	-	-	-	-	16	8/3/12	Coal Creek
P029	+	+	+	-	+	+	+	6	9/7/12	Little Snake River #1
P032	-	-	-	-	+	-	-	8	9/19/12	Eagle River #1
P033	-	-	-	-	-	-	-	15	7/24/12	Spring Creek
P034	-	-	-	-	-	-	-	8	9/20/12	Crystal River #2
P037	-	-	-	-	-	-	-	15	7/26/12	M. Fork Piedra R.
P038	-	-	-	-	-	-	-	7	8/1/12	Gill Creek
P045	-	-	-	-	-	-	-	16	9/4/12	Alfalfa Run
P046	-	-	-	-	-	-	-	18	9/28/12	Burro Creek
P047	-	-	-	-	-	-	-	6	10/3/12	Beaver Creek Big
P048	-	+	-	-	+	+	-	6	9/6/12	Milk Creek
I020	-	-	-	-	-	-	-	6	5/21/13	Sand Wash
I030	-	-	-	-	-	-	-	7	5/30/13	Bull Creek
I031	-	-	-	-	-	-	-	6	5/20/13	Douglas Creek
I038	-	-	-	-	-	-	-	6	6/18/13	Fourmile Creek
I052	-	-	-	-	-	-	-	6	6/17/13	Little Beaver Creek
I057	-	-	-	-	-	-	-	6	6/20/13	Deep Channel Creek
P051	-	-	-	-	+	-	-	16	7/2/13	Leroux Creek
P053	-	+	+	-	+	+	-	6	6/17/13	Milk Creek
P054	-	+	+	+	+	-	-	15	7/30/13	Rio Blanco #1

Table 11. Continued.

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
P056	-	-	-	-	+	-	-	10	6/19/13	Trout Creek #1
P062	-	-	-	-	-	-	-	15	5/14/13	McElmo Creek
P063	+	+	+	-	+	-	-	6	7/24/13	Little Snake R #1
P064	-	-	-	-	-	-	-	6	6/4/13	Steward Gulch Mid Fk
P068	-	-	-	-	+	-	-	6	5/22/13	Fortification Cr
P069	-	-	+	-	-	-	-	18	7/12/13	West Creek
P070	-	-	-	-	-	-	-	18	5/28/13	Loutsenhizer Arroyo
P072	-	-	-	-	+	-	-	6	6/18/13	Elkhead Creek #3
P074	-	-	-	-	-	-	-	6	6/4/13	Fawn Creek
P076	-	-	-	-	-	-	-	9	6/19/13	Un-named
P078	-	-	-	-	-	-	-	8	7/26/13	Eagle River #2
P079	-	-	-	-	-	-	-	6	5/23/13	Piceance Creek
P080	-	-	-	-	-	-	-	18	5/16/13	Cottonwood Creek
P081	+	+	+	-	-	-	-	18	6/5/13	Escalante Cr
P083	-	-	-	-	+	-	-	15	7/11/13	Stollsteimer Creek
P084	-	-	-	-	-	-	-	6	7/25/13	Deer Creek
P088	-	-	-	-	-	-	-	15	10/28/13	Mancos River #2
P089	+	+	+	-	-	-	-	6	7/24/13	Little Snake R #1
P093	-	-	+	-	-	-	-	7	6/3/13	Divide Creek West
P096	-	-	-	-	-	-	-	18	8/28/13	Peach Valley
P099	-	-	-	-	-	-	-	7	9/3/13	Salt Creek East
P101	-	-	+	-	+	-	+	15	7/31/13	Piedra River #1
P106	-	-	+	-	+	-	-	15	8/1/13	Spring Creek
P109	-	-	+	-	-	-	-	15	7/9/13	Dolores River West Fk
P112	-	-	-	-	-	-	-	15	10/28/13	Mancos River #2
P117	-	-	-	-	+	-	-	18	7/2/13	Wise Creek
P124	-	+	-	-	-	-	-	6	7/23/13	Piceance Creek
P150	-	-	-	-	-	-	-	15	8/1/13	Turkey Creek
P159	-	-	-	-	-	-	-	10	8/13/13	Foidel Creek
P160	-	-	-	-	-	-	-	10	8/14/13	Willow Cr #2
P161	-	-	-	-	-	-	-	7	9/6/13	Salt Creek
P163	-	-	-	-	-	-	-	18	8/29/13	Dry Creek
P166	-	-	-	-	+	-	+	10	8/13/13	Fish Creek #1 (Milner)
H001	-	-	+	-	-	-	-	15	6/20/14	Yellowjacket Canyon
H002	-	-	+	-	-	-	-	15	7/22/14	Rio Blanco #1
H003	-	-	-	+	-	-	-	7	5/8/14	East Creek
H004	-	-	-	-	-	-	-	7	5/22/14	Dry Owens Creek
H005	-	-	-	-	-	-	-	15	8/5/14	Dolores River #4
H006	-	-	-	+	-	-	-	10	6/24/14	Elkhead Creek #1
H009	+	+	+	+	+	-	-	7	11/12/14	Badger Wash
H010	-	-	-	-	-	-	-	6	5/23/14	Piceance Creek
H012	-	-	-	-	+	-	-	18	5/9/14	Montrose Arroyo

Table 11. Continued.

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
H013	+	-	-	-	-	-	-	15	10/22/14	Mancos River #2
H014	-	-	-	-	-	-	-	6	7/15/14	Milk Creek
H015	-	-	+	-	-	-	-	18	7/8/14	Cimarron R, Little
H016	-	-	+	-	-	-	-	15	6/3/14	Mancos River #3
H017	-	-	-	-	-	-	-	7	7/16/14	Divide Creek, East
H018	-	-	+	-	-	-	-	18	5/14/14	Tabeguache Creek
H019	-	-	-	-	-	-	-	6	7/29/14	Miller Creek
H020	-	-	-	+	-	-	-	7	11/21/14	Mack Wash
H023	-	-	-	-	-	-	-	7	5/15/14	Hightower Creek
H026	-	-	+	-	+	-	-	7	7/7/14	Buzzard Creek #1
H027	+	+	+	+	-	-	+	18	6/30/14	San Miguel R #1
H028	-	-	+	-	-	-	-	18	5/30/14	Naturita Creek
H029	-	-	+	-	-	-	-	8	7/22/14	Dry Fork Cabin Creek
H031	-	-	+	-	-	-	-	7	9/8/14	Roan Creek
H032	-	-	+	-	-	-	-	7	7/9/14	Buzzard Creek #2
H035	-	-	-	-	-	-	-	10	9/25/14	Elk River #1
H036	-	-	-	-	+	-	+	15	7/23/14	Rock Creek
H037	-	-	-	-	-	-	-	15	6/18/14	Lightner Creek #1
H038	-	-	-	-	+	+	+	6	9/24/14	Williams Fk Y
H039	-	-	-	-	+	-	-	15	7/23/14	Piedra River #1
H041	-	-	-	-	-	-	-	6	6/25/14	Milk Creek
H043	-	-	+	-	-	-	-	18	6/11/14	Potter Creek
H044	+	+	+	+	+	-	-	7	9/8/14	Roan Creek
H045	+	+	-	-	+	+	+	7	11/13/14	Persigo Wash
H047	+	+	+	-	-	-	-	7	11/13/14	Salt Creek
H048	-	+	+	-	-	-	-	18	8/4/14	San Miguel R #1
H050	-	-	-	-	-	-	-	9	9/10/14	Rock Creek
H051	-	-	-	-	-	-	-	18	7/10/14	Cow Creek
H056	+	+	+	+	+	+	+	6	9/22/14	Little Snake R #1
H057	+	+	+	+	+	+	-	7	11/12/14	Salt Wash, Big
H058	-	-	-	-	-	-	-	6	7/14/14	Piceance Creek
H059	-	-	+	-	-	-	-	15	8/6/14	Yellowjacket Canyon
H060	+	-	-	-	+	-	+	7	7/25/14	Rifle Creek
H062	-	-	+	-	-	-	-	15	10/1/14	Long Hollow Creek
H063	-	-	+	-	+	-	+	6	9/26/14	Milk Creek
H064	+	-	-	-	-	-	-	15	10/22/14	Mancos River #2
H066	-	-	+	-	-	-	-	18	7/31/14	Tabeguache Creek
H067	-	-	-	-	-	-	-	6	7/29/14	Vermillion Creek
H068	-	-	-	-	+	-	-	7	7/28/14	Garfield Creek
H069	-	-	+	+	+	-	+	18	8/21/14	Cimarron River
H070	+	+	+	-	-	-	-	18	8/18/14	Escalante Creek
H071	-	-	-	-	-	-	-	18	10/23/14	Dallas Creek

Table 11. Continued.

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
H073	+	+	+	-	+	+	+	18	7/18/14	Dry Creek
H074	-	-	+	-	-	-	-	7	8/19/14	Grove Creek
H075	-	-	-	-	-	-	-	15	8/5/14	Cherry Creek
H076	+	+	+	-	+	+	-	7	9/9/14	Plateau Creek #1
H079	-	-	-	-	-	-	-	15	9/30/14	Junction Cr #1
H002	-	-	+	-	-	-	-	15	9/30/15	Rio Blanco #1
H016	-	-	+	-	-	-	-	15	8/25/15	Mancos River #3
H018	-	-	+	-	-	-	-	18	4/22/15	Tabeguache Cr
H018	-	+	+	-	-	-	-	18	7/23/15	Tabeguache Cr
H018	-	-	+	-	-	-	-	18	9/11/15	Tabeguache Cr
H032	-	+	+	-	-	-	-	7	7/28/15	Buzzard Creek #2
H041	+	+	+	-	+	-	-	6	10/7/15	Milk Creek
H043	-	-	+	-	-	-	-	18	7/29/15	Potter Creek
H056	+	+	+	+	+	+	+	6	8/20/15	Little Snake R #1
H058	-	-	+	-	-	-	-	6	8/19/15	Piceance Creek
H073	-	-	-	-	+	-	-	18	9/28/15	Dry Creek
H081	-	+	+	-	-	-	+	18	8/17/15	Naturita Creek
H112	+	-	+	-	+	+	-	18	4/28/15	Dominguez Creek, Big
H112	+	-	-	-	-	-	-	18	8/24/15	Dominguez Creek, Big
H114	-	-	+	-	-	-	-	7	9/29/15	Owens Creek
H126	-	+	-	-	-	-	-	6	10/26/15	Douglas Creek
H142	+	+	+	-	-	-	-	18	4/15/15	Tabeguache Cr
H142	+	+	+	-	-	-	-	18	4/22/15	Tabeguache Cr
H142	+	+	+	-	-	-	-	18	6/3/15	Tabeguache Cr
H142	-	+	-	-	-	-	-	18	9/11/15	Tabeguache Cr
H209	-	-	+	-	+	-	+	6	9/23/15	Williams Fk Yampa
H278	-	+	+	-	-	-	-	18	4/14/15	Potter Creek
H278	+	+	-	-	-	-	-	18	4/21/15	Potter Creek
H278	-	-	+	-	-	-	-	18	4/30/15	Potter Creek
H278	+	+	+	-	-	-	-	18	5/14/15	Potter Creek
H278	+	+	+	+	-	-	-	18	6/2/15	Potter Creek
H278	+	-	+	-	+	-	-	18	6/17/15	Potter Creek
H303	-	+	+	-	+	-	-	6	10/7/15	Milk Creek
H311	-	+	+	-	-	-	-	6	8/19/15	Piceance Creek
H701	+	+	+	+	+	+	+	18	8/27/15	Roubideau Cr
H702	+	+	+	+	+	+	+	18	5/6/15	Escalante Creek
H702	+	+	+	+	+	+	+	18	5/20/15	Escalante Creek
H703	+	+	+	-	-	-	-	18	8/31/15	Escalante Creek
H704	+	+	+	+	-	-	-	18	8/6/15	Escalante Creek
H705	+	-	+	-	+	-	+	18	5/12/15	Cottonwood Creek
H705	+	-	+	-	-	-	-	18	5/22/15	Cottonwood Creek

Table 11. Concluded.

