Three Species Investigations

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Project Title: <u>Three-Species Investigations</u>

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, 2013 to December 30, 2013

Introduction

The so-called three-species assemblage comprises flannelmouth sucker *Catostomus latipinnis*, bluehead sucker *Catostomus discobolus,* and roundtail chub *Gila robusta*. Natives of the Colorado River basin, each species is estimated to occupy only 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. 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 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.

The genetic fingerprints of individuals were analyzed for 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. commersoni,* 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 biologists' 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. We report the results of the latter analysis. Comparing field identifications to genetic identifications, we considered a pure species field identification to be correct if the genetic analysis estimated ≥ 95% the same species. We considered a hybrid call to be correct if the genetic analysis estimated ≥ 95% the same species as called, in combination. We considered a hybrid call incorrect if the genetic analysis identified a different species mix than the field identification, or if there were additional species represented in the specimen other than those called in the field when the 95% rule was not met.

A second genetic method is generally recommended as a means of confirming results. In addition to microsatellite analysis, we chose to conduct analysis of the mitochondrial ND2 gene from 463 fish representing pure specimens as well as specimens field- and microsatellite-identified as hybrids. As mitochondrial genes are passed only through the maternal line, this method allows a check that will provide a definitive answer if an unexpected species assignment is given for a specific fish.

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 student Liz Mandeville is conducting 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 include 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 her project 649 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.

Results and Discussion

Genetic purity - Eight of the 96 samples selected as potential members of the reference populations were excluded from the STRUCTURE analysis due to one or more of the following reasons: Null allele calls (PCR priming site not in the genome), DNA degradation, low DNA concentration, or overly diluted PCR product. For the remaining 88 samples, STRUCTURE was able to differentiate the six sucker species without any population information (all samples treated as unknowns) using the alleles scored for each sample at the six loci (Figure 1). Nine of the 88 samples were predominantly assigned by STRUCTURE to species inconsistent with the original identification of the sample. One of the inconsistencies involved a purported razorback larval sucker that displayed bluehead sucker microsatellite alleles; the other eight are evident in Figure 1 (razorback not displayed). These eight samples were checked for ambiguities in the raw genotyping data and for possible sample identification errors during the sample logging or DNA handling procedures. No errors were detected, so the original identifications in the field were scrutinized for reliability. In all cases evaluators of those collections indicated there was a reasonable probability that such samples had been mis-identified when collected. All the mis-assigned samples were collected early in the process when collecting biologists were less comfortable and experienced with sucker identifications. Moreover, the samples in question all came from waters with non-native suckers and their hybrids present, further clouding the reliability of visual identification in the field. Consequently these samples were eliminated from the candidate pool. Six additional samples were also eliminated based on admixture levels that support the possibility that they were truly hybridized – four bluehead suckers showing more than 3% purported admixture, the longnose sucker at position 44(3) in Figure 1 and the white sucker at position 69(6).

The remaining samples were deemed pure enough to represent the respective species and became the initial reference population set. To these, an additional 19 mountain sucker samples from

Vermillion Creek were added because the mountain suckers in that stream produce genetic signals that appear quite unique compared to those obtained in other northwest Colorado collection locations. These reference populations each represented widespread locations across the Northwest Region of Colorado Parks and Wildlife. The total numbers of fish in each initial reference population were 11 bluehead suckers, 13 flannelmouth suckers, 10 longnose suckers, 35 mountain suckers, and 14 white suckers. The razorback sucker reference population is not presently being used because the 11 razorback samples involved consisted of just seven hatchery samples and four larvae so geographic representation is poor.

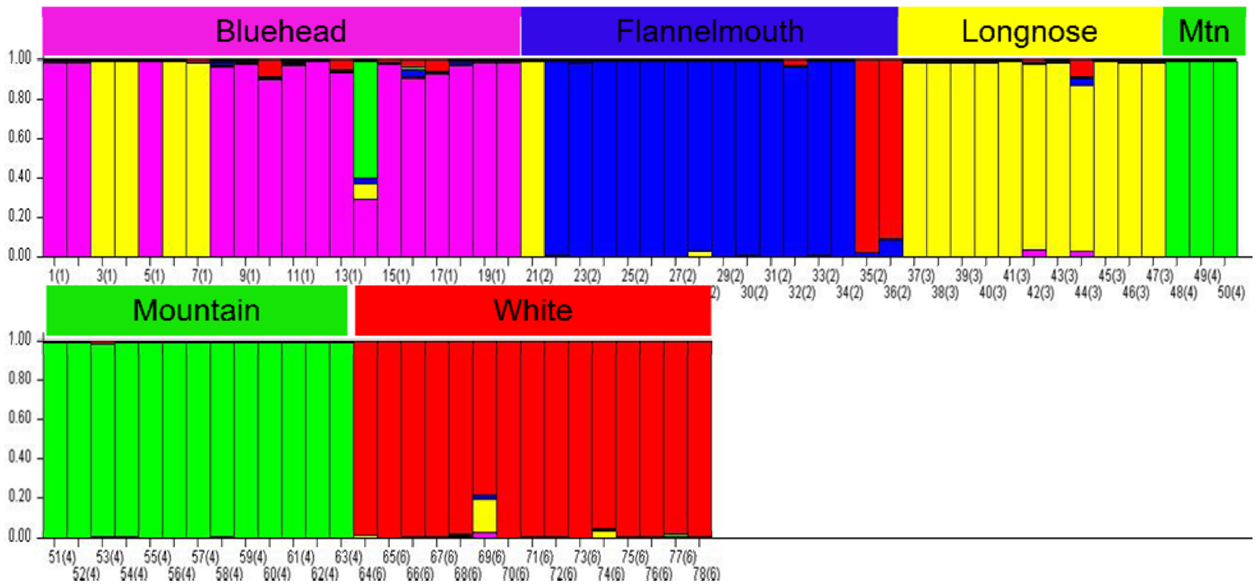

Figure 1. Species assignment based on analysis of six microsatellite loci of 78 sucker specimens selected as candidates for reference populations (razorback sucker not displayed).

The purity analysis for 137 purported bluehead suckers revealed 9 specimens that were predominantly assigned to species or species mixes that were not bluehead. These specimens were all collected in 2010 and 2011 (prior to the sucker and hybrid sucker identification workshop), and likely were mis-identifications in the field. Among the remaining samples, 12 exhibited 5 – 15% admixture. Otherwise, pure bluehead suckers were found in nearly all sampled drainages, with Milk Creek being the exception. This result does not mean that there are no pure bluehead suckers in Milk Creek, since sampling was limited and only 5 individuals were included. Nor does it mean that other drainages are free of hybridization, since sampling was not conducted randomly and these fish were thought to be bluehead suckers at the time of collection.

The purity analysis for 139 flannelmouth suckers following establishment of the reference populations revealed only three fish with significant admixtures of other species, suggesting that field identification of flannelmouth suckers for this data set was more reliable than for bluehead suckers. An important note on one of those admixed fish, however, is its origin in the Dolores River above Disappointment Creek. There are few records of non-native suckers in the Dolores, but this result establishes a new one.