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
H705	+	+	+	-	-	-	-	18	6/18/15	Cottonwood Creek
H706	+	+	+	-	-	-	-	6	9/2/15	Little Snake R. #1
H707	-	-	+	-	-	-	-	18	4/22/15	Tabeguache Cr
H707	-	-	-	-	-	-	-	18	6/3/15	Tabeguache Cr
H001	+	+	-	-	-	-	-	15	9/29/16	Yellowjacket Canyon
H004	-	-	+	-	+	-	-	7	6/28/16	Owens Creek, Dry
H016	-	-	+	-	-	-	-	15	6/29/16	Mancos River
H018	-	+	+	-	-	-	-	18	6/2/16	Tabeguache Creek
H018	-	+	+	-	-	-	-	18	8/2/16	Tabeguache Creek
H018	-	+	+	-	-	-	-	18	9/21/16	Tabeguache Creek
H036	+	+	+	-	-	-	-	15	11/7/16	Rock Creek
H053	+	-	+	-	-	-	-	15	9/27/16	Yellowjacket Canyon
H056	+	+	+	-	+	-	-	6	9/8/16	Little Snake River
H058	-	-	+	-	-	-	-	6	6/22/16	Piceance Creek
H058	-	+	+	-	-	-	-	6	9/7/16	Piceance Creek
H068	-	-	+	-	+	-	-	7	9/6/16	Garfield Creek
H073	-	+	+	-	+	-	-	18	7/27/16	Dry Creek
H076	+	+	+	-	+	+	+	7	9/14/16	Plateau Creek
H080	+	+	-	-	-	-	-	15	9/26/16	Yellowjacket Canyon
H081	-	+	+	-	-	-	+	15	8/3/16	Naturita Creek
H082	-	-	+	-	-	-	-	15	10/13/16	Divide Creek,West
H085	+	+	+	-	-	-	-	15	9/28/16	Yellowjacket Canyon
H093	+	-	+	-	-	-	-	15	6/29/16	Weber Canyon Creek
H093	+	-	+	-	-	-	-	15	7/19/16	Weber Canyon Creek
H112	-	-	-	-	-	-	-	18	4/7/16	Big Dominguez Creek
H114	-	-	-	-	-	-	-	7	6/28/16	Owens Creek
H125	-	-	+	-	-	-	-	7	10/12/16	Divide Creek, West
H187	+	+	+	-	-	-	-	15	9/28/16	Yellowjacket Canyon
H188	-	+	+	-	+	+	+	7	9/14/16	Plateau Creek
H258	-	-	-	-	+	-	-	7	9/6/16	Garfield Creek
H262	+	-	-	-	-	-	-	18	6/1/16	Roubideau Creek
H278	+	+	+	-	-	-	-	18	5/3/16	Potter Creek
H278	-	+	+	-	-	-	-	18	5/11/16	Potter Creek
H278	+	+	+	-	-	-	-	18	5/17/16	Potter Creek
H278	+	+	+	+	-	-	-	18	5/25/16	Potter Creek
H278	-	-	-	-	-	-	+	18	6/1/16	Potter Creek
H278	+	+	+	+	-	-	-	18	9/29/16	Potter Creek
H311	-	+	-	-	-	-	-	6	6/23/16	Piceance Creek
H341	+	+	+	+	-	-	-	18	8/30/16	Escalante Creek
H354	+	+	+	+	-	+	+	7	10/13/16	Divide Creek, West
H702	-	+	+	+	+	+	+	18	5/5/16	Escalante Creek
H703	+	+	+	-	-	-	-	18	7/28/16	Escalante Creek

Conclusions and Recommendations

After the first two seasons of field work, we encountered three-species fishes at just two random intermittent water sites. Those were Kannah Creek and Douglas Creek, and an argument could be made for both at the respective sampling sites that they are perennial or nearly so. Moreover, many intermittent sites were dry even though time of visitation was limited to the runoff season when they would most likely carry water. Thus these sites were relatively uninformative. Although we know from experience that three-species fishes use intermittent waters, efforts to find new three-species sites should focus primarily on perennial waters. Even then, agency personnel should remain aware that certain ephemeral waters, like Cottonwood Creek, may host meaningful spawning populations of one or more of the three-species fish.

The occupancy modeling conducted to date demonstrates conclusively that stream gradient is an important predictor of occupancy for all of the three-species. Therefore, any search for new sites hosting these species would be more efficient if potential sampling sites were screened by site gradient prior to visiting them. Little would be gained by searching sites where stream gradient exceeds 4.0%, and most productive would be those sites or stream sections where gradient is less than 2.0%.

The shift toward historic three-species sites in 2014 demonstrated that maintaining a sampling program in these waters will be important, particularly since the rate of occupancy in randomly selected, spatially balanced historic sites was not particularly high given that the target sites were presumed or demonstrated to be occupied circa 1979-1980. Also, there is a need to continue expanding sampling efforts during times of the year often neglected with respect to this species assemblage. Although good information has been obtained regarding seasonal changes in site use, there remain questions about the extent to which certain life stages use tributary systems throughout the year.

Job No. 4
Job Title: Improving genetic integrity of sucker spawning runs by mechanical removal of non-native and hybrid spawners.

Job Objective: Determine whether physical removal of non-native suckers and their hybrids from spawning tributaries results in the production of more pure native sucker larvae.

Period Covered: January 1, 2014 to December 31, 2016

Introduction

The “three species” assemblage comprises Flannelmouth Sucker *Catostomus latipinnis*, Bluehead Sucker *C. discobolus,* and Roundtail Chub *Gila robusta*. Natives of the Colorado River basin, they each occupy only 45 – 55% of their historic native range in the upper Colorado River basin (Bezzerides and Bestgen 2002; the upper basin includes the Colorado River and its tributaries from Glen Canyon Dam upstream). Of the three, Roundtail Chub is considered a species of special concern by Colorado, whereas the two sucker species hold no special status. For all three, there is concern that populations are exhibiting downward trends. Collectively, the three species are the subjects of a Rangewide Conservation Agreement and Strategy (UDWR 2006, hereafter Rangewide Agreement), to which Colorado is a signatory. Genetic and morphological characterization of existing populations is an important conservation action listed in the Rangewide Agreement so that threats of hybridization can be measured.

Perhaps the most insidious threat to the continued integrity of the Bluehead and Flannelmouth Suckers is the presence and spread of non-native species with which the native suckers hybridize. The native suckers are prone to hybridization with sucker species not native to the Colorado River basin. Primarily, these non-natives are White Sucker *C. commersonii* and Longnose Sucker *C. catostomus* in Colorado, but also such species as Utah Sucker *C. ardens* in other parts of the upper Colorado River Basin. The range and relative numbers of these non-native suckers has greatly expanded in western Colorado over the last 30 years. Hybrid suckers have been more readily observed and identified in recent times. Wilson (1992) suggested that 38% of North American freshwater fish could be threatened by hybridization, and certainly these native western suckers should be counted among them. Continued hybridization and introgression could even result in the eventual extinction of the native species as we know them today (*sensu* Rhymer and Simberloff 1996). Therefore it is important to continue refining the baseline data on genetic purity and diversity among the three species.

Once present in a river basin, it is very difficult to prevent movement and range expansion of non-native suckers, and mitigating management options are limited. Because the native suckers are known as “big river” fish, opportunities to segregate pure populations of native suckers from invading non-natives, à la the cutthroat trout model, by translocation or barrier erection will be very limited. Habitat disturbance has been identified as a pathway to hybridization, at least in the botanical realm (Wolf et al. 2001). However it can be readily argued that aquatic habitat disturbance within the native range of the three species – dams, irrigation withdrawals, temperature and sediment regime changes, conversion of lotic to lentic habitats – have paved the way for thriving populations of non-native suckers (e.g. Chart and Bergersen 1992, Martinez et al. 1994, Collier et al. 1996). Most such habitat disturbances are unlikely to be reversed because they are the foundation of societal infrastructure in the arid west.

A lack of opportunities for segregation or habitat restoration leads to consideration of a third option – removal of non-native suckers and hybrids. However, such an approach is unlikely to be executed on a scale as broad as the present range of non-native suckers in upper Colorado River basin. Moreover, attempts to suppress fish populations may result in demographic or life history responses on the part of the removal target species that counter the removal efforts (Brodeur et al. 2001, Zipkin et al. 2009). Removals to benefit three-species fishes have been suggested or attempted in smaller drainages (Rawson and Elsey 1948, Compton 2007, Garner et al. 2010). So, although attempts to remove non-native suckers in a large river basin are unlikely to be successful and may even be counter-productive, perhaps focusing on the spawning run in a smaller tributary would allow success on a smaller scale that would have implications for the larger river basin.

Here, we would test the hypothesis that mechanical removal of non-native suckers and their hybrids from an important spawning tributary of the Gunnison River would result in detectable changes in the proportion of pure native suckers drifting to the Gunnison River from the tributary. If non-natives can be successfully repressed to the advantage of native suckers, progeny produced in that stream would result in more pure fish in the Gunnison River. While such a strategy would not result in the disappearance of non-native suckers from the entire Gunnison basin, it may provide an avenue toward ensuring that the native species persist in the Gunnison basin. If successful, this strategy could be implemented in other river basins on appropriate tributaries as well.

Specific Objectives:

1. Conduct tagging operations using PIT tags in spawning tributaries of the Gunnison River that host large sucker spawning runs.
2. Install a stationary PIT tag antenna (passive interrogation array; PIA) in Roubideau Creek to increase future tag detections and refine arrival timing estimates.
3. Conduct a removal of non-native and clearly hybridized and introgressed suckers from the spawning run in Cottonwood Creek, tributary to Roubideau Creek, over several years to assess the effect on genetic purity of the larval drift and on composition of future spawning runs.
4. In the longer term, PIT tag detections on the Roubideau PIA will allow annual survival to be estimated for PIT-tagged fish populations.

Methods

Two tributaries of Roubideau Creek (itself a tributary of the Gunnison River) were identified as study streams. Potter Creek was chosen to serve as the control and the ephemeral stream Cottonwood Creek as the treatment.

Commencing in 2014, during select electrofishing surveys in the Gunnison River basin, and during spring fish-trapping operations, suckers ≥ 150 mm in total length were tagged with a Passive Integrated Transponder (PIT) tag measuring 12.5 mm long and 2 mm diameter. All suckers encountered that exceed 150 mm TL are scanned for the presence of a previously implanted tag using a handheld reader (Biomark model 601, Boise, ID). New tags were inserted intra-peritoneally slightly left of the abdominal midline and about 50-60% the length of the pelvic fin behind the left pelvic fin insertion. All fish were identified to species or suspected hybrid combination and measured (total length, mm, and weight, gm), and then released back to the stream upstream of the weir. The number of PIT tags deployed was limited by budget, and the tagging of new fish ceased if annual provisions of tags were depleted. The deployment of PIT tags is intended to allow assessment of spawning stream fidelity, and eventually to allow estimation of survival rates of these native fishes.

A Passive Interrogation Array (PIA) was installed in Roubideau Creek in February 2015. The array consists of four individual antennas that span the entire channel in two locations, allowing increased detection probabilities and the possibility of discerning direction of movement of individual fish in many cases. The system is solar-powered, and the antennae are linked to a multiplexing receiver which stores the data. Data are downloaded about weekly during the spawning season from mid-March to late June, and about monthly during other times of the year. This PIA operates continuously.

A fish weir was used to conduct the spring fish trapping commencing in 2015. The weir consisted of two stream-spanning aluminum fences (with 2.22 cm spaces between vertical bars) that funneled fish into two trap boxes. One trap captured upstream migrants, and one trap captured downstream migrants. The traps were aluminum box-frames (76.2 × 76.2 × 152.4 cm) with 2.54 × 1.27 cm PVC-coated 14 gauge wire mesh panels, and funneled entrances 7 – 7.5 cm wide. Vertical bar spacing was designed to preclude passage of fish measuring about 220 mm total length or longer, based on measurements of head width over a range of fish lengths. The fish weir was installed at a time thought to be early enough to capture the earliest spawning immigrants, and was intended to be held in place throughout the spawning run.

Every migrating sucker entering the trap was identified to putative species or hybrid mix using a morphological characteristics matrix and accompanying photographs assembled by staff from Colorado State University's Larval Fish Laboratory, the Upper Colorado River Endangered Fish Recovery Program, and CPW (unpublished data). Those deemed to be pure Flannelmouth Sucker or Bluehead Sucker were released upstream after work-up. Those deemed to be hybrids or pure non-native White Sucker or Longnose Sucker were released downstream of the trap if they were PIT tagged (either historically or on the present occasion), but most often removed from the population if not PIT tagged. In 2016, we randomly selected putative pure native suckers for genetic analysis in order to determine the accuracy of identification and the level of potential indiscernible non-native sucker genetic influence due to introgression, because the genetic purity of putative pure native sucker individuals affects the purity of larval drift from the tributary.

Larval fish produced in the spawning runs in both tributaries were collected with a combination of drift nets and hobby aquarium hand nets. Larval fish were preserved in 95% non-denatured ethanol and shipped to the Museum of Southwestern Biology, Fishes, and the University of New Mexico (UNM) for curation and genetic analysis. Beginning in 2016, larval fish were identified to genus level and shipments of fish to UNM were limited to purported *Catostomus* to reduce the incidence of *Gila* in the collections. Our goal was to provide 120-150 specimens from each study tributary for genetic analyses each year.

Genetic analyses used six microsatellite DNA markers to evaluate spatial (between streams) and temporal (within streams, among years) variation in the genetic contribution of four sucker species to larval drift in the two streams (Carson et al. 2016). Genomic DNA was isolated using the E.Z.N.A.® Tissue DNA Kit (Omega-biotek corp.) according to the manufacturer's instructions. Species contribution diagnoses were based on microsatellite data from reference samples of Flannelmouth Sucker (N=25), Bluehead Sucker (N=25), White Sucker (N = 25), Longnose Sucker (N

= 25), and Mountain Sucker (N = 12), provided by Pisces Molecular, and Razorback Sucker (N = 25), provided by Thomas E. Dowling (UNM). The same methods were used to assess purity of the randomly collected putative pure native suckers sampled at the weir in 2016.

Short term PIT tag retention – In 2016 we took advantage of the stream trapping and PIT tagging to examine short-term retention of PIT tags in native suckers over the spawning season. An evaluation of tag retention is particularly important in light of the future goal of estimating survival in native sucker populations. All fish receiving a newly implanted PIT tag as they ascended Cottonwood Creek were also given a second mark consisting of a 6.35-mm hole punch in the dorsal lobe of the caudal fin. After exhausting the supply of tags designated for Cottonwood Creek in 2016, we applied hole punches to the ventral lobe of the caudal fin to differentiate these fish from PIT-tagged fish. Fish ascending Cottonwood Creek that carried PIT tags implanted in previous years were given no hole punch marks since they were already identifiable as having passed the weir.

Results and Discussion

2014 – In 2014 both streams were repeatedly electrofished and were found to host spawning suckers. Cottonwood Creek was sampled on May 5, 6, and 19. Potter Creek was sampled on April 9 (no spawning fish present) and on May 2, 12, and 19. Of those occasions, the most fish in spawning condition were found on May 5 and 6 in Cottonwood Creek and on May 12 in Potter Creek. We tagged 397 suckers in Potter Creek, and 296 in Cottonwood Creek (Table 12).

Table 12. PIT tags implanted in several sucker species and Roundtail Chub captured in streams of the Gunnison River basin since 2014 (with additional tags from 2005 in the Gunnison River implanted by former CDOW researcher Rick Anderson).

Stream	Year	BHS	FMS	LGS	WHS	Hybrids	RTC
Big Dominguez Ck	2015	7					1
Cottonwood Ck	2014	63	175		49	9	42
	2015	570	4	1		19	77
	2016	2249	399		18	227	2
Escalante Ck	2014	364	123	1	12	96	43
	2015	123	50		11	25	88
	2016	201	71		9	58	71
Gunnison River	2005	540	286				136
	2014	100	66			47	13
	2015	462	214	2	87	111	155
Potter Ck	2014	211	169		1	16	5
	2015	87	2			1	14
	2016	109	10			1	2
Roubideau Ck	2014	406	90			17	50
	2015	356	27			5	20
	2016	2	1				10
Total		5850	1687	4	187	628	729

Larval fish collections resulted in successful amplification of microsatellite markers for 157 specimens from Potter Creek and 79 specimens from Cottonwood Creek. Admixture analyses revealed that the tributaries differed greatly in the genetic identity of the tested larvae (Figure 13). Potter Creek larvae were predominated by pure native suckers and hybrids thereof, with very little White Sucker representation. Cottonwood Creek larvae were predominated by White

Sucker and Flannelmouth Sucker alleles, and about 20% of the larvae samples were hybrids between these two species. One caution pertaining to these results is that many of the Cottonwood Creek larvae were collected on a single occasion and in a single location. Considering the larval drifting life history of these fishes, self-mixing should alleviate concerns over sampling a sibling group, but it remains a possibility for these samples.

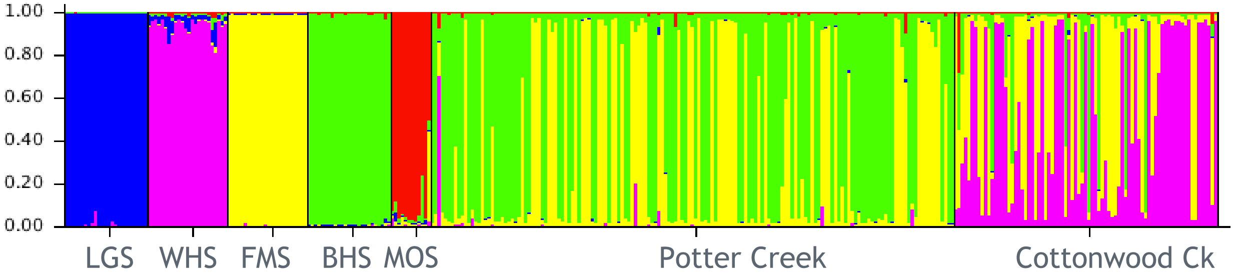

Figure 13. Structure analysis of admixture for larval samples collected from Potter (n=157) and Cottonwood (n=79) creeks in 2014, and reference samples for five species of sucker tested for in the analysis. Colors represent each species' genetic representation in a fish specimen, and each column of the chart displays the results for a single fish. LGS = Longnose Sucker *Catastomus catastomus*, WHS = White Sucker *C. commersonii*, FMS = Flannelmouth Sucker *C. latipinnis*, BHS = Bluehead Sucker *C. discobolus*, and MOS = Mountain Sucker *C. platyrhynchus*.

Both study streams were sampled for drifting sucker larvae in 2014; Cottonwood Creek contained large numbers of non-native and hybrid sucker larvae resulting in a situation where we may anticipate creating an effect using spawner removal. In contrast, the higher elevation Potter Creek contained a higher proportion of native sucker larvae.

2015 – The poor snowpack of 2015 affected this study greatly. Cottonwood Creek was still dry at its mouth in early April, and the only snow telemetry (snotel) site informing runoff from the Uncompahgre Plateau was at that time reporting near zero snow water equivalent at the site (Figure 19). Consequently we deployed the trap in Roubideau Creek on April 20, 2015, 2.0 Km downstream of the Potter Creek confluence. In late April, the rains commenced that later gave rise to the moniker "miracle May" with respect to the effects on Colorado's runoff experience that year. These heavy rains necessitated the removal of the weir on May 6. It was deployed in Cottonwood Creek on May 11 and removed on May 22. Officials at the adjacent Delta Correctional Center indicated that Cottonwood Creek began to flow at the mouth on May 6, the same date the weir was rendered nonfunctional in Roubideau Creek. This resulted in missing the first five to six days of flow in Cottonwood Creek, and in fact the majority of the fish captured from Cottonwood Creek in 2015 were exiting, not entering the stream. Emigrating fish were also predominated by Bluehead Sucker and Roundtail Chub. Flannelmouth Sucker, which likely spawn at the coolest temperatures and therefore earliest, were poorly represented in Cottonwood Creek during 2015, perhaps indicating that they had already accomplished spawning activities elsewhere in the Roubideau drainage.

A second factor impacting this study in 2015 and 2016 was an irrigation diversion in Roubideau Creek 6.8 miles downstream of Potter Creek that was rebuilt prior to spring 2015 runoff. Notably, the electrofishing catch rate in Potter Creek plummeted from 3.0 fish/minute in 2014 to 0.44 fish/minute in 2015, and the proportion of Flannelmouth Sucker was greatly diminished also, dropping from 41.9% of the catch in 2014 to 1.3% in 2015. Only later did we discover the rebuilt diversion. The new diversion was built with 27 interlocking concrete barrier blocks, resulting in a very formidable fish passage obstacle. The diminished catch rates during the sucker spawn in Potter Creek suggest strongly that fish passage in general was inhibited. Further, that the passage of Flannelmouth Sucker was particularly strongly inhibited.

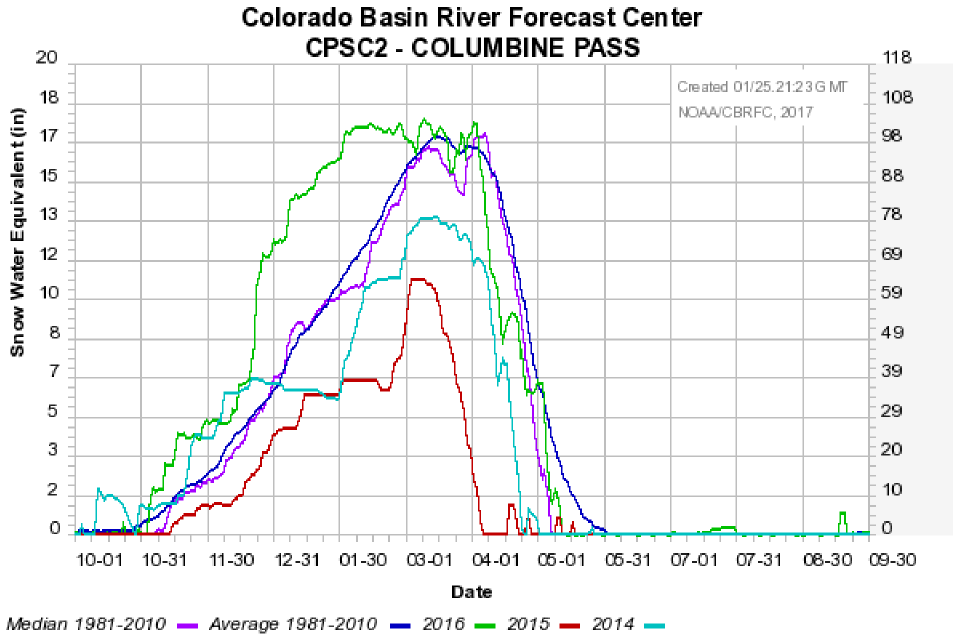

Figure 14. Columbine Pass (Uncompahgre Plateau) snow telemetry data chart showing long term median and average snowpack along with the individual water years 2014 – 2016. Retrieved from https://www.cbrfc.noaa.gov/lmap/lmap.php?interface=snow, accessed 01/25/2017.

The combined trapping and electrofishing efforts resulted in the PIT tagging of 594 suckers in Cottonwood Creek, 388 suckers in Roubideau Creek, and 90 suckers in Potter Creek (compare to 397 in Potter Creek in 2014). Species composition was dramatically different in both tributaries compared to 2014. Flannelmouth Suckers were nearly absent from both tributaries, a circumstance likely explained by the diversion in Roubideau Creek and its effect on Potter Creek access, and by the lateness of the runoff with respect to Cottonwood Creek access. Cottonwood Creek was dominated by Bluehead Suckers in 2015, which usually spawn a little later than Flannelmouth Sucker. White Sucker specimens were not encountered in Cottonwood Creek in 2015, whereas they were fairly common in 2014.

These changes in the spawning fish population species composition were reflected in the genetics of the larvae collected. A total of 124 larvae from Cottonwood Creek and 84 larvae from Potter Creek were identified as catostomids based on microsatellite genetic analyses. Potter Creek larvae were exclusively Bluehead Sucker. Cottonwood Creek larvae were dominated by Bluehead Sucker (n = 95, 76.6%) but also included Flannelmouth Sucker (n = 7, 5.6%), White Sucker (n = 12, 9.7%), and hybrids (n = 10, 8.1%). Hybrids were primarily Bluehead x White Sucker, but included one Flannelmouth x White Sucker hybrid.

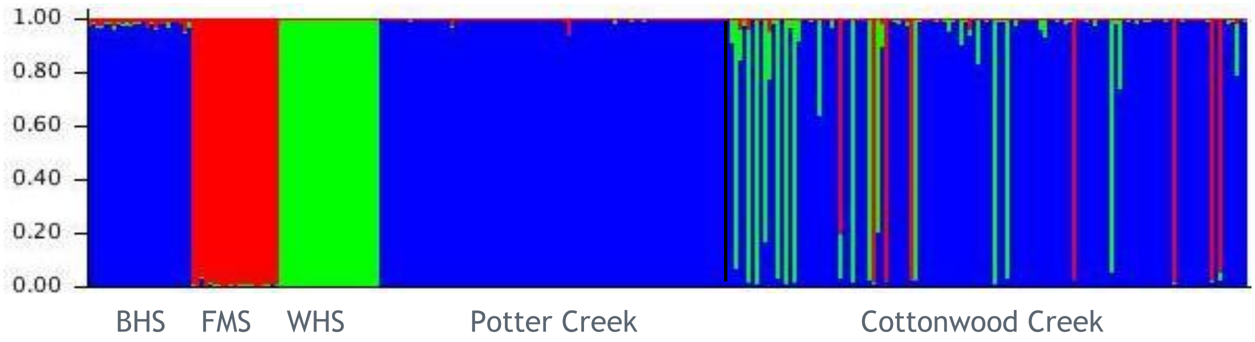

Figure 15. Structure analysis of admixture for larval samples collected from Potter (n=84) and Cottonwood (n=124) creeks in 2015, and reference samples for three species of sucker tested for in the analysis. Colors represent each species' genetic representation in a fish specimen, and each column of the chart displays the results for a single fish. WHS = White Sucker *C. commersonii*, FMS = Flannelmouth Sucker *C. latipinnis*, and BHS = Bluehead Sucker *C. discobolus*.

2016 – The snowpack in 2016 was much higher than 2015 and produced ample runoff in Cottonwood Creek (Figure 14). Prior to the installation of the weir and trap boxes, a submersible PIT tag antenna was placed in Cottonwood Creek between the weir site and the mouth of the stream to detect potential early arrival of tagged fish. None were detected before weir placement.