The results of these initial genetic analyses suggested CPW should acquire just one additional broodstock of bluehead suckers, to represent the Southwest Region. Thirty fish were collected for this purpose in May 2012 from the lower San Miguel River, a location with very few indications of non-native sucker invasion. However, one fish was collected during this effort was identified as a white-flannelmouth hybrid which was later confirmed genetically. Additionally, Shiozawa et al. (2003) mention observing one white sucker in the San Miguel near Tabeguache Creek. Although all 30 fish exhibit bluehead sucker phenotypes, the genetic analysis of this set of fish revealed six fish with ≥ 5% admixture with other species (one fish nearly 50% admixture). Among these six most admixed fish, white sucker was the predominant foreign signal in two fish, longnose sucker predominated the foreign signal in three fish, and mountain sucker was the predominant foreign signal in the last fish. Of these admixtures, mountain sucker seems the least probable.
These results raised concerns, since all 30 fish were phenotypically bluehead sucker. Perhaps the assignment test is less reliable for these fish because the Dolores River basin was represented by only three fish in the original reference population. With few records of any non-native or hybrid suckers in the basin, this seemed reasonable. To test this, we added 18 of the candidate brood fish showing ≥ 98.5% purity to the reference population temporarily and re-ran the analysis. This resulted in the upgrade of a single fish to the same level of purity, which was added to the reference population and the analysis re-ran. This exercise was repeated six times. No more fish attained ≥ 98.5% purity after six iterations, but four additional fish reached 98.4% purity. This left three fish still testing as strongly admixed (> 5%), and the fish that tested ~ 50% impure exhibited no significant movement. This problem has still not been fully resolved, but we are currently revising all reference populations to include greater geographic representation, as well as transitioning from identifying microsatellite peaks by human observer to identifying them with program TANDEM designed for this task. Our testing has shown that the new method will change species apportionments somewhat, but has the distinct advantage of true objectivity among analyses and the power to deal efficiently with large numbers of fish in a single analysis.

An additional possibility is that the suite of reference population species is not diverse enough. The program STRUCTURE is required to apportion sample fish into the five available species, so if there were to be genetic material present from a different species it would be forced to assign it to the “closest match”. Both Shiozawa et al. (2003) and Douglas et al. (2007) described the finding of bluehead haplotypes in the upper Colorado River basin that most closely match desert sucker sequences, suggesting that desert sucker, and perhaps other geographically nearby species, should be included in our reference populations. Outreach to colleagues to obtain desert sucker DNA has thus far been unsuccessful, but there is opportunity in 2014 to obtain fresh tissue samples from investigators in Arizona and Nevada.

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. Such a result was good, but not definitive since there were just 30 specimens. If introgression has 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.

Measures were taken in 2013 to expand the reference populations. After the purity analysis for bluehead sucker, 56 specimens were added to that reference population from streams across the western slope, enlarging it to 67 individuals. Additionally, 80 purported white sucker and 40 purported longnose sucker specimens were collected from sites in eastern Colorado (n = 10 per site for each species) to bolster reference populations for these species with fish from within the native range for each. These samples have been submitted, DNA extracted, and are undergoing analysis as of March 2014. We also continue to seek geographic near-neighbor species samples.

The 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 (see Table 1). If hybrid field identifications are recognized as correct even when the specific species mix isn't accurate, the level of congruent results rises to 86.2%. The best rates of accurate identification are among the three-species suckers, and the greatest difficulties are encountered with mountain and white suckers. Of note, though, is that half of the white sucker field identifications not corroborated by microsatellite assignment were from eastern Colorado streams where CPW biologists collected potential white sucker and longnose sucker reference population fish, and half of those came from a single stream. This suggests two scenarios. First, that white sucker – longnose sucker hybridization is common in eastern Colorado and second, that these hybrids require some care to differentiate from pure parental species.

Table 1. Comparison of field identification of sucker specimens with microsatellite genetic assignment using TANDEM automated binning software and STRUCTURE for assignment.

Field identification	Microsatellite identification	N	% correct
BHS	BHS	93	90.3
	Hybrid	10	
FMS	FMS	63	94.0
	Hybrid	4	
LGS	LGS	37	84.1
	Hybrid	7	
MOS	MOS	24	63.2
	Hybrid	13	
	BHS	1	
WHS	WHS	67	72.8
	Hybrid	24	
	LGS	1	
Hybrid	Hybrid – correct	30	44.1
	Hybrid – incorrect	31	
	BHS	1	
	FMS	1	
	LGS	3	
	MOS	1	
	WHS	1	

Among hybrids, despite the poor rate of congruent identifications, 89.7% of specimens identified as hybrid in the field were actually hybridized fish. This demonstrates that it is often difficult to identify parental species in the field, and frequently genetic analyses show contributions from more than two species (Table 2), indicating the back-crossing of hybrid individuals. Many of the hybrid identifications labeled incorrect actually had the species represented, but additional species as well. Most of the hybrid sucker congruent results (29 of 30) were represented by those identified in the field as WXF, FXB, and WXB. Seven fish genetically called pure were called hybrids in the field (1.7%) and 58 fish genetically called hybrids were called pure in the field (14.1%).

Table 2. Details of incongruent identifications showing the field identification as column headings, and in each column the microsatellite species assignments for those field identifications. Numbers of occurrences are in parentheses, unless n = 1, as are species combination requiring more than one row to display. A given species was added to the species mix for a hybrid sucker when it exceeded an estimated 2.5% of genetic representation.

BHS	FMS	LGS	MOS	WHS	FxB	WxF	WxB	LxF	WxM	WxL
FxB (4)	FxB	BxL (5)	MxL (3)	WxL (12)	BxL	WxFxB	WxF	MxL	WxB (3)	WxF (2)
WxB (3)	WxF (4)	MxL (2)	MxB (4)	WxB (3)	WxL	(WxFxBxL) (2)	WxBxL (2)	FxB	MxL	WxB
FxBxW (2)		FxB	WxB	WxF (3)	FxBxL (2)		WxMxB			
		WxL	WxM	BxL	WxBxF (2)	(WxFxBxMxL) (2)	(WxBxFxL) (2)		(WxMxFxL)	(WxBxF)
			WxBxL	WxFxB (2)	(WxBxFxL)		(WxBxFxMxL)			(WxBxFxM)
			WxMxF (2)	WxMxL (2)						
				(WxLxMxB)						
				(WxLxFxB)						

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.

Genetic diversity – No additional genetic diversity analyses were conducted in 2013 with microsatellites. However, the ND2 analysis will provide an additional frame of reference for diversity as haplotypes are identified and examined for basin presence and frequency of occurrence. Those identifications of haplotype are underway.

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. Potential additions would comprise desert sucker and Utah sucker. Acquisition of genetic material representing these additional species is being pursued.

Accurate field identification of suckers and sucker hybrids will continue to be a problem, 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. As specimens have been added, it would be advantageous to facilitate another sucker identification workshop.

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, 2013 to December 30, 2013

Investigators

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Introduction

State and federal agencies are concerned with the declines of flannelmouth sucker *Catostomus latipinnis*, bluehead sucker *Catostomus discobolus* and roundtail chub *Gila robusta* (herein after referred to as the three-species). The three-species were once widespread and abundant in the Upper Colorado River Basin. In 2002, a review of available literature and collection records suggested each species presently occupied 50% or less of its historical range and extant populations were declining in many localities (Bezzerides and Bestgen 2002).

The declines of the three-species can be attributed to anthropogenic changes in their ecosystem. Non-native predatory fish threaten native fish species; they prey upon and compete for resources with adult, juvenile and larval native fish (Martinez et al. 1994; Bestgen et al. 2006). Hybridization between the non-native white sucker *Catostomus commersonii* and both the bluehead sucker and flannelmouth sucker is likely the biggest threat to the genetic integrity of these species (Quist et al. 2009). The construction of dams has altered runoff patterns, changed thermal regimes, fragmented fish populations and retained sediment throughout their habitat (Martinez et al. 1994; Collier et al. 1996; Ward et al. 2002).

Temperature and flow cues are likely to influence spawning events for the three-species (Zelasko et al. 2011) and may cue movement patterns for adult and juvenile three-species fishes. To investigate the impacts of flow and temperature we chose the Upper White River because it is undammed, has a natural hydrograph, and all of the three-species present (Chart and Bergersen 1992).