The weir and traps were installed in Cottonwood Creek on April 5 in low, clear water. Migrating suckers arrived at the weir on April 8 with increasing discharge (and turbidity). The weir was in place until May 6, but there were a number of occasions when debris load compelled the removal of picket rods from parts of the weir resulting in the loss of control over hybrid and non-native sucker immigration. This, of course, defeated the primary objective of excluding all such fish from participating in the spawning run. The weir was re-deployed in Cottonwood Creek from May 23 to 25, during which time 4,433 emigrating suckers were captured. Included in this number were both White Suckers and hybrid suckers – 212 fish that had not been previously handled and 42 fish that had been encountered attempting the upstream migration while the weir was in place. The latter group had been tagged and released back into Roubideau Creek downstream of the Cottonwood confluence, but subsequently returned to Cottonwood Creek.

Fish-trapping and electrofishing efforts in 2016 resulted in the PIT tagging of 2,893 suckers in Cottonwood Creek and 120 suckers in Potter Creek. The numbers of suckers ascending Cottonwood Creek were much higher than anticipated based upon the 2015 experience, and in fact 2,660 native suckers were passed upstream without having a PIT tag implanted, but rather a ventral lobe caudal fin batch mark. On the outmigration from May 23 -25, 3,046 unmarked suckers were handled in addition to recaptured fish with both dorsal and ventral caudal punches. Therefore about 8,599 individual suckers were handled during the trapping operation, and many more than that were in the stream as evidenced by the numbers of fish we were unable to handle during outmigration. Tagged suckers continued to be detected on the submersible antenna for several days following the removal of the weir and downstream trap.

In comparison to 2015, fewer Roundtail Chub were handled at the weir (Table 13). Only three Roundtail Chub were caught on the upstream migration, but 92 were captured during the downstream migration, suggesting that this species commenced upstream migration at a later date than the sucker species, after the weir had to be removed.

Catch rates and overall numbers of fish captured in Potter Creek remained substantially reduced from 2014, with the rebuilt diversion in Roubideau Creek still in place. The CPUE for suckers sampled in Potter Creek totaled over five occasions from May 3 to June 1, 2016 was 0.51, compared to 0.44 in 2015.

We refined our larval selection procedures in 2016 by learning to distinguish *Catostomus* larvae from *Gila* larvae, and submitted 150 specimens from both Cottonwood and Potter creeks to collaborators at UNM for genetic analysis. As of this writing, results on the 2016 larvae are unavailable, but it is anticipated that a higher proportion of submitted larvae will amplify *Catostomus* microsatellite markers.

A significant development after the 2016 season was the total removal of the diversion that hindered access to Potter Creek in 2015-16. Removal was completed February 28, 2017, more than two weeks prior to the earliest PIT tag detections on the Roubideau PIA in 2015 and 2016. Therefore, these fish populations should have full access to Potter Creek and other upper tributaries in the Roubideau drainage.

Table 13. Numbers (proportion) of Catostomid suckers and Roundtail Chub captured in trapping operations in Roubideau and Cottonwood creeks, 2015 – 2016. This table includes all fish encounters; many fish were captured more than once.

Stream	Year	BHS	FMS	LGS	WHS	Hybrids	RTC
Roubideau	2015	347 (0.90)	14 (0.04)	0	1 (<0.01)	9 (0.02)	13 (0.03)
Cottonwood	2015	648 (0.61)	5 (<0.01)	2 (<0.01)	11 (0.01)	77 (0.07)	313 (0.30)
Cottonwood	2016	7689 (0.74)	1794 (0.17)	2 (<0.01)	166 (0.02)	639 (0.06)	94 (0.01)

Short term PIT tag retention – The evaluation of short term PIT tag retention in 2016 proved to be a success. We PIT tagged and dorsal caudal-punched a total of 2,249 Bluehead and 396 Flannelmouth Suckers. Bluehead Suckers ranged from 204 to 505 mm (mean = 360.1 mm; SE = 0.62) and Flannelmouth Suckers from 182 to 535 mm (mean = 454.3 mm; SE = 1.64). We recaptured 730 individual dorsal-caudal-punched fish, a mark indicating the fish should have a tag. Only one Bluehead Sucker had lost a PIT tag resulting in an overall retention rate of 99.9% (99.9% and 100% for Bluehead and Flannelmouth Suckers, respectively). An additional 245 White Suckers and hybrid suckers were PIT tagged and dorsal caudal-punched. Of these, 153 were recaptured, and we documented a single lost tag resulting in an estimated retention rate among these suckers of 99.3%.

Of the Bluehead and Flannelmouth Suckers we marked, 3.0% and 8.6%, respectively, were confirmed females (expressing eggs), and 40.7% and 34.6%, respectively, were suspected females (expressing no eggs, but exhibiting no spawning tubercles nor expressing milt). No confirmed or suspected females lost PIT tags. The only tag loss occurred in a confirmed male Bluehead Sucker. Among the white and hybrid tagged suckers, 71.8% of fish were confirmed or suspected females. One suspect female hybrid sucker was recaptured without a PIT tag. Together, these rates of retention were achieved over an average of 36 days at large, and through a spawning event, suggesting strongly that suckers are not prone to expelling tags during spawning activities even when they are implanted intraperitoneally posterior to the pelvic girdle.

A publication more fully describing the study of short-term PIT tag retention in Bluehead and

Flannelmouth Suckers has been published and is available online and open-access at http://www.tandfonline.com/doi/full/10.1080/02755947.2017.1303008.

Conclusions and Recommendations

Having completed fish weir operations over two spawning seasons, it is apparent that the primary challenge encountered in this study is maintaining the integrity of the picket weir and traps during spates of high runoff. Thus far we have not succeeded in fully controlling a spawning run, and the genetic data lag enough that we cannot yet say anything about the level of success achieved in 2016. Nevertheless, there are important things to be gleaned from this project so far.

These native suckers are very opportunistic in taking advantage of available spawning habitat. This was demonstrated by the rapid entry of Bluehead Suckers into Cottonwood Creek in 2015 when heavy rains initiated stream flow at the mouth, and apparently by the paucity of Flannelmouth Suckers in that same event. The latter presumably had accomplished spawning in the mainstem of Roubideau Creek or Buttermilk Creek, another tributary accessible below the diversion on Roubideau Creek. Then, in 2016, with ample streamflow, thousands of Bluehead Suckers and hundreds of Flannelmouth Suckers used Cottonwood Creek. It will be interesting to see if those numbers diminish in 2017 when access to points upstream is available once again.

It is important to stress that these large spawning runs in Cottonwood Creek are in a stream that does not flow at the mouth during much of the year. A stream such as this would be likely to receive little attention or consideration under ordinary circumstances from fish managers, yet they may be heavily used for certain aspects of native fish life history. As such, we ought to view such streams through a new lens, recognizing the possibility that even snowmelt ephemeral streams could be very important to the conservation of the three-species fishes.

References

Bestgen, K.R., P. Budy, and W.J. Miller. 2011. Status and trends of Flannelmouth Sucker *Catostomus latipinnis*, Bluehead Sucker *Catostomus discobolus*, and Roundtail Chub *Gila robusta*, in the Dolores River, Colorado, and opportunities for population improvement: Phase II Report. Final report submitted to the Lower Dolores Plan Working Group-Legislative Committee. Larval Fish Laboratory Contribution 166 and Intermountain Center for River Rehabilitation and Restoration 2011(2): 1-55, + appendices.

Bezzerides, N., and K. Bestgen. 2002. Status Review of Roundtail Chub *Gila robusta*, Flannelmouth Sucker *Catostomus latipinnis*, and Bluehead Sucker *Catostomus discobolus* in the Colorado River Basin. Colorado State University Larval Fish Laboratory, Final Report submitted to U.S. Department of Interior Bureau of Reclamation Division of Planning Salt Lake City, Utah. Contribution 118:81.

Brodeur, P., P. Magnan, and M. Legault. 2001. Response of fish communities to different levels of White Sucker (*Catostomus commersoni*) biomanipulation in five temperate lakes. Canadian Journal of Fisheries and Aquatic Sciences 58:1998-2010.

Burnham, K.P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer-Verlag,New York, New York, USA.

Carson, E.W., M.R. Schwemm, M.J. Osborne, and T.F. Turner. 2016. Genetic contribution of native and non-native suckers to larval drift in two streams of the Gunnison River Basin in 2015. Final Report for study year 2015, to Colorado Parks and Wildlife.

Chart, T.E., and E.P. Bergersen. 1992. Impact of mainstream impoundment on the distribution and movements of the resident Flannelmouth Sucker (Catostomidae: *Catostomus latipinnis*) population in the White River, Colorado. Southwestern Naturalist 37(1):9-15.

Collier, M., R.H. Webb, and J.C. Schmidt. 1996. Dams and Rivers: Primer on the Downstream Effects of Dams. U.S. Geological Survey Circular 1126.

Compton, R.I. 2007. Population fragmentation and White Sucker introduction affect populations of Bluehead Suckers, Flannelmouth Suckers, and Roundtail Chubs in a headwater stream system, Wyoming. Master's Thesis, University of Wyoming.

Compton, R.I., W.A. Hubert, F.J. Rahel, M.C. Quist, and M.R. Bower. 2008. Influences of Fragmentation on Three Species of Native Warmwater Fishes in a Colorado River Basin Headwater Stream System, Wyoming. North American Journal of Fisheries Management 28(6):1733-1743.

Falush, D., M. Stephens, and J.K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574-578.

Federal Resister, 2012. Volume 77, number 225, November 21, 2012.

Fraser, G.S. 2015. Movement patterns, reproduction, and potential impacts of climate change on three native fishes in the Upper White River drainage, Colorado. Master's Thesis, Colorado State University, Fort Collins.

Fraser, G.S., D.L. Winkelman, K.R. Bestgen, and K.G. Thompson. *In Press*. Tributary use by imperiled Flannelmouth and Bluehead Suckers in the Upper Colorado River Basin. Transactions of the American Fisheries Society.

Garner, B., E. Gardunio, R. Keith, R. Compton, and C. Amadio. 2010. Conservation efforts for the Three Species in the Green River region of Wyoming. Wyoming Game and Fish Department Administrative Report, Cheyenne.

Hopken, M.W., M.R. Douglas, and M.E. Douglas. 2013. Stream hierarchy defines riverscape genetics of a North American desert fish. Molecular Ecology 22:956-971.

Koch, J.D., and M.C. Quist. 2007. A technique for preparing fin rays and spines for age and growth analysis. North American Journal of Fisheries Management 27:782-784.

MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248-2255.

MacKenzie, D.I., J.D. Nichols, J.A. Royle, K.H. Pollock, L.L. Bailey, and J.E. Hines. 2006. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Academic Press.

Martinez, P.J., T.E. Chart, M.A. Trammell, J.G. Wullschleger, and E.P. Bergersen. 1994. Fish species composition before and after construction of a main stem reservoir on the White River, Colorado. Environmental Biology of Fishes 40(3):227-239.

Matschiner, M., and W. Salzburger. 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics 25:1982-1983.

Osmundson, D.B., R.J. Ryel, V.L. Lamarra, and J. Pitlick. 2002. Flow-sediment-biota relations: implications for river regulation effects on native fish abundance. Ecological Applications 12:1719-1739.

Ptacek, J.A., D.E. Rees, and W.J. Miller. 2005. Bluehead Sucker (*Catostomus discobolus*): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available at: http://www.fs.fed.us/r2/projects/scp/assessments/Blueheadsucker.pdf [Accessed October 19, 2010].

Rawson, D.S., and C.A. Elsey. 1948. Reduction in the Longnose Sucker population of Pyramid Lake, Alberta, in an attempt to improve angling. Transactions of the American Fisheries Society 78:13-31.

Quist, M.C., Z.J. Jackson, M.R. Bower, and W.A. Hubert. 2007. Precision of hard structures used to estimate age of riverine catastomids and cyprinids in the upper Colorado River Basin. North American Journal of Fisheries Management 27:643-649.

Rees, D.E., J.A. Ptacek, R.J. Carr, and W.J. Miller. 2005a. Flannelmouth Sucker (*Catostomus latipinnis*): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available at: https://www.fs.usda.gov/detail/r2/landmanagement/?cid=stelprdb5208300 [Accessed March 28, 2016].

Rees, D.E., J.A. Ptacek, and W.J. Miller. 2005b. Roundtail Chub (*Gila robusta robusta*): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available at: https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5206785.pdf [Accessed March 28, 2016].

Shiozawa, D.K., M.D. McKell, B.A. Miller, and R.P. Evans. 2003. Genetic assessment of four native fishes from the Colorado River drainages in western Colorado: the results of DNA analysis. Final Report to Colorado Division of Wildlife.

Stanford, J.A., and J.V. Ward. 2001. Revisiting the serial discontinuity concept. Regulated Rivers: Research and Management 17:303-310.

Theobald, D.M., Stevens, Jr, D.L., White, D., Urquhart, N.S., Olsen, A.R., and Norman, J.B., 2007. Using GIS to generate spatially balanced random survey designs for natural resource applications. Environmental Management 40:134-146.

Thompson, K.G. 2014. Three Species Investigations. Progress Report, Colorado Parks and Wildlife, Fort Collins, CO.

Tranah, G.J., J.J. Agresti, and B. May. 2001. New microsatellite loci for suckers (*Catostomidae*): primer homology in *Catostomas, Chasmistes*, and *Deltistes*. Molecular Ecology Notes 1:55-60.

Utah Division of Wildlife Resources (UDWR). 2006. Range-wide conservation agreement and strategy for Roundtail Chub (*Gila robusta*), Bluehead Sucker (*Catostomus discobolus*), and Flannelmouth Sucker (*Catostomus latipinnis*). Publication Number 06-18. Prepared for Colorado River Fish and Wildlife Council. Utah Department of Natural Resources, Division of Wildlife Resources, Salt Lake City, Utah.

Ward, J.V., and J.A. Stanford. 1983. The serial discontinuity concept of lotic ecosystems. Pages 29-42 *in* T.D. Fontaine and S.M. Bartell (editors). Dynamics of lotic ecosystems. Ann Arbor Science Publishers, Ann Arbor, Michigan.

White, G.C. and K.P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, 120-138.

Zipkin, E.F., C.E. Kraft, E.G. Cooch, and P.J. Sullivan. 2009. When can efforts to control nuisance and invasive species backfire? Ecological Applications 19:1585-1595.

Appendix Table 1. Sampling conducted under the three-species research program from 2011 – 2014. Site Type column is filled only for sites that were part of dual frame sampling scheme, where "Int" = intermittent stream random site, "Per" = perennial stream random site, and "His" = historically sampled site that was randomly selected. In the species columns, "N" indicates the species was not captured and "Y" indicates the species was captured.

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
2011								
Dolores River Basin								
Blue Creek	U.S. of Culvert @ 19 5/10 Rd	9/21/2011		1-Pass	Backpack	N	N	N
Coyote Wash	U.S. of Dolores R confluence	4/21/2011		Spot Check	Dip nets, Seine	N	N	N
Disappointment Creek	James Ranch bridge	9/20/2011		1-Pass	Backpack	N	N	N
Disappointment Creek	Township 42N R16W Section 8	9/20/2011		1-Pass	Backpack	N	N	N
North Fork Mesa Creek	D.S. of Mesa Ck Temp. logger	9/21/2011		1-Pass	Backpack	N	N	N
Tabeguache Creek	300 ft U.S. of San Miguel River	9/21/2011		1-Pass	Backpack	Y	Y	Y
Tabeguache Creek	0.28 mi U.S. of Bridge	9/21/2011		1-Pass	Backpack	N	Y	Y
Tabeguache Creek	0.31 mi U.S. of Bridge	9/21/2011		1-Pass	Backpack	N	Y	Y
West Creek	Adjacent to West Creek Day Use Area	9/22/2011		1-Pass	Backpack	N	N	N
Gunnison River Basin								
Dry Creek	0.19 mi U.S. of Cushman Ck	9/16/2011		Spot shock	Backpack	N	N	Y
Dry Fork Escalante Creek	0 .77mi U.S. of Escalante Ck Rd ford	9/15/2011		Net Set	Net	Y	Y	N
Escalante Creek	At Walker Cabin, Escalante SWA	9/15/2011		1-Pass	Backpack	N	N	Y
Escalante Creek	At Smith Cabin, Escalante SWA	9/15/2011		1-Pass	Backpack	Y	Y	Y
Escalante Creek	2.77 mi U.S. of turn for Pothole parking lot	9/15/2011		1-Pass	Backpack	N	N	N
Escalante Creek	0.17 mi D.S. of wash N of "Potholes" parking lot	9/26/2011		1-Pass	Backpack	Y	Y	Y
Escalante Creek	At Walker Cabin, Escalante SWA	9/27/2011		2-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	D.S. of McCarthy ditch diversion dam	10/3/2011		1-Pass	Bank Shocker	Y	Y	Y
Muddy Creek	1.2 mi U.S. of Dyke Ck confluence	7/26/2011		1-Pass	Backpack	N	Y	N
Potter Creek	Station GU0820 above Roubideau confl.	9/14/2011		1-Pass	Backpack	Y	Y	N
Potter Creek	430 ft D.S. of Monitor Ck confluence	9/14/2011		1-Pass	Backpack	Y	Y	N
West Muddy Creek	0.12mi D.S. of 265 Rd crossing	7/26/2011		1-Pass	Backpack	N	Y	N
West Muddy Creek	U.S. of 704 Rd bridge	7/26/2011		1-Pass	Backpack	N	Y	N
White River Basin								
Coal Creek	confluence with White River	5/3/2011		1-Pass	Backpack	Y	N	N
Coal Creek	1.47mi U.S. of CR 34-CR 15 intersection	5/3/2011		1-Pass	Backpack	N	N	N
Coal Creek	Between CR 8 and confluence with White River	5/26/2011		1-Pass	Bank Shocker, Trammel net	Y	Y	N

Coal Creek	U.S. of CR 8	5/27/2011		1-Pass	Bank shocker, Trammel net	Y	Y	N

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Coal Creek	0.57 mi D.S. of CR 8 crossing	7/7/2011		Net Set	Trap net	N	N	N
Coal Creek	0.14 mi U.S. of CR 8 crossing	7/7/2011		Net Set	Trap net	Y	Y	N
Coal Creek	0.11 mi U.S. of CR 8 crossing	7/7/2011		Net Set	Trap net	N	N	N
Coal Creek	0.57 mi D.S. of CR 8 crossing	7/8/2011		Net Set	Trap net	Y	Y	N
Coal Creek	0.14 mi U.S. of CR 8 crossing	7/8/2011		Net Set	Trap net	Y	Y	N
Coal Creek	0.11 mi U.S. of CR 8 crossing	7/8/2011		Net Set	Trap net	Y	Y	N
Coal Creek	0.42 mi U.S. of CR 8 crossing	7/13/2011		Net Set	Trap net	Y	Y	N
Coal Creek	412 ft U.S. of CR 8 crossing	7/13/2011		Net Set	Trap net	N	N	N
Coal Creek	0.41 mi U.S. of CR 8 crossing	7/13/2011		Net Set	Trap net	N	N	N
Coal Creek	65 ft U.S. of Little Beaver Ck confluence	7/13/2011		Net Set	Trap net	N	N	N
Coal Creek	0.51 mi U.S. of confluence with White River	7/14/2011		Net Set	Trap net	N	N	N
Coal Creek	0.42 mi U.S. of CR 8 crossing	7/14/2011		Net Set	Trap Net	N	Y	N
Coal Creek	0.12 mi U.S. of confluence with White River	7/14/2011		Net Set	Trap net	Y	N	N
Coal Creek	0.51 mi U.S. of confluence with White River	7/15/2011		Net Set	Trap net	Y	N	N
Coal Creek	147 ft U.S. of CR 6 crossing	7/15/2011		Net Set	Trap net	N	N	N
Coal Creek	0.47 mi U.S. of CR 8 crossing	7/15/2011		Net Set	Trap net	Y	Y	N
Coal Creek	0.51 mi U.S. of confluence with White River	7/19/2011		Net Set	Trap net	N	N	N
Coal Creek	0.12 mi U.S. of confluence with White River	7/19/2011		Net Set	Trap net	Y	N	N
Coal Creek	0.17 mi U.S. of confluence with White River	7/19/2011		Net Set	Trap net	N	N	N
Coal Creek	0.12 mi U.S. of confluence with White River	7/20/2011		Net Set	Trap net	Y	N	N
Coal Creek	0.42 mi U.S. of CR 8 crossing	7/20/2011		Net Set	Trap net	N	Y	N
Coal Creek	0.42 mi U.S. of CR 8 crossing	7/21/2011		Net Set	Trap net	N	Y	N
Coal Creek	0.17 mi U.S. of confluence with White River	7/21/2011		Net Set	Trap net	N	N	N
Coal Creek	333 ft U.S. of confluence with White River	8/2/2011		1-Pass	Bank Shocker	Y	N	N
Coal Creek	176 ft U.S. of CR 8 crossing	8/2/2011		1-Pass	Bank Shocker	N	N	N
Coal Creek	0.54 mi U.S. of CR 8 crossing	8/2/2011		1-Pass	Bank Shocker	N	N	N
Crooked Wash	0.56 mi U.S. of White River confluence	5/6/2011		1-Pass	Backpack	Y	N	N
Crooked Wash	0.24 mi D.S. of old BLM Rd 1728 crossing	5/6/2011		1-Pass	Backpack	Y	N	N
Curtis Creek	0.25 mi U.S. of White River confluence	6/24/2011		1-Pass	Backpack	N	N	N
Flag Creek	At confluence with White River	4/13/2011		1-Pass	Backpack	Y	Y	N
Flag Creek	237 ft D.S. of CR 13 crossing	4/13/2011		1-Pass	Backpack	N	N	N
Flag Creek	confluence with White River	5/4/2011		1-Pass	Backpack	Y	N	N
Flag Creek	237 ft D.S. of CR 13 crossing	5/4/2011		1-Pass	Backpack	N	N	N
Flag Creek	Below private bridge U.S. of CR 13	5/4/2011		1-Pass	Backpack	N	N	N
Flag Creek	Above private bridge U.S. of CR 13	5/4/2011		1-Pass	Backpack	N	N	N

Flag Creek	0.4 mi U.S. of CR 36 - CR 13 intersection	5/5/2011		1-Pass	Backpack	N	N	N
Miller Creek	390 ft U.S. of confluence with White River	5/2/2011		1-Pass	Backpack	N	N	N
Piceance Creek	Gauging Station D.S. of 1st CR 5 Bridge	4/14/2011		1-Pass	Backpack	Y	N	N
Piceance Creek	2.14 mi U.S. of 1st CR 5 Bridge	4/15/2011		1-Pass	Backpack	Y	N	N

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Piceance Creek	Pump station 0.4 miles U.S. CR 20	4/15/2011		1-Pass	Backpack	N	N	N
Piceance Creek	Below Gauge Station D.S. of 1st CR 5 Bridge	6/8/2011		1-Pass	Backpack	Y	N	N
Piceance Creek	175 ft U.S. of 1st CR 5 Bridge	6/8/2011		1-Pass	Backpack	Y	N	N
Piceance Creek	2.14 mi U.S. of 1st CR 5 Bridge	6/8/2011		1-Pass	Backpack	N	N	N
Piceance Creek	Pump station 0.4 miles U.S. CR 20	6/23/2011		1-Pass	Backpack	N	N	N
Piceance Creek	Piceance SWA, Square S Campground	6/23/2011		1-Pass	Backpack	Y	N	N
White River	5 mi U.S. of Kenney Res.	7/12/2011		1-Pass	Boat Shocker	Y	Y	N
White River	5 mi U.S. of Kenney Res.	8/3/2011		3-Pass	Boat Shocker	Y	Y	Y
Yellow Creek	confluence with White River	5/5/2011		1-Pass	Backpack	Y	N	Y
Yellow Creek	0.23 mi D.S. of Hwy 64 crossing	5/5/2011		1-Pass	Backpack	Y	N	N

2012

Colorado River Basin

Crystal River	0.9 mi U.S. Hays Creek confluence	9/20/2012	Per	2-Pass	Bank Shocker	N	N	N
Dry Hollow Creek	13S 267609 4363481	5/31/2012	Int	2-Pass	Backpack	N	N	N
Eagle River	BLM boat launch 4.9mi U.S. of Colo River	9/19/2012	Per	2-Pass	Boat Shocker	N	N	N
Roaring Fork River	Below 3-Mile Ck	6/20/2012	Per	1-Pass	Boat Shocker	N	Y	N

Dolores River Basin

Big Bear Creek	1.0 mi up Rd 60M	6/18/2012	Per	2-Pass	Backpack	N	N	N
Dolores River	Boxelder Rec. Site Reach	8/28/2012		Seining	Seine	N	Y	Y
Dolores River	James Ranch to Slick Rock	8/29/2012		Seining	Seine	N	N	Y
Dolores River	Big Gypsum Reach	8/30/2012		Seining	Seine	Y	N	Y
San Miguel River	At Uravan	5/24/2012		1-Pass	Boat Shocker	Y	Y	Y

Green River Basin

Vermillion Creek	1.4 mi D.S. of Hwy 318 crossing	6/28/2012	Per	2-Pass	Backpack	N	N	N

Gunnison River Basin

Alfalfa Run	0.5 mi U.S. of Main St, Austin CO	9/4/2012	Per	1-Pass	Backpack	N	N	N
Burro Creek	1.35 mi U.S. of Cow Ck	9/28/2012	Per	2-Pass	Backpack	N	N	N
Coal Creek	0.2 mi U.S. of Cascade Ck	8/3/2012	Per	2-Pass	Bank Shocker	N	N	N
East Fork Spring Creek	1.82 mi U.S. of the East Fk & Middle Fk confluence	4/17/2012	Per	2-Pass	Backpack/Seine	N	N	N
Escalante Creek	D.S. of McCarthy ditch diversion dam	5/4/2012		1-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	D.S. of McCarthy ditch diversion dam	5/23/2012		1-Pass	Backpack	Y	Y	Y