Background and Life history – The flannelmouth sucker is a member of the family Catostomidae. It is differentiated from other suckers by its lack of a scraper plate, large fleshy lobes on its upper and lower lips, narrow caudal peduncle and small scales along the lateral line. Typical adults are green or bluish-grey dorsally, yellow to dark-orange ventrally and are typically measured between 400-500 mm in total length (TL) (Bezzerides and Bestgen 2002).
The bluehead sucker is also a member of the family Catostomidae. It is characterized by its prominent scraper plates on the upper and lower jaws, blue head, notches on the lateral margins of the upper and lower lips and lack of papillae on the anterior face of the upper lip. This benthic fish is typically 300-450 mm TL but may vary in size depending on the river.

The roundtail chub is a member of the *Gila* genus. It is a highly variable fish morphologically. Roundtail chubs can be readily identified by a combination of morphologic characteristics, and by the lack of other definitive characteristics of similar Gila species, such as the nuchal hump of the humpback chub *Gila cypha* and the thin elongated caudal peduncle of the bonytail *Gila elegans*. Typical roundtail chubs are between 200-300 mm TL but can be over 500 mm TL. Non-breeding adult roundtail chubs are green to bluish-grey dorsally and silvery white ventrally. Breeding adults express tubercles and red-orange coloring along their ventro-lateral surface and on all fins except the dorsal fin.

All three-species share some similar life history characteristics. They all are long-lived (Bezzerides and Bestgen 2002), which may enable populations to withstand poor reproductive years. They all evolved in systems characterized by highly variable runoff patterns, high turbidity and few predators (Bestgen et al. 2006). They are all migratory species (Compton et al. 2008; Breen and Hedrick 2009; Budy et al. 2009), spawn over gravel bars, their eggs are sticky and demersal (Bezzerides and Bestgen 2002).

Specific Objectives and Study Questions:

1) Determine the current distribution of the three-species in the Upper White River.
2) Is distribution related to temperature and flow?
3) What is the timing and location of reproduction?
4) Is reproduction correlated to temperature and flow?
5) Do the three-species exhibit site fidelity to spawning reaches?

Methods

In April 2011, we placed HOBO thermal loggers throughout the study area in both the mainstem White River and tributaries. Temperature data from the HOBO thermal loggers will provide fine scale temperature data and were programmed to record one temperature reading every hour. Data from the HOBO readers was downloaded in autumn 2011-13; temperature monitoring will continue in the White River for the remainder of this project.

We began fish sampling in May of 2012 and 2013 by placing four hoop nets in Coal Creek, a tributary to the White River, CO, and at least three nets remained in Coal Creek until June. Trapping ceased when the catch rate dropped to zero for seven consecutive days. All fish were tagged with Oregon RFID passive integrated transponder (PIT) tags inserted into the body cavity and released back into Coal Creek. Trapping was conducted in Piceance Creek as well.

Two remote antennae arrays were placed in Coal Creek. The arrays were constructed as loops located on the bottom of Coal Creek and spanning the entire stream width. Each array consisted of two separate antennae, separated by about 10 feet. Placing two antennae at each location allowed us to detect the direction of movement of tagged fish. One array was located near the mouth of Coal Creek (referred to as "Nelson") while the other was located 1.1 miles upstream (referred to as "Strang"). A third antennae array was assembled and activated in Piceance Creek, another major tributary to the White River. The Piceance Creek array is located approximately 1.6 miles upstream from the mouth of Piceance Creek.

We completed five raft electrofishing surveys on the mainstem White River, which included sampling of three reaches, two reaches sampled twice and one reach sampled once. Electrofishing surveys were accomplished with two inflatable rafts, with one raft sampling on each side of the river.

Larval samples were collected in 2012 and 2013 (Figure 2). In addition to early life stage fish sampling at fixed stations, in 2012 we completed six surveys of the mainstem White River using inflatable rafts. Each raft survey consisted of three sections of the White River that were each floated twice and a total of 77 samples were collected.

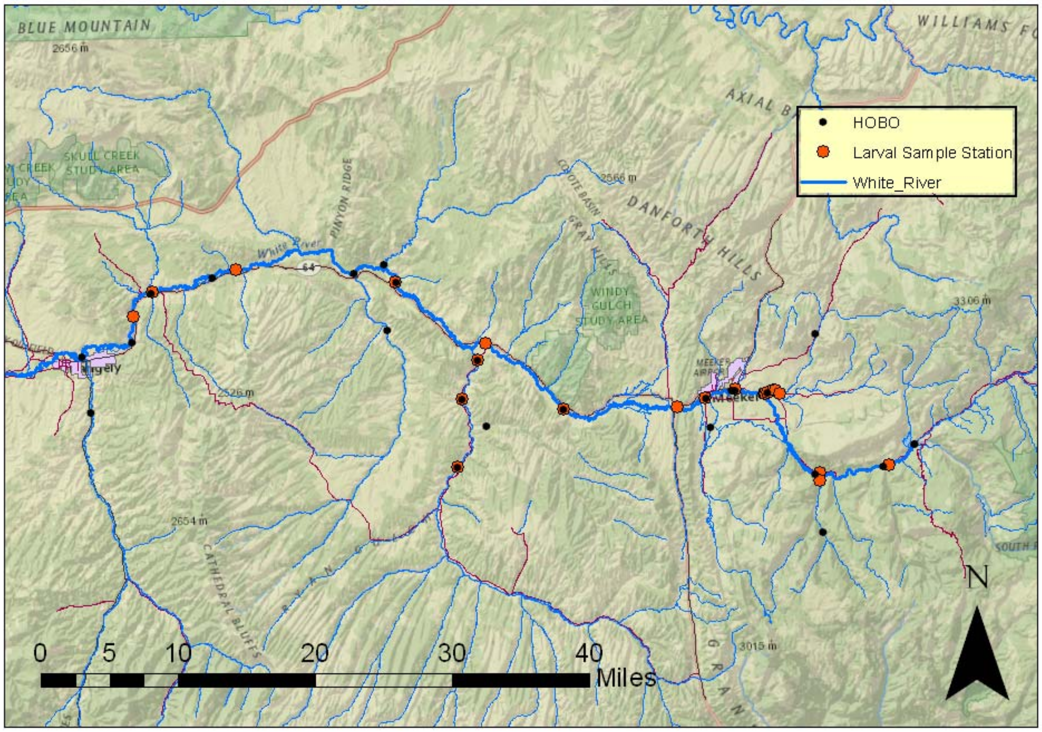

Figure 2. The study site for this project, the upper White River, CO, showing the locations of larval shore sampling sites and HOBO thermal loggers.

Results and Discussion

We tagged flannelmouth suckers (FMS) and bluehead suckers (BHS) in Coal Creek from 2011-2013 and in Piceance Creek from 2012-2013. Piceance Creek flows were very low in both years and few fish were marked in that stream or detected by the Piceance antenna, so the Piceance Creek data are not presented. Recapture numbers were relatively low for fyke nets in Coal Creek but they did indicate a low level of site fidelity from year to year (Table 3). The Coal Creek antenna arrays detected more fish than fyke netting and indicated return rates of 53% for fish tagged in 2011 and 21.5% for fish tagged in 2012 (Table 4).

Table 3. Fyke net capture-mark-recapture data for flannelmouth and bluehead suckers in Coal Creek.

Year	Number Marked	Recaptures				
		2011	2012	2013	Total Unique Tags	% Unique Tags
2011	75	6	7	7	14	18.7
2012	275		3	7	7	2.5
2013	184			6	--	--

PIT tag detections on the antenna array clearly show that the majority of suckers leave Coal Creek very soon after they are captured and released (Table 5). In 2012, 84% left within 24 hours and 90% in 48 hours. We observed a similar result in 2013. This behavioral response to tagging has implications for monitoring sucker spawning and population numbers. Clearly, making population estimates in Coal Creek will not be possible using traditional mark-recapture techniques. Additionally, mark-recapture electrofishing efforts in the mainstem White River may also be biased due to emigration caused by handling. Our recapture numbers were low, which could be due to behavioral responses to handling (Table 6).