Escalante Creek	2.83 mi SE from BLM Boundary	6/15/2012	Per	2-Pass	Backpack	N	N	N
Escalante Creek	Smith Cabin, Escalante SWA	7/12/2012		Seining	Seine	N	N	Y
Escalante Creek	Walker Cabin, Escalante SWA	7/12/2012		Seining	Seine	Y	N	Y
Escalante Creek	Smith Cabin, Escalante SWA	9/24/2012		2-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	Walker Cabin, Escalante SWA	9/24/2012		2-Pass	Bank Shocker	Y	Y	Y

Stream	**Station**	**Date**	**Site Type**	**Method**	**Sample Gear**	**FMS**	**BHS**	**RTC**
Gill Creek	0.8 mi U.S. of confluence with Kannah Ck	8/1/2012	Per	2-Pass	Backpack	N	N	N
Kannah Creek	860 m above G S Road	4/18/2012	Int	2-Pass	Backpack/Seine	N	Y	N
La Fair Creek	1.34 mi S on Divide Rd from T S Rd Turnoff	5/10/2012	Per	2-Pass	Backpack	N	N	N
Leroux Creek	At 3100 Rd crossing	10/4/2012		2-Pass	Backpack	N	N	N
Muddy Creek	Just Above confluence of Dugout Ck	7/17/2012	Per	2-Pass	Backpack	Y	Y	N
Potter Creek	Station GU0820 above Roubideau confl.	7/12/2012		Seining	Seine	Y	N	N
Roubideau Creek	Near confluence of Cottonwood Ck	7/12/2012		Seining	Seine	Y	Y	Y
Roubideau Creek	1.6 mi D.S. of confluence of Potter Ck	7/12/2012		Seining	Seine	Y	N	Y
Roubideau Creek	Escalante SWA, Youth Access parcel	7/12/2012		Seining	Seine	Y	N	Y
San Juan River Basin								
Cherry Creek	4.3 mi U.S. of CR 100 crossing	7/23/2012	Per	2-Pass	Backpack/Seine	N	Y	N
Mancos River	Trail Canyon	9/25/2012		2-Pass	Bank Shocker	N	N	N
Mid Fork Piedra River	1.1 mi above Confl. with East Fk Piedra River	7/26/2012	Per	2-Pass	Backpack	N	N	N
Piedra River	At Hwy 160 Bridge	9/26/2012	Per	2-Pass	Bank Shocker	N	N	N
Spring Creek	1.1 mi NW of NM state line on Hwy 4	7/24/2012	Per	2-Pass	Backpack	N	N	N
White River Basin								
Big Beaver Creek	0.85 mi U.S. of Big Beaver Basin Trl crossing	10/3/2012	Per	2-Pass	Backpack	N	N	N
Coal Creek	0.45 mi U.S. of White River	5/8/2012		1-Pass	Bank Shocker	N	Y	N
Douglas Creek	Below Hwy 64	4/7/2012	Int	2-Pass	Backpack/Seine	Y	N	N
Piceance Creek	3.5 mi U.S. From First Culvert	6/26/2012	His	2-Pass	Backpack	Y	N	N
Piceance Creek	Piceance SWA, Square S Campground	6/26/2012		2-Pass	Backpack/Seine	Y	Y	N
Spring Creek	At confluence of East and West Forks	6/25/2012	Per	2-Pass	Backpack/Seine	N	N	N
Yampa River Basin								
Cottonwood Creek	3.2 mi up CR 11 from Hwy 13	5/8/2012	Int	2-Pass	Backpack	N	N	N
Elk River	1.75 mi D.S. Deep Ck confluence	9/13/2012	Per	2-Pass	Backpack	N	N	N
Little Snake River	0.5 mi U.S. of Red Wash confluence	9/7/2012	Per	2-Pass	Backpack/Seine	Y	Y	Y
Milk Creek	BLM D.S. Hwy 13	9/6/2012	Per	2-Pass	Backpack/Seine	Y	N	N
Mill Creek	0.3 mi N of Cottonwood Ck State Wildlife Land	6/27/2012	Per	2-Pass	Backpack	N	N	N
Slater Creek	0.5 mi inside Forest Boundary	5/9/2012	Per	2-Pass	Backpack	N	N	N
Trout Creek	0.5 mi U.S. Yampa River confluence	6/27/2012	Per	2-Pass	Backpack/Seine	N	N	N

2013

Colorado River Basin

Bull Creek	Culvert off of KE Rd	5/30/2013	Int	2-Pass	Backpack	N	N	N
Eagle River	1.02 mi U.S. of Hwy 131 crossing	7/26/2013	Per	2-Pass	Bank Shocker	N	N	N
East Salt Creek	0.41 mi U.S. of 9 ¼ Road crossing	9/3/2013	Per	1-Pass	Backpack	N	N	N
Stream	**Station**	**Date**	**Site Type**	**Method**	**Sample Gear**	**FMS**	**BHS**	**RTC**
Salt Creek	1 mi U.S. of confluence with Plateau Ck	9/6/2013	Per	2-Pass	Backpack	N	N	N
Un-Named Creek	0.15 mi U.S. of Diamond Creek	6/19/2013	Per	2-Pass	Backpack	N	N	N
West Divide Creek	Bridge 1 mi S. of Maxfield Rd on CR 311	6/3/2013	Per	2-Pass	Bank Shocker	N	Y	N
		Dolores River Basin						
McElmo Creek	3.91 mi D.S. of Stinking Springs Canyon	5/14/2013	Per	2-Pass	Backpack	N	N	N
Tabeguache Creek	0.45 mi U.S. from Hwy 141	5/29/2013		1-Pass	Bank Shocker	N	Y	Y
Tabeguache Creek	300 ft D.S. of 1st stream ford, U19 Rd	5/29/2013		1-Pass	Bank Shocker	N	Y	Y
West Creek	2.06 mi U.S. from Dolores River confluence	7/12/2013	Per	2-Pass	Backpack	N	Y	N
West Fork Dolores River	2.27 mi U.S. of Fish Ck confluence	7/9/2013	Per	2-Pass	Backpack	N	Y	N
		Gunnison River Basin						
Cottonwood Creek	10.5 mi on 25 Mesa Rd from Hwy 348	5/16/2013	Per	2-Pass	Backpack	N	N	N
Dry Creek	Upstream of Holly Road	5/15/2013		2-Pass	Bank Shocker	Y	N	Y
Dry Creek	Vernal pools Cushman Ck to Piney Ck	8/29/2013		1-Pass	Backpack	N	N	Y
Dry Creek	0.2 mi U.S of Piney Ck confluence	8/29/2013	Per	2-Pass	Backpack	N	N	N
Escalante Creek	D.S. of McCarthy ditch diversion dam	5/6/2013		1-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	U.S. of Smith Cabin, Escalante SWA	6/5/2013	Per	2-Pass	Bank Shocker	Y	Y	Y
Leroux Creek	0.21 mi D.S. of East,West Leroux confluence	7/2/2013	Per	2-Pass	Backpack	N	N	N
Loutzenhiser Arroyo	0.2 mi U.S. of N. River Rd crossing	5/28/2013	Per	2-Pass	Backpack	N	N	N
Peach Valley Creek	1.1 mi D.S. of Peach Valley Rd crossing	8/28/2013	Per	1-Pass	Backpack	N	N	N
Potter Creek	U.S. of confluence with Roubideau Ck	5/7/2013		2-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	U.S. of Potter Creek confluence	5/7/2013		2-Pass	Bank Shocker	Y	Y	Y
Wise Creek	1.2 mi U.S. of Buttermilk Creek	7/2/2013	Per	2-Pass	Backpack	N	N	N
		San Juan River Basin						
Piedra River	At Boat Ramp at Navajo State Park	7/31/13	Per	2-Pass	Bank Shocker	N	Y	N
Rio Blanco River	At confluence with San Juan River	7/30/2013	Per	2-Pass	Bank Shocker	Y	Y	N
Spring Creek	0.58 mi U.S. of Navajo River	8/1/2013	Per	2-Pass	Backpack	N	Y	N
Stollsteimer Creek	Next to Capote Lake	7/11/2013	Per	2-Pass	Backpack	N	N	N
Turkey Creek	3.73 mi U.S. of Hwy 160	8/1/2013	Per	2-Pass	Backpack	N	N	N
Mancos River	Ute Mountain Ute Reservation	10/28/13	Per	2-Pass	Backpack	N	N	N
Mancos River	Ute Mountain Ute Reservation	10/28/13	Per	2-Pass	Backpack	N	N	N

		White River Basin						
Deep Channel Creek	0.88 mi U.S. of Twin Wash	6/20/2013	Int	2-Pass	Backpack	N	N	N
Douglas Creek	0.16 mi U.S. of Philadelphia Ck	5/20/2013	Int	2-Pass	Backpack	N	N	N
Fawn Creek	4.06 mi U.S. of Black Sulphur Ck	6/4/2013	Per	2-Pass	Backpack	N	N	N
Little Beaver Creek	0.30 mi U.S. of Milk Ck	6/17/2013	Int	2-Pass	Backpack	N	N	N
Middle Fork Steward Gulch	On boundary Oil Shale Corp. and BLM land	6/4/2013	Per	2-Pass	Backpack	N	N	N
Piceance Creek	0.25 mi D.S. of Cole Gulch	5/23/2013	Per	2-Pass	Backpack	N	N	N
Piceance Creek	0.56 mi U.S. of CR 24 crossing	7/23/2013	Per	2-Pass	Backpack	Y	Y	N

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
		Yampa River Basin						
Deer Creek	1.59 mi U.S. of Moody Gulch	7/25/2013	Per	2-Pass	Backpack	N	N	N
Elkhead Creek	1.75 mi SW on Trail from CR 80	6/18/2013	Per	2-Pass	Backpack	N	N	N
Fish Creek	0.18 mi D.S. of CR 37 crossing	8/13/2013	Per	2-Pass	Backpack	N	N	N
Foidel Creek	0.52 mi U.S. of CR 27 crossing	8/13/2013	Per	2-Pass	Backpack	N	N	N
Fortification Creek	4.12 mi U.S. of E Victory Way crossing	5/22/2013	Per	2-Pass	Bank Shocker	N	N	N
Fourmile Creek	1.3 mi from Hwy 13 crossing	6/18/2013	Int	2-Pass	Backpack	N	N	N
Little Snake River	5.36 mi U.S. of Hwy 318 crossing	7/24/2013	Per	2-Pass	Backpack	Y	Y	Y
Little Snake River	7.65 mi D.S. of Hwy 318 crossing	7/24/2013	Per	2-Pass	Backpack	Y	Y	Y
Milk Creek	0.6 mi on Rd Across from CR 51	6/17/2013	Per	2-Pass	Backpack	Y	Y	N
Sand Wash	1.43 mi D.S. of Dugout Draw	5/21/2013	Int	2-Pass	Backpack	N	N	N
Trout Creek	0.6 mi U.S. of 2nd CR 179 crossing	6/19/2013	Per	2-Pass	Bank Shocker	N	N	N
Willow Creek #2	1.02 mi U.S. of CR 62 crossing	8/14/2013	Per	2-Pass	Backpack	N	N	N

2014

		Colorado River Basin						
Badger Wash	At confluence with West Salt Creek	11/12/2014	His	2-Pass	Bank Shocker	Y	Y	Y
Big Salt Wash	~76ft U.S. of W I-70 bridge	11/12/2014	His	2-Pass	Bank Shocker	Y	Y	Y
Buzzard Creek	Fairgrounds, 76 ft D.S. of Rodeo Rd	7/7/2014	His	2-Pass	Backpack	N	Y	N
Buzzard Creek	100 ft U.S. of Forest Service Rd 266	7/9/2014	His	2-Pass	Backpack	N	Y	N
Dry Fork Cabin Creek	.54 mi U.S. of Cabin Creek	7/22/2014	His	2-Pass	Backpack	N	Y	N
Dry Owens Creek	188 ft D.S. of P E Rd	5/22/2014	His	2-Pass	Backpack	N	N	N
East Divide Creek	.15 mi U.S. of Road Gulch	7/16/2014	His	2-Pass	Backpack	N	N	N
Garfield Creek	1.13 stream miles D.S. of Baldy Creek	7/28/2014	His	2-Pass	Backpack	N	N	N
Grove Creek	At 58 7/10 Rd crossing	8/19/2014	His	2-Pass	Backpack	N	Y	N
Hightower Creek	430 ft D.S. of 71 4/10 Rd crossing	5/15/2014	His	2-Pass	Backpack	N	N	N
Mack Wash	23 ft U.S. of R Road culvert	11/21/2014	His	2-Pass	Bank Shocker	N	N	N
Persigo Wash	U.S. side of W I-70 bridge	11/13/2014	His	2-Pass	Bank Shocker	Y	N	Y
Plateau Creek	.7 stream mi U.S. of Little Wash confluence	9/9/2014	His	2-Pass	Bank Shocker	Y	Y	Y