Table 4. Number of PIT tags detected in 2012 and 2013 in Coal Creek at two antenna arrays. The last two columns show only data for years subsequent to the year fish were marked. Nelson refers to the lower antenna and Strang to the upper antenna.

Year Marked	Number Marked	Recaptures					
		2012 Nelson	2012 Strang	2013 Nelson	2013 Strang	Unique Tags	% Unique Tags
2011	75	23	20	31	26	40	53.3
2012	275	270	82	59	48	59	21.5
2013	184			181	121	--	--

The antenna arrays in Coal Creek were efficient at detecting tags, indicated by the detections of marked suckers leaving Coal Creek soon after marking. Moreover, 100% of the 2012 tagged fish and 99.5% of the 2013 tagged fish were eventually detected leaving Coal Creek.

Netting and antenna data indicate that suckers entered Coal Creek early in May and moved back to the White River by late June (Figure 3 and 4, antenna data not shown). Suckers that were not trapped or handled (antenna data only) remained in Coal Creek for about 14 days but the range of residence time was large (Table 7). Peak spawning appears to be earlier for FMS but both species co-occur in Coal Creek during the spawning season (Figure 3 and 4).

Table 5. The number of suckers marked in 2012 and 2013 subsequently detected leaving Coal Creek on the Nelson array within 24 and 48 hours of being implanted with a PIT tag.

	Marked	24-hr (N)	24-hr (%)	48-hr (N)	48-hr (%)
2012	275	230	84	18	7
2013	184	149	81	9	5

Table 6. The number of suckers marked and recaptured during electrofishing sampling of three separate sections on the mainstem White River.

	2012		2013	
Location	Mark	Recap	Mark	Recap
Tux - Cox	216	21	152	13
Schultz-2x	207	4	--	--
Bailey-RV	329	18	237	6

Table 7. Average and range of the number of days that flannelmouth (FMS) and bluehead (BHS) suckers remained in Coal Creek for antenna-detected, PIT-tagged fish that were not handled.

	BHS		FMS	
	Average	Range	Average	Range
2012	14.44	(1-27)	9.70	(1-16)
2013	14.15	(1-30)	13.29	(1-32)

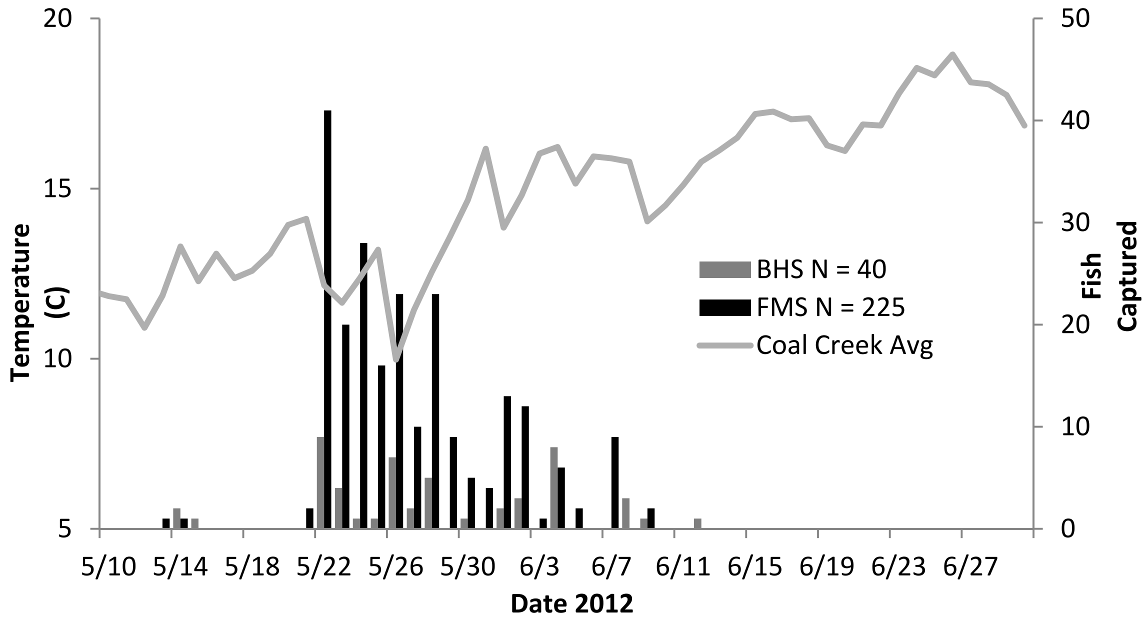

Figure 3. The number of flannelmouth and bluehead suckers captured in fyke nets in Coal Creek with the Coal Creek daily average temperature in 2012.

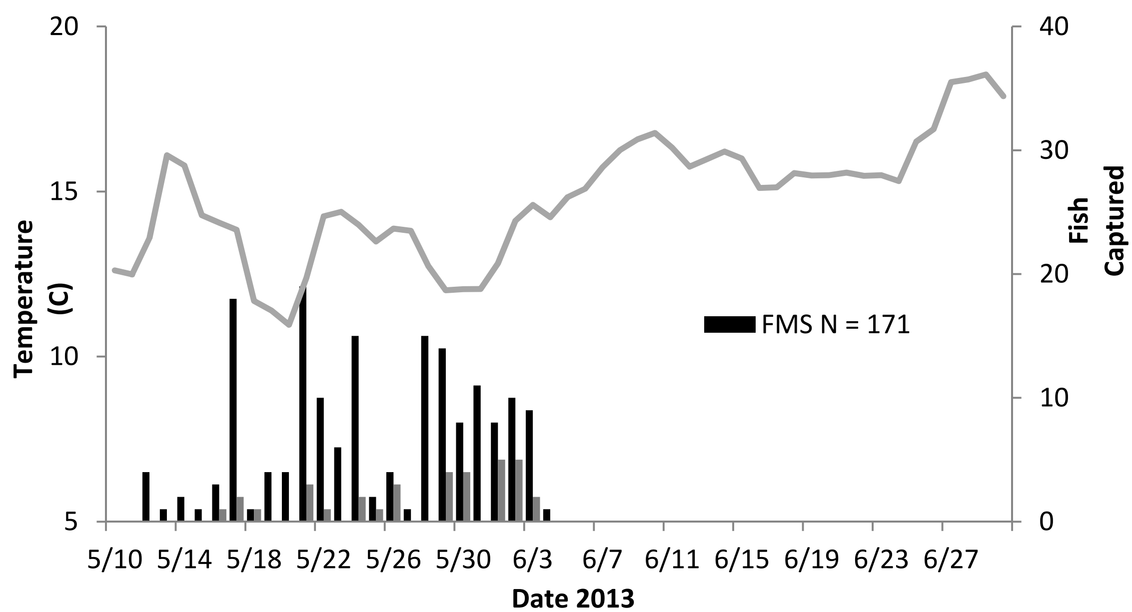

Figure 4. The number of flannelmouth and bluehead suckers captured in fyke nets in Coal Creek with the Coal Creek daily average temperature in 2013.

Average temperature in Coal Creek is higher than in the White River (Figure 5 and 6). Whether temperature differences contribute to differences in spawning timing and duration are currently being assessed using larval fish collections and back-calculated spawning dates estimated from otolith aging. These analyses are ongoing and should be completed by May 2014. We are also developing a predictive model for water temperature in the White River. We will use the model to predict changes in water temperature that may occur due to climate change and use these predictions to assess the potential changes in spawning distributions and timing. These analyses are ongoing and should be completed by June 2014.

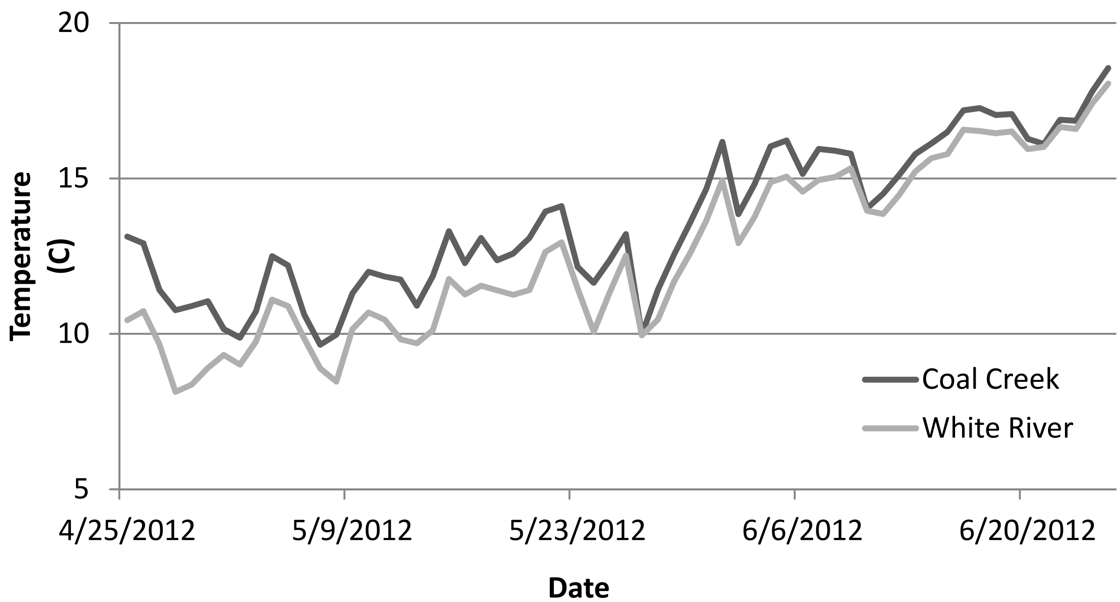

Figure 5: The daily average temperature in Coal Creek and the mainstem White River near Coal Creek during the sucker spawning period in 2012.

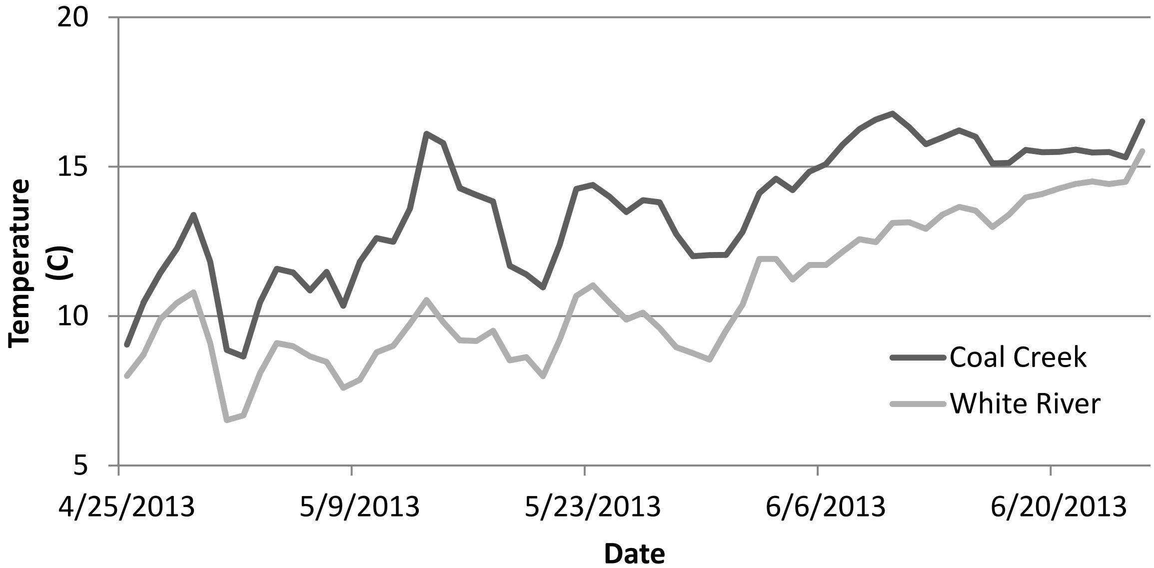

Figure 6: The daily average temperature in Coal Creek and the mainstem White River near Coal Creek during the sucker spawning period in 2013.

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, 2013 to December 30, 2013

Introduction

The so-called three-species assemblage comprises flannelmouth sucker *Catostomus latipinnis*, bluehead sucker *Catostomus 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 3 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 1/8, 4/19 and 9/25 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 1200 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 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 7). 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.

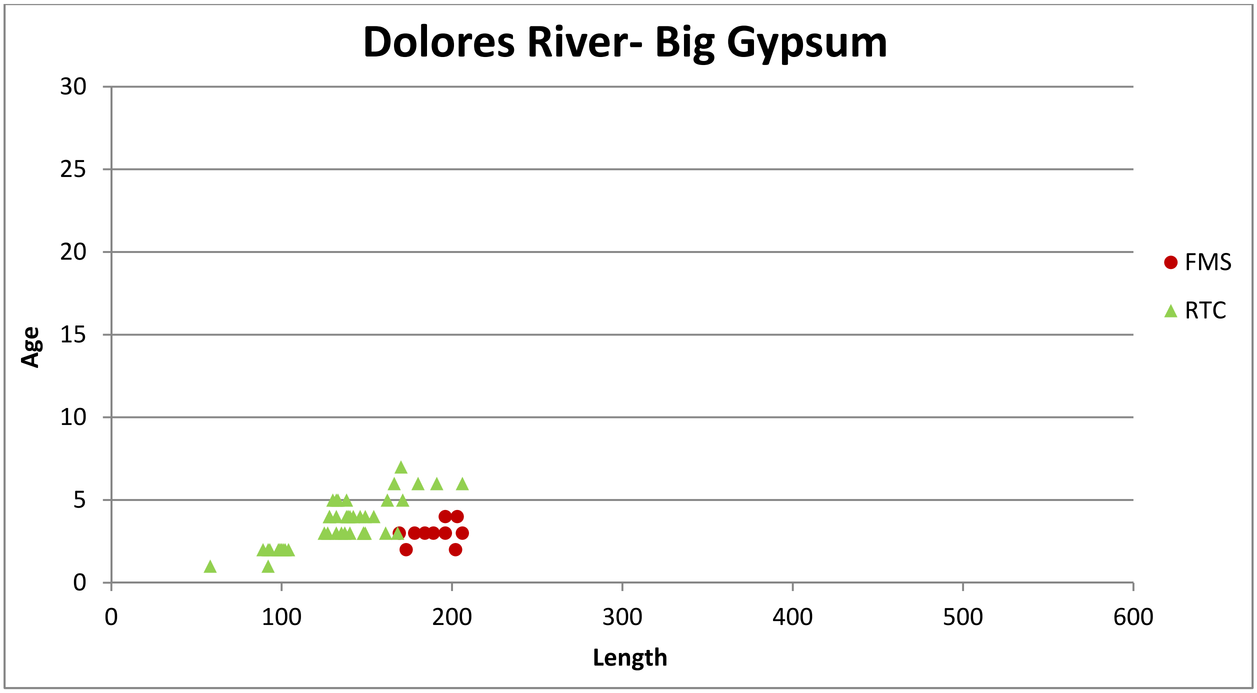

Figure 7. Age estimates of fish from the Dolores River, by length. Collected August 2013.