Rifle Creek	At Centennial Parkway Bridge	7/25/2014	His	2-Pass	Backpack	N	N	Y
Roan Creek	1.46 stream mi D.S. Carr Creek Confluence	9/8/2014	His	2-Pass	Backpack	N	Y	N
Roan Creek	D.S. side of 2nd St bridge	9/8/2014	His	2-Pass	Bank Shocker	Y	Y	Y
Rock Creek	At Hwy 131 crossing	9/10/2014	His	2-Pass	Backpack	N	N	N
Salt Creek	~1.05 mi from I-70 on RR access road, U.S. side of pedestrian bridge	11/13/2014	His	1-Pass	Bank Shocker	Y	Y	Y
Dolores River Basin								
Disappointment Creek	James Ranch Bridge	4/28/2014		1-Pass	Backpack/Seine	Y	N	Y
Dolores River	Joe Davis Canyon	3/28/2014		2 Nets	Trammel nets	Y	N	Y

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Dolores River	.6 stream miles U.S. of Rd 36 crossing	8/5/2014	His	2-Pass	Bank Shocker	N	N	N
Naturita Creek	.84 Stream miles D.S. of Ii35 Rd crossing	5/30/2014	His	2-Pass	Backpack	N	Y	N
N. Fork Mesa Creek	At ford on P12 Road	4/22/2014		1-Pass	Backpack	N	N	N
San Miguel River	Uravan to Dolores confluence	6/30/2014	His	2-Pass	Boat Shocker	Y	Y	Y
San Miguel River	.23 stream mi D.S. private driveway bridge	8/4/2014	His	2-Pass	Bank Shocker	Y	Y	N
Tabeguache Creek	At confluence with San Miguel River	3/29/2014		1-Pass	Bank Shocker	Y	N	Y
Tabeguache Creek	480 ft. D.S. of bridge	3/29/2014		1-Pass	Bank Shocker	N	N	Y
Tabeguache Creek	300 ft. below first ford	3/29/2014		1-Pass	Bank Shocker	Y	Y	Y
Tabeguache Creek	.20 Rd miles U.S. of v19 Rd Bridge	5/14/2014	His	2-Pass	Bank Shocker	N	Y	N
Tabeguache Creek	.21 miles U.S. of first ford	5/14/2014		1-Pass	Backpack	Y	Y	N
Tabeguache Creek	.44 miles U.S. of first ford	5/14/2014		1-Pass	Backpack	Y	N	Y
Tabeguache Creek	FS trail 500, 1.27 mi from FS Rd 660.1a	7/31/2014	His	2-Pass	Backpack	N	Y	N
Green River Basin								
Vermillion Creek	D.S. of pool below Vermillion Falls	7/29/2014	His	2-Pass	Backpack	N	N	N
Gunnison River Basin								
Cimarron River	400 ft D.S. of Red Bridge Ranch bridge	8/21/2014	His	2-Pass	Bank shocker	N	Y	N
Cottonwood Creek	10 Rd Bridge	5/5/2014		2-Pass	Bank shocker	Y	Y	Y
Cottonwood Creek	.24 mi U.S. of Roubideau Creek	5/6/2014		1-Pass	Backpack	Y	Y	N
Cottonwood Creek	.32 mi U.S. of Roubideau Creek	5/6/2014		1-Pass	Backpack	Y	Y	Y
Cottonwood Creek	.38 mi U.S. of Roubideau Creek	5/6/2014		1-Pass	Backpack	Y	Y	Y
Cottonwood Creek	.43 mi U.S. of Roubideau Creek	5/6/2014		1-Pass	Backpack	Y	Y	Y
Cottonwood Creek	10 Rd Bridge	5/19/2014		1-Pass	Bank shocker	N	Y	Y
Cow Creek	Top of station at Hwy 550 bridge	7/10/2014	His	2-Pass	Backpack	N	N	N
Dallas Creek	0.11 mi D.S. of CR24a road crossing	10/23/2014	His	2-Pass	Bank Shocker	N	N	N
Dry Creek	155 ft U.S. of Hwy 348 crossing	7/18/2014	His	2-Pass	Backpack	Y	Y	Y
East Creek	2.87 road miles from Gunnison River	5/8/2014	His	2-Pass	Backpack	N	N	N
Escalante Creek	Smith Cabin, Escalante SWA	1/24/2014		1-Pass	Backpack	Y	Y	Y
Escalante Creek	Walker Cabin, Escalante SWA	1/24/2014		1-Pass	Backpack	Y	Y	Y

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Escalante Creek	Smith Cabin, Escalante SWA	4/10/2014		1-Pass	Backpack/Seine	Y	Y	Y
Escalante Creek	600 ft U.S. Smith Cabin, Escalante SWA	4/10/2014		1-Pass	Backpack/Seine	Y	Y	Y
Escalante Creek	Below McCarty Ditch diversion (barrier)	5/1/2014		2-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	600 ft U.S. Smith Cabin, Escalante SWA	5/1/2014		1-Pass	Bank Shocker	Y	Y	Y
Escalante Creek	Below McCarty Ditch diversion (barrier)	5/7/2014		2-Pass	Backpack	Y	Y	Y
Escalante Creek	At 23 Rd (Escalante Rim Rd) ford	8/18/2014	His	2-Pass	Backpack	Y	Y	Y
Kannah Creek	860 m above G S Road	8/19/2014	Int	2-Pass	Backpack	N	N	N
Little Cimarron River	D.S. of Pleasant Valley Store bridge	7/8/2014	His	2-Pass	Bank Shocker	N	Y	N
Montrose Arroyo	.19 stream miles U.S. of 12th St	5/9/2014	His	2-Pass	Backpack	N	N	N
Potter Creek	At confluence with Roubideau	4/9/2014		1-Pass	Backpack	N	N	N
Potter Creek	At confluence with Roubideau	5/2/2014		1-Pass	Bank Shocker	Y	Y	Y
Potter Creek	At confluence with Roubideau	5/12/2014		1-Pass	Backpack	Y	Y	Y
Potter Creek	At confluence with Roubideau	5/19/2014		1-Pass	Backpack	Y	Y	Y
Potter Creek	5.5 mi U.S. of Monitor Creek confluence	6/1/2014	His	2-Pass	Backpack	N	Y	N
Roubideau Creek	.32 mi U.S. Sawmill Mesa Road Bridge	4/9/2014		1-Pass	Backpack	Y	Y	Y
Roubideau Creek	4.5 mi U.S. of 25 Mesa Road	4/9/2014		1-Pass	Backpack	Y	Y	Y
Roubideau Creek	Above Potter Creek confluence	4/9/2014		1-Pass	Backpack	Y	N	N
Roubideau Creek	4.32 mi U.S. of 25 Mesa Road	5/2/2014		1-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	350 ft below Potter Ck confluence	5/2/2014		1-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	100 ft below Potter Ck confluence	6/12/2014		1-Pass	Backpack	N	Y	Y
Roubideau Creek	.32 mi U.S. Sawmill Mesa Road Bridge	7/3/2014		2-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	At Cottonwood Creek confluence	7/17/2014		2-Pass	Backpack	Y	Y	Y
Roubideau Creek	.32 mi U.S. Sawmill Mesa Road Bridge	9/4/2014		2-Pass	Bank Shocker	Y	Y	Y
		San Juan River Basin						
Cherry Creek	U.S. side of Hwy 160 culvert	8/5/2014	His	2-Pass	Backpack	N	N	N
E. Fork Cherry Creek	Just above Cherry Ck confluence	8/5/2014		2-Pass	Backpack	N	Y	N
Junction Creek	Above dam ~200ft U.S. of Animas River	9/30/2014	His	2-Pass	Backpack	N	N	N
Lightner Creek	300 ft D.S. of Deep Creek confluence	6/18/2014	His	2-Pass	Backpack	N	N	N
Long Hollow Creek	Top of Station at CR131 culvert	10/1/2014	His	2-Pass	Backpack	N	Y	N
Mancos River #2	2.34 stream mi U.S. of Trail Canyon	10/22/2014	His	2-Pass	Bank Shocker	N	N	Y
Mancos River #2	0.11 mi D.S. of Lewis creek	10/22/2014	His	2-Pass	Bank Shocker	N	N	Y
Mancos River #3	At Beech St Bridge	6/3/2014	His	2-Pass	Backpack	N	Y	N
Piedra River #1	1.57 stream miles U.S. of Stollsteimer Ck.	7/23/2014	His	2-Pass	Backpack	N	N	N
Rio Blanco #1	.12 mi D.S. of Blanco Dam	7/22/2014	His	2-Pass	Backpack	N	Y	N
Rock Creek	267 ft U.S. of hwy 172 crossing	7/23/2014	His	2-Pass	Backpack	N	N	N
Yellowjacket Canyon	190 ft D.S. of Sandstone Canyon confluence	6/20/2014	His	2-Pass	Backpack	N	Y	N
Yellowjacket Canyon	5.5 road miles U.S. of Creek 21 Road	8/6/2014	His	2-Pass	Backpack	N	Y	N

White River Basin								
Miller Creek	.16 stream miles U.S. of White River	7/29/2014	His	2-Pass	Backpack	N	N	N
Piceance Creek	.23 mi U.S. of CR 22 crossing	5/23/2014	His	2-Pass	Backpack	N	N	N
Piceance Creek	.41 road miles U.S> of CO Rd 20	7/14/2014	His	2-Pass	Backpack	N	N	N
Yampa River Basin								
Elk River	.93 road miles U.S. of Mad Ck USFS TR 1100	9/25/2014	His	2-Pass	Bank shocker	N	N	N
Elkhead Creek	U.S. Hwy 40 bridge	6/24/2014	His	2-Pass	Backpack	N	N	N
Little Snake River	.12 mi D.S. of CR 26 crossing	9/22/2014	His	2-Pass	Bank shocker	Y	Y	Y
Milk Creek	580 ft above confluence with Yampa River	7/14/2014	His	2-Pass	Backpack	N	N	N
Milk Creek	1.39 stream miles U.S. of Yampa River	6/25/2014	His	2-Pass	Backpack	N	N	N
Milk Creek	160 ft U.S. of CR 15 bridge	9/26/2014	His	2-Pass	Backpack	N	Y	N
Williams Fork Yampa River	8.7 rd mi on Hwy 317 from Hwy 13	9/24/2014	His	2-Pass	Bank shocker	N	N	N

Appendix Table 2. Sampling conducted under the three-species research program from 2015 - 2016. Site Type column is filled only for sites that were part of dual frame sampling scheme, where “Int” = intermittent stream random site, “Per” = perennial stream random site, and “His” = historically sampled site (not randomly selected in 2015 or 2016). In the species columns, “N” indicates the species was not captured and “Y” indicates the species was captured.

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
2015								
Colorado River Basin								
Buzzard Creek	100 ft U.S. of Forest Service Rd 266	7/28/2015	His	2-Pass	Backpack	Y	Y	N
Owens Creek	~0.6 Stream Mi US of Dry Owens Creek	9/28/2015	His	2-Pass	Backpack	N	Y	N
Dolores River Basin								
Naturita Creek	100 ft U.S. of HWY 141 bridge	8/17/2015	His	2-Pass	Backpack	Y	Y	N
San Miguel River	~0.45 Road Mi on Y11 Rd from HWY 141	4/15/2015		1-Pass	Bank Shocker	Y	Y	N
San Miguel River	~0.6 Road mi on Y11 Rd from HWY 141	4/15/2015		1-Pass	Bank Shocker	Y	Y	N
Tabeguache Creek	At confluence with San Miguel River	4/15/2015	His	1-Pass	Backpack	N	Y	Y
Tabeguache Creek	At confluence with San Miguel River	4/22/2015	His	1-Pass	Backpack	Y	Y	Y
Tabeguache Creek	At confluence with San Miguel River	6/3/2015	His	2-Pass	Backpack	Y	Y	Y
Tabeguache Creek	At confluence with San Miguel River	9/11/2015	His	2-Pass	Backpack	Y	N	N
Tabeguache Creek	300 ft. below first ford	4/22/2015	His	1-Pass	Backpack	N	Y	N
Tabeguache Creek	300 ft. below first ford	6/3/2015	His	2-Pass	Backpack	N	N	N
Tabeguache Creek	.20 Rd miles U.S. of v19 Rd Bridge	4/22/2015	His	1-Pass	Backpack	N	Y	N
Tabeguache Creek	.20 Rd miles U.S. of v19 Rd Bridge	4/23/2015	His	2-Pass	Backpack	Y	Y	N
Tabeguache Creek	.20 Rd miles U.S. of v19 Rd Bridge	9/11/2015	His	2-Pass	Backpack	N	Y	N