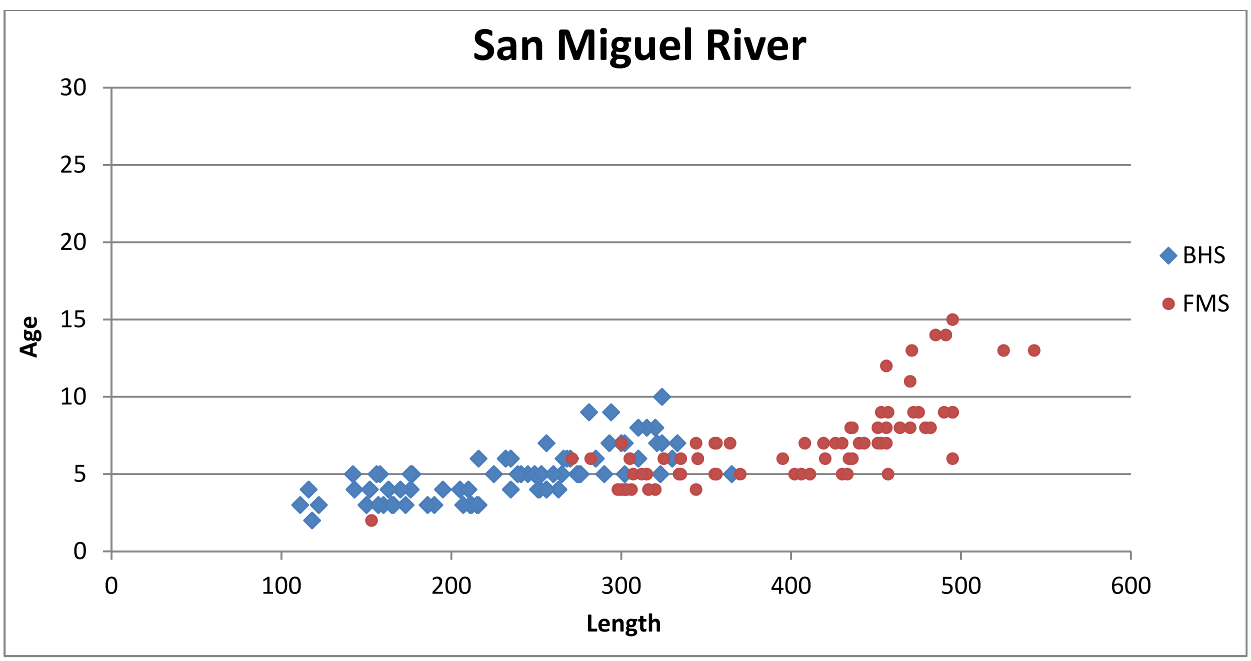

Figure 8. Age estimates of fish from the San Miguel River, by length. Collected May 2012.

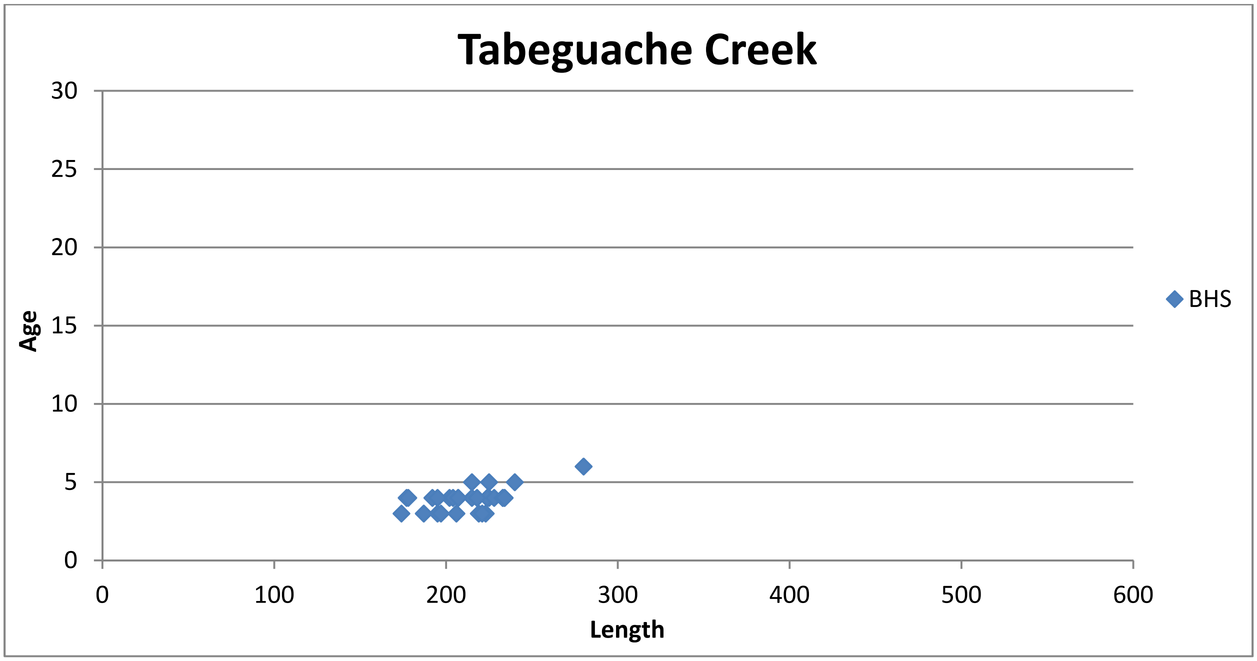

Figure 9. Age estimates of fish from Tabeguache Creek, by length. Collected May 2013.

Gunnison River Basin

Fin ray collections were obtained from three Gunnison River tributaries during spring spawning runs, Escalante Creek (Figure 10) and Roubideau and Potter creeks (Figure 11, 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 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.

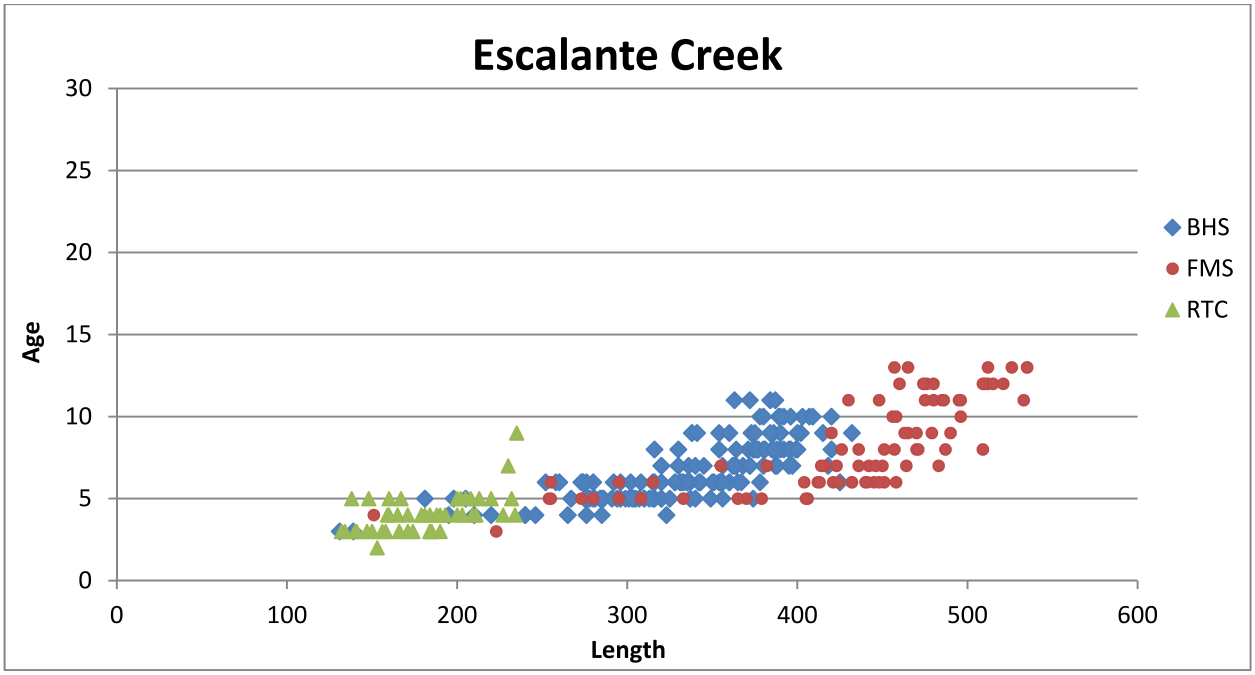

Figure 10. Age estimates of fish from Escalante Creek, by length. Collected May, 2012 and 2013.

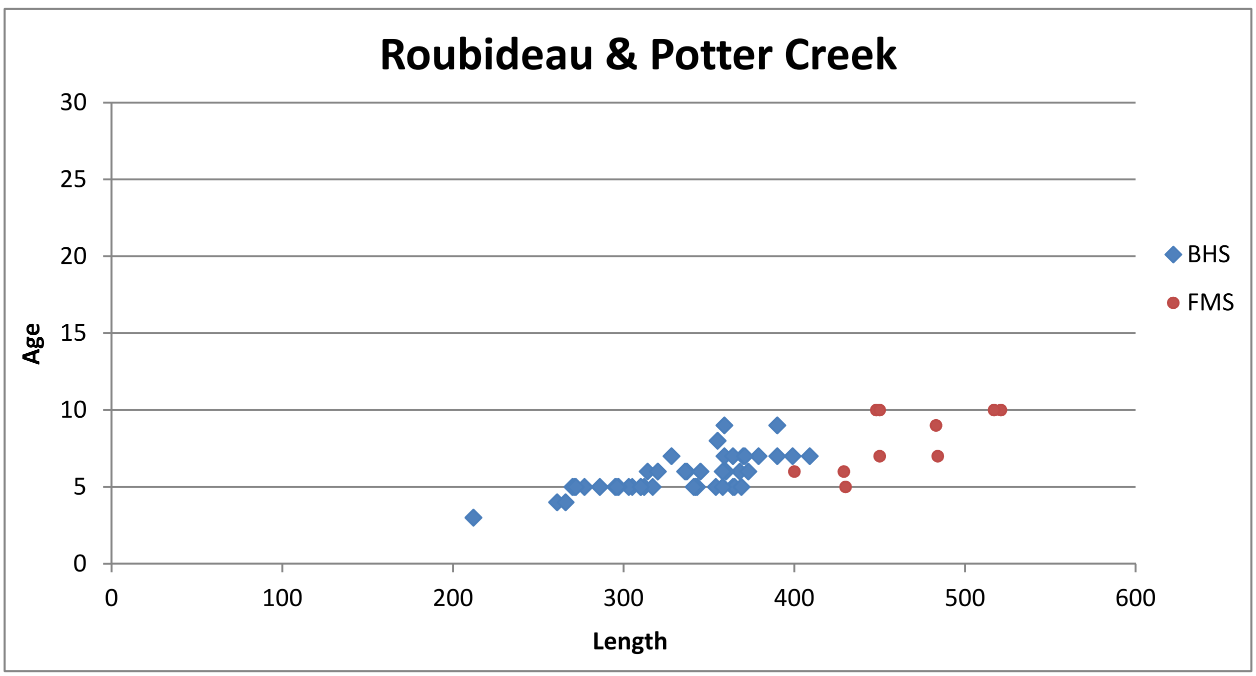

Figure 11. Age estimates of fish from Potter and Roubideau creeks, by length. Collected May 2013 at the confluence of the two streams.

White River Basin

Fish were aged from two locations in the White River, a section in Meeker (Figure 12) and another about 5 miles above Kenney Reservoir (Figure 13). 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 et al. 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 will be 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=09) 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 strongly suggest 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.

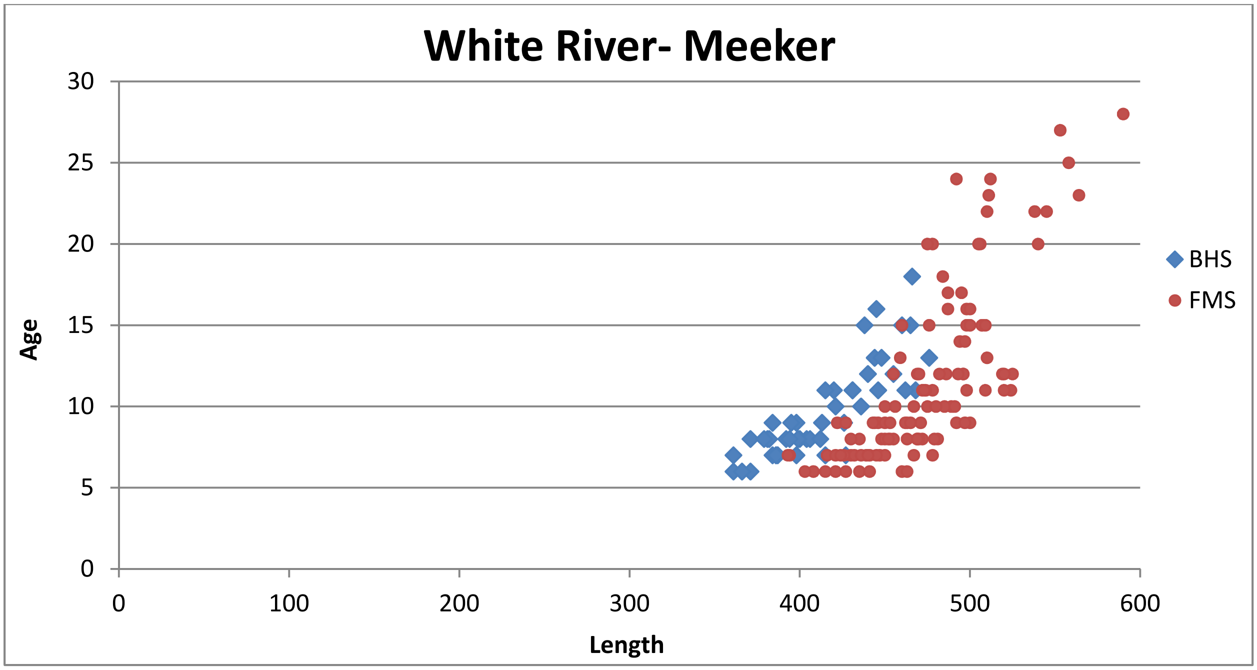

Figure 12. Age estimates of fish from the White River at Meeker, by length. Collected June 2012 and June 2013.