Tabeguache Creek	.18 miles U.S. of first ford	4/22/2015		1-Pass	Backpack	N	N	N
Gunnison River Basin								
Big Dominguez Creek	At Confluence with Gunnison River	4/28/2015	His	1-Pass	Backpack	N	Y	Y
Big Dominguez Creek	At Confluence with Gunnison River	8/24/2015	His	2-Pass	Backpack	N	N	Y
Cottonwood Creek	10 Rd Bridge	5/12/2015	His	1-Pass	Backpack	N	Y	Y
Cottonwood Creek	10 Rd Bridge	5/22/2015	His	2-Pass	Backpack	N	Y	Y
Cottonwood Creek	10 Rd Bridge	6/18/2015	His	2-Pass	Backpack	Y	Y	Y
Dry Creek	155 ft U.S. of Hwy 348 crossing	9/28/2015	His	1-Pass	Backpack	N	N	N
Dry Fork Escalante Creek	BLM Boundary to diversion below petroglyphs	4/29/2015		1-Pass	Backpack	N	Y	N
Dry Fork Escalante Creek	Diversion to petroglyphs	4/29/2015		1-Pass	Backpack	N	N	N
Escalante Creek	600 ft U.S. Smith Cabin, Escalante SWA	8/6/2015	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	Walker Cabin, Escalante SWA	8/31/2015	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	Below McCarty Ditch diversion (barrier)	5/6/2015	His	1-Pass	Backpack	Y	Y	Y
Escalante Creek	Below McCarty Ditch diversion (barrier)	5/20/2015	His	1-Pass	Backpack	Y	Y	Y
Stream	**Station**	**Date**	**Site Type**	**Method**	**Sample Gear**	**FMS**	**BHS**	**RTC**
Gunnison River	Delta to Escalante SWA	7/27/2015		1-Pass	Raft Shocker	Y	Y	Y
Potter Creek	At confluence with Roubideau	4/14/2015	His	1-Pass	Backpack	Y	Y	N
Potter Creek	At confluence with Roubideau	4/21/2015	His	1-Pass	Backpack	Y	N	Y
Potter Creek	At confluence with Roubideau	4/30/2015	His	1-Pass	Backpack	N	Y	N
Potter Creek	At confluence with Roubideau	5/14/2015	His	2-Pass	Backpack	Y	Y	Y
Potter Creek	At confluence with Roubideau	6/2/2015	His	1-Pass	Backpack	Y	Y	Y
Potter Creek	At confluence with Roubideau	6/17/2015	His	2-Pass	Backpack	N	Y	Y
Potter Creek	.23 River miles above Roubideau Confluence	4/24/2015		1-Pass	Backpack	N	N	N
Potter Creek	1.27 River miles above Roubideau Confluence	4/24/2015		1-Pass	Backpack	N	N	N
Potter Creek	Above Roubideau Confluence at temp logger	5/13/2015		1-Pass	Backpack	Y	Y	Y
Potter Creek	5.5 mi U.S. of Monitor Creek confluence	7/29/2015	His	2-Pass	Backpack	N	Y	N
Roubideau Creek	.32 mi U.S. Sawmill Mesa Road Bridge	8/27/2015	His	2-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	1.08 Riv. miles above confluence with Potter	4/23/2015		1-Pass	Backpack	N	N	N
Roubideau Creek	Above Potter Creek confluence	4/14/2015		1-Pass	Backpack/Seine	Y	Y	Y
Roubideau Creek	4.32 mi U.S. of 25 Mesa Road	4/14/2015		1-Pass	Backpack/Seine	Y	Y	Y
Roubideau Creek	0.7 River Mi above confluence with Potter	4/22/2015		1-Pass	Backpack	Y	Y	N
Roubideau Creek	0.7 River Mi above confluence with Potter	5/14/2015		1-Pass	Backpack	Y	Y	N
Roubideau Creek	0.7 River Mi above confluence with Potter	7/7/2015		2-Pass	Backpack	Y	N	N
Roubideau Creek	1.7 Rd Mi from 25 Mesa Rd	4/14/2015		1-Pass	Backpack	Y	N	Y
Roubideau Creek	1.7 Rd Mi from 25 Mesa Rd	7/7/2015		2-Pass	Backpack	Y	N	Y
Roubideau Creek	1.4 River Mi above confluence with Potter	4/23/2015		1-Pass	Backpack	N	Y	Y

San Juan River Basin

Mancos River #3	At Beech St Bridge	8/25/2015	His	2-Pass	Backpack	N	Y	N
Rio Blanco #1	.12 mi D.S. of Blanco Dam	9/30/2015	His	2-Pass	Backpack	N	Y	N

White River Basin

Douglas Creek	Below HWY 64	10/26/2015	His	2-Pass	Bank Shocker	Y	N	N
Piceance Creek	.11 Road Miles D.S. from CR5 Bridge	8/19/2015	His	2-Pass	Backpack	Y	Y	N
Piceance Creek	.41 Road miles U.S of CO Rd 20	8/19/2015	His	2-Pass	Backpack	N	Y	N

Yampa River Basin

Little Snake River	.12 mi D.S. of CR 26 crossing	8/20/2015	His	2-Pass	Bank Shocker	Y	Y	Y
Little Snake River	1.25 Rd Mi from CR-4	9/1/2015		1-Pass	Bank Shocker	Y	Y	Y
Little Snake River	Below Lily Gauge	9/1/2015		1-Pass	Backpack	Y	Y	Y
Little Snake River	5.4 mi U.S. of HWY 318	9/2/2015	His	1-Pass	Backpack	Y	Y	Y
Milk Creek	1.39 Stream Mi above Yampa River	10/7/2015	His	2-Pass	Backpack	Y	Y	Y
Milk Creek	1.86 Miles above Yampa River	10/7/2015	His	2-Pass	Bank Shocker	Y	Y	N
Williams Fork Yampa River	2 Road Miles U.S. of HWY 13	9/23/2015	His	2-Pass	Bank Shocker	N	Y	N

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC

2016

Colorado River Basin

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Dry Owens Creek	190 ft. D.S. of PE Rd	6/28/2016	His	2-Pass	Backpack	N	N	N
Garfield Creek	0.31 Mi. above Unnamed Rd	9/6/2016	His	2-Pass	Backpack	N	N	N
Garfield Creek	0.2 Mi. U.S. Baldy Creek	9/6/2016	His	2-Pass	Backpack	N	N	N
Owens Creek	0.61 Mi. U.S. of Dry Owens Creek	6/28/2016	His	2-Pass	Backpack	N	N	N
Plateau Creek	0.7 Mi. U.S. of Little Wash	9/14/2016	His	Net	Seine	Y	N	N
Plateau Creek	0.7 Mi. U.S. of Little Wash	9/14/2016	His	1-Pass	Backpack	Y	Y	N
Plateau Creek	1 Mi. D.S. of Little Wash	9/14/2016	His	Net	Seine	N	N	N
Plateau Creek	1 Mi. D.S. of Little Wash	9/14/2016	His	1-Pass	Backpack	Y	Y	N
West Divide Creek	1 Mi. Above Little Muddy Gulch on USFS	10/12/2016	His	2-Pass	Backpack	N	Y	N
West Divide Creek	About 650 ft. above Maxfield Rd	10/13/2016	His	2-Pass	Backpack	N	Y	N
West Divide Creek	0.81 Mi. U.S. of Divide Creek	10/13/2016	His	2-Pass	Backpack	Y	Y	Y

Dolores River Basin

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
Dolores River	1.8 Rd Mi. D.S. of Dove Creek Pump Site	8/9/2016		Net	Seine	N	N	Y
Dolores River	1.8 Rd Mi. D.S. of Dove Creek Pump Site	8/9/2016		Net	Trammel	N	N	Y
Dolores River	2.2 Rd Mi. D.S. of Dove Creek Pump Site	8/9/2016		Net	Seine	N	N	Y
Dolores River	4.47 Rd Mi. D.S. of Dove Creek Pump Site	8/10/2016		Net	Seine	N	Y	Y
Dolores River	4.8 Rd Mi. D.S. of Dove Creek Pump Site	8/10/2016		Net	Seine	N	N	Y

Dolores River	5.74 Rd Mi. D.S. of Dove Creek Pump Site	8/10/2016		Net	Seine	Y	Y	Y
Dolores River	6.3 Rd Mi. D.S. of Dove Creek Pump Site	8/10/2016		Net	Seine	N	N	Y
Dolores River	6.9 Rd Mi. D.S. of Dove Creek Pump Site	8/10/2016		Net	Seine	N	N	Y
Dolores River	Big Gypsum Boat Launch	8/11/2016		Net	Seine	N	N	N
Dolores River	Big Gypsum Boat Launch	8/11/2016		Net	Trammel	N	N	Y
Dolores River	At Gypsum Valley Rd Bridge	8/11/2016		Net	Seine	N	N	N
Dolores River	At Gypsum Valley Rd Bridge	8/11/2016		Net	Trammel	N	N	N
La Sal Creek	CO-UT State line	9/27/2016		2-Pass	Backpack	N	Y	N
La Sal Creek	0.4 Mi. D.S. of Spring Creek	9/27/2016		2-Pass	Backpack	N	Y	N
La Sal Creek	0.44 Mi. U.S. of Dolores River	9/28/2016		2-Pass	Backpack	N	Y	Y
Naturita Creek	At HWY 141 Bridge	8/3/2016	His	2-Pass	Backpack	Y	Y	N
Roc Creek	2.5 Mi. above HWY 141	10/4/2016	His	2-Pass	Backpack	N	Y	N
Tabeguache Creek	0.20 Rd miles U.S. of v19 Rd Bridge	6/2/2016	His	2-Pass	Bank Shocker	Y	Y	N
Tabeguache Creek	0.20 Rd miles U.S. of v19 Rd Bridge	8/2/2016	His	2-Pass	Backpack	Y	Y	N
Tabeguache Creek	0.20 Rd miles U.S. of v19 Rd Bridge	9/2/2016	His	2-Pass	Backpack	Y	Y	Y
Tabeguache Creek	0.1 Mi. U.S. of 26-24 Rd junction	9/13/2016		Net	Seine	Y	Y	Y
Tabeguache Creek	0.7 Mi. U.S. of 26-24 Rd junction	9/13/2016		Net	Seine	Y	Y	Y

Stream	Station	Date	Site Type	Method	Sample Gear	FMS	BHS	RTC
		Gunnison River Basin						
Big Dominguez Creek	At Confluence with Gunnison River	4/7/2016	His	1-Pass	Backpack	N	N	N
Cottonwood Creek	About 100 ft. U.S. of 10 Rd Bridge	4/15/2016		1-Pass	Backpack	Y	Y	N
Cottonwood Creek	About 1.25 Mi. U.S. of 10 Rd Bridge	5/9/2016		1-Pass	Backpack+Seine	Y	Y	N
Cottonwood Creek	10.5 Mi. On 25 Mesa Rd from HWY 348	5/10/16	Per	2-Pass	Backpack	N	N	N
Cottonwood Creek	10 Rd Bridge	5/19/2016		1-Pass	Bank Shocker	Y	Y	Y
Cottonwood Creek	About 1 Mi U.S. of 10 Rd Bridge	5/19/2016		1-Pass	Backpack	Y	Y	Y
Dry Creek	155 ft. U.S. of HWY 348 Crossing	7/27/2016	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	1.5 Mi. NE from Cottonwood Spring Crossing	4/7/2016	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	Below McCarty Ditch diversion (barrier)	5/5/2016	His	1-Pass	Bank Shocker	Y	Y	N
Escalante Creek	Walker Cabin	7/28/2016	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	1 Mi. U.S. of Tatum Gulch	8/30/2016	His	2-Pass	Backpack	Y	Y	Y
Escalante Creek	Above Smith Cabin Diversion	9/12/2016		Net	Seine	Y	Y	Y
Potter Creek	At Confluence with Roubideau Creek	5/3/2016	His	1-Pass	Bank Shocker	Y	Y	Y
Potter Creek	At Confluence with Roubideau Creek	5/11/2016	His	1-Pass	Backpack	Y	Y	N
Potter Creek	At Confluence with Roubideau Creek	5/17/2016	His	1-Pass	Backpack	Y	Y	Y
Potter Creek	At Confluence with Roubideau Creek	5/25/2016	His	1-Pass	Backpack	Y	Y	Y
Potter Creek	600 ft. U.S. of Roubideau Creek	6/1/2016		1-Pass	Backpack	N	N	N

Potter Creek	At Confluence with Roubideau Creek	9/29/2016	His	2-Pass	Backpack	N	Y	Y
Roubideau Creek	Montrose/Delta County Line	5/3/2016		1-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	Montrose/Delta County Line	8/30/2016		2-Pass	Bank Shocker	Y	Y	Y
Roubideau Creek	Above Potter Creek	6/1/2016		1-Pass	Backpack	N	N	Y
San Juan River Basin								
Mancos River	At Beech St. Bridge, Mancos	6/29/2016	His	2-Pass	Backpack	N	Y	N
Weber Canyon Creek	1.3 Mi. U.S. of East Canyon	6/29/2016	His	2-Pass	Backpack	N	Y	Y
Weber Canyon Creek	1.3 Mi. U.S. of East Canyon	7/19/2016	His	2-Pass	Backpack	N	Y	Y
White River Basin								
Piceance Creek	0.41 Road miles U.S. of CO Rd 20	6/22/2016	His	2-Pass	Backpack	Y	Y	N
Piceance Creek	Below gauging station to bridge	6/23/2016	His	2-Pass	Backpack	Y	N	N
Piceance Creek	0.41 Road miles U.S. of CO Rd 20	9/7/2016	His	2-Pass	Backpack	Y	Y	N
Yampa River Basin								
Little Snake River	0.12 Mi. D.S. of CR26 crossing	9/8/16	His	2-Pass	Bank Shocker	Y	Y	Y
Little Snake River	5.36 mi U.S. of Hwy 318 crossing	9/8/16	His	2-Pass	Backpack	Y	Y	Y