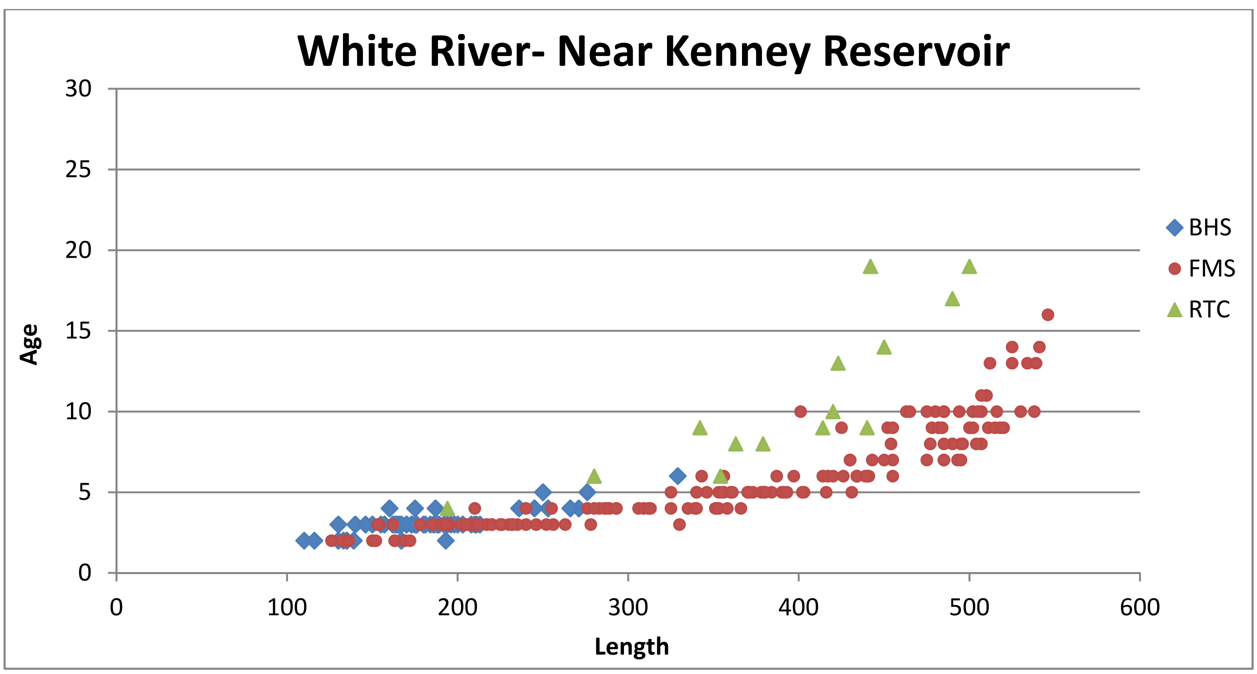

Figure 13. Age estimates of fish from the White River above Kenney Reservoir, by length. Collected June 2012 and June 2013.

Job No. 3

Job Title: Current Distribution of the Three-Species

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

Period Covered: January 1, 2013 to December 30, 2013

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 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 8).

Table 8. 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, denied permission, 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 3.9% 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. 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 three-species fishes that were not captured with electrofishing.

Initial site occupancy and capture probability estimates for each of the three-species for 2012 were obtained using occupancy models available in program Presence (Hines 2006). Data acquired in 2013 were analyzed cumulatively with the 2012 random site data in program MARK (White and Burnham 1999). Occupancy models have been a useful tool in ecological research since being developed and first applied to amphibians (MacKenzie et al. 2002). This analysis framework allows a wide variety of approaches to problems where target organisms are detected imperfectly and issues of site colonization or extinction are in play (MacKenzie et al. 2006).

Results and Discussion

Six intermittent sites and 36 perennial stream sites were sampled in 2013. Six additional intermittent sites were visited but not sampled because they were dry. Nine additional perennial sites were visited but not sampled. In some cases these "perennial" stream segments were dry, in others there was simply not enough water to sample. All waters sampled under this research project in 2013, as well as those sampled in 2011 and 2012, are listed in Appendix 1.

Representatives of the three-species were found at 11 of the 36 perennial random sites, but at none of the six intermittent sites (Table 9). All three species were detected at the same site in Escalante Creek (1 site) and the Little Snake River (2 sites).

Occupancy modeling for combined 2012 and 2013 data indicated that detection probabilities (p) for the three species (p, given presence at the site) were 0.76 (se 0.08) for bluehead sucker, 0.73 (se 0.10) for flannelmouth sucker, and 0.92 (se 0.08) for roundtail chub. These probabilities of detection are based on general models incorporating all the random sites sampled to date. Site occupancy estimates (Ψ) in perennial random sites were 0.25 (se 0.06) for bluehead sucker, 0.16 (se 0.05) for flannelmouth sucker, and 0.07 (se 0.03) for roundtail chub. Among intermittent random sites, Ψ = 0.11 (se 0.10) for both the bluehead and flannelmouth suckers, and Ψ = 0 for roundtail chub (which were not detected at any intermittent random site sampled).

The application of a stream gradient filter in 2013 removed many sites from the sampling schedule, but did not greatly increase the rate at which sites sampled were found to be occupied. Considering perennial and intermittent sites, we handled three-species fishes at 24% of sampled sites 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. Future occupancy modeling will incorporate stream gradient as a site covariate, as it is likely that even a 3.9% gradient restriction may too liberal, given how few ADAMAS records for three-species fishes are attached to stream locations where gradients exceed 2.0%.

Conclusions and Recommendations

After two seasons of field work, we have 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 have been dry even though time of visitation was limited to the runoff season when they would most likely carry water. Thus these sites have been uninformative and have cost a lot of time that could otherwise have been spent visiting additional perennial sites. Although we know from experience that three-species fishes use intermittent waters, I think our search efforts would be better spent on perennial waters in 2014.

In 2014 attention will shift toward historic waters, although some new random perennial sites as well as some where we encountered three-species fishes over the last two years will be visited. Our list of historic sampling stations was assembled, subjected to the same filter system and randomized with the same algorithm as the random waters. The randomization was recommended rather than opportunistically taking advantage of biologist-collected data at historic sites because with the three-species not all historic sites can be visited, so this will achieve the spatial balance in the same fashion as we did for the random sites.

Table 9. Summary of the three-species, white suckers, and select sucker hybrids detected at each random site sampled in 2012 and 2013 (dry sites excluded). SITE codes incorporate an initial letter to describe site type: "I" = intermittent, and "P" = perennial. A "+" indicates that species or hybrid was found at the site and a "-" indicates it was not detected. Area is the CPW 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

SITE	RTC	FMS	BHS	FXB	WHS	WXF	WXB	Area	Date	Stream
P051	-	-	-	-	+	-	-	16	7/2/13	Leroux Creek
P053	-	+	+	-	+	+	-	6	6/17/13	Milk Creek
P054	-	+	+	+	+	-	-	15	7/30/13	Rio Blanco #1
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)

Job No. 4

Job Title: Technical Assistance

Job Objective: Provide information on three-species research or boreal toad research to Colorado Parks and Wildlife Management and Hatchery Sections and to other interested agencies or publics.

Period Covered: January 1, 2013 to December 30, 2013

Corresponded via email with Kevin Wheeler of Utah Division of Wildlife Resources to provide assistance as requested, March - April 2013:

"Utah Division of Wildlife is creating a Captive Management Plan for our refuge Paunsaugunt Plateau Population of boreal toads, and I'd asked Ted Smith a couple questions, to which he responded that you may be able to answer them better. Here are my questions:

1) Has Colorado determined a target number of lots (or individual toads) to represent each wild population that you've brought into captivity?

2) What information led you to determine that target number? Do you think it represents your wild population genetically?

Thanks in advance for any info you can provide, and I appreciate all your facility has done to help conserve our Utah boreal toad population."

Several follow-up emails were exchanged, and I reviewed the "Draft Paunsaugunt Plateau Boreal Toad Captive Management Plan" for Kevin Wheeler.

Continued in 2013 to perform budget analysis to estimate funds needed for chytrid testing. After field season, collated and submitted boreal toad *Batrachochytrium dendrobatidis* samples, and dispersed results appropriately.

Manuscript review for Northwest Science on a submitted paper examining *C. Shasta* invertebrate host populations following high flow events. November 2013.

Provided CSU PhD candidate Brittany Mosher with *B. dendrobatidis* database of results. This student is studying chytrid fungus – boreal toad dynamics and will be using our extensive *B. dendrobatidis* data in her studies. CPW stands to benefit from her research using these data.

Consulted with Brian Holmes, CPW Conservation Biologist, Meeker, on fisheries sampling stations and fisheries data from Flag Creek. This stream is a tributary to the White River in Meeker, and fishery data were requested in support of a proposed conservation easement.

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Appendix I

Sampling Conducted Under the Three-Species Research Program 2011 - 2013

Site Type column is filled only for sites that were part of dual frame sampling scheme, where “Int” = intermittent stream and “Per” = perennial stream. 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 Pothole 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
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
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
Big Beaver Creek	0.85 mi U.S. of Big Beaver Basin Trl crossing	10/3/2012	Per	2-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
Gill Creek	0.8 mi U.S. of confluence with Kannah Ck	8/1/2012	Per	2-Pass	Backpack	N	N	N
Kannah Creek	1.3 mi on G.S. Rd from Lands End Rd	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						
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
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
		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